



Estimating the diversity of tropical anurans in fragmented landscapes with acoustic monitoring: lessons from a sampling sufficiency perspective

Paula Ribeiro Anunciação^{1,2} · Larissa Sayuri Moreira Sugai^{3,4} · Felipe Martello⁵ · Luis Marcelo Tavares de Carvalho¹ · Milton Cezar Ribeiro²

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Abstract

Determining the distribution and abundance of populations is the first step toward assessing biodiversity conservation status. This step is based on field observations that are largely influenced by the sampling method employed. Autonomous Recording Units (ARUs) are tools developed to improve species monitoring that employ acoustic communication. Although largely employed, the efforts required to achieve good diversity estimates with this technique are still unknown. We investigate the use of ARUs in estimating species richness of anuran assemblages in a tropical region, aiming to determine the sampling sufficiency of species richness at local and regional levels, analyze whether the asymptote point is related to forest cover, and investigate the influence of subsampling type over time on species richness estimates. We monitored amphibians in 14 streams embedded in landscapes representing a gradient from 20 to 70% native forest coverage. We detected a total of 14 species, with the regional sampling sufficiency of total species richness reached in 3448 min and influenced mainly by the terrestrial species' presence. Forest coverage had no influence on the minimum audio processing time required to achieve local asymptote. The subsampling schemes (temporally stratified and randomly assigned) had similar efficiency when using 5 min/h or more sample efforts. Our findings indicate that passive acoustic monitoring can adequately represent local anuran richness, focusing especially on the arboreal guild. Sampling effort can be optimized, with a 5 min/h duty cycle being sufficient to recover detection of most species, saving up to 75% of the effort devoted to auditing the acoustic dataset.

Keywords Amphibia · Survey methods · Acoustic surveys · Anura conservation · Tropical ecosystems

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✉ Paula Ribeiro Anunciação
paulaelvel@yahoo.com.br

Extended author information available on the last page of the article

Introduction

Conservation decisions and species management often require data on species presence and abundance over large spatio-temporal scales (Pereira et al. 2013). While field observation constitutes the bulk of ecological data used in biodiversity monitoring programs (Proença et al. 2017), obtaining standardized data using traditional techniques over larger spatio-temporal scales is challenging (Koblitz et al. 2017; Gotelli and Colwell 2001), and increasing sampling effort in field biodiversity surveys can become prohibitive (Sugai et al. 2020). In hyperdiverse systems, such as tropical forests, estimating species diversity usually requires intensive field data collection to adequately represent local and cross-site variation in species diversity (Rödel and Ernst 2004). However, species diversity estimates can be biased when surveys fail to consider the structure of habitats, which directly influences species detectability (Doan 2003). In this sense, understanding sampling sufficiency for different taxa and landscape contexts remains an important component in biodiversity survey and monitoring.

The Atlantic Forest is an endangered hotspot of biodiversity, with more than 80% of its territory altered by anthropogenic pressures (Ribeiro et al. 2009) and 72% of Brazil's population within its boundaries (IBGE 2010; Tabarelli et al. 2010). Even with only few remnants of fragmented Atlantic Forest, it is considered the Brazilian biome with the highest number of expected future species discoveries (Moura and Jetz 2021), with estimates pointing 25% of amphibian species still undescribed (Guerra et al. 2020a, b). In Brazil is the leading biome in terms of amphibian diversity (Vancine et al. 2018). In general, native-forested areas hold the highest levels of species diversity (Hilton-Taylor et al. 2009), with up to 82% of amphibian species being forest dependents (Stuart et al. 2004). Amphibian populations are undergoing sharp declines worldwide mainly due to habitat loss, fragmentation, chytridiomycosis, and climate change (Stuart et al. 2004), and these impacts are particularly severe in tropical biomes, as for the Brazilian Atlantic Forest (Becker and Zamudio 2011; Becker et al. 2007; Eterovick et al. 2005). Therefore, there is an urge in advancing monitoring techniques for threatened taxa such as tropical amphibians, which can be promoted by new technologies aimed to improve biodiversity monitoring at scale.

Autonomous recording units (ARUs) have emerged as a tool to complement field observation and increase the spatio-temporal capacity of biodiversity monitoring (Gibb et al. 2019; Sugai et al. 2019a). For vocalizing species such as birds, frogs, primates, and insects, passive acoustic monitoring (PAM) may be more efficient when compared to active sampling methods, with the benefit of reduced fieldwork effort (Melo et al. 2021; Darras et al. 2018; Shearin et al. 2012; Dorcas et al. 2009). PAM uses audio devices to record sounds from a specific area, enabling the assessment of species incidence at high temporal resolution (Blumstein et al. 2011; Obrist et al. 2010). The use of ARUs in biodiversity monitoring has been advocated as an important complementary survey technique (Parris et al. 1999; Mack and Alonso 2000; Montambault and Missa 2002). With the release of commercial and low-cost ARUs, PAM quickly became a trending sampling technique for biodiversity research worldwide (Sugai et al. 2019b; Browning et al. 2017; Wimmer et al. 2013; Blumstein et al. 2011; Bardeli et al. 2010; Acevedo and Villanueva-Rivera 2006). PAM offers advantages for continuous and simultaneous biodiversity sampling with reduced human interference in animal behavior (Digby et al. 2013; Laiolo 2010) and better cost-benefits for surveying remote areas (Wrege et al. 2017; Hutto and Stutzman 2009). Additionally, PAM generates permanent registers of vocalizing animals associated with a specific place and time, which can provide key information in the long-term to support conservation and

mitigation measures for the future (Sugai and Llusia 2019; Acevedo and Villanueva-Rivera 2006).

Despite the rapid adoption of ARUs in ecological research, the appropriate balance between survey time, cost, and the proper characterization of biodiversity patterns is still highly debated (Francomano et al. 2020; Sugai et al. 2020). While most investigations on PAM sampling sufficiency were based on bird diversity (Araújo et al. 2020; Wimmer et al. 2013), we still know little about anuran sampling sufficiency based on PAM. Anuran calling behavior differs substantially from birds, being characterized by stereotyped calls with varying diel and seasonal activity patterns (Guerra et al. 2020a, b; Sugai et al. 2021). Anuran calls have an overall simple structure and are species-specific, which facilitates the process of manually detecting and identifying species calls (Köhler et al. 2017). In this context, recording schedules can be adapted to ease data management and manual analysis, in addition to being a strategy for cost-effective sampling designs (Sugai et al. 2019a, b, 2021). However, determining an appropriate recording regime is not trivial, as different settings might exclude crucial information and result in a distorted characterization of the vocalizing species (Thomisch et al. 2015).

In this study, we surveyed anuran communities in the Atlantic Forest and investigated the effectiveness of distinct ARUs temporal samplings in estimating species richness. We used passive acoustic monitoring to investigate communities over a gradient of landscapes with different percentages of remaining forest coverage and inspected audio recordings manually. We were interested in answering: (i) how many minutes are required to achieve the asymptote of species richness (for all species, and for arboreal and terrestrial guilds) at the local (alpha) and regional (gamma) levels? (ii) does landscape forest coverage influences the amount of minutes needed to reach species richness asymptote? (iii) how do different types of sampling over time (i.e., stratified vs. random) influence the estimates of species richness? We expected that anuran communities from landscapes with a higher proportion of forest coverage would be more species-rich and consequently, require longer sampling efforts to reach asymptotes (Carey et al. 2020; MacArthur and Wilson 1967). We also expected differences in the sampling sufficiency of arboreal and terrestrial guilds due to their habitat affinities and distinct detectability (Ramalho et al. 2021). For the different types of temporal sample (hereafter referred to as “subsampling type”), we expected that random subsampling would be more efficient to represent the diversity of anurans because of the remarkable differences in silent periods between species, as part of their calling strategy (Emmrich et al. 2020).

Material and methods

Study area

The study region comprises the Cantareira-Mantiqueira Mountain ranges continuum (from 846 to 926 m.a.s.l.) in São Paulo State, southeastern Brazil. Originally covered by the Atlantic Forest, the region has undergone intense resource exploitation and conversion of natural vegetation into pastures, eucalyptus plantations, croplands, and urban settlements (Muylaert et al. 2018; Morellato and Haddad 2000). Many of the natural vegetation remnants are forests in intermediate stage of succession (Ribeiro et al. 2009).

In an area of approximately 1900 km², we selected 14 permanent streams separated at a minimum of 2.9 km ($\bar{x}$ = 27 km, Fig. 1). We selected streams that were characterized by the

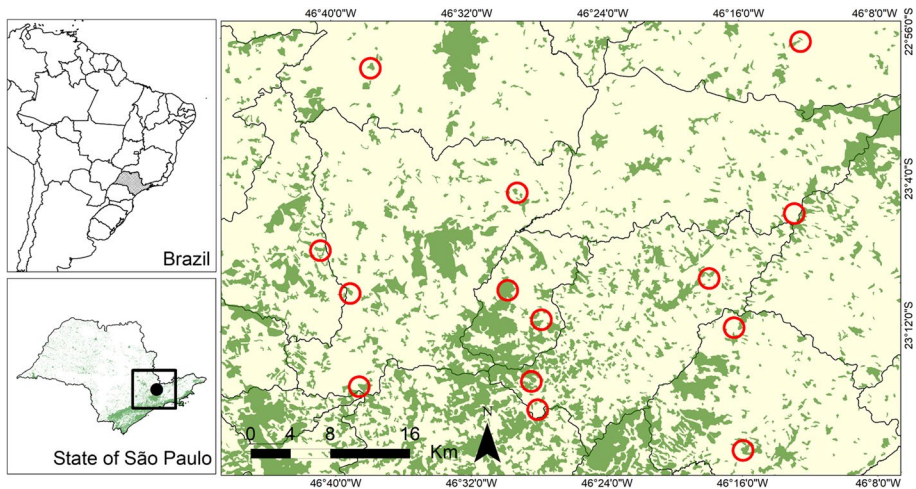


Fig. 1 Study region in southeastern Brazil, with the 14 1-km radius circular sampling units (red circles). The green patches and background represent native forest cover and the non-forest matrix, respectively

following habitat features used by anuran species in the Atlantic Forest biome: (i) streams less than 5-m wide, (ii) slow running streams, (iii) presence of aquatic and riparian vegetation, and (iv) streams located within forest patches embedded in landscapes over a gradient of forest cover, from 20 to 70% (Vasconcelos and Rossa-Feres 2005; Kluber et al. 2008).

In each site, we calculated the percent forest coverage within a 1000 m radius buffer based on a 0.3-m spatial resolution remote sensing imagery (ArcGIS 10.3 basemap imagery, DigitalGlobe satellites 2010–2011; scale of 1:5000). We used this buffer because it comprises the average displacement for amphibians (Guerry and Hunter 2002; Wagner et al. 2014) and is reported to be an adequate scale to investigate anuran occupancy and diversity (Guerry and Hunter 2002; Van Buskirk 2012).

Data sampling and species identification

We installed an ARU (Song Meter SM3, Wildlife Acoustics, Inc.) in each of the 14 sampling units for a total of four months that comprise the rainy and warmer season (December 2015 to March 2016), which coincides with the breeding season for anurans in tropical regions (Ferreira et al. 2016; Watanabe et al. 2005). Each recorder was deployed in trees, at 1.5 m height, located no more than 3 m away from the streams. We used a continuous schedule recording between 6:30 PM (UTC-3) and midnight to coincide with the peak calling activity of anurans (Prado and Pombal Jr 2005). Audio recordings were registered at a sample rate of 48 kHz, in stereo mode using the two built-in omnidirectional microphones, using default gain levels, and powered by four D-cell alkaline batteries. All files were stored in uncompressed “.wav” format and saved on four 32 GB SD memory cards. Memory cards and batteries were replaced every 15 days.

From a total of 271,830 min obtained (c.a. 4530 h), we randomly selected a maximum of 480 min/site over eight distinct days to manually inspect the recordings for anuran vocalizations. Calling activity of forest anurans is relatively stable throughout the breeding season and, in a diel cycle, usually peaks from dusk to midnight (Rocha et al. 2015; Prado and Pombal Jr 2005). To represent different night periods in each

day, we selected 20 min for three time slots: 6:40–7:00 PM, 9:00 PM–9:20 PM, and 11:00 PM–11:20 PM. The acoustic recordings were inspected by the first author and species-specific calls were identified by listening to the recordings and/or using spectrograms (Raven Pro version 1.4). The incidence of a vocally active species was assigned to each 1-min from the 20 min slots.

Analyses

To answer (i) how many minutes are required to achieve the asymptote of species richness at the local (alpha) and regional (gamma) levels, we used the Hill number interpolation and extrapolation method (Chao et al. 2014) from the iNEXT package (Hsieh et al. 2016) implemented in R (R Core Team 2020). We estimated the asymptote of species richness considering three approaches: only arboreal species, only terrestrial species, and all species. For the following tests, we limited to use only the approach considering all species registered locally.

To test if (ii) total forest coverage inside the sampling units influences the amount of minutes needed to reach species richness asymptote, we fitted a general linear model with the number of minutes required to achieve local species richness asymptote as the response variable and the percentage of forest coverage as the predictor. Normality assumptions were confirmed by inspecting residual heteroscedasticity and diagnostic QQ plots.

Finally, to answer (iii) how different temporal sampling efforts and types (random or stratified) affect species richness estimates, we simulated two types of recording schedules (subsampling types): temporally stratified and randomly assigned. For different sample efforts, represented by the amount of audited minutes (j , with j ranging from 1 to 20), the temporally stratified type had j minutes selected starting from the first and following the sequential j minutes of each hour monitored. The randomly assigned type had j minutes randomly sampled from a total of 20. In terms of total sample effort, with $j=1$ min, we would have a total of 24 min ($1 \text{ min} \times 3 \text{ h} \times 8 \text{ days}$). Increasing sample effort to $j=2$ min would lead to a total of 48 min. For each combination of sample effort and subsampling type, we calculated the percentage of species richness that each subsampling scheme retrieved from the total information at each site (i.e., from the 480 min per landscape) (Fig. 2). To test for differences between the two subsampling types, we tested if the percentage of total species richness retrieved differed between the subsampling types using a two-tailed t-test. We performed all analyses in R (R Core Team 2020).

Results

A total of 14 anurans species were detected in audio recordings with PAM, with a varying number of species across sites (from 1 to 8 species, $\bar{x}=4.75 \pm 1.93 \text{ SD}$, Table 1). The audited time required to reach the asymptote of all species at the local scale was considerably variable among sites, ranging from 28 to 960 min ($\bar{x} = 426.64 \pm 272.34 \text{ SD}$, Fig. 3; Table 1). Considering only arboreal species, the number of audited minutes varied from 17 to 480 min ($\bar{x}=270.7 \pm 202.5 \text{ SD}$), while for terrestrial species the mean value was higher ($\bar{x}=301.5 \pm 170.7 \text{ SD}$), ranging from nine to 480 min (Table 1; Fig. 4).

At the regional level (gamma), the total minutes processed to reach the regional species richness asymptote for all species was 3448 min, which corresponded to ~50% of the

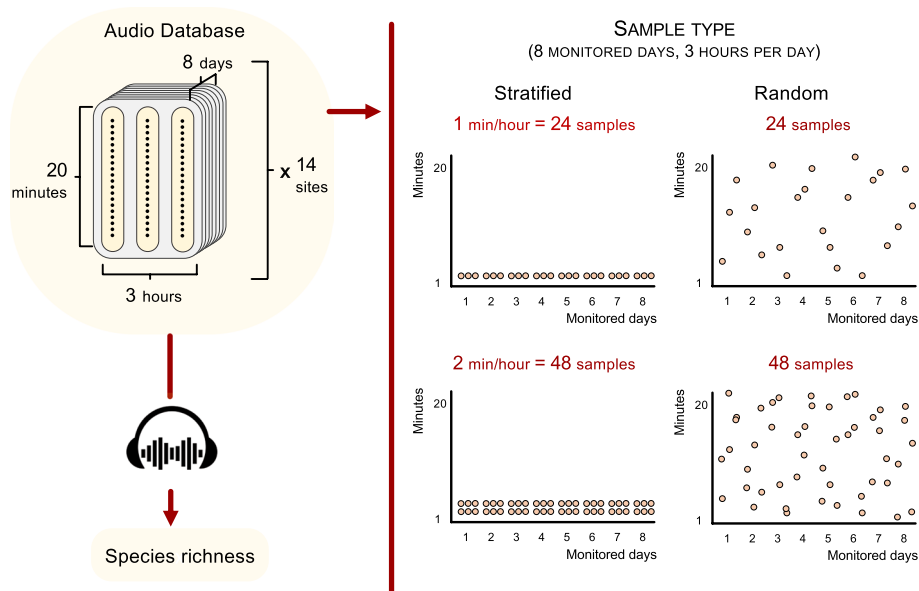


Fig. 2 Structure of the audio database (14 sites, 8 days, 3 h from each day, 20 min from each hour). Species richness was determined for each 1-min from the total dataset for each site (480 min/site). To test if distinct subsampling of the total dataset could optimize manual analysis, we took distinct number of minutes in a stratified (i.e., first n minutes of each hour) or randomized manner

Table 1 Summary of the accumulation curves of the 14 sampling units for arboreal and terrestrial guilds, and for all species

Forest cover (%)	Arboreal		Terrestrial		All species	
	S	Asymp-tote (min)	S	Asymp-tote (min)	S	Asymp-tote (min)
21.7	3	344	3	442	6	463
24.5	1	480	4	480	5	960
28	0	NA	1	9	3	199
32.5	2	470	2	223	4	469
32.8	2	470	2	480	4	960
40	3	81	2	49	6	81
53.9	0	NA	2	143	4	408
54	3	211	2	480	6	480
57.1	3	480	2	344	7	480
57.9	2	480	0	NA	2	460
58.7	1	27	0	NA	1	28
61.9	1	17	2	442	5	459
76.8	1	27	3	272	4	272
77.7	5	161	2	254	8	254

S observed amphibian richness, min minutes

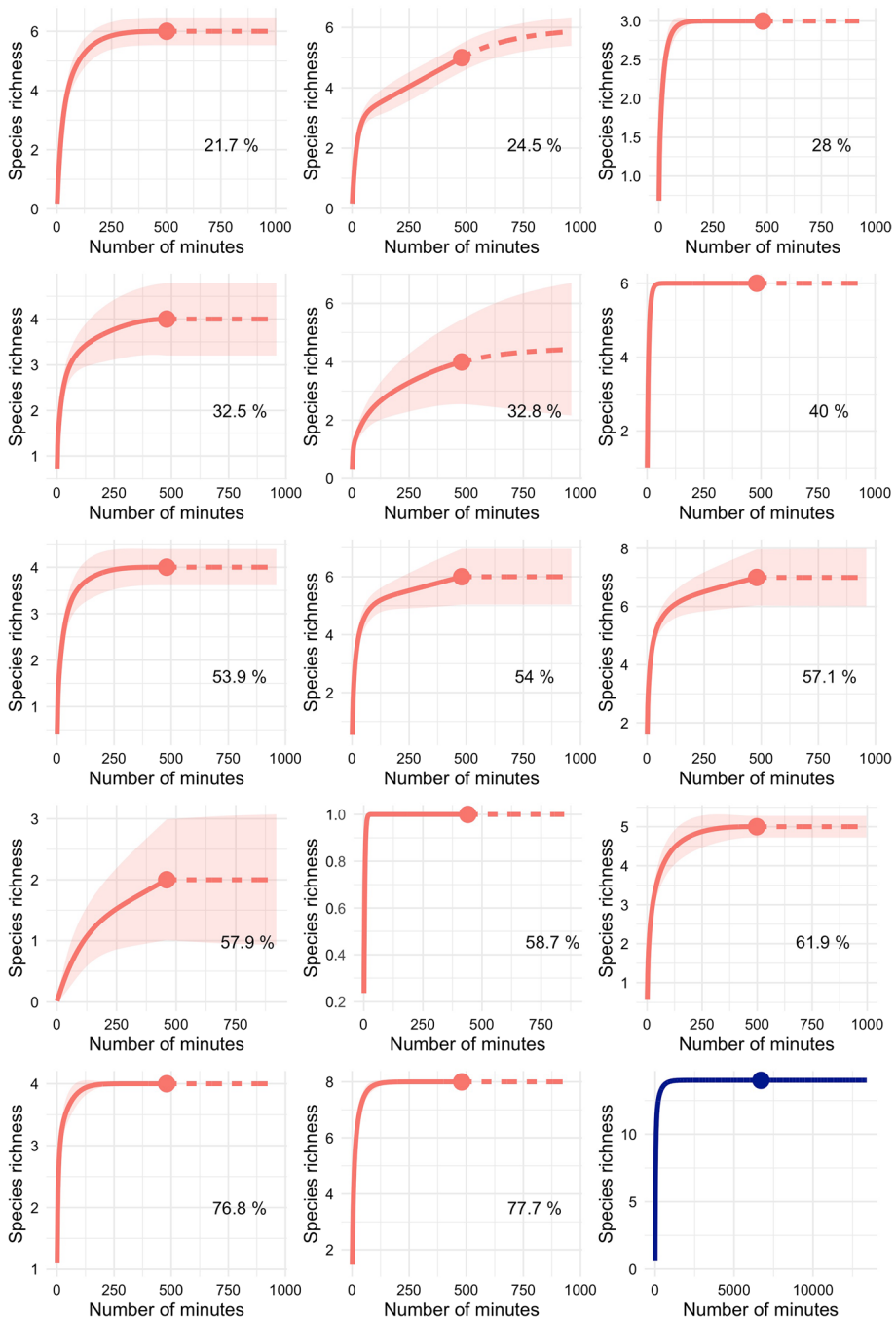


Fig. 3 Accumulation curves of amphibian richness over 14 SUs with different forest cover percentages (red) and for the regional pool (blue) located at the Atlantic Forest in Brazil. Solid lines = interpolated method; dotted lines = extrapolated method; red dots = total sample, red area around the curves = confidence intervals. Numbers in the bottom right corner are the proportion of forest cover (%)

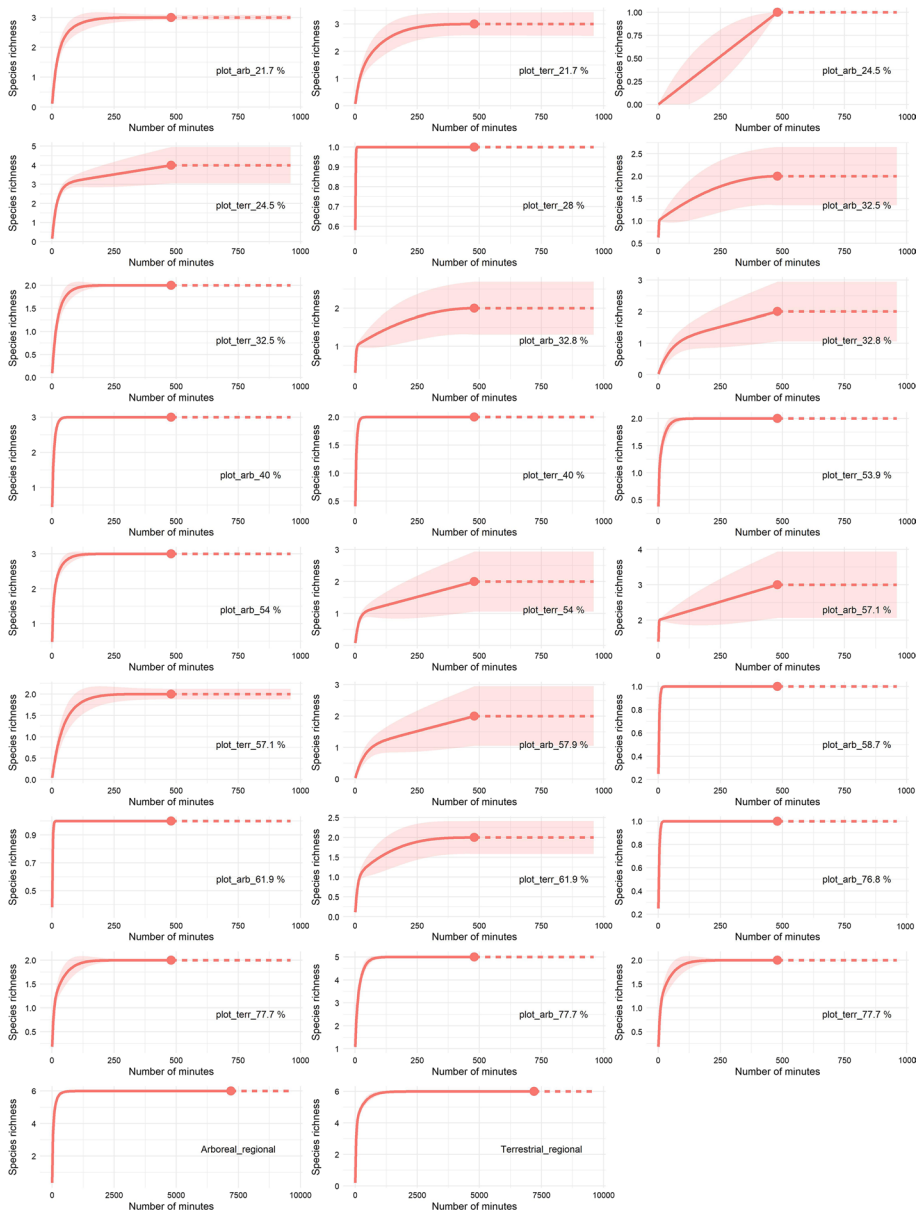


Fig. 4 Accumulation curves of arboreal and terrestrial species richness over 14 SUs with different forest cover percentages, and for the regional pool (last two graphs on the bottom) located at the Atlantic Forest in Brazil. Solid lines = interpolated method; dotted lines = extrapolated method; red dots = total sample, red area around the curves = confidence intervals. Codes in the bottom right corner of each graph are the proportion of forest cover (%) for arboreal (plot_arb) and for terrestrial guilds (plot_terr)

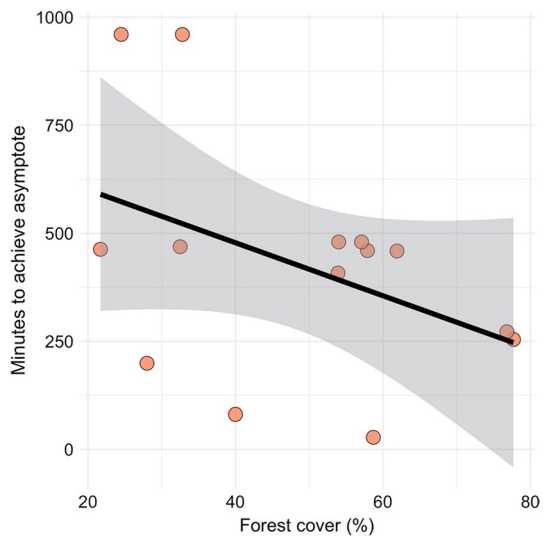
total analyzed (Fig. 3). When we considered only the arboreal or terrestrial species, a total of 1180 and 2450 min were required to achieve the regional species richness asymptote, respectively (Fig. 4).

We found no relationship between the sample effort to achieve the asymptote of local species richness and the percentage of remaining forest coverage in the landscapes ($F=2.52$, $df=12$, $p=0.138$; Fig. 5). From the fitted relationship, a wide variability in the number of minutes required to reach the asymptote of species richness was observed when communities are located in sampling units with a lower percent of forest coverage, while in sampling units with increasing forest coverage, the variability of sample effort shrinks (Fig. 6).

When sample effort was assigned with either temporal stratified or randomly assigned minutes, the differences between the percentage of species retrieved from the total were negligible (Table S2, Fig. 6). With small sampling efforts (< 3 min/hour), the percentage of retrieved species richness was largely underestimated, regardless of subsampling type (Fig. 6). Starting from 3 min/hour, the percentage of retrieved species richness increases steadily and reaches 75% quickly. We found that increasing sample effort using the random subsampling type is more efficient to retrieve higher percentages of total species richness. For instance, all sites had more than 75% of total species richness retrieved with 10 min/h effort with the random subsampling, while for the temporally stratified one, the percentage in some sites only increased only after 15–18 min/h (Fig. 6).

When we inspected the variability in the percentage of species richness retrieved from total considering different sample efforts, we observed that overall, the communities located in landscapes with less than 60% forest coverage showed wider variability in the number of minutes needed to retrieve high percentages of total species richness in comparison with landscapes with more than 60% forest coverage. For instance, a three minute/hour sampling effort on the anuran community in the landscape with 32.5% forest cover will detect about 75% of the total species richness for both random and stratified methods (Fig. S1). On the other hand, the same effort for the community located in the landscape with the highest forest coverage detects nearly 100% of the total species richness (Fig. S1).

Fig. 5 Relationship between time to reach the asymptote of amphibian richness (estimated with interpolation and extrapolation of Hill numbers) and forest cover of 14 SUs localized in streams of Atlantic Forest remnants, state of São Paulo, Brazil



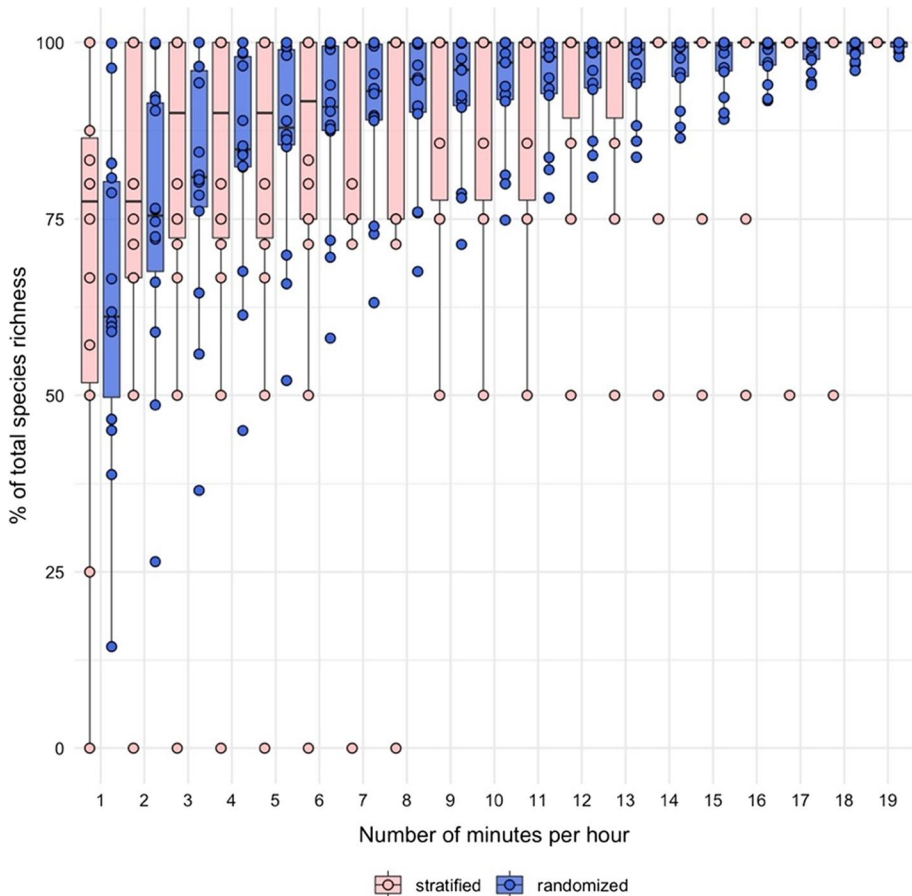


Fig. 6 Percentage of species richness from the total from 14 landscapes localized around streams of Atlantic Forest remnants, state of São Paulo, Brazil. Percentages were estimated with different sampling efforts (number of minutes per hour, x-axis) and two subsampling types (stratified: pink, random: blue)

Discussion

Using amphibian communities distributed over landscapes comprising a gradient of remaining forest coverage in the Atlantic Forest biome, our findings indicate that investigating local and regional patterns of species richness using passive acoustic monitoring can be underestimated if using a single protocol that fails to consider differences in the detectability of species and the landscape context. Neither total species richness nor the percentage of remaining forest coverage were factors related to the sampling effort required to achieve the asymptote of species richness. However, our findings suggest that higher sample efforts (inspected audio files) are required to estimate the asymptote of total species richness when communities have a larger contribution of terrestrial species in their composition. Furthermore, by showing how different sample efforts retrieve local species richness, small sample efforts (i.e., 5 min/h) are capable to provide reasonable estimates of species richness for these tropical amphibian communities, regardless of whether using temporally stratified samples or random sample selection.

Standardized sampling efforts are crucial to obtaining reliable and effective data on amphibians in auditory surveys. For traditional field auditory research, the standardized survey protocol used by the North American Amphibian Monitoring Program (NAAMP) consists of five minutes in length at every road point near breeding sites (Weir and Mossman 2005). Pierce and Gutzwiller (2004) proposed 10–15 min to detect at least 90% of amphibian species. In Brazil, it is common to perform auditory surveys of amphibians starting at sunset and ending at midnight (Benício and Silva 2017; Maffei et al. 2015; Campos et al. 2013; Zina et al. 2012), which means 6 continuous hours in total. The miscellaneous of biodiversity sampling protocols urge standards in amphibian sampling. For this purpose, an important advantage of ARUs is the possibility of increasing sampling time without significant impacts on fieldwork expense (Melo et al. 2021; Acevedo and Villanueva-Rivera 2006; Dorcas et al. 2009).

Halving the total sample effort would be enough to estimate the regional asymptote of species richness in our study. Previous research in the region using active search methods over a variety of vegetation types (including open canopy areas) registered 13 species (Sabbag and Zina 2011), 24 species (Zina et al. 2007), and 29 species (Costa et al. 2013). In this sense, it is likely that some species were unable to be registered in our study. An important shortfall in passive acoustic monitoring is the lack of standardized sampling procedures that enable to represent distinct ecosystems and taxonomic groups with accuracy while maximizing cost benefits, such as reducing costs with time-consuming acoustic analysis (Sugai et al. 2019; Gibb et al. 2019). In this study, this could be related to the temporal subsampling of audio files. A possibility would be to use 50% of the sample effort to inspect recordings from other days of the monitoring period and investigate whether local and regional species accumulation curves are improved. Alternatively, a rotation procedure with the automated acoustic recorders could improve the spatial coverage of the regional pool of species, so that the surplus sample effort could be destined for audio samples from different sites.

Our findings indicate that 5 min/h survey would be enough to represent local anuran richness, as studies using traditional survey methods in the same region revealed similar or even greater species richness, considering only species found inside forest patches (Sabbag and Zina 2011; Costa et al. 2013, both found six species). Importantly, one of the studies did not specify which habitat type the species were found in (e.g.: forest or open areas, Zina et al. 2007) and none of the studies specified which type of sampling (acoustic or visual encounter surveys) was used to register anurans in the field. Therefore, if the species were found only with acoustic sampling and within forest patches in these studies, our findings would be presumably consistent, with similar levels of species richness registered. These results can orient passive acoustic monitoring studies mainly in the southeastern Atlantic Forest, but we contend that additional testing is still required for other fragmented tropical areas and considering different vocal species groups. For instance, recording schedules ranging from 2 to 10 min/h were already used to sample tropical amphibian anurans (e.g.: Alvarez-Berríos et al. 2016; Ulloa et al. 2019; Sugai et al. 2020, 2021), and birds (Araújo et al. 2020; Wimmer et al. 2013), although none of the studies formally tested the adequacy of the recording schedules to represent the communities.

The absence of a relationship between the percentage of remaining forest coverage with either species richness (see Fig. S2—supplementary material) and the sample effort to achieve the asymptote of species richness (Fig. S3) suggests that this variable might be inadequate to represent a range of habitat conditions for different guilds. A consequence of this is that the patterns of richness can mask guild specific responses to disturbance (Gardner et al. 2007). The spatial scale, degree of habitat degradation, and relative habitat

use can influence the occurrence of different guilds (Ramalho et al. 2021). Forests also play an important role as nonbreeding habitats for several generalist species (supporting growth and survival during high solar incidence) and as corridors, by facilitating the movement between breeding sites (Houlahan and Findlay 2003; Becker et al. 2007). The strong dependence on forests combined with a high sensitivity to human impacts makes forest-specialist amphibians to be important bioindicators of habitat disturbance (Storfer 2018; Buckley et al. 2012). Conversely, impact-tolerant species can be bioindicators of disturbed areas, and such sites can harbor a significant richness of anuran amphibians (D’Anunção et al. 2013; Ruffel et al. 2017). This might be the particular case of our study, as there was no clear relationship between species richness and percentage of forest coverage (Fig. S2).

When landscapes had a lower percentage of forest coverage, the sample effort to retrieve high percentages of the total species richness was higher compared to landscapes with a high percentage of forest coverage. This may be attributed to lower species equitability since communities composed of species with low abundance will generally have the plateaus of their cumulative curves reached with more sample effort compared with communities composed of high abundance species (see Fig. S4, Sgarbi et al. 2020; Thompson and Withers 2003; Magurran 2003).

Although there were no differences between the number of arboreal and terrestrial species between sampling units, arboreal species were detected more quickly, resulting overall in reaching species richness asymptote earlier. Hylidae, the family that includes all the arboreal species in our study, is the most species-rich family of anurans in Brazil and populations are commonly abundant, which probably contributed to reaching the sampling sufficiency faster than the terrestrial group (Segalla et al. 2021). Different methods are more or less appropriate to sample distinct anuran groups (Rödel and Ernst 2004; Doan, 2003), and previous studies have shown that PAM was successfully used to survey arboreal species (Madalozzo et al. 2017; Willacy et al. 2015; Sinsch et al. 2012). The arboreal guild could represent a suitable group for rapid inventories aimed at quickly and efficiently assessing the importance of priority areas for conservation in tropical fragmented regions. Populations in these areas usually have undergone declines and some species were even extinct (Almeida-Gomes and Rocha 2015; Eterovick et al. 2005). For instance, the endemic amphibian *Phrynomedusa fimbriata*, previously found in the Atlantic Forest of southeastern Brazil, now is considered extinct (Baêta et al. 2016; Cruz and Pimenta 2004). Therefore, passive acoustic monitoring has the potential to optimize the survey efforts especially when communities are largely composed of arboreal species.

Moreover, although degraded forests and agricultural landscapes can still provide conditions to maintain anuran diversity (D’Anunção et al. 2013; Ruffel et al. 2017), they have reduced micro-habitat quality and availability and reinforce isolation among preferential habitat patches. Such conditions would benefit impact-tolerant species (e.g., *Dendropsophus minutus*, *Scinax crospedospilus*, *Leptodactylus latrans*, and *Physalaemus cuvieri* in the current study, Table S1). The occurrence of more generalists across many communities contributes to the homogenization of communities (Martello et al. 2018), which impact negatively local species richness and abundance and presumably, the sampling effort required to represent these communities. It is expected that the arboreal species (e.g., *Aplastodiscus leucopygius*), due to their general habitat affinity for shrubs and trees, where this group finds reproductive and food resources, to be the most affected by deforestation and land-use change, appearing to have higher risks of declining (Sodhi et al. 2008; Ramalho et al. 2021).

By contrasting different recording schedules and sampling types, it is possible to explicitly perform a cost–benefit analysis and assess the limitations of different sampling designs.

We show that an effort of five minutes per hour (random or stratified) can detect above 90% of the total species detected. At the cost of underestimating 10% of the total species, the listening effort is reduced by 75%. This finding must be carefully interpreted in light of the goals of the surveys. For complete inventories, increasing sampling efforts would enable most species to be registered. For long-term monitoring, optimizing the use of recorders and the costs associated with maintenance should be considered to enable long-term servicing. In order to optimize listening effort, Araújo et al. (2020) concentrated efforts over diel periods with the highest species detection. Being aware that manually analyzing any amount longer than 200 min is prohibitively expensive and time-consuming, our results indicate that optimized analysis can be a cost-effective alternative even when the goal is to retrieve most of the species from communities, as for wildlife assessment or for cross-site comparisons (Pieretti et al. 2015; Weir and Mossman 2005).

The anuran communities registered in our study site on the Atlantic Forest remnants were composed of both explosive and prolonged breeders, which usually engage in calling activity simultaneously in the breeding season (Table S1, Bertoluci and Rodrigues 2002). Given the persistent activity throughout the night and the simple and stereotyped calls, tropical anuran's detectability is easier compared to species-rich communities of vocalizing animals with complex acoustic signals, such as dawn birds (Brauer et al. 2016; Dorcas et al. 2009). Our findings that sampling effort is more important than subsampling type (stratified versus random) likely reflect the pattern of anuran calling behavior. However, a random selection of minutes can be more efficient for detecting species with median sampling effort (in our study, estimates of 9 to 18 min/sampling hour). It is likely that silent intervals between calls may influence detectability, as calls with long silence periods are less probable to be detected over consecutive minutes. The opposite is true for species with temporally aggregated calling emissions sampled with a random scheme (Emmrich et al. 2020). However, temporally stratified, or random subsamples have negligible effects on the detectability, as the probability of sample non-silent minutes is positively correlated with the effort.

Standardized and effective species diversity sampling methods support the task of identifying core areas in human-dominated landscapes, especially in tropical regions experiencing the highest rates of habitat loss (Arntzen et al. 2017; Gardner et al. 2007; Cushman 2006; Rödel and Ernst 2004). Furthermore, most of the forest remnants are found in remote or difficult to access areas (Ribeiro et al. 2009), and acoustic monitoring is a highly cost-effective alternative to describe population changes over time, especially in these types of areas mentioned above (Ribeiro et al. 2017). Our findings also shed light on strategies for amphibian conservation. In addition, can be less time-consuming and effective when specific guilds are considered (e.g., the arboreal group in the current study) and successfully indicate important areas to protect. Taken together, acoustic monitoring is an important tool to expand our understanding of the ecology and diversity of such an important and threatened group, which, in turn, can better inform conservation actions and the mitigation of anthropogenic-related impacts.

Conclusion and future studies recommendations

The diversity of tropical anuran communities in fragmented landscapes can be adequately assessed through passive acoustic monitoring. Using sampling sufficiency analysis, we show that sample effort can be optimized by inspecting at least five minutes

per monitored hour, preferentially randomly assigned. Such a strategy would provide reliable information about the total amount of species richness detected at each site and at the regional level for acoustic monitoring research. Our findings indicate that forest cover did not influence species richness and the sampling effort needed to reach the asymptote of species richness. However, when different guilds are considered, we observed that a higher sample effort was required to achieve good estimates when communities had larger contribution of terrestrial species. This indicates that species biological aspects, detectability and landscape context should be considered when defining the appropriate sampling method.

Reducing the listening effort in manual analysis by up to 75% can still represent 90% of the regional species richness while greatly reducing costs with data storage, time spent auditing data, as well as promoting the monitoring of communities located in remote areas or of difficult access. Due to funding limitations in ecological research, sampling techniques that allow for more efficient and accurate population monitoring are indispensable to ensure the effectiveness of management and conservation strategies, especially in low-income countries (Willacy et al. 2015).

We further suggest future studies to explore these analytical frameworks over different ecosystems and focal taxa. Such efforts would enable cross-taxon and cross-biome synthesis that could guide more cost-effective standard passive acoustic sampling.

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Author contributions PRA, MCR, and LMTC conceived the ideas and the study design. PRA and LSMS reviewed the original ideas and hypotheses. PRA acquired field data and analyzed the acoustic data. FM curated the sound data. PRA and LSMS analyzed the data and wrote the results. PRA led the original draft and LSMS reviewed all drafts. All authors consented to the final draft.

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Data availability The data that support the findings of this study are available from the corresponding author, PRA, upon reasonable request.

Declarations

Conflict of interest The authors have no conflict of interest to declare.

Ethical Approval No animals were collected or sacrificed in this study.

Consent to participate All authors agree have reviewed and agree with the submission of this version of the manuscript.

Consent to publish All authors accept the responsibility for releasing the material included in this manuscript.

Clinical trials registration It does not apply to this manuscript.

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Authors and Affiliations

Paula Ribeiro Anunciação^{1,2}  · Larissa Sayuri Moreira Sugai^{3,4} · Felipe Martello⁵ · Luis Marcelo Tavares de Carvalho¹ · Milton Cezar Ribeiro²

¹ Conservation and Ecology Department, UFLA – Universidade Federal de Lavras, Lavras, Minas Gerais 37200-000, Brazil

² Spatial Ecology and Conservation Lab (LEEC), Department of Ecology, Bioscience Institute, UNESP - Universidade Estadual Paulista, Rio Claro, Rio Claro, São Paulo 13506-900, Brazil

³ Departamento de Ecología, Facultad de Ciencias, Universidad Autónoma de Madrid, Ciudad Universitaria de Cantoblanco, C/ Darwin, 2, Edificio de Biología, C-211, 28049 Madrid, Spain

- ⁴ Cornell Lab of Ornithology, K. Lisa Yang Center for Conservation Bioacoustics, Cornell University, 159 Sapsucker Woods Road, Ithaca, NY 14850, USA
- ⁵ Biodiversidade e Serviços Ecosistêmicos, Instituto Tecnológico Vale - Desenvolvimento Sustentável, Boaventura da Silva, 955, Belém, Pará 66055-090, Brazil