Received: 16 May 2023 | Revised: 30 January 2024 | Accepted: 31 January 2024

DOI: 10.1111/btp.13307

bioTROPICA ASSOCIATION FOR TROPICAL BIOLOGY AND CONSERVATION WILEY

ORIGINAL ARTICLE

Acoustic monitoring of anurans and birds in tropical biomes

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Funding information
Consejo Nacional de Investigaciones Científicas y Técnicas; Fundação Araucária; Conselho Nacional de Desenvolvimento Científico e Tecnológico; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

Associate Editor: Jennifer Powers
Handling Editor: Michael Staab

Abstract

Passive acoustic monitoring (PAM) is increasingly popular in ecological research, but recording and analyzing large amounts of data is still a critical bottleneck for the long-term monitoring of multiple species. We evaluated how temporal and spatial sampling effort affects species diversity estimates using a set of 14,045 1-min recordings from various neotropical birds and anuran communities. Our goals were to evaluate (i) the daily vocal activity cycle of birds and anurans, (ii) the effect of temporal structure (e.g., number of minutes listened each hour; continuous versus intermittent recordings) on determining the species composition, and (iii) the species–area relationship, and how the number of recorders affects species richness estimates. Based on sampling coverage and completeness, we (iv) evaluate manual inspection schedules for birds and anurans across four biomes of Brazil. We found marked diel variation in vocal activity between taxonomic groups, indicating that birds and anurans are more efficiently detected during early periods of the day and night, respectively. For proper diversity estimates, biomes with higher biodiversity required longer inspecting periods and a larger number of replicates, irrespective of taxa. Although fewer recordings per hour are less informative than full-hour sampling, species diversity is better estimated when inspected minutes are interspersed over longer periods than inspecting minutes

recorded over shorter timespans. Based on our findings, we recommend how to set PAM programs over highly diverse ecosystems.

Abstract in Portuguese is available with online material.

KEYWORDS
bioacoustics, inspecting schedules, passive acoustic monitoring, sampling completeness, sampling coverage, sampling effort, species–area relationship

1 | INTRODUCTION

Passive acoustic monitoring (PAM) has become a valuable tool in ecology and conservation while providing basic information on biological diversity and species composition (Alquezar & Machado, 2015; de Araújo et al., 2021; Sugai & Llusia, 2019), populations dynamics (Bezerra et al., 2019; Lees et al., 2021; Pérez-Granados & Traba, 2021), and environmental integrity (Alquezar et al., 2020; de Camargo et al., 2019; Gasc et al., 2017). The acoustic monitoring of biodiversity is a robust, efficient, and non-invasive method for the detection of terrestrial and aquatic fauna, which often results in data acquisition at enormous temporal and spatial scales (Sugai et al., 2019; Sugai & Llusia, 2019). The capability of sampling multiple localities simultaneously makes PAM especially suitable for studies addressing cross-scale comparisons.

While PAM has proven to be an invaluable tool for evaluating the hyperdiverse communities found in the Neotropics, obtaining biological data from sound recordings is a complex task when the goal is to assess species diversity (Gibb et al., 2019). Processing large datasets is time-consuming (de Araújo et al., 2021), and automatic classification algorithms often yield inaccurate results when signals are faint or mixed with background noise (Kahl et al., 2021). Training personnel to inspect sound recordings and identify vocally active species based on the unique signal structure might be an alternative (Alquezar & Machado, 2015; de Araújo et al., 2020; Wimmer et al., 2013). Although trained personnel can detect signals with great precision within sound-rich Neotropical soundscapes (de Araújo et al., 2021; Vielliard, 2004), the amount of data that trained staff can parse is a limiting factor (Gibb et al., 2019). One possibility for increasing sampling efficiency is to focus sampling efforts on the period of highest acoustic activity (de Araújo et al., 2021; Metcalf et al., 2022; Wimmer et al., 2013).

Individuals are not evenly distributed across species in any given community of organisms. Rather, a small proportion of species tend to be much more abundant than the rest (Magurran, 2013). In the case of birds in tropical forests, it is widely recognized that substantial field efforts are required to detect rare species, which often constitute a much larger proportion of the communities (Parker, 1991). While it is relatively easy to detect the abundant species, or the species with highest vocal activity, it can be very challenging to detect the rare species of a community. This is especially true for highly diverse ecosystems such as the Neotropics, where many rare species are expected to occur, and larger samples are often required to ensure their detection. In that sense, PAM can increase the amount of data collected and improve the chances of detecting rare species. Nevertheless, the collection and manual analysis of acoustic datasets can be a bottleneck and should ideally be optimized. Among the metrics used to evaluate the quality of sampling, the proportion of individuals belonging to the species already detected (sampling coverage) can be informative and describes how well data capture the true diversity of the community (Roswell et al., 2021). Additionally, the proportion of species detected in relation to the estimated number of species of a community (i.e., sampling completeness) can also provide valuable information on sampling quality. Another aspect of interest when studying communities is the temporal variation in bird species detection in terms of richness and composition. Regarding this matter, prior research on bird communities in the Neotropical region, using techniques like point counts or transects, has revealed that the number of detected species tends to decrease from early to late morning, and that species exhibit distinct diurnal patterns of activity (Blake, 1992).

Efforts to standardize recording schedules for large-scale sampling are still in their infancy (Cifuentes et al., 2021; Sugai et al., 2020), hampering cross-site comparisons. Therefore, ecological research can greatly benefit from a PAM framework that allows standardized sampling. Here, we investigate how sampling efforts can be optimized to improve inspecting schedules in PAM. We have compiled a unique, large-scale set of annotated Passive Acoustic Monitoring datasets that reports the presence of birds and anurans in 14,045 1-min recordings. The data come from four Brazilian biomes: Caatinga, Cerrado, Amazonia, coastal and inland Atlantic forests. Using this dataset, we first describe bird and frog communities' activity patterns and explore how species detectability varies during a diel cycle. This approach sheds light on setting inspecting efforts to periods when the species are most active, optimizing overall detection rates. As already mentioned in literature, we expected birds to sing at higher rates during early mornings and late afternoons, while anuran, on the other hand, should call at higher rates during early nights, regardless of the biome. Secondly, we investigate the influence of temporal sampling strategy (e.g., varying sample duration and the interval between samples, inspection rates) on species diversity estimates. Thirdly, we use the species–area relationship to evaluate how the number of recorders (i.e., sampling area) affects species diversity

estimates, as expanding the sampled areas should have a positive effect on the number of detected species. Finally, we use these results to suggest optimized inspecting schedules for birds and anurans.

2 | MATERIALS AND METHODS

2.1 | Datasets of acoustic recordings

We collectively analyzed 14,045 1-min sound recordings from nine datasets and annotated the composition of species in each recording. These datasets encompass a wide range of habitats, including open xeric Caatinga, open seasonal, and dense tropical Amazon and Atlantic rainforests (Carvalho & Almeida, 2011). Table 1 describes the sensors and setup used in each dataset. Bird data sets were recorded continuously for 24 h, while anuran datasets were only recorded during nocturnal periods (18:00–06:00). The data can be found within http://doi.org/10.5281/zenodo.10556619.

Four out of the nine datasets used here were recorded at a (generic) rate of 6 min/h (1 min every 10) for at least 24 h (resulting in 144 1-min recordings per day). These recordings were set to gather comparable samples of environmental sounds between 20 and 20,000 Hz emitted within the detection area of audio recorders.

2.2 | Diel activity patterns

We used the generic (recorded at a 6 min/h rate) birds (datasets #1, #3, and #7) and anurans (#8) datasets to describe the general vocal activity of these groups. To investigate the consistency of species composition throughout the day, we examined the species incidence during each hour and used it to calculate Morisita Indexes. The Morisita index measures the similarity between communities and varies from 0 (no similarity) to 1 (complete similarity). We used the results to build clusters using an Unweighted Pair Group Method (UPGMA), and examine periods of similar vocal activity (e.g., nocturnal, diurnal, or crepuscular birds). We calculated the average detection rates for birds and anurans (number of species detected per unit of minute) at each hour and compared it across biomes to examine how vocal activity varies throughout the day. By identifying the periods of highest detection rates, we could focus inspecting efforts into the most informative periods of the day.

2.3 | Evaluating inspecting rate

Subsampling large datasets is often necessary in order to estimate species diversity. Yet, the selection of subsamples can affect the results and should consider aspects such as the duration of each sample or inter-sample interval. Inspecting two consecutive minutes might provide less information than inspecting two 1-min samples, separated by long intervals. Because we expect to register less diversity in a contiguous 2-min recording than in two 1-min (intermittent) recordings, we tested if diversity estimated using 87 pairs of samples was higher when (1) recorded continuously (a contiguous 2-min file) or (2) intermittently (two 1-min files recorded using an inter-sample interval of 10 min; dataset #9). We used a Wilcoxon paired test to evaluate differences between continuous and intermittent inspecting schedules.

To address the effects of inspecting rates (i.e., the number of listened subsamples per unit of time) on diversity estimates, we used the concept of sampling coverage, which measures the proportion of the total number of individuals in an assemblage that belong to the species represented in the sample (Chao et al., 2020). By comparing how bird diversity accumulates with inspecting rates of 1 min/h (first minute of each hour), 2 min/h (first and fourth), 3 min/h (first, third, and fifth), or 6 min/h (first-sixth minutes), we could determine the effect of inspecting rates to species completeness and sampling coverage. We expected that the further apart the samples are in time, the more species would be recorded. Given a fixed number of samples (inspecting effort), lower inspecting rates (i.e., 1 min/h) should cover local biodiversity faster than higher inspecting rates (6 min/h). We analyzed datasets #1, #3, #5, #6, and #7 using the package iNext (Hsieh et al., 2016).

2.4 | The species–area relationship

Acoustic signals naturally decay as they propagate through space due to spherical spreading and atmospheric absorption (Feynman et al., 1963). Therefore, automated recording units (ARU) can capture species' sounds up to a distance where the acoustic energy remains sufficient for detection, and beyond this point, the call will no longer be detectable (Bistafa, 2011). In this sense, while a single ARU can survey species over a fixed area A, two independent ARUs can survey species in two fixed areas (2A), and so on. Therefore, the species–area relationship can be a simplified approach to assess how the number of ARUs affects overall species detection. The species–area relationship is one of the oldest macroecological patterns, suggesting that the number of species (S) increases with additional sampling area (Arrhenius, 1921), as follows:

$$S = cA^z \quad (1)$$

or

$$\log(S) = \log(c) + z.\log(A) \quad (2)$$

where S—species richness; A—sampled area; c and z—model constants.

The detection range of the set of ARUs (area A) can be affected by several factors, such as local habitat structure (Chapuis, 1971), sound propagation (Parris & McCarthy, 2013; Wiley & Richards, 1978), call characteristics (Darras et al., 2018; Yip et al., 2020), atmospheric conditions (Wiley & Richards, 1978), and equipment settings

TABLE 1 Description of the nine datasets from Passive Acoustic Monitoring of birds and anurans in distinct Neotropical biomes in Brazil.

Dataset	Type	Recorder	Rec settings	Minimum distance	Gain	Period	Sunrise/sunset	Target	#ARUs	min/h	#h/day	#days	Minutes
Caatinga (1)	Generic	Sony IPX-240	MP3 192 kbps	200 m	Standard	24 h	05:15/17:41 UTC-3	Birds	11	6	24	~1.84	2572
Caatinga (2)	Focused	Sony IPX-240	MP3 192 kbps	200 m	Standard	5 h	05:15/17:41 UTC-3	Birds	32	4	5	3	1619
Inland AF (3)	Generic	SM4	24 bits 48 kHz	200 m	Gain 16 dB; Preamp 26 dB	24 h	05:55/18:52 UTC-3	Birds	1	6	24	5	705
Inland AF (4)	Focused	SM4	24 bits 48 kHz	200 m	Gain 16 dB; Preamp 26 dB	6 h	05:55/18:52 UTC-3	Birds	8	6	5	~4.67	1120
Coastal AF (5)	Focused	Audio Moth	24 bits 48 kHz	200 m	Medium gain	24 h	5:12 to 6:04/19:03 to 18:54 UTC-3	Birds	48	6	24	5	1054[a]
Cerrado (6)	Focused	SM2	16 bits 48 kHz	200 m	Standard	3.5 h	5:00/–	Birds	12	30	3.5	2	2226
Amazon (7)	Generic	Audio Moth	24 bits 48 kHz	250 m	Medium gain	24 h	06:15/18:48 UTC-3	Birds	20	6	24	1	2388[b]
Coastal AF (8)	Generic	Sony IPX-240	MP3 192 kbps	1 h	Standard	13 h	5:30/17:10 UTC-3	Anurans	5	6	24	1	2100
Inland AF (9)	Focused	SM4	24 bits 48 kHz	800 m	Standard	7 h	06:15/18:48 UTC-3	Anurans	6	3	8	2	261
TOTAL									144				14,045

Note: Datasets were considered as generic ($n = 4$) when sampling used a 6 min/h rate over at least a 24 h period, or as focused ($n = 5$), when sampling aimed at specific time periods of the day. For each dataset, the following information is provided: the type of audio recorders, file format, bit depth, sample rate (kHz), minimum distance between recorders, gain (dB), daily period of recording, biological group, number of Automated Recording Units used (#ARU), the recording rate (min/h), number of hours sampled each day (#h/day), number of sampling days (#days), and total sampling time.

[a]Data were randomly inspected from nearly 34,000 samples.

[b]Variations in autonomy and malfunctioning reduced the final amount of recordings.

(e.g., microphone sensitivity and type, presence of gain or pre-amplification, self-noise). Nevertheless, factors such as equipment settings and habitat structure should change little within the datasets presented in this study, and even though a wide range of vegetation types can be found in each biome, deploying an additional ARU should in average increase the sampling area by A.

The parameters of the species–area curves can provide valuable insights. For instance, when a single ARU (A = 1) is deployed, log(A) equals to 0, and according to Equation (2), constant c will represent the expected richness (S) of a single ARU. Constant z can be seen as a beta diversity metric representing the rate at which new species are detected in space. The higher the value of z, the larger the number of ARUs necessary to sample a study area.

We listened to 144 min per ARU (6 min/h generic inspecting rate) to build the species–area curves, treating each ARU as spatial replicates in a log-linear regression of the species–area curve (Equation 2). We selected a variable number (i) of ARUs randomly (i = 1, 2, *n*), and calculated species richness at each set of (i). This process was randomly repeated 100 times for each dataset (Caatinga #1, Atlantic Forest #3, and Amazon #7), and we used the results to fit a linear regression on the logarithmic form of the species–area curve (Equation 1), and retrieve parameters z and c.

2.5 | Inspecting effort optimization

To assess how well rare and abundant species are represented in each dataset, we constructed accumulation curves for two of Hill's diversity numbers (^{0}D and ^{2}D) using the iNext package (Hsieh et al., 2016). By assigning different weights to rare species, the Hill Numbers (^{q}D) simultaneously assess how abundant and rare species are represented in the datasets. While species richness (^{0}D) considers all species to have equal importance, the inverse Simpson (^{2}D) gives more weight to species with higher incidences (Hill, 1973; Hsieh et al., 2016). Therefore, Hill's numbers will accumulate differently, and as abundant species should be much easier to detect, ^{2}D should stabilize much faster than ^{0}D. We examined the sampling completeness over Hill numbers of different orders (q = 0, 1, and 2) to evaluate how optimizing inspecting schedules would alter Hill's numbers completeness. Sampling completeness is the ratio between the detected and estimated diversity and indicates the proportion of detected species (Chao et al., 2020).

We also determined the sampling coverage of the studied communities, which represents the chance of detecting a new species with an additional detection (Chao & Jost, 2012). For instance, under a sampling coverage of 0.99, we would have a 1% chance of detecting a new species (1–0.99) for each new detection. Comparing datasets of similar coverage has many advantages and has been recently advised as a universal method for biodiversity comparisons (Chao et al., 2020; Roswell et al., 2021). We compared coverage across biomes to assess whether the number of minutes sampled was suitable to accurately represent the given community. All statistical approaches were implemented in R (R Core Team, 2022).

3 | RESULTS

3.1 | Diel activity patterns

Birds and anurans exhibit distinct patterns of vocal activity during specific periods of the day (Figure 1). Despite variations of sunset and sunrise due to latitude and longitude differences (Table 1), birds form three distinct groups along the day: (i) diurnal singers that were active during the day, (ii) nocturnal singers active only at night, and (iii) crepuscular birds that sing around both sunrise and sunset (Figure 1a–c). Similarly, we identified three groups of anurans: (i) early singers that were active before 21:00, (ii) late singers active after 21:00, and (iii) crepuscular species active both early in the morning and late in the afternoons (Figure 1d). Even though species composition varies across biomes, birds and anurans exhibited similar patterns of activity.

We also observed a clear pattern of vocal activity across biomes. Specifically, we found that birds were most active during the early morning hours (05:00–07:00), with detection rates exceeding six species per minute in inland Atlantic Forests (Figure 2a; #3). Despite we expected a peak of activity in late afternoons (dusk), our data revealed that higher bird detections were associated with early morning hours (dawn) but not dusk. In fact, detection rates across the late mornings (10:00–12:00) were higher than late afternoons in all biomes. As for anurans, we observed that they were highly active between 17:00 and 03:00 in lentic environments, with detection rates of 3–4 species per minute. In contrast, lotic environments exhibited consistently lower acoustic activity, with only one species detected per minute (Figure 2b).

The diversity of bird communities in Caatinga (#1) was low, with species accumulation curves stabilizing rapidly after approximately 1000 min of sampling (Figure 2c). Conversely, despite we have listened to 4000 1-min samples from the Amazon Rainforest, larger sampling efforts were still required for species accumulation curves to stabilize in this species-rich biome. Amazon (#7) and Atlantic forests (#3) exhibited much higher diversity values than those in the Caatinga (Figure 2c; #1). In lentic environments, overall anuran diversity reached the asymptote after 500 min of sampling. The anuran communities in lotic environments from the Coastal Atlantic Forest (#8) could benefit from further sampling, as these communities were sampled only for a month using 5 ARUs (Figure 2d). Additionally, within Coastal AF (#8) most species were found in both lentic and lotic habitats, although four species were exclusive to lotic environments, and only one was exclusive to lentic environments (Simões, 2014).

3.2 | Inspecting rates

Our results indicate that using different temporal sampling strategies had no effect on estimating anuran species composition. We found no significant differences in diversity estimates using either two continuous minutes or two intermittent 1-min sample types

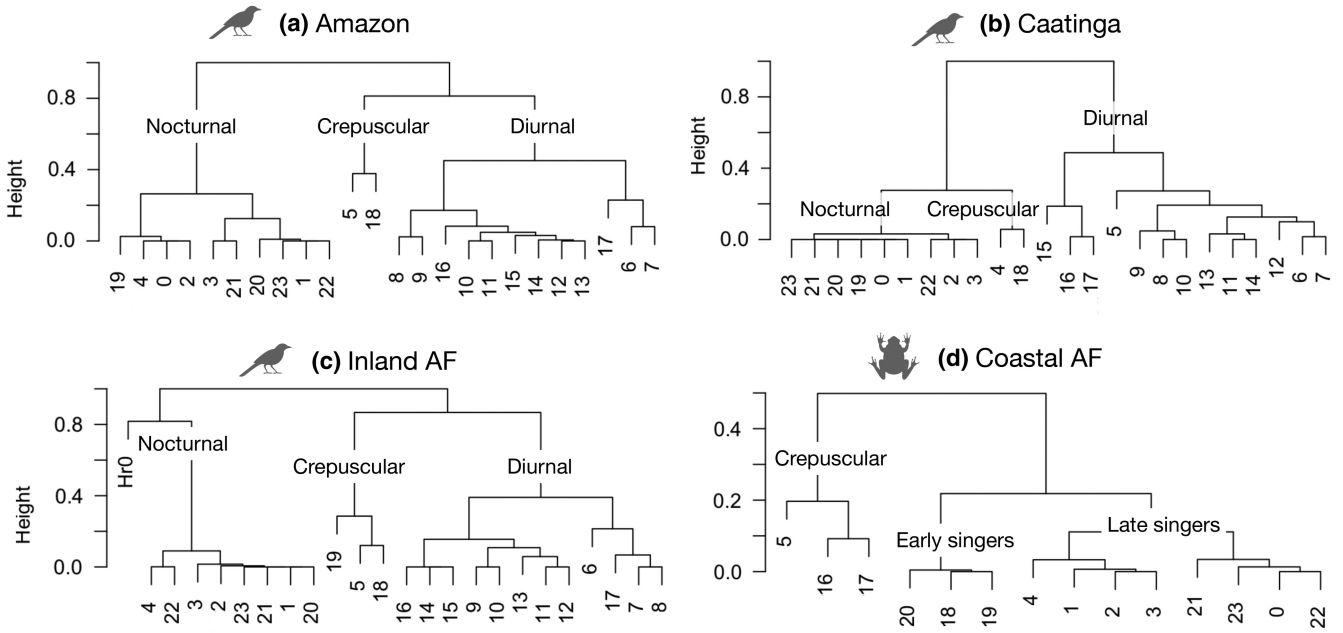

FIGURE 1 Results of the unweighted pair group method with arithmetic mean (UPGMA) show that similar compositions of vocalizing species are found at specific day periods. The numbers represent the period of the day of the sample, where 1 represents 1 am, 2 represents 2 am, and so on. Data were collected in the Amazon (a), Caatinga (b), Coastal Atlantic Forest (for birds, c), and Coastal Atlantic Forests (Amphibians, d) using passive acoustic monitoring.

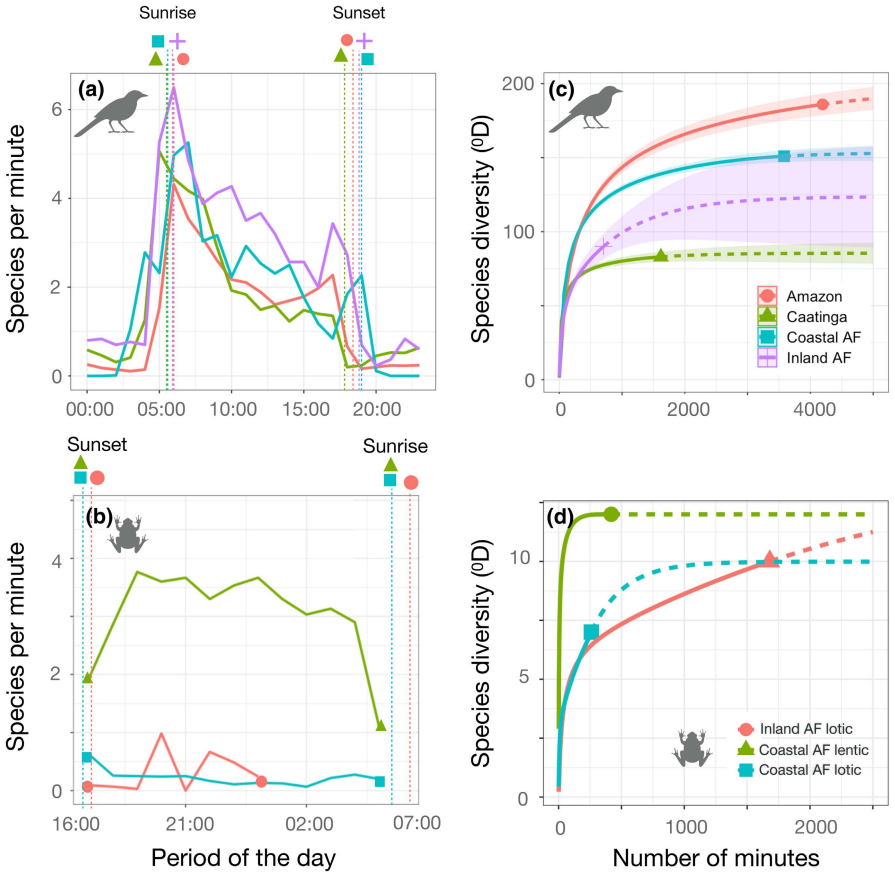

FIGURE 2 Left: The species detection rates along the day are shown for birds (a) and anurans (b). Vertical dotted lines represent the local time of sunset and sunrise for each dataset. Right: the plot shows the species richness (^{0}D) rarefaction and extrapolation curves of birds (c) and anurans (d). Contiguous and dashed lines represent interpolated and extrapolated values, respectively.

(Wilcoxon V = 44, p = .708). For birds, however, given a fixed number of analyzed minutes (i.e., inspection effort), inspecting to the soundscape using a 1 min/h inspecting rate (or 2 min/h) resulted in faster sampling coverage of birds in the Atlantic Forests compared to rates of 6 min/h (Figure 3). This suggests that distributing 1-min recordings over different hours instead concentrating them in a

single hour may result in a faster accumulation of sampling coverage for birds.

3.3 | The species–area relationship

The species–area curve was a good fit for our data, presenting coefficients of determination values above 85% (Table 2). Based on the species–area relationship, a single ARU using a standard schedule of 144 min per day (6 1-min files recorded at each hour over a single 24-h cycle) is expected to detect 59 bird species in the Amazon and 43 bird species in the Caatinga. The same schedule yields intermediate values of 53 bird species for Atlantic Forests. As Amazon rainforests exhibit nearly double the beta diversity (z) found in the Caatinga, a second ARU installed in the Amazon provides an even larger number of species (81) compared to a second ARU installed in the Caatinga (52).

3.4 | Inspecting effort and optimization

Our findings indicate that inspecting effort, here defined as the total number of 1-min files inspected, is largely variable compared among biomes. The number of subsamples should be about 10 times higher in the Amazon to achieve a similar sample coverage in Caatinga. For birds of Caatinga, a coverage value of 0.995 is achieved before inspecting 500 min, while a similar value was achieved at 4000 1-min samples in the Amazon (Figure 4a). Estimates of anuran diversity are largely dependent on the type of environment, and coverage accumulates much faster in lentic environments compared to lotic ones. While 500 min was sufficient to achieve 0.999 coverage in lentic environments, in some Atlantic Forests (AF) lotic areas, a sample of 5000 min would still not be enough to cover the local anuran diversity (Figure 4b).

We explored how an optimized inspecting schedule focusing on the most informative periods could improve the sampling completeness of Caatinga and Atlantic Forests. For birds, inspecting 1-min samples from the 5 most informative hours increased sampling completeness from 0.7 (Figure 4c, gold dashed line) to nearly 0.97 in Caatinga (solid gold line) and from 0.72 (pink dashed line) to nearly 0.9 (solid pink line) in inland Atlantic Forests (Figure 4c, q = 0). We show that the diversity indexes that give more weight to the abundant species stabilize faster. Hill numbers of higher q orders, such as ^{2}D, achieve sampling completeness much faster than lower q orders, which require larger samples. Specifically, we found that species richness (^{0}D) had a completeness of around 0.7 in both Caatinga and Atlantic Forests (Figure 4c, q = 0), while higher q orders provided completeness values greater than 0.95 for ^{1}D (Figure 4c, q = 1) and greater than 0.98 for ^{2}D (inverse Simpson; Figure 4c, q = 2).

4 | DISCUSSION

Our study provides insights on how to efficiently use PAM as a tool for unveiling the activity patterns and diversity of sonant animal communities. We found that birds were most active during early mornings, and contrary to our initial expectations, found no peak activity

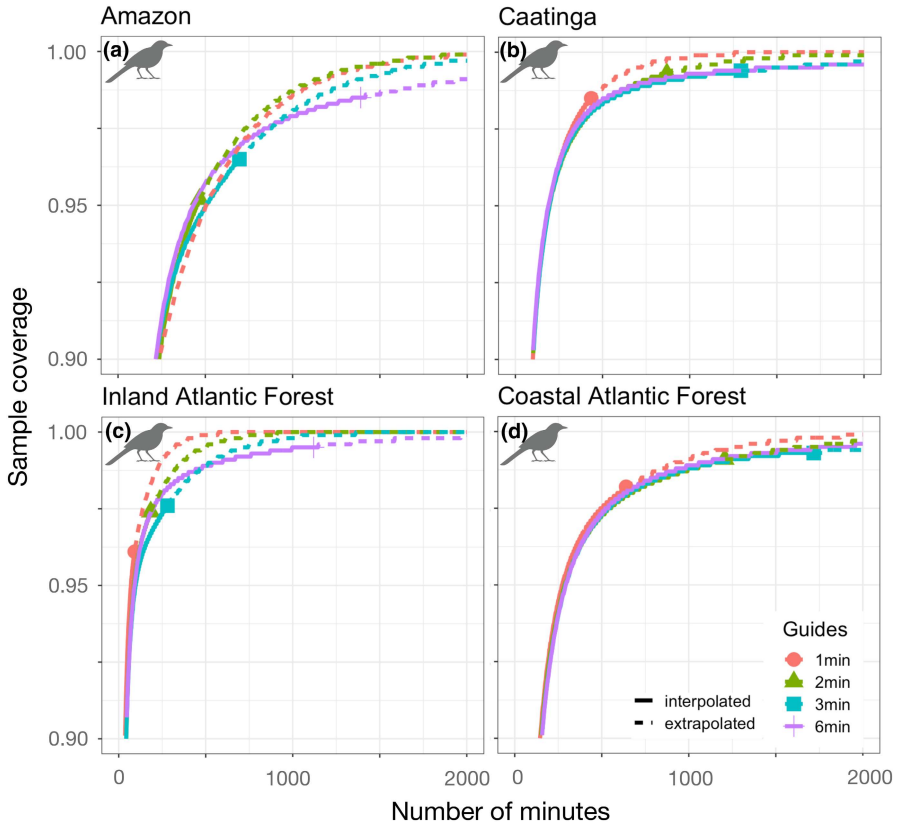

FIGURE 3 Sample coverage accumulation curves of birds of the samples attained from Amazon (a), Caatinga (b), inland (c) and coastal (d) Atlantic Forests. Contiguous and dashed lines represent interpolated and extrapolated values, respectively.

during late afternoons. Anurans exhibited the highest activity between 17:00 and 03:00 in lentic environments, while in lotic environments the acoustic activity was low during the entire night. For the same inspecting effort, inspecting rates of 1 min/h can provide faster sampling coverage compared to rates of 6 min/h for birds, and the longer inter-sample intervals (lower inspecting rates), the faster the sampling coverage accumulation. Bird sample coverage accumulates much faster in the Caatinga when compared to the Amazon. Biodiversity varies over time and space, making allocating resources effectively in both dimensions crucial. To develop an effective monitoring program, it is essential to understand how choosing different inspecting rates, recording periods, number of recorders, and total inspecting effort can influence the information gathered through PAM

Overall, PAM's efficiency seems comparable to traditional field techniques such as point counts, pitfalls, or active search (Alquezar & Machado, 2015; Darras et al., 2018). For instance, while 138 bird species were recorded over a 10-year sampling period of 30 points at the Iguaçú National Park (dataset #4; Oliveira & dos Anjos, 2022), a focused inspecting schedule on 8 ARUs recording for 5 days (a total 1120 min) detected 124 bird species at the same site (dataset #4; dos Anjos et al., 2022). PAM was able to capture at least 90% of the bird species in 18 h of sound recordings. Similarly, in the case of anurans, five pitfall and active search provided a list of 17 species (Simões, 2014), while a simultaneous PAM (dataset #8) using 5 ARUs detected a total of 15 species. Our findings provide additional support for PAM as an effective method for monitoring biodiversity. However, it is important to keep in mind that, beyond fieldwork, PAM will require additional inspecting time. Most terrestrial passive acoustic monitoring (PAM) uses recording rates of up to 3 min/h (Sugai et al., 2020), even though the effectiveness of such schedules has yet to be fully investigated. We found that sound recordings with long inter-sample intervals (lower inspecting rates) provide faster sampling coverage accumulation for birds, regardless of the biome. In contrast, we found no difference in the anuran diversity estimated using contiguous and intermittent sound recordings (10 min apart). Our findings suggest that sound recordings made in close succession are likely to have high similarities, as most species emit a sequence of calls, such as territorial, alarms, and contact calls. Therefore, sampling continuous minutes (or short inter-sample intervals) might not be the most efficient strategy. Instead, reduced inspecting rates over longer periods could provide better results. In fact, we found that inspecting rates of 1 min/h resulted in a much faster accumulation of bird species compared to higher rates of 6 min/h. By spreading out samples in the dataset, the sampling strategy approaches a random temporal selection, which has been shown to drive differences in estimates of species diversity (Anunciação et al., 2022). In summary, our findings suggest that an inspecting effort of 100 min using a rate of 1 min/h provides much more information compared to the same inspecting effort of 100 1-min files using an inspecting rate of 6 min/h. In the first scenario, the environment would be sampled for a period of 100 h, while in the second scenario, only 17. Therefore, reducing inspecting rates and increasing the overall inspecting periods can increase the efficiency and effectiveness of PAM (Anunciação et al., 2022; Metcalf et al., 2022).

By recording generic rates of 6 consecutive min/h in 24 h cycles, we were able to generate comparable datasets across a wide range of taxa and biomes. By considering that each ARUs can detect species within a fixed area A, we were able to use the species–area relationship to attain information on the number of species expected to be detected by a single (or multiple) ARUs, as well as the species turnover between ARUs. Using the species–area relationship, we predict that a single ARU recording at generic rates (144 min) would detect 59

TABLE 2 Vocal diversity in space in three biomes and the species detection rate.

Biome	C	z	*p*	r^2
Amazon	59.7	0.439	<.01	0.865
Atlantic forest	53.1	0.419	<.01	0.882
Caatinga	43.2	0.281	<.01	0.949

Note: C represents the number of species expected in a single ARU; z—the species detection rate; *p*—regression *p*-value; r^2—coefficient of determination.

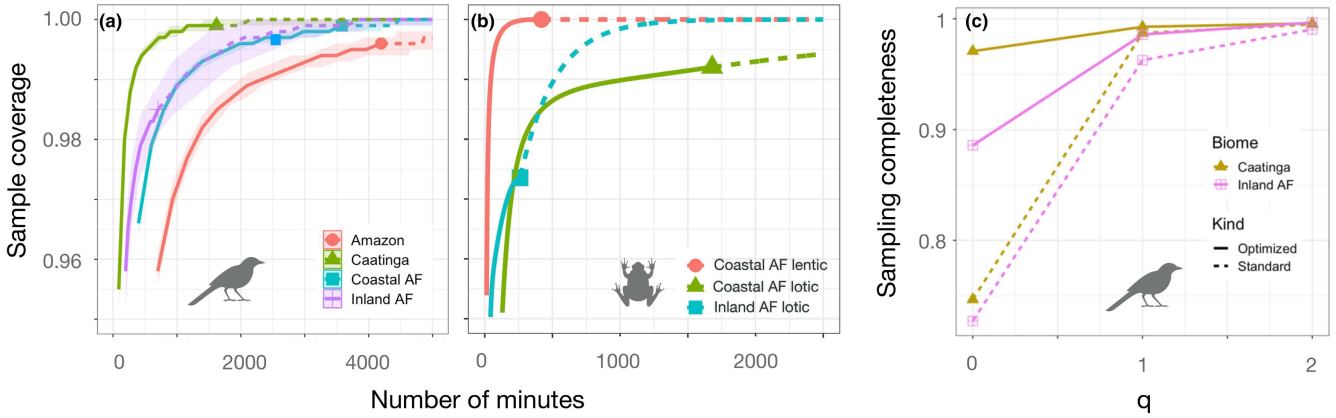

FIGURE 4 (a) Bird and (b) anuran sample coverage variation with the number of recorded minutes. Contiguous and dashed lines represent interpolated and extrapolated values, respectively. (c) Bird sampling completeness for Hill numbers of multiple orders (represented by order q) for generic (dashed lines) and optimized (solid line) datasets.

species in Carajás (#7) and 42 species in Assú (#1) during the first 24 h. Our results suggest that additional sampling efforts may be required in areas with high species richness, such as the Amazon Rainforest, as sampling coverage accumulates much slower in this biome compared to poorer biomes like the Caatinga. Therefore, to capture a representative sample of the acoustic biodiversity in the Amazon, researchers should consider increasing the number of ARUs, while in the Caatinga, an efficient monitoring program could be achieved with much less effort. Furthermore, as each recorder is installed at a fixed position in space, proper evaluations of the surrounding environment (e.g., canopy openness, temperature, and tree density) could enrich spatial data, allowing the use of statistical models that evaluate how these environmental variables affect biodiversity distribution.

Given the different levels of sensitivity to rare species, we anticipated that Hill numbers of higher orders (^{2}D) would accumulate and stabilize faster than those of lower orders ^{0}D. Achieving an optimal balance to optimize the manual inspection of subsamples of audio is critical for successful passive acoustic monitoring (PAM) of multiple species, and Hill numbers of higher orders will provide reliable estimates at much smaller sample sizes. The application of Hill numbers in PAM is understudied (but see Anunciação et al., 2022), and there is a significant gap in knowledge about optimizing spatial and temporal efforts for PAM at different orders (q) of sensitivity to rareness.

We found multiple opportunities to improve the efficiency of inspecting schedules for passive acoustic monitoring. Focusing on diurnal birds and inspecting files during periods of the day when detection rates are highest (e.g., 06:00–08:00) delivers higher completeness values. In the Caatinga (#1), we were able to increase species completeness from 70% to over 95%, even though we reduced overall inspecting effort from 2904 to 1920 min. Despite the strategy was not as effective in the Atlantic Forest, we still observed a sample completeness improvement from 70% to nearly 90%. By identifying the most informative periods and species for a given ecosystem, researchers can optimize their inspecting schedules and achieve higher completeness rates with less effort, thereby increasing the cost-effectiveness of passive acoustic monitoring.

Using our result as a starting point to evaluate how aspects such as diel activity patterns, inspecting rates, recording periods, number of recorders, or total inspecting effort affects biodiversity estimates, we propose four steps toward optimizing inspecting efforts of birds and anurans in high diversity areas. We hope the following steps can help researchers plan the spatial and temporal designs of PAM, as shown in the Box, while having some expectancy on sampling efficiency.

4.1 | Four steps toward a framework for optimizing manual analysis

4.1.1 | Inspecting effort (a)

The first step is to estimate a target number of minutes to be used for analyses, the inspecting effort (Figure 5). The inspecting effort depends on the amount of time available to analyze sound recordings and researchers' efficiency in doing so. Even though this might sound counterintuitive, an important bottleneck of PAM is the amount of time required to inspect sound recordings and identify the species within. It is very important to determine a maximum inspecting effort upfront, even though it might be wise to start inspecting a smaller number of subsamples that represents different times and places of monitoring, making it possible to evaluate the best subset to estimate the number of minutes it will take to reach the asymptote, and if the value falls within the maximum inspecting effort. In general, as a greater number of rare species are expected within biomes of greater biodiversity, a larger number of samples will be needed to capture the species composition in such biomes. This can be accomplished by employing a greater number of spatial and temporal replicates. Considering the experience of the specialist labeling the data is also an important component, as experienced biodiversity experts should be more efficient. Additionally, observer fatigue might also affect the output. As the observer gets tired or distracted, it might take longer to inspect a given set of sound recordings. From our experience with dataset #7, a well-trained observer (MB, who has over 10 years of ornithological experience at the study site) took about 3 min to inspect and annotate the bird composition of a 1-min sound recording.

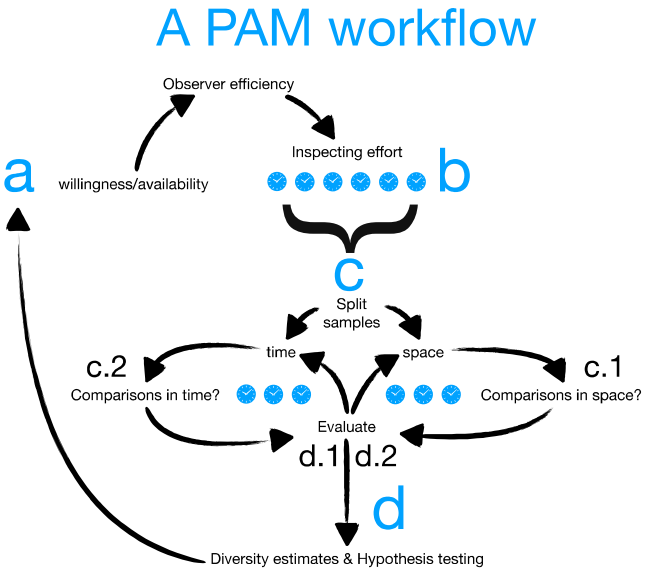

FIGURE 5 Four-step toward an efficient PAM monitoring program.

4.1.2 | Number of samples (b)

Our results suggest that initial sampling targets for birds in the Neotropical region should range from 1000 to 4000 min (Figures 2 and 4), which would take an experienced ornithologist some 60 to 200 h of work. Anurans might be sampled with smaller inspecting efforts, and as diversity is usually lower in frog assemblages compared to birds, observer efficiency is much higher. Optimizing inspecting effort to daily periods of increased acoustic activity can be a good

TABLE 3 Suggested inspecting schedules for efforts varying from 900 to 1600 min.

Taxa	Target	#ARU	Min/h	#h	Period	#days	Min/ARU	Minutes
Birds	Early birds	50	2	4	05:00–08:59	4	31	1600
Birds	Diurnal/nocturnal	20	4	5	04:00–08:59	4	80	1600
Birds	Diurnal/nocturnal	20	1	5	06:00–07:59	60	80	1600
Birds	Feeding activity	10	1	2	05:00–08:59	60	80	1600
Frogs	Season	40	2	2	19:00 and 22:59	6	24	960
Frogs	Rainy season	10	6	4	19:00–22:59	4	96	960
Frogs	Rainy season	5	1	3	19:00–21:59	60	180	900
All	Generic	10	6	24	24 h cycle	1	144	1440

strategy, but it is still important to consider that it might take additional time to annotate all the species with high activity, as the overall number of tags increases (Figure 2a,b). For less experienced personnel, we suggest annotating the amount of time taken to analyze ~30 files and evaluate steps (a) and (b).

4.1.3 | Split subsamples into time and space (c)

Passive acoustic monitoring (PAM) should be tailored to achieve specific objectives and scaled to the tested hypotheses, considering the fundamental limitations of inspecting effort. In that sense, inspected subsamples (1-min files) should be split in time and space, depending on the hypothesis to be tested. If, on the one hand, the number of audio samples at each site should be enough to provide good local diversity estimates. On the other, the number of spatial replicas should be proportional to the number of spatial treatments or habitat-use categories (c.1). For example, a study designed to evaluate differences in the diversity of five physiognomies should possess enough samples for each sampling site, but also enough replicas at each of these physiognomies. Lastly, recorders should be placed at a certain distance from one another which guarantee independence by assuring each call can only be detected by a single recorder. Point counts on Atlantic Forests consider sites 200 m apart to be independent (e.g., Oliveira & dos Anjos, 2022), even though some species might be detected at 500 m on sound recordings in the mixed-wood sites of Alberta (Yip et al., 2020). In addition to evaluating biodiversity changes in space, it might be interesting to evaluate changes in diversity over time. For instance, inspecting periods and rates cannot be considered apart from the hypothesis being tested.

4.1.4 | Evaluate local estimates (d)

The data collected can be used to evaluate sampling quality by assessing the completeness and/or coverage at each sampling point. At this step, is not only important to evaluate how the number of temporal replicates influences diversity estimates locally (d.1), but also to determine whether the number of recorders is sufficient to assess regional diversity estimates (d.2). The goal at this point is to determine whether the initial inspecting effort is adequate and distributed evenly across space, or whether additional inspecting is necessary before any hypotheses can be tested. Table 3 suggests examples of inspecting protocols designed for scenarios such as sampling large low-diversity areas, sampling the entire avian community over short or long periods, or targeting specific groups during biological phenomena. Our intention is not to establish a fixed sampling protocol, but rather to provide examples of how a similar inspecting effort between 900 and 1600 min can be distributed over time and space to enable testing a wide variety of hypotheses. These examples can serve as a starting point that can be adjusted to suit any particular need.

Altogether, we provide insights into the temporal and spatial aspects of passive acoustic monitoring (PAM) in the Neotropics, bridging a gap between community ecology and bioacoustics. Using the concept of call specificity by which each species possess a singular acoustical code (Florence, 1831), we identified vocally active species and compared sampling designs using sample coverage and completeness. Our systematic approach to data collection, using a generic recording rate of 6 min/h over 24 h, enabled us to compare data across several biomes of South America. Nevertheless, we found that optimizing inspecting schedules for specific groups can enhance sampling completeness. While we suggest recording and storing soundscapes using a rate of 6 min/h, focusing inspecting efforts on specific periods of interest, depending on the question and group being studied, can improve the quality of the diversity estimates and comparisons.

ACKNOWLEDGMENTS

CBA thanks CAPES PNPD program (financial code 001) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) of Argentina for Post Doctoral fellowships. EG, DV, CBA, and JPZ thank grant PUE #22920160100130CO from Consejo Nacional de Investigaciones Científicas y Técnicas of Argentina. RDA thanks doctoral scholarships provided by CAPES and CNPq. LdA (process 307643/2018-2) and RBM (process 311116/2022-1) receive grants from CNPq. ALR was supported by Fundo Brasileiro para Biodiversidade (FUNBIO)/Instituto Humanize. GLMR thanks Fundação Araucária for the NAPI Biodiversidade post-doctoral fellowship. We thank Dr. Larissa Sugai for her suggestions on earlier versions of this manuscript.

CONFLICT OF INTEREST STATEMENT

On behalf of all authors, the corresponding author confirms that there have been no interests that might affect the work reported here, including financial personal, political, or religious interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Zenodo at [http://doi.org/10.5281/zenodo.10556619].

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How to cite this article: De Araújo, C. B., Lima, M. R., Albuquerque, P., Alquezar, R. D., Barreiros, M., Jardim, M., Gangenova, E., Machado, R. B., Phalan, B. T., Roos, A. L., Rosa, G. L. M., Saturnino, N., Simões, C. R., Torres, I. M. D., Varela, D., Zurano, J. P., Marques, P. A. M., & dos Anjos, L. (2024). Acoustic monitoring of anurans and birds in tropical biomes. *Biotropica*, *00*, e13307. https://doi.org/10.1111/btp.13307