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# Optimizing bat bioacoustic surveys in human-modified Neotropical landscapes

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**Abstract.** During the last decades, the use of bioacoustics as a non-invasive and cost-effective sampling method has greatly increased worldwide. For bats, acoustic surveys have long been known to complement traditional mist-netting, however, appropriate protocol guidelines are still lacking for tropical regions. Establishing the minimum sampling effort needed to detect ecological changes in bat assemblages (e.g., activity, composition, and richness) is crucial in view of workload and project cost constraints, and because detecting such changes must be reliable enough to support effective conservation management. Using one of the most comprehensive tropical bat acoustic data sets, collected in the Amazon, we assessed the minimum survey effort required to accurately assess the completeness of assemblage inventories and habitat selection in fragmented forest landscapes for aerial insectivorous bats. We evaluated a combination of 20 different temporal sampling schemes, which differed regarding number of hours per night, number of nights per site, and sampling only during the wet or dry season, or both. This was assessed under two different landscape scenarios: in primary forest fragments embedded in a matrix of secondary forest and in the same forest fragments, but after they had been re-isolated through clearing of the secondary forest. We found that the sampling effort required to achieve 90% inventory completeness varied considerably depending on the research aim and the landscape scenario evaluated, averaging ~80 and 10 nights before and after fragment re-isolation, respectively. Recording for more than 4 h per night did not result in a substantial reduction in the required number of sampling nights. Regarding the effects of habitat selection, except for assemblage composition, bat responses in terms of richness, diversity, and activity were similar across all sampling schemes after fragment re-isolation. However, before re-isolation, a minimum of four to six sampling hours per night after dusk and three to five nights of sampling per site were needed to detect significant effects that could otherwise go unnoticed. Based on our results, we propose guidelines that will aid to optimize sampling protocols for bat acoustic surveys in the Neotropics.

**Key words:** acoustics; Amazon; Chiroptera; echolocation; habitat use; monitoring; rainforest; sampling design.

## INTRODUCTION

The use of bioacoustics has massively increased in recent years as a non-invasive and cost-effective method to answer ecological questions, address biodiversity

conservation issues or improve habitat management (Froidevaux et al. 2014, Bradfer-Lawrence et al. 2019), especially for echolocating bats (e.g., Kunz et al. 2007, Rodhouse et al. 2011, Frick 2013). Bioacoustic sampling has great potential to overcome limitations of conventional biodiversity sampling approaches (Deichmann et al. 2018, Burivalova et al. 2019). However, megadiverse regions such as the Amazon basin, while hotspots for biodiversity, lag far behind other regions in terms of

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research and conservation programs (Wilson et al. 2016), and information on more elusive taxonomic groups remains scarce due to limitations inherent to commonly employed sampling approaches.

For bats, acoustic surveys can effectively complement traditional mist-netting (Flaquer et al. 2007, Walters et al. 2013), vastly increasing inventory completeness in bat assemblage studies, especially in the species-rich tropics (MacSwiney et al. 2008, Furey et al. 2009, Silva and Bernard 2017). Acoustic surveys can cover large temporal and spatial scales, even in habitats where particular environmental conditions such as vegetation clutter, strong winds or large areas covered by water (e.g., lakes and ponds) make mist-netting inefficient or unfeasible (Murray et al. 1999, MacSwiney et al. 2008, Torrent et al. 2018, Wordley et al. 2018).

Relatively low-cost passive detectors (Hill et al. 2018) now allow researchers to automatically survey bats for extended periods of time, accumulating increasingly larger acoustic data sets (Towsey et al. 2014, Adams et al. 2015). In response to this growth in data availability, automatic classification algorithms have been developed and are increasingly being used to aid in the daunting task of processing and identifying echolocation calls (e.g., Zamora-Gutiérrez et al. 2016, López-Baucells et al. 2019). Due to these advances, and despite certain limitations (e.g., dubious species identification or impossibility to quantify individuals and thus obtaining true abundance data; Kunz and Parsons 2009, Adams et al. 2015, Barré et al. 2019), over the past two decades bat acoustic surveys have become increasingly popular.

Amongst the limitations of acoustic surveys, variability in bat activity levels and imperfect detection due to several environmental factors such as weather or vegetation clutter are generally difficult to overcome (Duchamp et al. 2006). Bat activity can substantially vary in space and time and is molded by ecological patterns such as reproductive cycle, proximity of roosts, seasonal migration, swarming activity, moonlight, habitat clutter, or insect abundance (Murray and Kurta 2004, Dzal et al. 2009, Piksa et al. 2011, Adams et al. 2015, Appel et al. 2017). A number of studies have already addressed these problems with regard to mist-netting and provided sampling design recommendations as to how best to cope with them (Hayes 2000, Duchamp et al. 2006, Yates and Muzika 2006, Gorresen et al. 2008, Fischer et al. 2009). However, whereas sampling design optimization has been an active area of research for these more conventional techniques (e.g., Weller and Lee 2007, Marques et al. 2013, Trevelin et al. 2017), similar comprehensive assessments for bioacoustics are limited (but see Rodhouse et al. 2011, Froidevaux et al. 2014, Pieretti et al. 2015). Different acoustic sampling protocols to estimate bat species richness and activity have been evaluated in temperate areas (Skalak et al. 2012, Froidevaux et al. 2014). Some of these focused on the effects of the position, orientation and number of detectors (Weller and Zabel 2002), while others examined the

representativeness of sampling during the dusk peak of bat activity (Froidevaux et al. 2014). Similar assessments are lacking for tropical regions, where the use of passive detectors is rapidly expanding (Silva and Bernard 2017, Arias-Aguilar et al. 2018, Burivalova et al. 2019).

Choosing an effective and statistically robust acoustic sampling protocol is a fundamental issue that researchers must address at the early stages of any bat monitoring or survey project due to time/cost constraints (Rodhouse et al. 2011, Law et al. 2015, Meyer 2015). Limited resources force researchers to optimize sampling, taking into consideration the trade-off between temporal vs. spatial replication or the minimum number of seasons, years, or sites required to answer specific ecological questions. Since the accuracy of results depends on how well the real activity patterns of the animals are captured in the data collected (Froidevaux et al. 2014), establishing a priori a minimum sampling effort for acoustic surveys to reliably detect ecological changes and disturbance impacts on ecosystems is crucial.

The overall aim of this study was to optimize acoustic bat sampling protocols in Neotropical regions, using one of the most comprehensive Neotropical bat acoustic data sets, collected during three years of sampling in the Central Amazon using automatic detectors. The two main goals were to assess the minimum survey effort required to evaluate (1) the completeness of assemblage inventories and (2) habitat selection in fragmented forest landscapes for aerial insectivorous bats (species richness, activity levels, species diversity, and assemblage composition). To address the aforementioned objectives, we compared different survey efforts with their results accuracy, under 20 different temporal sampling schemes, considering different time windows or data subsets (i.e., varying the number of hours of sampling per night, the number of consecutive nights of sampling per site, and sampling only during the wet or dry season, or both).

Moreover, the performance of all sampling schemes was assessed under two different landscape scenarios: (1) a combination of continuous primary forest, and primary forest fragments embedded in a matrix of old secondary forest (all surveyed areas being forests), hereafter referred to as “Scenario A,” and (2) a combination of continuous primary forest and forest fragments surrounded by forest clearings and open areas (surveyed areas including forests and small vegetation clearings) hereafter referred to as “Scenario B” (see *Study area* for more information).

## MATERIAL AND METHODS

### *Study area*

The study was conducted at the Biological Dynamics of Forest Fragments Project (BDFFP), located ~80 km north of Manaus (2°30' S, 60° W; Laurance et al. 2018). The forest in the area was experimentally fragmented during the 1980s, which resulted in a mosaic landscape

containing about 1,000 km<sup>2</sup> of continuous primary terra firme forest, secondary forests, and a series of different-sized forest fragments. Canopy height in continuous forest is about 30–37 m, and annual precipitation ranges from 1,900 to 3,500 mm, with a dry season between July and October and a wet season from November to June (Ferreira et al. 2017). Forest fragments were initially surrounded by cattle pasture (early 1980s), which due to abandonment regenerated into tall secondary forest (Farneda et al. 2018, Rocha et al. 2018). However, fragment isolation was experimentally preserved by periodically clearing a 100 m wide strip around each of the fragments, leaving relatively large areas (6.4–42 ha) of open grass- or shrubland (Rocha et al. 2017). The most recent fragment re-isolation occurred between November 2013 and March 2014, and the acoustic data used herein were collected before (from June 2011 to September 2013) and after this (from April to October 2014). Prior to fragment re-isolation, we sampled bats in the interior of continuous primary forest ( $N = 9$  sites) and forest fragments ( $N = 8$ ) as well as in the adjacent secondary forest matrix ( $N = 8$ ; Scenario A). The same sites were sampled following fragment re-isolation, placing the detectors in the exact same locations (therefore the sampling points in the secondary forest corresponded to the new open areas or clearings; Scenario B). Due to logistical constraints, after re-isolation, only six of the control sites were sampled.

#### *Acoustic sampling*

At each sampling site, one SM2Bat detector with an omnidirectional ultrasonic SMX-US microphone (Wildlife Acoustics, Maynard, MA, USA) was placed ~1.5 m above the ground. Detectors recorded from sunset to sunrise, at 384 kHz sampling rate in full spectrum with 16-bit resolution. The high pass filter was set at 12 kHz (fs/32), with a trigger level of 18SNR. At each site, we recorded bats during five (2012–2013) and three (2014) consecutive nights, always twice during the dry and wet seasons. We standardized our sampling unit using bat passes, which were defined as 5-s long sequences with a minimum of two distinguishable pulses of a certain species (e.g., Millon et al. 2015, Torrent et al. 2018, Kemp et al. 2019). Acoustic data sets are archived in the Dryad Digital Repository (López-Baucells et al. 2021).

#### *Echolocation call analysis*

All bat passes were analysed using Kaleidoscope v.4.0.4 software (Wildlife Acoustics). Classification was done manually to species/sonotype level following López-Baucells et al. (2016). Therefore, the shortcomings of our classification method and potential bias in bat species identification can be considered constant. A sonotype was defined as a category that grouped species with indistinguishable calls (Appendix S1: Table S1). Additionally, some calls that were difficult to identify

were compared to those from a local reference call library compiled by the authors during this study (A. López-Baucells, *unpublished data*). This led to a total of 1,088,940 analyzed recordings, and ~640,000 identified bat passes from a total of 21 species/sonotypes.

#### *Statistical analysis*

##### *Protocol optimization for species richness evaluation.*

To identify the minimum sampling effort required to determine species inventory completeness before and after forest fragment re-isolation, we compared the results of species accumulation curves (SAC), considering sampling nights as the unit of sampling effort (Moreno and Halffter 2000, Froidevaux et al. 2014, Law et al. 2015). To achieve this, we pooled together sampling nights in continuous forest, forest fragments, and secondary forests/clearings. Then we split the full data set to reflect a set of different temporal sampling schemes, varying (1) the number of hours of sampling per night, (2) the number of consecutive full nights of sampling per site, and (3) sampling only during the wet or dry season, or both (also using full nights).

Different time windows were chosen from 18:00 to 6:00 by gradually increasing the number of recording hours (e.g., 18:00–18:59, 18:00–19:59, 18:00–20:59..., 18:00–06:00; Banks-Leite et al. 2012). SACs were computed (with randomization of the samples; 100 permutations) using the specaccum function from the R package vegan (Oksanen et al. 2015). Because achieving 100% inventory completeness usually requires a prohibitively large sampling effort (Moreno and Halffter 2000), we compared the number of sampling nights per site required to reach 90% of the estimated total number of species in the survey area, calculated with the Jackknife estimator (jack1) using the specaccum function in the R package vegan (e.g., Skalak et al. 2012, Froidevaux et al. 2014). The Jackknife estimator uses subsets by successively deleting individuals from the main data set and it is widely used in ecological studies (Gotelli and Colwell 2011, Chao and Chiu 2016).

##### *Protocol optimization to assess habitat selection.*

We modeled the effect of habitat type (predictor variable) upon richness, bat activity, diversity, and assemblage composition (response variables) for each of the temporal sampling schemes previously described. Richness was considered as the number of species/sonotypes present at a sampling site per night. Bat activity was defined as the number of bat passes per night. Species diversity was assessed with the Simpson index (calculated using the R package vegan), which combines species richness and evenness (Borcard et al. 2011). Assemblage composition comparisons were performed using the Bray-Curtis index as an abundance-based measure of dissimilarity (Borcard et al. 2011). In order to use this index to characterize each sampling site, we first calculated a baseline assemblage composition by

pooling the data from all continuous forest interiors. Then we quantified similarity in species composition between each site and this reference assemblage using the function `vegdist` (Bray-Curtis distance metric) from the R package `vegan` (Jost et al. 2011). To model the effect of habitat type on the aforementioned response variables, we performed Monte Carlo Markov Chain generalized linear mixed models using the R package `MCMCglmm`, specifying a Poisson (for bat activity) or Gaussian (for richness, Simpson diversity, and assemblage composition) error distribution. When the response variable showed signs of overdispersion or zero-inflation, the priors were set to be weakly informative in order to deal with over-dispersion (inverse gamma parameters:  $\nu = 0.002$  and  $V = 1$ ; Kryvokhyzha et al. 2016, Hadfield 2017). Habitat type was specified as a categorical fixed effect (categories: continuous forest, forest fragments, and secondary forest/clearings), and sampling site as a random effect (Adams et al. 2015). Models were run with 50,000 iterations, and the first 10,000 were discarded from the results.

All statistical analyses were carried out using R software, version 3.2.4. (R Core Team 2017), and all plots built with the `ggplot2` package (Wickham 2009).

## RESULTS

### *Protocol optimization for species richness evaluation*

Based on the whole data set, `jack1` estimated a total of 21 different species/sonotypes to occur in the study area before and after fragment re-isolation. When assessing different temporal sampling schemes, the SACs varied considerably between Scenario A and B (Fig. 1A). The rate of decrease in the estimated sampling effort required with increasing number of hours of recording varied markedly, being much more gradual in Scenario A than in B (Fig. 1B). When recordings from only the first three hours after sunset were considered, ~150 nights in Scenario A and ~50 nights in Scenario B were needed to achieve 90% inventory completeness (Fig. 1B). Increasing the number of hours of recording after sunset to four

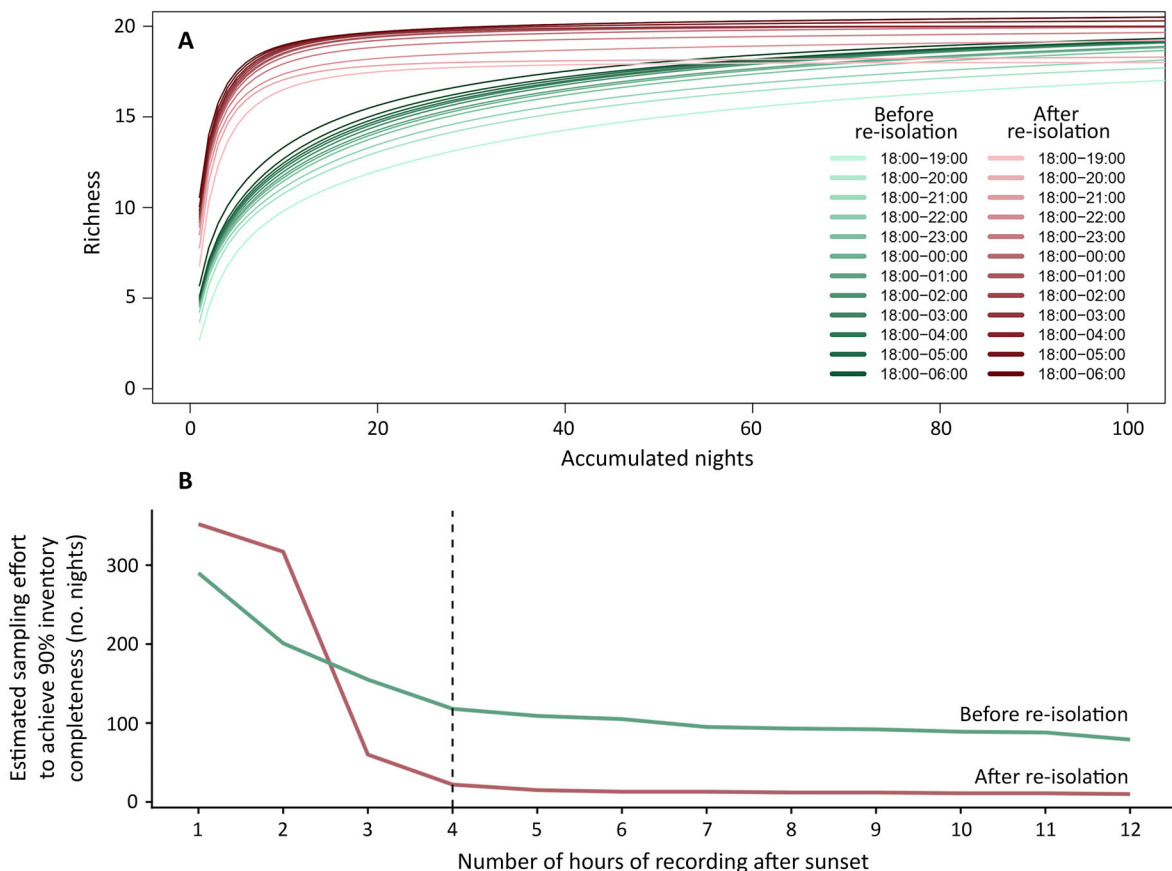


FIG. 1. (A) Species accumulation curves for different temporal sampling schemes using a varying number of hours of recording per night. (B) Estimated sampling effort measured as total number of nights required to detect 90% of the species present in the study area. Green lines show data collected before the re-isolation in continuous forest, primary forest fragments, and secondary forest (scenario A); red lines show data collected after the re-isolation in continuous forest, forest fragments, and clearings (scenario B). Vertical line in B at  $x = 4$  h of recording.

reduced the sampling effort needed to achieve 90% inventory completeness to ~80 and 10 nights, respectively. However, further increases in the number of recorded hours after sunset did lead to further decreases in the number of sampled nights needed to achieve a 90% inventory completeness (Fig. 1B). In terms of the number of consecutive nights per site, under both landscape scenarios, sampling only for one night was sufficient to achieve 90% inventory completeness. In fact, recording more consecutive nights per site did not significantly improve the rate of new species detections (Table 1). In the seasonal analysis, in Scenario A almost no differences were found between dry and wet season data sets, while in Scenario B, the estimated sampling effort for the wet season was almost double that required during the dry season (Table 1). When pooling the data from both seasons, the minimum estimated sampling effort was reduced by one-third or by one-half, respectively.

#### *Protocol optimization to assess habitat selection*

Although for some response variables the model results were quite consistent for different temporal sampling schemes (e.g., bat activity in forest fragments or richness in the clearings, in Scenario B), effect sizes for other response variables varied substantially (e.g., for Simpson diversity for both Scenario A and B, Fig. 2). This variability sometimes led to contradictory results with regard to significance of the effect (e.g., the effect upon Simpson diversity in secondary forests in Scenario A).

In Scenario A, a minimum of four hours was necessary to detect significantly lower richness and species diversity (Simpson) in secondary forest relative to continuous forest. In contrast, assemblage composition (Bray-Curtis) of the secondary forest was significantly distinct from continuous forest only when it was

evaluated with the full night data set. In Scenario B, except for species composition, the effect of the clearings on all response variables examined was very consistent as in all cases there were significant differences between clearings and continuous forest, independently of the sampling scheme.

Using different numbers of consecutive nights per site resulted in consistent effects for some response variables (e.g., all models for the Scenario B), whereas there were large inconsistencies for others (e.g., mostly in Scenario A) (Fig. 2). In Scenario A, up to five consecutive nights were needed to detect significant differences in richness and species diversity between secondary and primary forest. Also, in the secondary forest, with more than two nights, the significant effects detected for bat activity with smaller data sets disappeared (Fig. 2).

Results based on data sets collected during different seasons were quite consistent (Fig. 2). In all models for Scenario B, the direction of the effect was always the same, independently of the sampling scheme (except for assemblage composition in the forest fragments). Pronounced differences between subsets were only found for some particular cases in Scenario A (e.g., bat activity in the forest fragments for which using only data from the dry season resulted in a non-significant effect, as opposed to a significant positive response when using data from both seasons or the wet season only) (Fig. 2).

#### DISCUSSION

Analysis of over 640,000 bat passes from one of the most diverse tropical bat assemblages revealed that the sampling effort required to achieve species inventories at 90% completeness for aerial insectivorous bats varies substantially with landscape context, being about eight times higher in forest fragments embedded in a matrix of old secondary forest than in the same forest fragments re-isolated by clearing the surrounding secondary forest. We showed that recording longer than 4 h after sunset per night did not significantly reduce the number of nights required to inventory 90% of the species present in the study area. Moreover, the rate of decrease in the estimated sampling effort required varied considerably between Scenario A and B, being much more gradual in the former compared to the very marked decline in the latter. Our results indicate that, for inventories, the sampling season and the number of consecutive nights per site are less important than the nightly time window during which sampling is conducted. When modeling habitat selection for aerial insectivorous bats, if forest and clearings are surveyed together, the significance and direction of the effect was consistent across all sampling schemes (except for assemblage composition). However, if only forests (primary and secondary) are surveyed, choosing the most appropriate temporal sampling scheme was crucial to detect significant effects that otherwise might go unnoticed.

TABLE 1. Estimated sampling effort (number of full nights of recording) required to reach 90% inventory completeness under different subsampling schemes, varying the number of consecutive nights of sampling per site and sampling only during the wet or dry season, or both.

Scheme	Estimated sampling effort to reach 90% inventory completeness (no. nights)	
	Scenario A (primary and secondary forest surveyed)	Scenario B (primary forest and clearings surveyed)
1 night/site	59	9
2 nights/site	74	10
3 nights/site	80	10
4 nights/site	81	NA
5 nights/site	87	NA
Dry season	127	8
Wet season	128	14
Both seasons	80	10

Note: NA, not applicable.

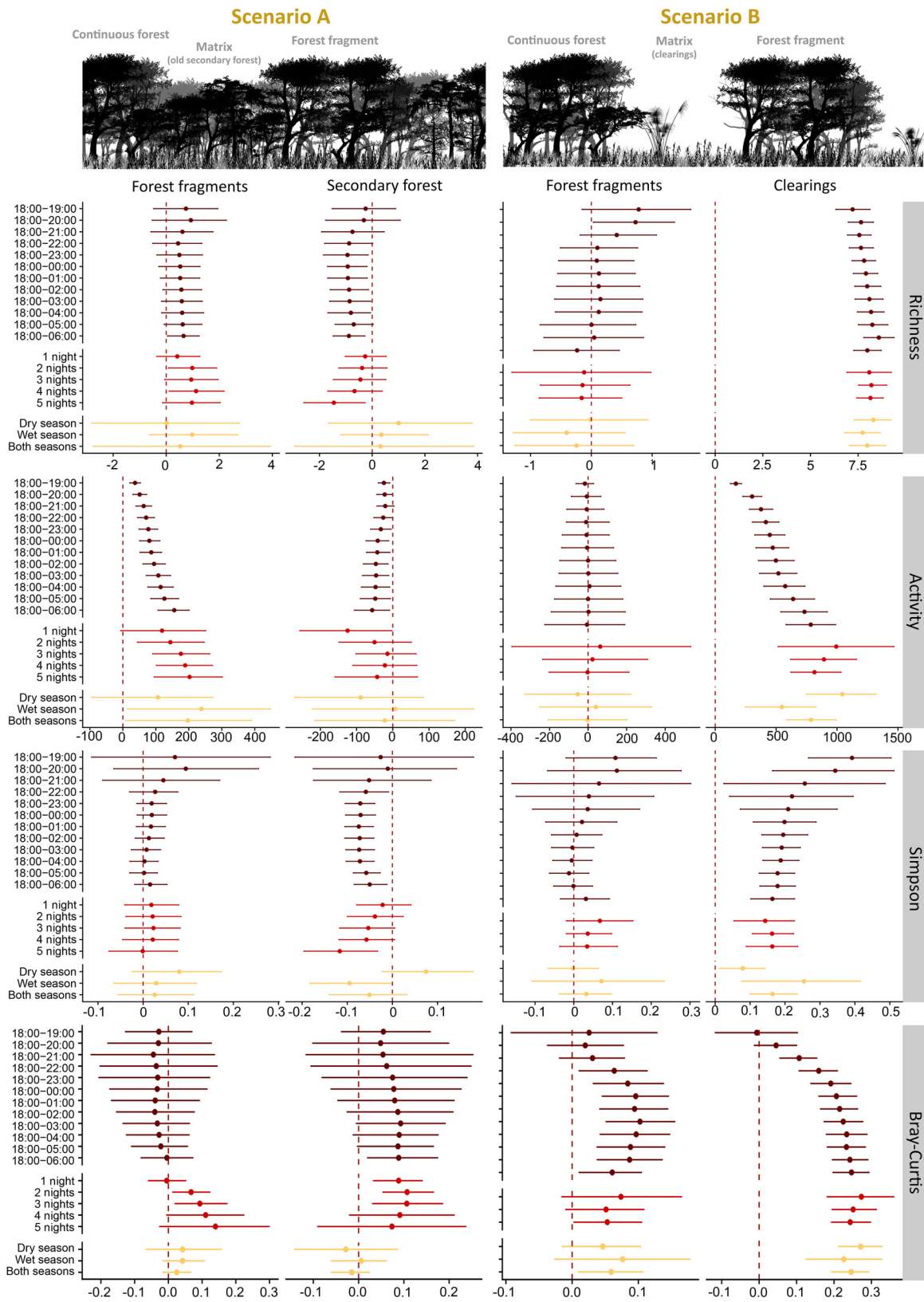




FIG. 2. Effects of habitat modification on richness, activity, diversity, and assemblage composition of aerial insectivorous bats before fragment re-isolation (2012–2013; scenario A) and after fragment re-isolation (2014; scenario B), evaluated using generalized linear mixed models. The effects of habitat modification are calculated as the magnitude of change between the disturbed habitat types (forest fragments, secondary forests, and clearings) and continuous forest interiors. Effect estimates are considered as the fixed effect posterior distribution, characterized by its mean (dot) and credible intervals (95% CI, lines).

#### *Protocol optimization for species richness evaluation*

In order to detect at least 90% of the species present, our analyses indicated a minimum sampling requirement of ~80 nights (when only primary and secondary forests are surveyed; Scenario A) but only ~10 nights (if clearings are also included; Scenario B). Similar results were obtained by Froidevaux et al. (2014) in temperate habitats where diversity in forest gaps was much more easily assessed than in forest interiors. This is explained by the fact that open or edge space foragers, which usually are rare in the cluttered forest interiors, seemingly increase in activity when the forest is cleared as they become more easily detected (Estrada-Villegas et al. 2010, Torrent et al. 2018). Alternatively, insect abundance might have substantially increased in the cleared areas, resulting in a localized burst in bat activity (Salvarina et al. 2018).

Aerial hawking bats tend to be more active during the first hours of the night due to the peak in aerial insect availability, during what is commonly known as the highest nocturnal activity peak (Bernard 2002). Sometimes researchers assume that sampling during this period is sufficiently representative to describe the diversity of aerial insectivorous bats (Stahlschmidt and Bruhl 2012). Froidevaux et al. (2014) evaluated the differences between three sampling schemes in Europe, full night, 4 h after sunset, and 2 h after sunset plus 2 h before sunrise, and found the best outcome was achieved when recording the entire night. Trevelin et al. (2017), who assessed the effectiveness of capturing phyllostomids with mist-nets during the first 6 h in the Neotropics, obtained similar results. Our study is the first to address similar questions for Neotropical aerial insectivorous bats, evaluating how completeness changed in response to cumulative increases in effort (i.e., number of sampling hours) instead of discrete recording time windows as used by Froidevaux et al. (2014). The fact that variability in the results stabilizes after using the first 4 h suggests that this time window is the minimum necessary sampling effort to ensure an adequate representativeness (90%) of these bat assemblages. If sampling effort needs to be optimized for Neotropical bat inventories, reducing the nightly time window appears more effective than reducing the number of consecutive nights. This is especially important now that low-cost passive detectors such as AudioMoths (Hill et al. 2018) are increasingly used, and for which battery life is a particularly limiting factor.

In terms of sampling locations, Law et al. (2015) recommended to repeat the number of visits in certain

sampling points rather than distributing the equivalent sampling effort in new sampling sites. The spatial dimension of their study is an important aspect that we were not able to address due to the fact that the BDFFP landscape offers limited scope for spatial replication. Likely the number of sampling nights presented above can be considerably reduced when multiple detectors/site are used. In fact, studies suggest that several detectors recording simultaneously are more efficient than a single detector recording in the same location for several nights (Froidevaux et al. 2014). Law et al. (2015) also found that to adequately capture spatial heterogeneity it is always preferable to use up to three detectors for two nights rather than using one single detector for six nights, prioritizing spatial over temporal replication. Our results are nevertheless relevant given that the number of detectors available to researchers for long-term studies is often limited. It would be important to extend our present analyses by using several detectors within the same sampling sites, and by substantially increasing the number of sampling sites across the study area, as Law et al. (2015) did in Australia and Froidevaux et al. (2014) in Europe.

In terms of seasonal sampling window, especially before the re-isolation, pooling of the data from both dry and wet season allowed us to record significantly more species using less sampling effort than using data from only one season. We recorded fewer species in the dry season (although at higher rates of detection) than in the wet season, probably due to reduced insect availability (Taylor et al. 2013). This implies that to accurately describe bat assemblages in the Neotropics, inventory studies should cover both seasons.

#### *Protocol optimization to assess habitat selection*

Choosing the right sampling scheme to accurately describe ecological patterns and to detect the consequences of habitat modification on wildlife is more important in a conservation context, although inherently more difficult than choosing the right protocol for inventories. We showed that with inappropriate sampling designs, existing differences can not only become more difficult to detect but can also erroneously point into the opposite direction, therefore potentially undermining conservation efforts. Full and accurate knowledge of the responses of animal communities to habitat alteration is needed to ensure appropriate conservation actions.

In Scenario A, different nightly sampling schemes provided contradictory results, underlining the importance of not restricting the time of recording within the night



if weak ecological patterns are to be detected. Variability in model effect sizes was sometimes so large that significant results were only found with full-night sampling. Similarly, when we considered data sets from five entire nights of recording, some significant responses detected with smaller data subsets turned out to be non-significant. The large variability in the model estimate can potentially mask significant effects, which makes the interpretation of some specific results quite difficult. In some cases, increasing the number of consecutive nights per site up to five substantially increased the accuracy of our model estimates. This was especially true for secondary forest (e.g., richness, bat activity, species diversity, and assemblage composition), which suggests that the number of consecutive nights might be more important for detecting ecological patterns than for inventories. In agreement with Law et al. (2015), we found that night-to-night variation in activity was high, however, we argue that within-night variation might even exceed any bias linked to the former. The fact that, in Scenario A, we did not find clear differences between model results that considered both seasons either together or separately, suggests that those ecological patterns might be consistent all year long.

In Scenario B, for almost all response metrics, robust results were already obtained with the smallest subsets evaluated (e.g., recording for 1 h after sunset, one season or one night per site). The only exception was for assemblage composition in forest fragments and clearings, for which four and three hours, respectively, were necessary to detect significant effects. This suggests that minimum sampling effort to answer specific ecological questions strongly depends on the variation in the variables studied and the landscape context (Bros and Cowell 1987, Chao et al. 2009, Johnson et al. 2014). Besides environmental heterogeneity, specific characteristics of local bat assemblages (e.g., the total number of species present in the sampling area, or the proportion of high-flying vs forest-dwelling species) will also influence the sampling effort required. More detailed information about species' phenology or foraging strategies of Neotropical bats would fill current knowledge gaps that currently hamper the reliability of monitoring programs.

### *Recommendations*

Optimizing sampling protocols in monitoring programs, surveys or ecological studies implies that sampling effort, methodology, equipment costs and workload associated with field work are carefully balanced (Guillera-Arroita and Lahoz-Monfort 2012) as time dedicated to field work and data analysis is directly linked to project costs (Gardner et al. 2008, Froidevaux et al. 2014). Sampling design will mainly depend on the scientific questions that the study aims to address. Based on our findings, we propose guidelines that can aid to streamline bat acoustic surveys in the Neotropics, by reducing project workload and costs, while maintaining

high accuracy and representativeness. We believe our following recommendations are valuable to guide future research, especially in the context of the funding crisis that science is experiencing:

#### 1) For species inventories

- a. We recommend reducing, in decreasing order of priority, the number of consecutive nights per site, the number of sampling hours per night; as well as distributing the sampling effort equally between both seasons.
- b. The minimum time window to detect at least 90% of bat species present in a particular study area should include the first 4 h after dusk. However, if rare or elusive species also are to be targeted, we recommend recording the complete night (Moreno and Halffter 2000).
- c. The total time required to achieve 90% complete inventories highly depends on the landscape context. Although in landscapes with low between-habitat structural contrast, as represented here by Scenario A, a total of ~80 entire nights spread over both seasons can be enough, in high-contrast landscapes such as Scenario B, ~10 entire nights would be sufficient.

#### 2) For studies on habitat selection

- a. We recommend reducing, in decreasing order of priority, the number of seasons sampled, the number of consecutive nights per site, and finally the number of sampling hours per night.
- b. To detect population- and assemblage-level responses to habitat modification in low-contrast landscapes such as Scenario A, recording the first 4–6 h after sunset and recording during three to five nights in the same sites is advisable. However, our results suggest that, in high-contrast landscapes such as Scenario B, the same responses can be detected with considerably less sampling effort (e.g., one night per site).

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#### SUPPORTING INFORMATION

Additional supporting information may be found online at: <http://onlinelibrary.wiley.com/doi/10.1002/eap.2366/full>

#### OPEN RESEARCH

Data (López-Baucells et al. 2021) are available in the Dryad Digital Repository: <https://doi.org/10.5061/dryad.2bvq83bq4>.