Community Ecology (2023) 24:47–60
https://doi.org/10.1007/s42974-022-00131-5

ORIGINAL ARTICLE

Complementarity between mist-netting and low-cost acoustic recorders to sample bats in Amazonian rainforests and savannahs

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Received: 18 February 2022 / Accepted: 14 December 2022 / Published online: 26 December 2022

Abstract

Effective survey methods are paramount to measure changes in species distribution, populations dynamics and to guide conservation. Mist-netting and passive acoustic monitoring are two of the most used techniques to sample bats assemblages. Yet, despite the great potential of low-cost autonomous ultrasound recorders in surveying bat assemblages, we lack thorough assessments of their performance in relation to more established survey methods. Taking advantage of the rich bat fauna of the northeastern Brazilian Amazon, we set out to i) investigate the complementarity of mist-netting and acoustic surveys in sampling bats in forest and savannah habitats in the Savannahs of Amapá and ii) undertake a cost-effectiveness evaluation of using one, two or three recorders per sampling site to simultaneously survey bat assemblages. The two methods show complementary, and overall species diversity recorded with mist nets was higher than with acoustic recorders. However, species diversity was higher with acoustic recorders than with mist nets when considering a reduced ($n < 3$) number of transects. In addition, we found a gain in species diversity when using more than one acoustic recorder in forest habitats, despite the low cost-effectiveness. However, there were no differences between the diversity using one, two or three acoustic recorders in savannah. Due to possible device malfunction, we recommend the use of at least two acoustic recorders in both habitats to reduce the likelihood of data loss. The use of low-cost bioacoustic recorders in bat surveys can help to address critical knowledge gaps for poorly known aerial-hawking insectivores and support evidence-based conservation strategies.

Keywords Acoustic monitoring · Audiomoths · Amazonian savannah · Chiroptera · Methods comparison · Neotropical bats

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Introduction

Quantifying changes in wildlife communities is a key challenge in the Anthropocene, requiring efficient and cost-effective survey methods (Hart et al. 2022). South and Central America constitute the planet's epicenter of bat diversity with over 300 species and 80 genera (Wilson & Mittermeier, 2019). Within this region, the Amazon is especially rich, harboring over one tenth of the world's known bat species (López-Baucells et al. 2018). Yet, comprehensive bat surveys covering the diversity of Amazonian habitats are both logistically challenging and costly.

Bats are key providers of multiple ecosystem services such as seed dispersal, pollination, and insect population suppression control (Aguiar et al. 2021; Kunz et al. 2011). According to their foraging strategy and feeding behavior, they can be classified into different ensembles, such as aerial-hawking insectivores (e.g., molossids and vespertilionids), which use echolocation to capture insects in the air (Arita & Fenton, 1997) or frugivorous and gleaning animalivores (e.g., most phyllostomids) which, in addition to echolocation, often rely on other senses to find fruit or capture prey (Joermann et al. 1988; Korine & Kalko, 2005; Tuttle et al. 1985). There are considerable interspecific differences in the efficiency of the distinct sampling methods used to survey bat assemblages (Meyer, 2015). While mainly frugivores, nectarivores and gleaning animalivores bats are easily captured using mist nets, the most popular sampling method across the tropics, other bat ensembles often go undetected in this kind of studies (Appel et al. 2021; Mancini et al. 2022; Silva & Bernard, 2017; Wordley et al. 2018). Aerial-hawking insectivorous bats are a conspicuous example, as most species are extremely difficult to capture with mist nets (Darras et al. 2021; López-Baucells et al. 2021; Silva & Bernard, 2017), and as a result, most of them remain poorly studied (e.g., Delgado-Jaramillo et al. 2020).

Multiple studies have investigated the complementarity of mist-netting and bioacoustic methods in detecting different bat species (e.g., Flaquer et al. 2007; Furey et al. 2009; MacSwiney et al. 2008; Pech-Canche et al. 2010). Yet, few were conducted in South America (but see, e.g., Pech-Canche et al. 2010; Silva & Bernard, 2017; Appel et al. 2021; da Silva et al. 2022; Mancini et al. 2022) and, to date, none simultaneously assessed the benefits of using multiple bat recorders per site or considering habitats with and without forest cover at the same time. In fact, most studies comparing survey methods in South America focused on birds, medium and large mammals, and amphibians (e.g., Carvalho et al. 2016a; Palmeirim et al. 2019; Ribeiro-Júnior et al. 2008; Rodrigues & Prado, 2018). Additionally, most comparisons between bat survey methodologies have investigated sampling efficiency in a single habitat, failing to address how habitat structure might influence the performance of the methods assessed (but see Wordley et al. 2018; López-Baucells et al. 2021).

In the Amazon, mist nets are the most used bat sampling methodology, and therefore, most available bat data are biased toward the family Phyllostomidae (Appel et al. 2021; Delgado-Jaramillo et al. 2020). With the recent increase in bioacoustic studies (e.g., Appel et al. 2021; Froidevaux et al. 2020; López-Baucells et al. 2022; Oliveira et al. 2015), this scenario has started to change, but there is still an immense gap in the knowledge of insectivorous bats throughout the Neotropics as a whole, mainly because of the high cost of acoustic devices. However, low-cost autonomous acoustic recorders, such as AudioMoth (Hill et al. 2018), are increasingly being used in bat studies (e.g., Ferreira et al. 2022; Froidevaux et al. 2020). Therefore, it is paramount that the performance and robustness of these novel devices are accurately assessed, especially in biomes with a high bat diversity (Delgado-Jaramillo et al. 2020) and extreme weather conditions (e.g., high temperature, rainfall, and relative humidity), such as the Amazon. Equally important is assessing the minimum number of acoustic recorders and sampling effort needed to maximise cost-effectiveness in detecting bat species, this being a type of methodological evaluation that is scarce when we consider autonomous acoustic recorders (Gardner et al. 2008; Meyer, 2015).

Amazonian savannahs are among the most heterogeneous Amazonian ecosystems, containing different habitats, ranging from open-canopy savannah to closed-canopy forest patches (Mustin et al. 2017; Prance, 1996). Due to this heterogeneity, the Amazonian savannahs can be used as excellent natural laboratories for ecological and methodological studies (e.g., Carvalho et al. 2020; Sousa et al. 2022). Ecological models have identified these Amazonian savannahs as a hotspot of bat diversity and priority areas for bat inventories (Aguiar et al. 2020). The state of Amapá, in the northeast of the Brazilian Amazon, is home to the fourth largest block of Amazonian savannah, known as Savannahs of Amapá (Carvalho & Mustin, 2017), which, due to its environmental complexity and heterogeneity, hosts over 50 bat species (Carvalho et al. 2021; Martins et al. 2022; Silva et al. 2013). However, bats are still one of the least sampled mammalian taxa in the region, and, to our knowledge, no acoustic bat survey has been conducted.

Here, we take advantage of the rich bat fauna of the northeastern Brazilian Amazon to investigate the complementarity of mist-netting and low-cost acoustic recorders in sampling Neotropical bats in open (savannah) and closed-canopy habitats (forest patches—hereinafter simply "forest"). Our aims were (i) to assess how metrics related to bat species richness and diversity change according to sampling method in savannah and forest; (ii) to test the benefits of simultaneously using one, two or three acoustic devices in the same transect in savannah and forest; and (iii) investigate the cost-effectiveness of using one, two or three acoustic recorders per transect. Regarding the latter, we used the cost-effectiveness ratio of registering each species per acoustic recorder to help select the minimum number of recorders used in each habitat.

Materials and methods

Study area

This study was carried out in the Savannahs of Amapá, an Amazonian savannah located on the eastern edge of the state of Amapá, northeastern Brazilian Amazon (Fig. 1). This region has a Tropical monsoon climate (Am – according to Köppen's climate classification), with rainfall of the driest month below 60 mm and annual rainfall ranging from 2,300 to 2,800 mm (Souza & Cunha, 2010). The rainy season runs from December to July, the dry season runs from August to

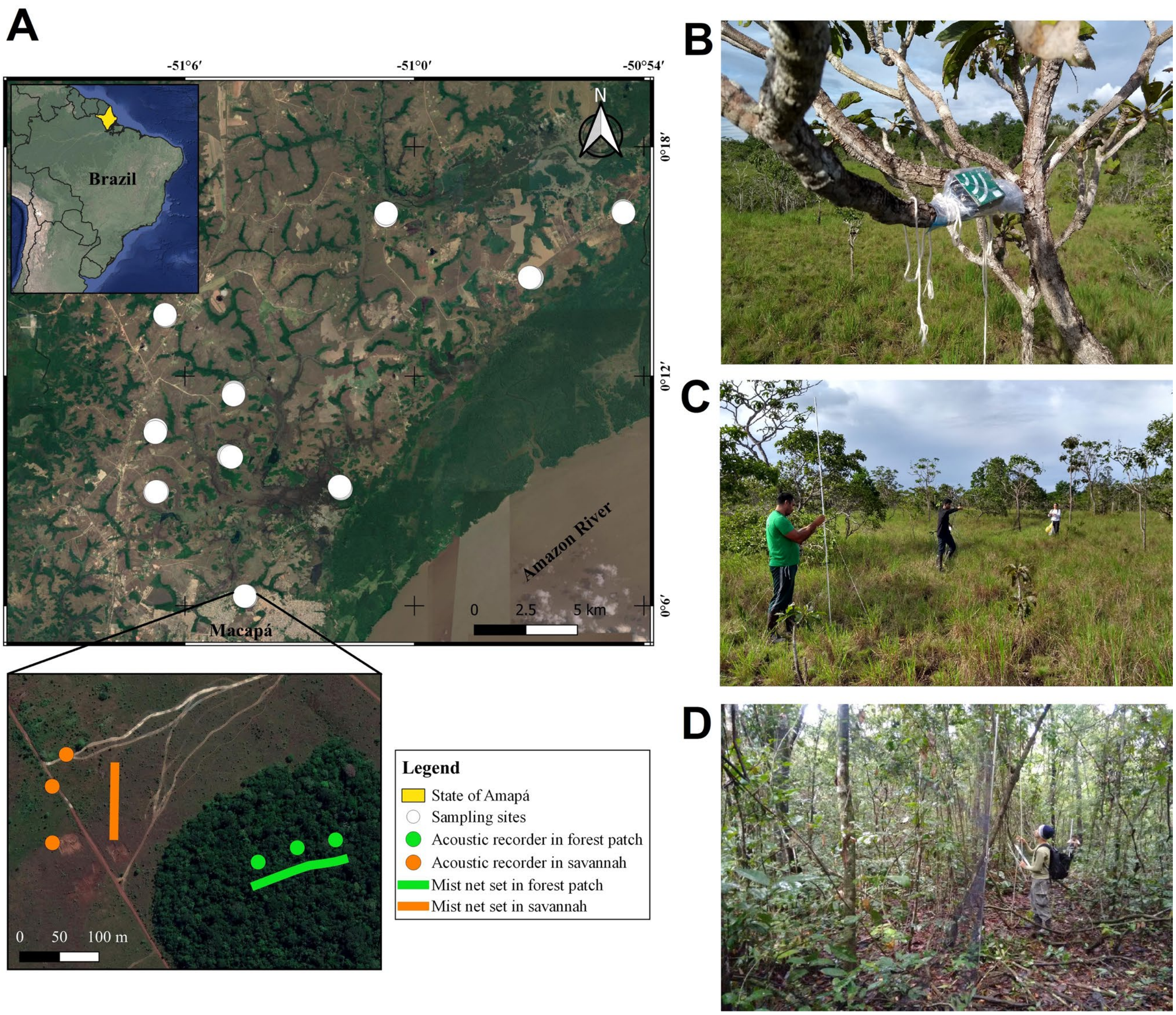

Fig. 1 Location of sampling sites in the Savannahs of Amapá, northeastern Brazilian Amazon (**A**). The image below shows how mist nets and acoustic recorders were arranged in both savannah and forest (**A**). AudioMoth inside a zip-lock bag on the branch of a tree in the savannah (**B**). Set of mist nets being mounted in savannah (**C**) forest (**D**) sampling sites

November, and the average air temperature has a low thermal range, oscillating around 27 °C (Tavares, 2014).

Sample design

In the southeastern of the area occupied by Savannahs of Amapá (Fig. 1), close to the state capital, Macapá, we selected ten sites to simultaneously survey bats using mist nets and passive ultrasound recorders. Each of these sites had one transect in the natural forest and one in the savannah matrix (Fig. 1), totalling 20 transects. All locations were selected in sites with as little human intervention as possible. All sites were dominated by park savannah (Mustin et al. 2017), characterised by an open tree story less than three meters high and a very open canopy (Costa-Neto, 2014). The forest patches are embedded in the savannah matrix and have a closed canopy with an average height of six meters with scattered trees reaching up to 25 m.

Bat sampling

We conducted two sampling nights during the rainy season between June and July 2018 at each site. The rainy season is the period with the highest bat capture rate in this region (Carvalho et al. 2018). We sampled the forest and savannah transects simultaneously at each site. This resulted in 20 nights of sampling in the forest and 20 nights in the savannah. Visits to the same site were spaced by at least 30 days to avoid net shyness (Marques et al. 2013).

In each transect, we used nine mist nets (12 × 3 m; 14 mm mesh size), set in the understory along a ~ 110 m trail, at least 30 m from the edge between the two habitats to minimise edge effects (López-Baucells et al. 2022; Meyer et al. 2016). In addition, three passive acoustic recorders (AudioMoth; Hill et al. 2018) were placed at approximately 1.5 m height, forming a line parallel to the mist nests (Fig. 1). Acoustic recorders were located at more than 50 m from the mist nets to minimise the risk of recording netted bats. In the forest, recorders were set in small clearings to minimise vegetation clutter and increase the probability of recording species that fly above the canopy (e.g., *Cormura brevirostris*, *Peropteryx kappleri*, *Molossus molossus* and *Promops centralis*; Marques et al. 2016). They were placed about 50 m from each other inside zip-lock bags (Fig. 1). Nets and recorders were operated for 6 h, from ~ 18:00 (i.e., 15 min before sunset) to 00:00. Mist nets were checked every ca. 20 min (Carvalho et al. 2016b). Total netting effort, calculated accordingly to Straube and Bianconi (2002), was 155.5 m^2*h, equally divided by the two habitats. The recorders were configured to record continuously, save a file every five minutes, and use a sampling rate of 384 kHz and medium gain (López-Baucells et al. 2022).

Captured bats were placed in cotton bags and identified according to Lim and Engstrom (2001), Reis et al. (2017) and López-Baucells et al. (2018). Nomenclature follows Garbino et al. (2020). Fieldwork followed the guidelines of the American Society of Mammalogists (Sikes et al. 2016). Recorded calls were identified using the software Kaleidoscope® (version 5.0.0, Wildlife Acoustics, USA). With this software, we ran an automatic process to split all the recording files, previously stored in five minutes Waveform Audio File Format (WAV) into multiple 5-s-long WAV files. To discard the files unlikely to include echolocation calls, we only used those that had at least two pulses between 10 and 200 kHz and with a duration between 2 and 500 ms, and a maximum inter-syllable gap of 500 ms. Two or more pulses of a single species/sonotype in a 5-s-long recording were considered a 'bat pass' (see, e.g., Yoh et al. 2022). Identification of the sonotype(s) was undertaken manually to the lowest taxonomic level possible (López-Baucells et al. 2019), following the acoustic keys in López-Baucells et al. (2018) and Arias-Aguilar et al. (2018). When possible, bat calls were assigned to a species, but otherwise they were assigned to sonotypes, i.e., groups of species that emit calls are very difficult or impossible to distinguish with acceptable certainty (Table S1, Supplementary Material 1). These species are often congeners but can also be phylogenetically distant.

Data analysis

Complementarity of mist nets and acoustic recorders

To investigate the complementarity of mist nets and passive acoustic surveys, we used a subsample with 22 nights, twelve in forest and ten in savannah. The different number of transects per habitat is because we only considered the nights when three recorders were simultaneously active. Using presence/absence data, we constructed rarefaction species accumulation curves in the 'iNEXT' package (Hsieh et al. 2016) for both habitats. We estimated the Hill numbers corresponding to the species richness ($q = 0$), the exponential of Shannon's entropy index ($q = 1$; Shannon diversity), and the inverse of Simpson's concentration index ($q = 2$; Simpson diversity) (Chao et al. 2014; Hill, 1973). Hill numbers are defined by the q parameter, which determines the sensitivity of the measure to relative species abundances and facilitates data comparison (Chao et al. 2014; Hill, 1973). For presence/absence data, Hill numbers estimate the value of q based on relative incidence in the assemblage (number of sample units in our case), being interpreted as the effective number of equally frequent species in the assemblage from which the sampling units are drawn (Chao et al. 2014). The rarefaction curves had a maximum of 12 and 10 samples, for forest and savannah, respectively, because that was the number of samples available for each habitat. We carried out all analyses for both habitats separately and together. The packages mentioned were loaded into the program R v.4.0.3 (R Core Team, 2020).

Sampling efficiency of multiple recorders

To investigate whether there is a relevant benefit in using more than one acoustic recorder per transect, we used 15 nights, eight in forest and seven in savannah. The different number of transects per habitat is because we only considered the nights when three recorders were simultaneously active. With these data, we analyzed rarefaction species accumulation curves for one, two and three recorders. The rarefaction curves had a maximum of eight and seven samples, for forest and savannah, respectively. To compare the curves, we used the overlap between the estimated confidence envelopes (see Chao & Chiu, 2016). Whenever the 84% confidence intervals did not overlap, the difference was considered significant at the $\alpha = 0.05$ level (Cumming & Finch, 2005; MacGregor-Fors & Payton, 2013).

Cost-effectiveness analysis

To assist in the selection of the appropriate number of acoustic recorders to be used to survey each habitat in a cost-effectively manner, we calculated the cost of identifying each species using one, two or three acoustic recorders per transect. To estimate the cost-effectiveness, we followed the procedure in Carvalho et al. (2016a). The expenses were divided into fixed and variable costs. In our case, the fixed expenses were those that did not vary depending on the number of acoustic recorders used per transect: per-diem for the researcher (scholarships and salary), field assistant, car rental, fuel, airline or bus tickets, per-diem for food, amortisation value of field material, safety equipment and first aid kit, computer, and field guides. As these costs were similar for any number of acoustic recorders, we focused our analysis on the variable expenses (see Carvalho et al. 2016a). The variable expenses are those that varied depending on the number of acoustic recorders used: acoustic recorders, plastic bags for environmental protection, alkaline batteries, digital memory (SD) cards, external hard disk (HD) for the storage of recordings, and daily salary for experts identifying the recordings (Table S2, Supplementary Material 1). Specifically, for acoustic recorders, we included one backup device to replace—if necessary—any glitched recorder. The costs were estimated in Brazilian Reais (R$) and then converted to Dollars ($), with a conversion rate of R$ 1.0 to $0.24. The conversion rate is equivalent to July 30, 2018, when the field sampling ended.

Results

Overview for acoustic recorders and mist nets

We obtained a total of 53 species/sonotypes (Table 1). Combining the species/sonotypes captured with mist nets with those registered with the acoustic recorders, we reached 40 species/sonotypes in savannah and 41 in forest. With the use of acoustic recorders, we identified 23,363 bat passes of 13 species and four sonotypes. Of these, 11,015 passes (47%) were recorded in forest and belong to 16 different species/sonotypes. The remaining 12,348 passes (53%) were recorded in savannah and included 16 species/sonotypes (Table 1). Using mist nets, we captured 322 bats, 228 (71%) in forest (26 species) and 97 (29%) in savannah (25 species).

Complementarity of mist nets and acoustic recorders

Of the 53 species recorded, only *Saccopteryx bilineata* and *Saccopteryx leptura* were registered with both methods. All species/sonotypes registered by the acoustic recorders were aerial insectivorous bats (Table 1). In forest, 13 species were registered only with mist nets, of which five were aerial insectivores (*Peropteryx leucoptera*, *P. pallidoptera*, *Rhynchonycteris naso*, *Saccopteryx leptura* and *Myotis riparius*) and eight belonged to the Phyllostomidae family. In savannah, 12 species were captured exclusively with mist nets, of which two (*Saccopteryx bilineata* and *Molossus molossus*) were aerial insectivores, and 10 belonged to the Phyllostomidae family (Table 1). Considering both habitats together, acoustic recorders alone recorded 15 species/sonotypes, 14 in forest and 14 in savannahs. *Diclidurus* sp. and *Eptesicus* sp. were only recorded by acoustic recorders in savannah and forest, respectively.

The diversity curves for both methods together and for mist nets did not show a stabilisation trend (Fig. 2). However, the diversity curves for acoustic recorders did (Fig. 2). Overall, mist nets registered a higher proportion of the estimated species richness, Shannon diversity, and Simpson's diversity than acoustic recorders (Figs. 2 and 3). This was true when pooling both habitats and when considering them separately, but the relative contribution of mist nets for Shannon and Simpson's diversity was slightly lower in savannahs than in forests (Figs. 2 and 3). Additionally, our results show that Simpson's diversity—the estimator most influenced by frequent species—led to smaller differences between the estimated species richness between the two methods evaluated (Fig. 3). However, it is important to note that this advantage of mist-netting

Table 1 Bat species captured with mist nets and species/sonotypes registered by acoustic recorders in the Savannahs of Amapá, northeastern Brazilian Amazon

Family and species/sonotype	Mist nets		Acoustic recorders	
	Forest	Savannah	Forest	Savannah
Emballonuridae				
Centronycteris centralis/maximiliani	–	–	X	X
Cormura brevirostris	–	–	X	X
Diclidurus sp.	–	–	-	X
Diclidurus albus/scutatus	–	–	X	X
Peropteryx sp.	–	–	X	X
Peropteryx leucoptera	X	–	–	–
Peropteryx pallidoptera	X	–	–	–
Rhynchonycteris naso	X	–	–	–
Saccopteryx sp.	–	–	X	X
Saccopteryx bilineata	–	X	X	X
Saccopteryx canescens/gymnura	–	–	X	X
Saccopteryx leptura	X	–	X	X
Molossidae				
Molossidae I	–	–	X	X
Molossidae II			X	X
Molossops sp.	–	–	X	X
Molossus molossus	–	X	-	-
Promops sp.	–	–	X	X
Phyllostomidae				
Ametrida centurio	–	X	–	–
Artibeus concolor	X	X	–	–
Artibeus lituratus	X	X	–	–
Artibeus obscurus	X	X	–	–
Artibeus planirostris	X	X	–	–
Carollia brevicauda	X	X	–	–
Carollia perspicillata	X	X	–	–
Chiroderma trinitatum	X	–	–	–
Artibeus cinereus	X	X	–	–
Artibeus gnomus	–	X	–	–
Desmodus rotundus	X	X	–	–
Glossophaga soricina	X	–	–	–
Lophostoma brasiliense	–	X	–	–
Lophostoma silvicola	X	–	–	–
Mesophylla macconnelli	–	X	–	–
Micronycteris microtis	X	–	–	–
Micronycteris minuta	–	X	–	–
Micronycteris schmidtorum	X	–	–	–
Gardnerycteris crenulatum	X	X	–	–
Phyllostomus elongatus	X	–	–	–
Phyllostomus hastatus	X	–	–	–
Platyrrhinus sp.	–	X	–	–
Platyrrhinus fusciventris	–	X	–	–
Platyrrhinus incarum	X	X	–	–
Rhinophylla pumilio	X	X	–	–
Sturnira lilium	X	X	–	–
Tonatia maresi	X	–	–	–
Trinycteris nicefori	–	X	–	–
Uroderma bilobatum	X	X	–	–

Table 1 (continued)

Family and species/sonotype	Mist nets		Acoustic recorders	
	Forest	Savannah	Forest	Savannah
Uroderma magnirostrum	–	X	–	–
Vampyriscus brocki	–	X	–	–
Vespertilionidae				
Vespertilionidae I	–	–	X	X
Vespertilionidae II	–	–	X	X
Eptesicus sp.	–	–	X	-
Myotis nigricans	–	–	X	X
Myotis riparius	X	–	–	–
Total number of species/sonotypes by method	26	25	16	16
Total number of species/sonotypes	38	17		

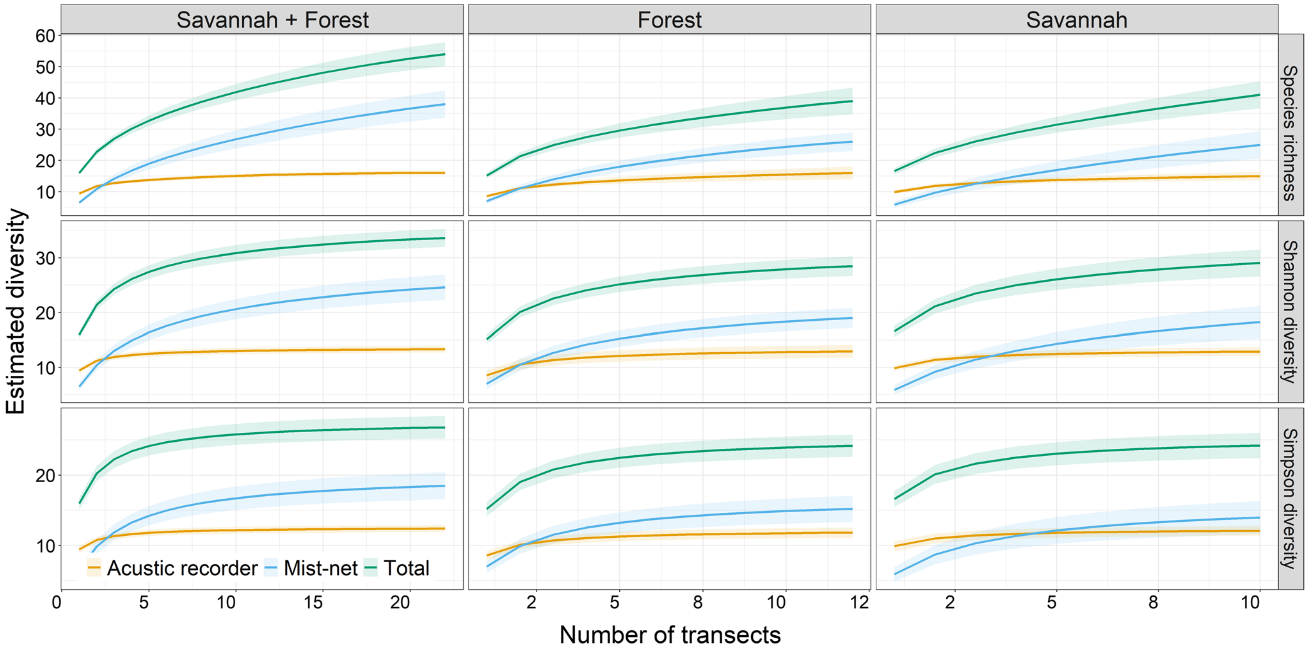

Fig. 2 Estimated species richness, Shannon and Simpson's diversity of bats sampled by mist nets and acoustic recorders in forest and savannah transects in the Savannahs of Amapá, northeastern Brazilian Amazon. The shaded areas represent the 84% confidence envelopes

was not observed when the number of sampled transects was very low (Fig. 2). The rarefaction curves in Fig. 2 show that, when using only 1 to ~ 3 sampled transects, the acoustic recorders registered the same number of species as mist-netting or even more.

Gain in species richness and diversity with the increase in the number of recorders

By pooling the data of forest and savannah, the estimated species richness was 14 species/sonotypes using one acoustic recorder per transect and 16 species/sonotypes using two or three acoustic recorders per transect (Fig. 4). For Shannon and Simpson's diversity, the values were slightly higher when using three acoustic recorders, followed by two acoustic recorders and one acoustic recorder (Fig. 4). However, the analysis of the overlaps between confidence envelopes indicates that only the differences between one and three recorders are statistically significant (Fig. 4).

None of the diversity curves showed a stabilisation trend, whether for one, two or three recorders. Considering the two habitats separately, in the forest, using three acoustic recorders led to higher estimated species richness, Shannon and Simpson's diversity than using one acoustic recorder (Fig. 4). The estimated species richness, Shannon and Simpson's diversity also increased with the use of two acoustic recorders. However, there was no difference between using two and three acoustic recorders. For the savannah,

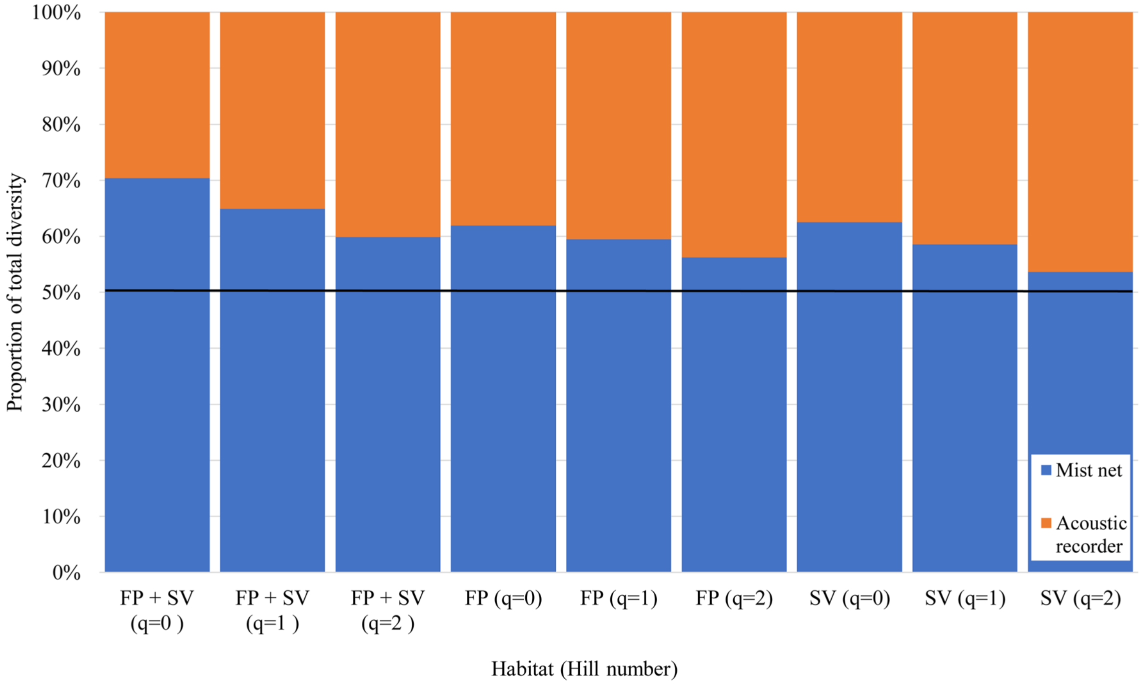

Fig. 3 Proportion of total diversity for estimated species richness ($q=0$), Shannon ($q=1$), and Simpson's diversity ($q=2$) of bats sampled by mist nets and acoustic recorders in forest and savannah, in the Savannahs of Amapá, northeastern Brazilian Amazon. FP—Forest patches (forest); SV—Savannah. The black horizontal line indicates the proportion of 50% of total diversity

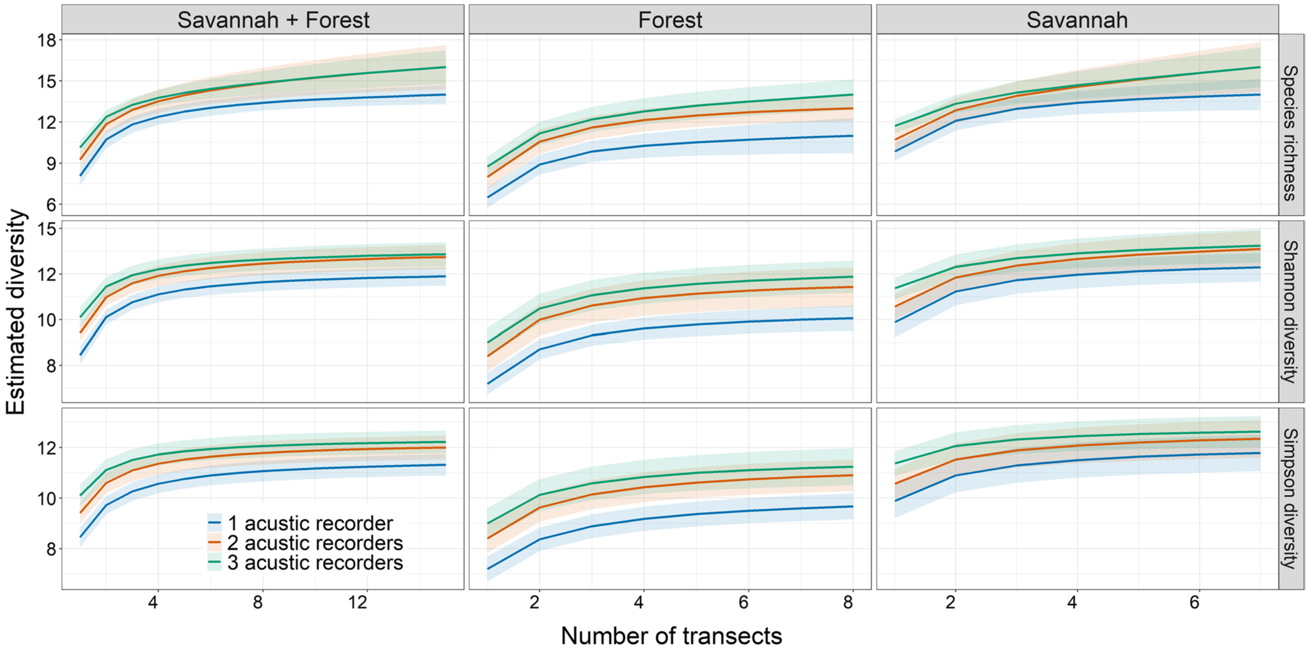

Fig. 4 Estimated species richness, Shannon and Simpson's diversity of bats sampled in transects with one, two or three acoustic recorders in forest and savannah transects in the Savannahs of Amapá, northeastern Brazilian Amazon. The shaded areas represent the 84% confidence envelopes

Table 2 Costs of surveying bats with one, two or three acoustic recorders in forest and savannah transects in the Savannahs of Amapá, northeastern Brazilian Amazon

	Forest – Total cost US$ (R$)			Savannah – Total cost US$ (R$)		
N° acoustic records	1	2	3	1	2	3
Total cost (20 days)	1,764.96 (7,354)	3,409.92 (14,208)	5,174.88 (21,562)	1,764.96 (7,354)	3,409.92 (14,208)	5,174.88 (21,562)
Per-day cost	88.25 (367.70)	170.49 (710.40)	258.74 (1,078.10)	88.25 (367.70)	170.49 (710.40)	258.74 (1,078.10)
Estimated richness	11	13	14	14	16	16
Per-species cost	160.45 (668.54)	262.30 (1,092.92)	369.63 (1,540.14)	126.06 (525.28)	213.12 (888)	323.43 (1,347.62)

all confidence intervals overlapped; thus, there were no significant differences between the diversity curves when using one, two or three acoustic recorders (Fig. 4). For all comparisons, the estimator that puts more weight in the most frequent species (i.e., Simpson's diversity) slightly increased the differences between the curves when using two recorders instead of three. However, this difference was not significant as there was a considerable overlap in the confidence intervals (Fig. 4).

Cost-effectiveness analysis

We found that the total cost to sample bats in 10 transects for 20 days was US$ 5,174 (R$ 21,562) when using three acoustic recorders, independently of the sampled habitat (Table 2). Reducing to two recorders, the total cost was 34% lower. Lastly, when using only one recorder, the total cost was 66% and 48% lower than using three and two acoustic recorders, respectively (Table 2).

The cost of sampling each species/sonotype was higher in forest than in savannahs (Table 2). In forest, when using two recorders, the cost per species/sonotype was 29% lower than using three recorders (Table 2). Considering only one recorder in forest, the cost of registering each species/sonotype was 56% and 39% lower when compared with three and two recorders, respectively. For savannahs, the cost of registering each species/sonotype with only two recorders was 34% lower compared with three acoustic recorders. Finally, with only one recorder in savannah, the cost of registering each species/sonotype was 61% and 41% lower compared to using three and two acoustic recorders, respectively (Table 2).

Discussion

This is the first study investigating the number of low-cost recorders in a single locality required to assess bat diversity in the Neotropics, specifically in the highly diverse Amazon rainforest. Sampling species-rich bat assemblages is often challenging due to the different methods that must be employed to survey the different bat guilds. In this context, we evidenced differences in the complementarity between mist nets and autonomous acoustic recorders in bat sampling between open (savannah) and closed (forest) Amazonian habitats. Our results are in line with previous studies (e.g., Appel et al. 2021; da Silva et al. 2022; Flaquer et al. 2007; Furey et al. 2009; MacSwiney et al. 2008; Mancini et al. 2022; Pech-Canche et al. 2010; Silva & Bernard, 2017; Wordley et al. 2018), that also combined methods to increase considerably the estimated species richness and diversity. Therefore, the simultaneous use of acoustic records and mist nets brings great benefits for the survey of bats in both open and close canopy habitats. We found that for better cost-effectiveness, researchers should use at least one recorder in habitats such as savannahs and two recorders in forests. Our results also highlight the importance of evaluating bat sampling methods, especially concerning the minimum number of recorders per transect, which can directly interfere with the estimated diversity.

When using all our transects, mist nets contributed more to the total registered richness and diversity than the recorders (Figs. 2 and 3). Other studies in the Neotropics also found a higher contribution of mist nets in relation to acoustic recorders for bat sampling (Appel et al. 2021; da Silva et al. 2022; Mancini et al. 2022; Silva & Bernard, 2017). This does not necessarily mean that the number of species recorded by sound recorders is lower than that captured by mist nets. The difference may be simply due to the difficulty, or even impossibility, of distinguishing the calls of some species, which are thus pooled in sonotypes. In contrast, virtually all bats captured in the mist nets can be identified to species. Our results show that, when using just a few transects, the recorders can be more efficient at recording species than mist-netting (Fig. 3). The rarefaction curves for recorders climb very quickly, reflecting the efficiency of this technique at detecting species with adequate vocalisations. However, the curves level off with just a few transects. This early stabilisation of the curves is presumably caused by the incapacity to distinguish species that has overlapping acoustic features (e.g., frequency of maximum energy). These are thus pooled in sonotypes that are treated as a single taxon in all analyses. That is, for example, the case of *Diclidurus albus/scutatus*, a sonotype that includes two species that occur in the study region (see Silva et al. 2013) but are acoustically indistinguishable. The curves for mist-netting climb more progressively, reflecting the capture of less abundant species (e.g., *Uroderma bilobatum* in forest and *Gardnerycteris crenulatum* in savannah).

The contribution of acoustic recorders to Shannon and Simpson's diversity in savannah was slightly higher than their contribution in forest (Fig. 2). Wordley et al. (2018) obtained similar results when comparing species richness of Indian bats using mist nets and acoustic recorders in open-canopy habitats (tea plantations) and closed-canopy habitats (forest fragments). Silva and Bernard (2017), working in open-canopy Caatinga, obtained virtually the same species richness values with both techniques. In Brazilian Cerrado, da Silva et al. (2022) registered more species with mist nets than with acoustic recorders, but the latter detected 10 species that were not captured by mist nets. Likewise, in our study, 15 species/sonotypes were only registered by the acoustic recorders. Altogether, this evidence indicates that, despite the difficulty of identifying some calls due to overlapping call parameters, call geographic variation, and lack of reference calls (Arias-Aguilar et al. 2018; López-Baucells et al. 2018), acoustic recorders are a powerful tool for surveying Neotropical bats. Consequently, whenever possible, acoustic recorders should be combined with mist nets to obtain better assessments of bat diversity.

Acoustic recorders were more efficient in savannah, due to its open canopy, than in forest. This may help explain why acoustic recorders made a proportionally higher contribution to species richness and diversity in savannah than in forest. Conversely, the lower contribution of mist-netting to the Shannon and Simpson's diversity recorded in savannah than that recorded in forest could also relate to genuine changes in species composition across habitats. Mounting evidence suggests that Neotropical bats present species-specific responses to habitat structure in both human-modified (e.g., Carrasco-Rueda & Loiselle, 2020; Carvalho et al. 2020; Yoh et al. 2022) and natural areas (e.g., Bernard & Fenton, 2002; Carvalho et al. 2021), partly reflecting that many aerial-hawking insectivores favor open, less structurally complex habitats (Marques et al. 2016). Less cluttered habitats (e.g., savannahs) might release aerial-hawking bats from restrictions imposed by a denser understory—e.g., due to masking effects of echoes reflected from the surrounding vegetation on the echoes originating in target prey (Schnitzler & Kalko, 2001)—and this might be reflected in an increase in the diversity of this ensemble in more open spaces (i.e., in the savannahs). So, the differences observed might be due to the reduced sampling efficiency of mist nets in open habitats (O'Farrell & Gannon, 1999; Wordley et al. 2018) and the higher sampling efficiency of acoustic recorders in more open spaces (López-Baucells et al. 2021; Patriquin et al. 2003).

A study on effort optimisation in acoustic bat surveys in the Amazon was recently published by López-Baucells et al. (2021). However, it only assesses the sampling effort needed for species identification in terms of sampling nights, nightly recording periods and sampled seasons. No assessment to date had yet investigated the effectiveness of having different numbers of recorders simultaneously recording bats within a single area in the Neotropics. We have addressed this gap, and since financial costs associated with fieldwork and bioacoustic analysis are often a limiting factor for bat surveys, we conducted a cost-effectiveness evaluation when using one, two or three recorders. In forest, there was a gain in diversity when using more than one recorder, but this was not reflected in the sampling cost-effectiveness per species/sonotype recorded. However, cost-effectiveness seemed to be influenced by the habitat's structural characteristics, with costs per species being slightly lower in savannahs (open habitat) than in forests (closed habitat), as suggested by López-Baucells et al. (2021). Thus, it can be inferred that for sampling Neotropical bats with a sampling scheme analogous to ours, one AudioMoth recorder can be used in open habitats akin to savannahs, as the results are likely to be similar to those obtained by using up to three devices. On the other hand, as using two recorders led to greater diversity in forest, habitats with higher vegetation structural complexity might attenuate echolocation calls and thus reduce the performance of acoustic recorders (Schnitzler & Kalko, 2001). Two AudioMoth recorders might thus offer a better assessment of bat diversity. However, setbacks—e.g., loss of recorders and damage of memory cards—must be considered, as it might be more expensive to repeat sampling nights than to have two or more recorders registering simultaneously, improving the cost-effectiveness of the project/study (Gardner et al. 2008; Meyer et al. 2015). Therefore, using at least two recorders in each habitat should be considered to reduce the likelihood of data loss due to device malfunction.

To our knowledge, this was the first study to sample Amazonian bats with AudioMoth acoustic recorders. Nonetheless, our results are comparable in terms of species richness and diversity to those recorded in previous assessments of bat diversity in natural Amazonian habitats (e.g., Appel et al. 2021; Barnett et al. 2006; Bernard & Fenton, 2002). The 53 species recorded here represent approximately 29% of the bat species known in Brazil (Garbino et al. 2020) and 60% of the species previously recorded in the Amapá state, which also has other ecosystems such as *terra firme* forest, *campinaranas* and mangroves (Carvalho et al. 2021; Martins et al.

2022; Silva et al. 2013). While only ca. 32% of the species/sonotypes surveyed in this study were detected with AudioMoths, these low-cost autonomous acoustic recorders have an immense potential to expand the known ranges of poorly known species. Moreover, the results indicate that such low-cost ultrasound recorders are a promising approach for the study of multiple aspects of the ecology of many bat species.

This study highlights the importance of simultaneously using mist nets and acoustic recorders for sampling Neotropical bats in both open- and closed-canopy habitats. From our results and from other studies (see Silva & Bernard, 2017; Appel et al. 2021; da Silva et al. 2022; Mancini et al. 2022), we know that both methods tend to sample different sets of Neotropical bat species. The use of only one method to sample Neotropical bats can bias the results of different studies or monitoring, biasing the bat assemblage sampled either toward Phyllostomidae (if using only mist nets) or toward aerial insectivores (if using only acoustic recorders). Therefore, to have more robust results, with direct consequences for species and habitat management, the use of both methods is strongly recommended. Considering the cost-effectiveness estimated here and the possibility of malfunctioning recorders, we recommend the use of at least two AudioMoth in non-forested (e.g., savannah, *campinas*, wetlands, fields, pastures, and non-forest plantations) and forested habitats (e.g., forest patches, *terra firme* forest, *campinarana*, and *várzea* forest) across the Amazon basin. We anticipate that using low-cost acoustic recorders such as AudioMoths will significantly enhance the number of bioacoustic bat surveys across Central and South America. Thus, we reiterate the need for the compilation of libraries of bat echolocation calls for the Neotropics and for more research on the identification to species level of these calls. The combined use of capture and bioacoustic methods will certainly provide evidence-based solutions for the challenges imposed by the current period of acute global, regional, and local changes.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42974-022-00131-5.

Acknowledgements William Douglas Carvalho was supported by post-doctoral (PNPD/CAPES) scholarships of the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil, until early 2020 (CAPES/PNPD—Finance Code 001). Currently, WDC is supported by ‘Ayudas Maria Zambrano’ (CA3/RSUE/2021-00197) funded by the Spanish Ministry of Universities. Bruna Xavier is supported by doctoral scholarships of the CAPES. Funding to Ricardo Rocha was provided by the Portuguese Foundation for Science and Technology (2020.01129.CEECIND/CP1601/CT0004). The fieldwork was authorised by the appropriate Brazilian authority, namely the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA; Collection licenses n° 55867-1). We are grateful to our field assistants Karen Mustin, Daniela Rato, Cledinaldo Marques, Cremilson Marques, Gustavo Silveira, Joandro Pandilha, Jackson Souza and Luís Miguel Rosalino. We also thank Dona Deusa, Dona Damiana, Dona Sônia (comunidade da Ressaca do Pedreira), Mateus (Quilombo do Curiaú), Márcilio e Ceará (Balneário do Alegre), Raizé Domingues, Juliano, José (comunidade do Abacate da Pedreira) e Ivori for allowing us to sample in their areas, for overnight stays and assistance during field activities.

Author contribution WDC, ALB, RR and JMP originally formulated the idea; WDC, JDM, BSX and IJC conducted fieldwork; JDM and ALB performed the acoustic identification; WDC and JDM performed statistical analyses and WDC, JDM and BSX wrote the drafts of the main manuscript and the online resources. All authors contributed critically to the manuscript and gave final approval for publication.

Data availability The datasets generated during and/or analyzed during the current study are available from the author on request.

Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

Ethics approval Not applicable.

Consent to participate All co-authors agree.

Consent for publication All co-authors agree.

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