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Use of complementary methods to sample bats in the Amazon

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Mist nets set at ground level is the traditional method of surveying bats and in the Amazon, almost half of the bat surveys used this methodology. The sole use of ground-level mist nets biases surveys because of the lack of records of aerial insectivorous bats, which forage above the canopy or in other open areas. Canopy mist nets, roost searches and acoustic surveys are methods to survey bat assemblages, but their efficiency compared to ground-level mist nets has not been fully evaluated in the Amazon, the world's largest tropical rainforest. Here, we test how the complementarity of sampling methods contributes to the number of species recorded in bat surveys in the Amazonian rainforest. We simultaneously sampled bats using ground mist nets and ultrasonic recorders at the Ducke Reserve (Central Amazon) in Brazil and did a literature review of bat surveys conducted in the Amazon to assess how these methods have been used in field research during the recent decades. Forty-three bat species were identified using ground mist nets, and seventeen species and five acoustic sonotypes were identified using ultrasonic recorders in Ducke Reserve. The combination of ground mist nets and acoustic recorders registered the largest number of bat species. However, for phyllostomid species the sole use of mist nets was efficient in recording the highest number of species, whereas for aerial insectivores acoustic surveys was the most effective. Of the 54 bat surveys made in the Amazon, 27 localities used complementary methods: roost search, canopy mist nets, harp traps and acoustic surveys. The combination of ground and canopy nets, and ground nets with roost search did not record more phyllostomid bat species than the use of ground nets alone. However, the sole use of acoustic surveys recorded more aerial insectivorous species than any other combination of sampling methods. Using mist nets and acoustic surveys simultaneously, as in our study, results in a dramatic increase in species diversity and different guilds than using only mist nets in the Amazon. Canopy nets and roost search did not increase the total number of species or the number of phyllostomid species in bat surveys. By combining different survey methodologies, we can optimize the recorded diversity of bats, especially using both mist nets and acoustic monitoring.

Key words: acoustic monitoring, canopy net, Chiroptera, mist nets, roost finding, ultrasonic detectors

Introduction

A major challenge in planning field surveys is to choose the most effective methods or combination of methods for the objectives of the study. When the objective is to compare assemblage structure, methods that detect most or all of the target species are preferred (Moreno and Halffter, 2000). Survey and monitoring protocols are often ineffective because sampling techniques vary in their ability to detect different species, and also the same species under different environmental conditions (MacSwiney *et al*., 2008; Lopéz-Baucells *et al*., 2019). For bats, a number of different detection methods are commonly used, such as mist nets, roost search, harp traps and ultrasonic recorders (Kunz and Parsons, 2009). In order to answer ecological questions about bat assemblages, and especially to implement bat monitoring studies, it is essential to survey them in a cost-effective manner (Hourigan *et al*., 2008).

In the Neotropics, bats constitute the most abundant, species-rich and ecologically diverse mammal assemblages (Voss and Emmons, 1996; Wilson and Mittermeier, 2019). Bats are most commonly sampled by ground-level mist netting (Kunz and Parsons, 2009; Marques *et al*., 2013). One of the advantages of capture methods, such as mist netting and harp-trap, is the possibility of the researcher to handle individual bats and conduct character-based species-level identifications, as well as collect biometric data, tissue and/or fecal samples, body fluids, ectoparasites and to collect voucher specimens for deposit in scientific collections (Flaquer *et al*., 2007). However, mist netting requires the presence of researchers during the entire sampling period (Murray *et al*., 1999), which limits the number of potential sampling sites. Captures conducted simultaneously in multiple locations require more time and/or people in the field, which increases the costs of research projects (MacSwiney *et al*., 2008).

Phyllostomid bats are comparatively easy to sample with ground mist nets as they use senses in addition to echolocation for primary orientation and hunting (Schnitzler *et al*., 2003; Denzinger and Schnitzler, 2013). Ground mist nets are not effective for capturing aerial insectivorous bats due to their foraging behavior in open spaces or above the canopy, their ability to detect and avoid the nets, and their capture is often considered 'accidental' (O'Farrell and Gannon, 1999). As Neotropical bat species vary in the use of vertical space (Marques *et al*., 2016; Silva *et al*., 2020), setting mist nets in higher forest strata can increase the probability of capturing additional phyllostomid species (Bernard, 2001) and also some aerial insectivorous bats (Kalko and Handley, 2001; Sampaio *et al*., 2003).

Passive continuous recordings of echolocation calls have been recognized as the most effective methods for detecting aerial insectivorous bat species (Froidevaux *et al*., 2014; López-Baucells *et al*., 2021) worldwide, but not for frugivorous or gleaning insectivorous species. Acoustic surveys are non-invasive methods that allow the identification of aerial insectivorous bat species by their species-specific calls, or the identification of sonotypes (species with similar calls that cannot be confidently distinguished) (Russo and Voigt, 2016; Zamora-Gutierrez *et al*., 2016). Passive acoustic recorders do not require the presence of the researcher during the whole sampling period and can be programmed to operate with sampling schedules, if the focus is based on specific species (Hintze *et al*., 2016; Hill *et al*., 2018). Although the advancement of acoustic methods has allowed researchers to address several ecological and taxonomic questions, acoustic studies provide little data for research on systematics, physiology and parasitology (Flaquer *et al*., 2007). However, differences in echolocation call structure between cryptic species (Jones and Van Parijs, 1993; López-Baucells *et al*., 2018), still require the capture of individuals to obtain morphological data, tissue samples and specimen vouchers for comprehensive taxonomy and systematics inferences (De Thoisy *et al*., 2014; Pavan *et al*., 2018).

Bats can also be detected by searching for roosts (Froidevaux *et al*., 2020*a*). In the Neotropics, several species and guilds have been recorded by searching caves, tree hollows and foliage (Rodríguez-Herrera *et al*., 2007; Lima *et al*., 2017; Appel *et al*., 2021*b*). For instance, some species of aerial insectivorous bats are also commonly found in man-made structures (buildings, bridges, culverts, mines, etc.). Therefore, roost searches can reveal species that are not usually captured in mist nets (Díaz and Linares García, 2012; Jung and Threlfall, 2018).

The Amazon harbours the world's largest tropical rainforest and is one of the biomes with the highest bat species richness; 117 species of bats can occur in sympatry in some Amazonian localities (Delgado-Jaramillo *et al*., 2020). Even in this species-rich biome, few studies have used different sampling methods to estimate local and regional bat richness (e.g., Voss and Emmons, 1996; Barnett *et al*., 2006; Moratelli *et al*., 2010; Tavares *et al*., 2017). The sole use of ground-level mist nets biases samples by the lack of records of aerial insectivores, resulting in the typical under-reporting of nearly half of the local diversity of potential species present. Although it is well known that acoustic survey complements the sampling of bats with mist nets (MacSwiney *et al*., 2008; Furey *et al*., 2009; Meyer *et al*., 2011; Trevelin *et al*., 2017), there has been no assessment of how different methods, such as canopy nets, roost searching and ultrasonic recorders complement captures with ground mist nets.

We test the complementarity of sampling methods for bat species by analyzing data collected by ground mist nets and ultrasonic recorders at the same plots in a 25-km^2 area of continuous forest in Central Amazon. With a comprehensive literature review of bat surveys conducted in the Amazon, we investigate which methods (ground mist nets, canopy mist nets, roost search, and acoustic recorders) and combination of methods recover most of the assemblages of bats, especially phyllostomid species and aerial insectivorous species.

Materials and Methods

Study Area

The study was conducted in the Adolpho Ducke Forest Reserve (02°55'–03°01'S; 59°53'–59°59'W), administered by the National Amazon Research Institute (INPA), located between the city of Manaus and the AM-110 Highway, Amazonas State, Brazil (Fig. 1). The Ducke Reserve has an area of 10,000 ha of low-altitude rainforest and is part of the Brazilian Long-Term Ecological Research Program of the Brazilian National Research Council (Programa de Pesquisas Ecológicas de Longa Duração - PELD/CNPq). The climate is tropical humid with two seasons: a rainy season between November and May and a dry season between June and October (Oliveira *et al*., 2008). The average annual temperature was 26.7ºC, the annual humidity was above 80% and the average precipitation was 2,093 mm during the rainy period of 2013 (Ferreira *et al*., 2012; Appel *et al*., 2019). The reserve has a trail system that forms a 64 km^2 (8 × 8 km) grid (Fig. 1). The grid has 72 permanent plots distributed uniformly with a minimum separation of 1 km (Oliveira *et al*., 2008). Each plot is 250 m long and follows an altitudinal contour of landscape to reduce variation in soil edaphic properties and consequently plant species composition (Magnusson *et al*., 2014). Plot width can depend on the habitat sampled with riparian plots dictated by the edges of streams instead of following altitudinal contours.

Mist Net Sampling

We captured bats using mist nets in 10 non-riparian plots and in 10 riparian plots (Fig. 1) between October 2013 and February 2014 (Capaverde Jr. *et al*., 2018; Pereira *et al*., 2019) using eight ground-level mist nets (12 × 3 m, 19 mm mesh — Ecotone®, Poland) per plot. The mist nets remained open between 18:00 and 00:00 h and were inspected every 15 minutes (Capaverde *et al*., 2018). Each plot was visited for three non-consecutive nights, totalling 2,880 mist net hours (1 mist net hour = one mist net opened for one hour) (Straube and Bianconi, 2002). The identification of bats was based on the dichotomous keys of Lim and Engstrom (2001), and descriptions of Simmons and Voss (1998) and Gardner (2007). The taxonomy followed Gardner (2007), Nogueira *et al*. (2014) and Garbino *et al*. (2020).

Ultrasonic Recorder Sampling

We used automatic Song Meter SM2BAT® recording stations coupled to SMX-US® ultrasonic omnidirectional microphones (Wildlife Acoustics, Maynard, Massachusetts) for the acoustic monitoring in Ducke Reserve. We installed

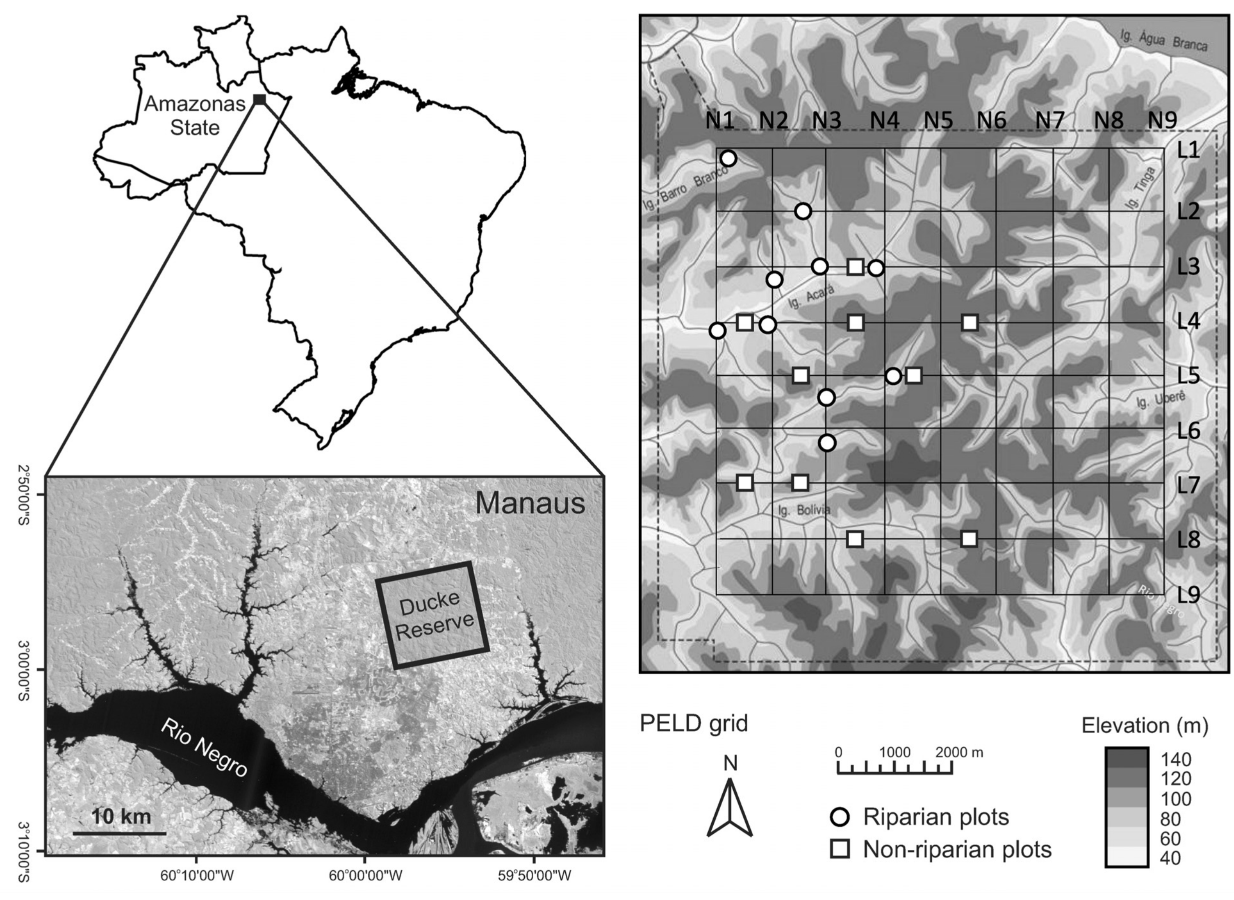

Fig. 1. Map of the distribution of sampled plots for bats in the Ducke Reserve grid, Central Amazon, Brazil

acoustic stations in the same 20 plots where the mist nets were opened (Fig. 1), during five months in the rainy season between January and May 2013 (de Oliveira *et al*., 2015; Appel *et al*., 2017). We placed the acoustic stations in the center of each plot and the microphones at a height of 1.5 m. In the riparian plots, the microphones were positioned over the streams. Recording started at 18:00 h and ended at 06:00 h, and each plot was sampled for four to six consecutive nights, totalling 101 sampling nights and 1,212 recording hours.

We programmed the SM2BAT to real-time recording at a sample rate up to 384 kHz (covering pulse frequencies from 16 to 192 kHz), 16-bit full spectrum resolution with 1 s of pre-trigger and 0.1 s of post-trigger. We set them to record in files of 30-minute intervals for 12 hours per night. All recordings were segmented into smaller files up to 5-s long and a bat pass was a sequence of 5-s duration that had a minimum of two recognizable search-phase calls per species (Torrent *et al*., 2018; Gomes *et al*., 2020). We used the program Kaleidoscope 3.1.1. (Wildlife Acoustics, Maynard, Massachusetts) to convert, segment, view and classify all the recordings. We identified the species manually by comparing the structure and frequency parameters of the search-phase calls such as: call shape, frequency of maximum energy (FME), start, end, maximum and minimum frequency, and call duration. We compared the parameters using the acoustic-identification key for the bats from the Amazon, and studies of spectrograms of tropical bat species (Jung *et al*., 2007, 2014; Barataud and Giosa, 2013; Arias-Aguilar *et al*., 2018). For sonotypes (species difficult to distinguish from only the calls) we used the same sonotype groups described by López-Baucells *et al*. (2016). *Pteronotus rubiginosus* (identified as *P. parnellii* in Ducke Reserve by de Oliveira *et al*., 2015) was identified by the taxonomic and acoustic description proposed by Pavan *et al*. (2018) (calls with frequency peak of 55 kHz, López-Baucells *et al*., 2018). We recorded 223 bat passes of Phyllostomidae, but due to the inability to differentiate species by call structures (Yoh *et al*., 2020), we did not use this data in further analyses.

Literature Review

We comprehensively searched the Web of Knowledge and Google Scholar for papers published between January 1979 and December 2019, but thesis and dissertations were not included. Published papers were selected based on the following search terms: 'bats' OR 'chiroptera' AND 'Amazon', 'Amazonian'; 'inventory' AND 'richness'. We searched for papers using the same words in Portuguese and Spanish. A total of 102 papers included bat surveys in the Amazon, but only 43 studies at 54 localities met our criteria and were used in the analyses (Supplementary Table S1). We only used bat-assemblage studies that identified the location of the capture, sampling method used, number of sampling nights, number of species, and names of the bat species recorded. For different papers conducted at the same location, we used the paper that reported the largest number of species. We selected articles that recorded at least 40% of the bat species presumed to be in the area to avoid studies with low sampling effort (Supplementary Table S2). To check this, we compared the bat species richness observed in each study with the estimated richness obtained in the IUCN distribution maps for bat species of the family Phyllostomidae (IUCN, 2020). As some aerial insectivorous species are data deficient in the IUCN distribution maps, we made the estimated richness only for Phyllostomidae species.

Data Analysis

Complementarity of methods in Ducke Reserve: mist nets and ultrasonic recorders

To assess if the combination of sampling methods increases the number of species recorded compared to that registered from the exclusive use of ground mist nets at the Reserva Ducke, we used Gardner-Altman estimation plots and non-parametric permutation tests with 1000 bootstrap samples to estimate effect sizes and 95% confidence intervals for the difference of means. Statistical significance of differences among the methods was determined based on the lack of overlap in the frequency distributions of the data sets (Ho *et al*., 2019). These analyses were done in R package 'dabestr' (Ho *et al*., 2019) and were made for assemblages of all bat species, phyllostomid species, and aerial insectivorous species.

Complementarity of methods in Amazonian bat studies

To assess if the combination of methods increases the number of bat species recorded compared to exclusive use of ground mist nets we evaluated using the Gardner-Altman analysis. This analysis is a non-parametric permutation that used 1000 bootstrap samples (Ho *et al*., 2019). Some combinations of methods were not included due to the low number of samples (e.g., acoustic survey with mist nets and harp traps).

Different sampling effort between studies (e.g., greater number of sampling nights) was balanced using Generalized Linear Models (GLM) with the number of bat species as the response variable, the methods employed as the predictor variable and the log-transformed number of sampling nights as offset in all models. For all bat species, phyllostomid species, and aerial insectivorous species, the results were the same for bootstrap analysis (Supplementary Table S3); therefore, we used the Gardner-Altman analysis to graph the results. All analyses were undertaken in R Studio version 1.2.5019 (RStudio Team, 2020).

Results

Complementarity of Methods in Ducke Reserve: Mist Nets and Ultrasonic Recorders

We registered 55 species and five sonotypes identified to genus using mist nets combined with ultrasonic recorders (Table 1). We captured 454 individuals with the mist nets representing 43 species from four guilds (nectarivores, frugivores, carnivores and gleaning insectivores). With the ultrasonic recorders, we registered 15,375 bat passes of 17 species and five generic sonotypes, all belonging to the aerial insectivorous guild. We recorded species of four families with both ground-level mist nets and ultrasonic recorders (Emballonuridae, Mormoopidae, Thyropteridae, and Vespertilionidae), one family exclusively in the ground-level mist nets (Phyllostomidae) and two families (Furipteridae and Molossidae) exclusively by ultrasonic recorders. Only *Carollia perspicillata* was recorded in all 20 plots by mist nets (n = 156). Three species were

TABLE 1. Bat species and guilds sampled using ground-level mist-nets and ultrasonic recorders at 20 plots in Ducke Reserve, Central Amazon, Brazil. Number of records using mist-nets and the number of bat passes using ultrasonic recorder with the number of plots each species/sonotype group was registered is given in parentheses. The bat guilds followed the classification proposed by Kalko *et al*. (1998) and Schnitzler and Kalko (2001)

Family/Species	Guilds	Mist-nets	Ultrasonic recorder
Emballonuridae			
Centronycteris maximiliani (Fischer, 1982)	Aerial insectivore		604 (14)
Cormura brevirostris (Wagner, 1843)	Aerial insectivore	1 (1)	2,685 (20)
Diclidurus ingens Hernandez-Camacho, 1955	Aerial insectivore		2 (1)
Peropteryx kappleri Peters, 1867	Aerial insectivore		14 (5)
Peropteryx leucoptera Peters, 1967	Aerial insectivore	2 (2)	
Peropteryx macrotis Wagner, 1843	Aerial insectivore		1 (1)
Peropteryx trinitatis Miller, 1899	Aerial insectivore		6 (4)
Saccopteryx bilineata (Temminck, 1838)	Aerial insectivore	1 (1)	3,351 (20)
Saccopteryx gymnura/canescens	Aerial insectivore		14 (6)
Saccopteryx leptura (Schreber, 1774)	Aerial insectivore		1,298 (18)
Phyllostomidae			
Anoura caudifer (É. Geoffroy, 1818)	Nectarivore	9 (6)	
Artibeus concolor Peters, 1865	Frugivore	2 (2)	
Artibeus gnomus Handley, 1987	Frugivore	3 (3)	
Artibeus lituratus (Olfers, 1818)	Frugivore	4 (4)	
Artibeus obscurus (Schinz, 1821)	Frugivore	11 (3)	
Artibeus planirostris (Spix, 1823)	Frugivore	14 (6)	
Carollia benkeithi Solari and Baker, 2006	Frugivore	16 (6)	
Carollia brevicauda (Schinz, 1821)	Frugivore	69 (16)	
Carollia perspicillata (Linnaeus, 1758)	Frugivore	156 (20)	
Choeroniscus sp.	Nectarivore	2 (2)	
Chrotopterus auritus (Peters, 1856)	Carnivore	1 (1)	
Glyphonycteris sylvestris Thomas, 1896	Gleaning insectivore	3 (2)	
Hsunycteris thomasi (Allen, 1904)	Nectarivore	35 (14)	
Lampronycteris brachyotis (Dobson, 1879)	Gleaning insectivore	1 (1)	
Lonchophylla sp.	Nectarivore	1 (1)	
Lophostoma carrikeri (Allen, 1910)	Gleaning insectivore	1 (1)	
Lophostoma schulzi Genoways e Williams, 1980	Gleaning insectivore	2 (2)	
Micronycteris hirsuta (Peters, 1869)	Gleaning insectivore	2 (2)	
Micronycteris megalotis (Gray, 1842)	Gleaning insectivore	3 (3)	
Micronycteris microtis Miller, 1898	Gleaning insectivore	5 (5)	
Micronycteris schmidtorum Sanborn, 1935	Gleaning insectivore	1 (1)	
Mimon crenulatum (É. Geoffroy, 1801)	Gleaning insectivore	15 (9)	
Phylloderma stenops Peters, 1865	Gleaning insectivore	1 (1)	
Phyllostomus elongatus (É. Geoffroy, 1810)	Gleaning insectivore	20 (9)	
Rhinophylla fischerae Carter, 1966	Frugivore	3 (3)	
Rhinophylla pumilio Peters, 1865	Frugivore	29 (11)	
Sturnira tildae de la Torre, 1959	Frugivore	1 (1)	
Tonatia saurophila Koopman and Williams, 1951	Gleaning insectivore	5 (5)	
Trachops cirrhosus (Spix, 1823)	Carnivore	4 (3)	
Trinycteris nicefori (Sanborn, 1949)	Gleaning insectivore	1 (1)	
Uroderma bilobatum Peters, 1866	Frugivore	1 (1)	
Vampyriscus bidens (Dobson, 1878)	Frugivore	9 (4)	
Vampyriscus brocki (Peterson, 1968)	Frugivore	1 (1)	
Mormoopidae			
Pteronotus gymnonotus (Wagner, 1843)	Aerial insectivore		1 (1)
Pteronotus rubiginosus (Wagner, 1843)	Aerial insectivore	8 (5)	3,506 (20)
Furipteridae			
Furipterus horrens (Cuvier, 1828)	Aerial insectivore		63 (12)
Thyropteridae			
Thyroptera lavali Pine, 1993	Aerial insectivore	2 (2)	
Thyroptera sp.	Aerial insectivore		25 (6)
Thyroptera tricolor (Spix, 1823)	Aerial insectivore	1 (1)	
Molossidae			
Cynomops abrasus/greenhalii	Aerial insectivore		12 (2)

TABLE 1. Continued

Family/Species	Guilds	Mist-nets	Ultrasonic recorder
Eumops sp.	Aerial insectivore		5 (1)
Molossus sinaloae/currentium/rufus	Aerial insectivore		333 (16)
Molossus molossus Pallas, 1766	Aerial insectivore		8 (1)
Vespertilionidae			
Eptesicus brasiliensis (Desmarest, 1819)	Aerial insectivore	1 (1)	6 (5)
Myotis albescens (É. Geoffroy, 1806)	Aerial insectivore	2 (2)	
Myotis nigricans (Schinz, 1821)	Aerial insectivore	1 (1)	
Myotis riparius Handley, 1960	Aerial insectivore	4 (4)	2,156 (19)
Myotis sp.	Aerial insectivore		436 (6)
Lasiurus ega/egregius/castaneus/atratus	Aerial insectivore		587 (16)
Rhogeessa io/Lasiurus blossevillii	Aerial insectivore		262 (11)
Number of species		43	22
Number of records		454	15,375

present in all plots sampled with the ultrasonic recorders (*Cormura brevirostris*, *Saccopteryx bilineata* and *Pteronotus rubiginosus*).

Most species were registered by only one of the sampling methods: 38 species solely with mist nets (60%), and 12 species and five sonotypes only with ultrasonic recorders (30.9%). Five species, all aerial insectivores, were recorded using both methods (9.1%) (Table 1) and in more than 90% of the sampled plots with acoustic records (with the exception of *Eptesicus brasiliensis*), but were recorded in only 25% of sampled plots with mist nets (Table 2).

For all bats, the combination of ground mist nets and acoustic recorders documented the largest number of bat species in the Ducke Reserve (Fig. 2A). The combination of methods recorded 2.2 times more species/species groups per plot on average than ground mist nets alone. Ultrasonic recorders registered more species/species groups per plot than mist nets. Only ground mist nets recorded phyllostomid bats (Fig. 2B). For aerial insectivorous bats (Fig. 2C), acoustic recorders sampled on average 10.4 times more species than ground nets.

Complementarity of Methods in Amazonian Bat Studies

The different bat sampling methods found in our review included ground and canopy mist nets, roost searches, harp traps, and acoustic recordings (Supplementary Table S1). Of the 54 localities, 51 (92.6%) used ground mist nets and 23 (42.3%) used exclusively this method (Table 3). Half of the localities used complementary methods and the most common combination was ground mist nets with roost searches (20.4%) followed by the combination of ground and canopy mist nets (18.5%) (Table 3). Only five localities (9.6%) used acoustic surveys, in which three localities used exclusively acoustics, and two used a combination of at least two methods (Table 3). Harp traps were used to sample Amazonian bats in only 3.7% of the localities. The combination of ground mist nets with canopy mist nets and ground mist nets with roost search did not really affect the total number of all bat species or number of phyllostomid species than that recorded with ground nets alone (Fig. 3A and B). The use of acoustic survey alone recorded the highest number of aerial insectivorous species compared to other methods and combinations among them (Fig. 3C).

DISCUSSION

This study is the most complete list of bat species made in the Ducke Reserve using ground-level mist nets and ultrasonic recorders (Capaverde Jr. *et al*., 2018; Appel *et al*., 2019). The inclusion of

TABLE 2. Number ($\bar{x} \pm$ SD) of bat species recorded in 20 plots using different methods (ground mist nets and ultrasonic recorders) in Ducke Reserve, Central Amazon, Brazil

Sampling methods	*n* plots	All species	Phyllostomidae	Aerial insectivores
Ground	20	43 (8.5 ± 2.6)	33 (7.6 ± 2.6)	10 (1.0 ± 0.9)
Acoustic	20	22 (10.4 ± 2.5)	0 (0 ± 0)	22 (10.4 ± 2.5)
Ground + Acoustic	20	60 (18.3 ± 4.0)	33 (7.6 ± 2.6)	17 (10.8 ± 2.4)

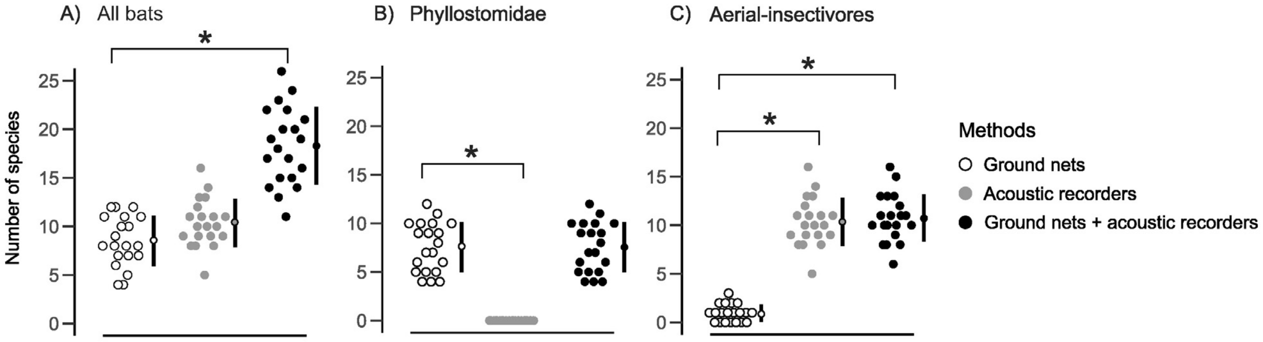

FIG. 2. Bat species recorded by different sampling methods in Ducke Reserve, Central Amazon, Brazil. Number of species sampled per plot for surveys based on ground mist nets (white circles), acoustic recorders (grey circles) and both methods combined (black circles) for all bats, phyllostomid bats, and aerial insectivorous bats. Mean (circles) ± SD (vertical lines) are beside the plots of each sampling method. '*' means that the difference between methods or combination of methods was statistically significant

ultrasonic recorders had a substantial impact on the number of bats recorded from Ducke Reserve, increasing by almost 45% the number of species we reported. Our analyses based on the literature review showed that the combination of two sampling methods other than acoustic recordings usually did not add more bat species, and exclusive use of ground-level mist nets resulted in the highest number of phyllostomid species and total bat species. Nevertheless, if the focus of the study is on aerial insectivorous bat species, the exclusive use of recorders is the most effective method.

In Ducke Reserve, the use of ground mist nets resulted in a higher number of phyllostomid species (60% of total) than any other group, which comprises the second largest family of Chiroptera (Wilson and Mittermeier, 2019). Phyllostomid species were not detected in our acoustic surveys because they emit similarly structured low intensity, multiharmonic and highly directional calls that make it almost impossible to identify differences among genera and species (Yoh *et al*., 2020).

The exclusive use of ground-level mist nets in Ducke Reserve recorded only a few species of aerial insectivorous bats per plot compared to acoustic recorders. It has been demonstrated previously that the employment of ultrasonic recorders is the most efficient method for sampling aerial insectivorous bats in tropical forests (e.g., Meyer *et al*., 2011; Britzke *et al*., 2013; Silva and Bernard, 2017). Our acoustic survey in the Amazon corroborates this finding and added 14 species/sonotypes to the previous list of seven aerial insectivorous bat species known from the Ducke Reserve (Appel *et al*., 2019). The increment of species by the use of acoustic surveys has been reported in other studies conducted in the Tropics, such as Venezuela (additional 11 species — Ochoa *et al*., 2000), Vietnam (additional four species and five acoustic groups — Furey *et al*., 2009), and in the Brazilian Caatinga (additional 22 species — Silva and Bernard, 2017). Only species from Noctilionidae and Natalidae were not detected in Ducke Reserve, probably because they are species associated with water bodies of open areas

TABLE 3. Number ($\bar{x}$ ± SD) of bat species recorded by different methods and combinations of methods found in a literature review of 54 localities of 43 studies carried out in the Amazon (see Supplementary Table S1). Methods are ground mist nets, ultrasonic recorders, roost searches, canopy mist nets, and harp traps

Sampling methods	*n* localities	All species	Phyllostomidae	Aerial insectivores
Ground	23	41.6 ± 12.8	34.6 ± 8.8	7 ± 6.2
Acoustic	3	26.3 ± 4	0 ± 0	26.3 ± 4
Ground + Roost	11	38.7 ± 8.1	29.5 ± 7.2	9.3 ± 5.7
Ground + Canopy	10	48.9 ± 13.5	38.9 ± 8.1	10 ± 7.3
Ground + Acoustic	1	56 ± 0	43 ± 0	22 ± 0
Ground + Canopy + Harp trap	1	71 ± 0	46 ± 0	25 ± 0
Ground + Canopy + Roost	2	50.5 ± 7.7	37.5 ± 6.4	13 ± 1.4
Ground + Roost + Harp trap	1	46 ± 0	32 ± 0	14 ± 0
Ground + Roost + Acoustic	1	49 ± 0	25 ± 0	24 ± 0
Ground + Canopy + Roost + Acoustic	1	78 ± 0	59 ± 0	19 ± 0

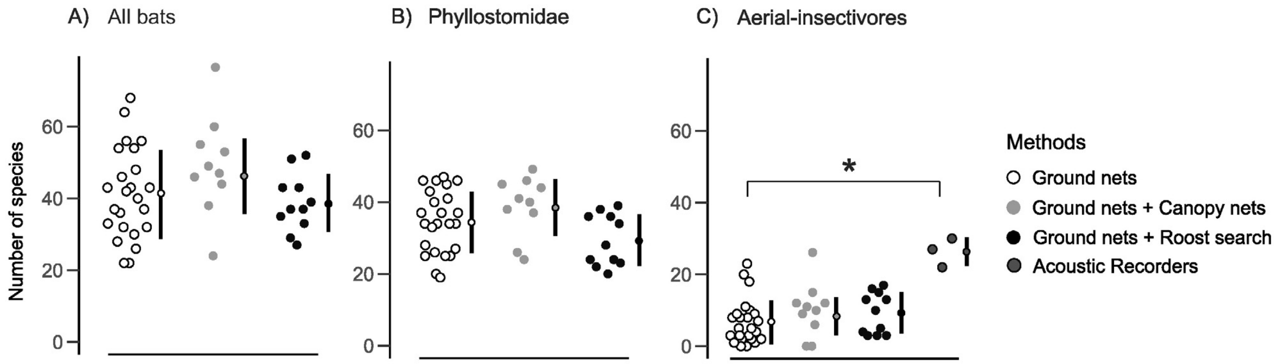

FIG. 3. Bat species recorded by different sampling methods in Amazonian bat studies compiled from the literature. Number of species sampled per study for surveys based on ground mist nets (white circles), ground nets with canopy nets (light grey circles), ground nets with roost search (black circles) and acoustic recorders (dark grey circles) for all bats, phyllostomid bats, and aerial insectivorous bats. Mean (circles) ± SD (vertical lines) are beside the plots of each sampling method. '*' means that the difference between methods or combination of methods was statistically significant

and caves that were not present in our study area (Voss *et al*., 2016; Tavares *et al*., 2017).

Pteronotus rubiginosus is the only aerial insectivore commonly included in community studies using ground mist nets in our study, and with its sister species *Pteronotus alitonus* in the forest of the Amazon (Peters *et al*., 2006; Bobrowiec and Gribel, 2010; Carvalho *et al*., 2018; Silva *et al*., 2020). Our study showed a discrepancy between sampling methods in relation to the number of plots in which this species was detected. Only 25% of plots sampled with ground mist nets registered *P. rubiginosus* in Ducke Reserve, against 100% of plots sampled with acoustic recorders (Table 2). This indicates that acoustic survey is the most suitable method for recording this species. However, there was sampling bias in that mist nets were only open during the first half of the evening on three nights, whereas acoustic monitoring was done throughout the evening on 4–6 nights. Notwithstanding this discrepancy, we recommend caution in using data on aerial insectivorous guilds for ecological studies when the records are based only on mist net surveys because distribution and abundance data can be underestimated by ground nets (Cunto and Bernard, 2012).

Our literature review confirmed that if the focus of the study is on phyllostomid species, ground mist netting is the most effective method and acoustic monitoring did not detect this family. By contrast, our study in Ducke Reserve indicates that ultrasonic recorders were an important sampling method for recording aerial insectivorous species that ground mist nets did not effectively capture. Therefore, if the focus of the study is overall bat diversity, the combination of the two methods is desirable to increase the number of species recorded. The combination of ground mist nets with canopy nets or searching for bats in their roosts did not increase the number of phyllostomid species or the total number of species in any substantive way. These methods probably are most effective in answering specific questions related to vertical stratification (Silva *et al*., 2020) and the role of roosts in bat biology (Vargas-Mena *et al*., 2020)

In terms of aerial insectivorous species, we found that the number of detected species was similar between the studies that used only ground nets and studies that used ground with canopy nets and roost searches. Canopy mist netting has been previously suggested as a potential method for capturing some species of aerial insectivores (Bernard, 2001; Marques *et al*., 2016; Gregorin *et al*., 2017; Silva *et al*., 2020). Although this might apply to specific habitats, we did not find such an effect in the Amazonian rainforests. Furthermore, canopy sampling requires specialized (usually more expensive) equipment or types of mist nets and a higher effort for installation in the field (Kalko and Handley, 2001; Hourigan *et al*., 2008), increasing project costs.

Regarding roost search, rocky outcrops found along the margins of streams, rivers and lakes (Voss *et al*., 2016; Tavares *et al*., 2017) and human-made constructions (Brosset *et al*., 1996; Capaverde Jr. *et al*., 2013) have been identified as important roosts for aerial insectivorous bats in the Amazon. However, these rocky outcrops are rare in the interior of Amazonian forest and are restricted to a few locations. Occupied roosts in foliage can be difficult to find because the lifetime of a tent can be short and the tent density can be lower in some forest areas (Timm, 1987).

For aerial insectivorous species, we found that acoustic surveys recorded the highest number of species. This finding was expected based on other studies that compared bat sampling methods in tropical forests (Flaquer *et al*., 2007; MacSwiney *et al*., 2008; Wordley *et al*., 2018). However, if the research objectives are beyond monitoring and species diversity, then the use of mist nets and searches for roosts can be important for sampling aerial insectivorous bats because they provide an opportunity to sample biological material, collect species vouchers, and measure morphological attributes that cannot be obtained with bat recorders (Flaquer *et al*., 2007). Gathering biological and morphological attributes is especially critical in the Amazon where such data is poorly known. In addition, the capture of aerial insectivorous bats allows recording of bat releases or zipline calls in order to expand and improve species-verified reference-call libraries (López-Baucells *et al*., 2014, 2018; Arias-Aguilar *et al*., 2018). Currently, this information for many species is lacking for the Amazon (López-Baucells *et al*., 2019; Froidevaux *et al*., 2020*b*).

Although ultrasonic recorders are known to be the best method for sampling insectivorous bats, acoustic surveys can be costly, time-consuming to identify the bat calls and requires highly trained researchers to classify the recordings (Fischer *et al*., 2009; Trevelin *et al*., 2017). However, ultrasonic recorders, power requirements (battery supplies), memory cards and hard drives are becoming increasingly cheaper (Hill *et al*., 2018). Automated identification software is not yet reliable for neotropical bats (Rydell *et al*., 2017; Menon *et al*., 2018) and the identifications need to be validated manually (Adams *et al*., 2012; Hintze *et al*., 2016; Lopéz-Baucells *et al*., 2019). Indeed, there are some limitations such as the imperfect detection of certain species (Duchamp *et al*., 2006; Torrez *et al*., 2017), the inability to quantify individuals and obtain bat abundance (Adams *et al*., 2015) and difficulties to separate sonotypes with similar echolocation call structure (Jung *et al*., 2007, 2014; López-Baucells *et al*., 2016). Despite this, passive ultrasonic recorders are quick to set up in the field, can be automated to run for the entire night and extended periods of time unsupervised, and can simultaneously sample a wide variety of habitats (Hourigan *et al*., 2008), including different forest strata (Gomes *et al*., 2020).

Different bat guilds have different detectability depending on the sampling techniques applied in the surveys (Meyer *et al*., 2011) and conclusions about ecological relationships for one guild should not be extrapolated to another without empirical justification. Different conclusions about ecological relationships may be obtained for the same guild, if different sampling techniques are used (Cardoso *et al*., 2011). Therefore, the ecological research question being examined will also determine the choice of technique to be used (Hourigan *et al*., 2008). Using multiple techniques may reduce sampling efficiency and increase required effort, but our study suggests that a combination of ground-mist nets and acoustical monitoring is the best option, if conclusions are to be made across most bat guilds and species. For the purpose of inventories and environmental impact studies, we encourage the combined use of ground mist nets and ultrasonic recorders. This provides a more complete database not only in terms of the number of bat species, but also of trophic guilds, habitat use, behavior and ecosystem services that bats perform. Our study provides to decision makers, consultants and environmental managers a reference source aimed at improving bat sampling in the Amazon and tropical forests elsewhere. The use of ultrasonic recorders is still uncommon for sampling bats in Brazil (Aguiar *et al*., 2020, Mendes and Srbek-Araujo, 2021), but that has been changing rapidly and contributing to our knowledge of the distribution and ecology of aerial insectivorous bats (Hintze *et al*., 2020; Appel *et al*., 2021*a*).

Supplementary Information

Contents: Supplementary Tables: Table S1. List of 54 localities of 43 Amazonian bat studies from a literature review; Table S2. List of selected papers that recorded at least 40% of the bat species presumed to be in the area; Table S3. Summary of GLM results comparing the number of bat species recorded with different combinations of methods in bat surveys conducted in the Amazon. Supplementary Information is available exclusively on BioOne.

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