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ECOACOUSTIC METHODS FOR MULTI-TAXA ANIMAL SURVEYS IN THE
AMAZON

by

Leandro A. Do Nascimento

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Ecology

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2020

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ABSTRACT

Ecoacoustic Methods for Multi-taxa Animal Surveys in the Amazon

by

Leandro A. Do Nascimento, Doctor of Philosophy

Utah State University, 2020

Major Professor: Dr. Karen H. Beard
Department: Wildland Resources

Ecoacoustics is a new discipline that investigates the ecological role of sounds in landscapes. The methods becoming available in this field have great potential for multi-taxa animal surveys and routine biodiversity assessments, a topic of great interest among the scientific community, general public, and governments around the world. Despite this potential, foundational assumptions of the field still need to be tested empirically, especially in tropical regions, where most of the world's animal diversity is located but where ecoacoustic studies rarely have been implemented. In this dissertation, I used ecoacoustic data collected over two years in the Brazilian Amazon to test the applicability of three different but complementary approaches to analyze large, audio files data sets (over 3000 hours of sound recordings). In Chapter 1, I provide a brief review of the ecoacoustic field and soundscapes. In Chapter 2, I confirm two central assumptions from the field of ecoacoustics, that habitats have unique and predictable acoustic signatures, and that soundscapes are intrinsically linked to changes in vegetation structure. In Chapter 3, I found that ecoacoustic surveys can be used to study the 24-h

calling behavior of howler monkeys, an animal producing a loud call that is a key component of Neotropical soundscapes, and identify key differences in their calls between day and night. In Chapter 4, I used ecoacoustic surveys and successfully employed automatic classifiers to retrieve information about two threatened bird species in the Amazon. In Chapter 5, I summarize my findings and discuss future research directions in the ecoacoustics field. The results from Chapters 2 to 4 confirm the great potential to establish ecoacoustic surveys and associated methods as a complementary strategy for multi-taxa animal surveys in the tropical region.

(136 pages)

PUBLIC ABSTRACT

Ecoacoustic Methods for Multi-taxa Animal Surveys in the Amazon

Leandro A. Do Nascimento

Tropical regions host most of the biodiversity found on Earth, but these species-rich areas are constantly threatened by human development and other disturbances that put this diversity of life forms at risk. To avoid extirpations of animal and plant species, scientists and managers rely on accurate monitoring techniques to retrieve information about population trends. This task is not easy, especially in the tropics, where there is often a lack of personnel to conduct surveys, a lack of funding, and the areas are so extensive that many countries need to be involved in monitoring (e.g., Amazon biome). For this reason, scientists are trying to take advantage of technological advancements to develop more cost-effective alternatives for multi-taxa surveys. While satellite imagery provides a richness of information about vegetation, it fails to provide direct measurement of the fauna. In this dissertation conducted in the Brazilian Amazon, I used passive acoustic recorders as a technique to collect reliable and verifiable information about the fauna. I show that the data collected with passive acoustic sensors is able to provide information about how the biodiversity of the Amazon changes with human disturbances, time of the day, and in different environments.

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Leandro A. Do Nascimento

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CHAPTER 1

INTRODUCTION

Ecoacoustics and the origin of sounds

The emerging field of ecoacoustics focuses on studying relationships between sounds and the environment over multiple spatial and temporal scales (Farina and Gage, 2017). Natural sounds may have a biological source, such as animal calls (biophony), or non-biological sources, such as rain and wind (geophony); while anthropogenic sounds are related to human-made machines (anthrophony) (Pijanowski et al., 2011). Together, these three sources of sound constitute the soundscape of a given region and at a given time (Pijanowski et al., 2011; Southworth, 1967). Soundscapes have been successfully used to investigate multiple ecological questions, ranging from evaluating restoration outcomes on islands (Borker et al., 2020) to the assessment of key ecosystem functions of coral reefs (Elise et al., 2019). Despite the large interest in soundscapes for biodiversity investigation, foundational assumptions of this new field still wait to be tested, and speciose locations in the tropical region, which would likely benefit the most from the methods and tools being developed, are rarely studied (Scarpelli et al., 2020). This dissertation advances this new ecological discipline by fulfilling some of these knowledge gaps in the Brazilian Amazon, the most biodiverse-rich region in the world.

Sounds are ubiquitous on Earth. From the deep-sea to rainforests, from deserts to urban centers, soundscapes are filled with a richness of euphonies and cacophonies. Apparently, it has been like this since the dawn of times. The first sound to likely ever exist was the sound of the Big Bang 13.8 billion years ago (Whittle, 2004). The Big Bang

likely produced such a low frequency sound that it was way out of the human and other animals' audible spectrum (Whittle, 2004). The ability to hear actually appeared on Earth only 400 million years ago in bony fishes that used a modified labyrinth organ to sense vibrations in the water (Christensen et al., 2015). When animals start migrating from water to land, these sensory organs were not as effective on air, and only millions of years later did eardrum organs evolve that allowed effective hearing in the air medium (Allin, 1975). Since then sounds became essential in animal communication and the established field of bioacoustics has been central to understanding how animals perceive and respond to acoustic signals that are omnipresent in soundscapes across the Earth (Bradbury and Vehrencamp, 1998). However, the study of sounds in bioacoustics is often restricted to single organisms and low level of organizations (Pijanowski et al., 2011).

The untapped potential of environmental sounds

Recently, researchers have realized the large and untapped potential of sounds emanating from landscapes and its potential to advance ecological and biological sciences (Pijanowski et al., 2011; Farina and Gage, 2017). Ecoacoustics have moved the study of sounds from the species level to the community-level (Pijanowski et al., 2011). This change in scale to higher levels of organization was only possible due to technological advancements in acoustic sensors, better analytical tools, and the recent decrease in costs to acquire audio recorders (e.g., Audio Moth recorders are as cheap as \$50 US dollars). There is large interest in conducting more efficient multi-taxa animal surveys (Yong et al., 2018) and several emerging technologies are being developed to achieve this goal (Pimm et al., 2015). For example, camera trapping has greatly advanced

our understanding of animal dynamics (Steenweg et al., 2017); similarly, metabarcoding can deliver reliable biodiversity assessments in the tropical region and beyond (Yu et al., 2012). I argue that all these tools are complementary to ecoacoustics surveys and together have great potential to advance our understanding of ecological systems and associated fauna (Deichman et al., 2018).

Threatens to the Amazon and the ecoacoustic solution

The Amazon biome harbors 10% of Earth's known biodiversity but it is also one of the most threatened ecosystems on Earth (Betts et al., 2008; Laurance et al., 2001). The Amazon occupies an area of 5,500,000 km² across nine different countries in South America (Soares-Filho et al., 2006). Brazil holds the largest portion of this biome and it is also the country that likely threatens the biome the most due a mix of urban development, politics, and illegal activities (Betts et al., 2008; Gerlak et al., 2020; Soares-Filho et al., 2006). Specifically, the activities directly impacting the largest rainforest in the world are cattle ranching, agriculture expansion, poaching, damming of rivers, illegal logging, and illegal mining (Asner et al., 2013; Betts et al., 2008; Gerlak et al., 2020; Soares-Filho et al., 2006). In Brazil, any new planned project, program, and legislative action must have their potential impact on the environment assessed (Ritter et al., 2017). This allows adverse effects to be mitigated and is particularly important in biodiverse-rich locations such as the Amazon.

The legal mechanism allowing such assessment in Brazil is the Environmental Impact Assessment (EIA) and the EIA report (in Portuguese, RIMA – *Relatório de Impacto Ambiental*). The drawbacks with this important legal mechanism are the lack of

standards in the assessments (Ritter et al., 2017) and a cost-effective way to survey the fauna (Ribeiro et al., 2017). Ecoacoustic methods could fulfill this niche because they allow for a rapid assessment of the vocalizing fauna and has the advantage of allowing the associated recordings to be archived for future validation purposes. In addition, the recordings could be analyzed by a plethora of different methods (the main methods are discussed in chapters 2 to 4). Ecoacoustic surveys could be an important and complementary mechanism for EIAs throughout the Amazon, but to date, this has been not explored to its fully potential and it is an open area for future research (Ribeiro et al., 2017)

Challenges

The main challenges for establishing ecoacoustic surveys as a method for routine biodiversity assessment (Gibb et al., 2019; Kissling et al., 2018) are similar to other big data fields (Deichman et al., 2018; Servick, 2014). Passive acoustic monitoring (PAM) is the main technique behind ecoacoustic surveys and has being used for a long time in marine ecology studies (McDonald and Fox, 1999), but only recently have we started to explore PAM in terrestrial ecosystems (Sugai et al., 2019). As such, we are way behind in establishing protocols and standards in the field for terrestrial habitats (Bradfer-Lawrence et al., 2019; Deichman et al., 2018; Sugai et al., 2019). PAM produces an enormous amount of data that should be collected in standardized ways, with associated metadata, and ideally be deposited online for verification and further applications (Bradfer-Lawrence et al., 2019; Deichman et al., 2018; Sugai et al., 2019). However, no public soundscape database exists for terrestrial environments and the creation of one is

paramount to further advance this new field (Deichman et al., 2018). Despite the challenges, soundscapes have proven useful for studying ecological systems, and the study of sounds has a long tradition in different disciplines that can help establish this new field as important in ecological science. Although technological advancements in sensors and better analytical tools are still needed to analyze the increasing amount of environmental recordings becoming available around the Earth, researchers and personnel working in the field have every reason to continue to collect acoustic data because they may prove to be extremely useful for future generations in answering several ecological questions and also as bioacoustic ‘time capsules’ of biodiversity (Deichman et al., 2018; Sugai and Llusia, 2019).

Objectives

The overarching goal of this dissertation is to test three different but complementary ecoacoustic approaches for multi-taxa animal surveys in the Amazon. In Chapter 2, I used acoustic metrics (proxies of biodiversity) to predict habitat type and vegetation structure across major habitat types of the Amazon. In Chapter 3, I used aural identification of calls in a large audio dataset to advance our understanding of the nocturnal behavior of howler monkeys and the function of their remarkable loud calls. In Chapter 4, I used passive acoustic monitoring and an automatic classifier to identify the presence or absence of two threatened birds across 60 sites and three different habitat types. In Chapter 5, I summarized my findings and I pointed to future research directions in the field of ecoacoustics.

REFERENCES

- Allin, E. F. (1975). Evolution of the mammalian middle ear. *Journal of Morphology*, *147*(4), 403-437.
- Asner, G. P., Llactayo, W., Tupayachi, R., & Luna, E. R. (2013). Elevated rates of gold mining in the Amazon revealed through high-resolution monitoring. *Proceedings of the National Academy of Sciences*, *110*(46), 18454-18459.
- Betts, R. A., Malhi, Y., & Roberts, J. T. (2008). The future of the Amazon: new perspectives from climate, ecosystem and social sciences. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1498), 1729-1735.
- Borker, A. L., Buxton, R. T., Jones, I. L., Major, H. L., Williams, J. C., Tershy, B. R., & Croll, D. A. (2020). Do soundscape indices predict landscape-scale restoration outcomes? A comparative study of restored seabird island soundscapes. *Restoration Ecology*, *28*(1), 252-260.
- Bradbury, J. W., & Vehrencamp, S. L. (1998). Principles of animal communication.
- Bradfer-Lawrence, T., Gardner, N., Bunnefeld, L., Bunnefeld, N., Willis, S. G., & Dent, D. H. (2019). Guidelines for the use of acoustic indices in environmental research. *Methods in Ecology and Evolution*, *10*(10), 1796-1807.
- Christensen, C. B., Christensen-Dalsgaard, J., & Madsen, P. T. (2015). Hearing of the African lungfish (*Protopterus annectens*) suggests underwater pressure detection and rudimentary aerial hearing in early tetrapods. *Journal of Experimental Biology*, *218*(3), 381-387.
- Deichmann, J. L., Acevedo-Charry, O., Barclay, L., Burivalova, Z., Campos-Cerqueira,

- M., d'Horta, F., ... & Linke, S. (2018). It's time to listen: there is much to be learned from the sounds of tropical ecosystems. *Biotropica*, *50*(5), 713-718.
- Elise, S., Urbina-Barreto, I., Pinel, R., Mahamadaly, V., Bureau, S., Penin, L., ... & Bruggemann, J. H. (2019). Assessing key ecosystem functions through soundscapes: a new perspective from coral reefs. *Ecological Indicators*, *107*, 105623.
- Farina, A., & Gage, S. H. (Eds.). (2017). *Ecoacoustics: The ecological role of sounds*. John Wiley & Sons.
- Gerlak, A. K., Saguier, M., Mills-Novoa, M., Fearnside, P. M., & Albrecht, T. R. (2020). Dams, Chinese investments, and EIAs: A race to the bottom in South America?. *Ambio*, *49*(1), 156-164.
- Gibb, R., Browning, E., Glover-Kapfer, P., & Jones, K. E. (2019). Emerging opportunities and challenges for passive acoustics in ecological assessment and monitoring. *Methods in Ecology and Evolution*, *10*(2), 169-185.
- Kissling, W. D., Walls, R., Bowser, A., Jones, M. O., Kattge, J., Agosti, D., ... & Denny, E. G. (2018). Towards global data products of Essential Biodiversity Variables on species traits. *Nature ecology & evolution*, *2*(10), 1531-1540.
- Laurance, W. F., Cochrane, M. A., Bergen, S., Fearnside, P. M., Delamônica, P., Barber, C., ... & Fernandes, T. (2001). The future of the Brazilian Amazon. *Science*, *291*(5503), 438-439.
- McDonald, M. A., & Fox, C. G. (1999). Passive acoustic methods applied to fin whale population density estimation. *The Journal of the Acoustical Society of America*, *105*(5), 2643-2651.

- Pimm, S. L., Alibhai, S., Bergl, R., Dehgan, A., Giri, C., Jewell, Z., ... & Loarie, S. (2015). Emerging technologies to conserve biodiversity. *Trends in ecology & evolution*, *30*(11), 685-696.
- Pijanowski, B. C., Villanueva-Rivera, L. J., Dumyahn, S. L., Farina, A., Krause, B. L., Napoletano, B. M., ... & Pieretti, N. (2011). Soundscape ecology: the science of sound in the landscape. *BioScience*, *61*(3), 203-216.
- Ribeiro, J. W., Sugai, L. S. M., & Campos-Cerqueira, M. (2017). Passive acoustic monitoring as a complementary strategy to assess biodiversity in the Brazilian Amazonia. *Biodiversity and Conservation*, *26*(12), 2999-3002.
- Ritter, C. D., McCrate, G., Nilsson, R. H., Fearnside, P. M., Palme, U., & Antonelli, A. (2017). Environmental impact assessment in Brazilian Amazonia: Challenges and prospects to assess biodiversity. *Biological Conservation*, *206*, 161-168.
- Scarpelli, M. D., Ribeiro, M. C., Teixeira, F. Z., Young, R. J., & Teixeira, C. P. (2020). Gaps in terrestrial soundscape research: It's time to focus on tropical wildlife. *Science of the Total Environment*, *707*, 135403.
- Servick, K. (2014). Eavesdropping on Ecosystems. *Science*, *343*(6173), 834-837.
- Soares-Filho, B. S., Nepstad, D. C., Curran, L. M., Cerqueira, G. C., Garcia, R. A., Ramos, C. A., ... & Schlesinger, P. (2006). Modelling conservation in the Amazon basin. *Nature*, *440*(7083), 520-523.
- Southworth, M. F. (1967). *The sonic environment of cities* (Doctoral dissertation, Massachusetts Institute of Technology).
- Sugai, L. S. M., Silva, T. S. F., Ribeiro Jr, J. W., & Llusia, D. (2019). Terrestrial passive acoustic monitoring: review and perspectives. *BioScience*, *69*(1), 15-25.

- Sugai, L. S. M., & Llusia, D. (2019). Bioacoustic time capsules: using acoustic monitoring to document biodiversity. *Ecological Indicators*, *99*, 149-152.
- Steenweg, R., Hebblewhite, M., Kays, R., Ahumada, J., Fisher, J. T., Burton, C., ... & Brodie, J. (2017). Scaling-up camera traps: Monitoring the planet's biodiversity with networks of remote sensors. *Frontiers in Ecology and the Environment*, *15*(1), 26-34.
- Whittle, M. (2004, June). Primordial Sounds: Big Bang Acoustics. In *Press release: American Astronomical Society meeting, June* (Vol. 1).
- Yong, D. L., Barton, P. S., Ikin, K., Evans, M. J., Crane, M., Okada, S., ... & Lindenmayer, D. B. (2018). Cross-taxonomic surrogates for biodiversity conservation in human-modified landscapes—A multi-taxa approach. *Biological conservation*, *224*, 336-346.
- Yu, D. W., Ji, Y., Emerson, B. C., Wang, X., Ye, C., Yang, C., & Ding, Z. (2012). Biodiversity soup: metabarcoding of arthropods for rapid biodiversity assessment and biomonitoring. *Methods in Ecology and Evolution*, *3*(4), 613-623.

CHAPTER 2
ACOUSTIC METRICS PREDICT HABITAT TYPE AND VEGETATION
STRUCTURE IN THE AMAZON¹

ABSTRACT

The rapidly developing field of ecoacoustics offers methods that can advance multi-taxa animal surveys at policy-relevant extents. While the field is promising, there remain foundational assumptions that need to be tested across different biomes before the methods can be applied widely. Here we test two of these assumptions in the Amazon: 1) that acoustic indices can be used to predict soundscapes of different habitat types, and 2) that acoustic indices are related to vegetation structure. We recorded soundscapes and collected vegetation data in 143 sites spanning six natural and two human-modified habitats in Viruá National Park, Roraima, Brazil. We grouped the eight habitats into three categories based on vegetative characteristics and flooding regime: open habitats, flooded-forests, and non-flooded forests. Thirteen acoustic indices were calculated from 92,283 one-minute recordings to describe the soundscapes of the habitats. We found that each habitat type had unique and predictable soundscapes. Random forest models were 74% accurate at predicting the eight habitats types and 87% accurate at predicting the three broader habitats categories. The most important acoustic indices to distinguish habitats were the third quartile and centroid. Canopy cover significantly affected 11 of 13

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acoustic indices, and while other vegetation variables (e.g., shrub cover and number of trees) appeared in top models for some indices, their effects were not significant. The best indices linking soundscapes to vegetation structure were the acoustic evenness index and skewness, with canopy cover explaining 81% and 52% of the variance in these indices, respectively. These results expand our knowledge regarding which acoustic indices best connect changes in habitats to changes in soundscapes. These findings are particularly important for diverse ecosystems, like the Amazon, which are known to have complex soundscapes with sound-producing animals that are difficult to detect with traditional survey methods (e.g., visual transects). Ultimately, our results suggest that soundscapes are able to track changes in biodiversity levels across major habitat types of the Amazon.

1. INTRODUCTION

The rapidly developing field of ecoacoustics offers tools to extract information quickly from large audio datasets and serves as a cost-effective way to monitor biodiversity and environmental change (Krause and Farina 2016; Farina and Gage, 2017; Pijanowski et al., 2011a; Rappaport et al., 2020). The field focuses on the investigation of natural and anthropogenic sounds (i.e., soundscapes) and their relationship with the environment over multiple spatial and temporal scales (Farina and Gage, 2017).

Soundscapes have been used in a variety of studies on topics ranging from describing biotic homogenization (Burivalova et al., 2019) to the impacts of mining and wildfire on ecological communities (Duarte et al., 2015; Gasc et al., 2018). While ecoacoustic methods are promising for ecological monitoring, several lingering knowledge gaps limit its widespread utility (Farina and Gage, 2017; Pijanowski et al., 2011b). For example,

more studies are needed to determine the ability of acoustic indices to differentiate habitat types in different biomes (Farina and Gage, 2017; Pijanowski et al., 2011b). Another point that remains largely unaddressed is how acoustic indices relate to vegetative habitat structure (Farina and Gage, 2017; Pijanowski et al., 2011b). The need to fill these gaps in knowledge is particularly pressing for tropical areas, where ecoacoustic monitoring holds great potential for species conservation, yet whose soundscapes are largely understudied (Scarpelli et al., 2020).

An almost overwhelming 60 acoustic indices have been created to describe soundscapes and represent faunal richness (Buxton, et al., 2018; Sueur et al., 2014). Acoustic indices are calculated using different patterns of soundscapes such as pitch, saturation and amplitude (Buxton, et al., 2018; Sueur et al., 2014). The theoretical underpinning of the application of acoustic indices for ecological monitoring is that acoustic diversity is positively associated with faunal species richness (Farina and Gage, 2017; Gage et al., 2001; Pijanowski et al., 2011). This positive relationship has been demonstrated through both empirical tests and computer simulations (Aide et al., 2017; Bradfer-Lawrence et al., 2020; Depraetere et al., 2012; Harris et al., 2016; Sueur et al., 2008a; Zhao et al., 2019), but in some cases no relationship was found (Gasc et al., 2015; Moreno-Gómez et al., 2019). As thousands of hours of soundscape recordings continue to accumulate globally, new indices continue to be developed that translate these data into ecological monitoring information, although often without sufficient tests for their ability to do so (Colonna et al., 2020; Gibb et al., 2019; Tuneu-Corral et al., 2020; Santiago et al., 2020).

One area which needs further investigation is the ability of acoustic indices to

distinguish soundscapes of different habitat types (e.g., anthropogenic versus natural). This area of research can improve biodiversity monitoring because if habitats have unique acoustic signatures we can use acoustic indices to monitor habitat change (Farina and Gage, 2017; Pijanowski et al., 2011b). Further, identifying the indices that correspond most closely with particular habitats across different ecosystems could reduce the computing burden of calculating several indices on large audio datasets (Bradfer-Lawrence et al., 2019; Buxton et al., 2018; Eldridge et al., 2018). To the best of our knowledge, only three studies have tested multiple acoustic indices to investigate habitat-specific soundscapes in terrestrial systems (Table 2.1). For example, Bormpoudakis et al. (2013) tested eight acoustic indices and found that the centroid index (CENT) performed best at distinguishing soundscapes of six habitat types in Greece, whereas Bradfer-Lawrence et al. (2019) tested seven acoustic indices across six habitats in Panama and found the acoustic complexity index (ACI) performed best. However, differences in sample sizes, acoustic indices used, and study regions limit the comparative and application value of these results in different ecosystems (Table 2.1).

A second area of research in ecoacoustics that requires further clarification is how vegetation structure influences acoustic indices (Farina and Pieretti, 2014; Farina and Gage, 2017; Pijanowski et al., 2011b). It is expected that habitats with greater vegetation structural complexity have higher species diversity leading to greater acoustic diversity (Farina and Pieretti, 2014; Fuller et al., 2015; Pijanowski et al., 2011b). Despite the centrality of this assumption, it has received limited empirical validation, likely due to the time-consuming task of collecting both vegetation structure and soundscape data (Table 2.1). This knowledge gap hinders our ability to build predictive models linking

changes in vegetation structure to acoustic diversity (Farina and Pieretti, 2014; Farina and Gage, 2017; Pijanowski et al., 2011b). In the few studies conducted on this topic, a relationship between vegetation structure and four acoustic indices [acoustic diversity index (ADI), acoustic evenness index (AEI), normalized difference soundscape index (NDSI), and total entropy (H)] was found across five habitats in Australia (Fuller et al., 2015; Ng et al., 2018), and between canopy cover and the bioacoustic index (BIO) in two habitats in Madagascar (Rankin and Axel, 2017). However, Tucker et al. (2013) suggested that landscape variables (e.g., patch size) were more important than vegetation structure in driving differences in one acoustic index (relative soundscape power; RSP) in Australia. Thus, it remains largely unknown if relationships between vegetation and soundscapes are a widespread phenomenon and which indices best connect vegetation structure to soundscapes.

Here, we investigate habitat-specific soundscapes and the relationships between vegetation structure and soundscapes in the Brazilian Amazon. We collected vegetation data and recorded soundscapes at 143 sites across eight habitat types (natural and anthropogenic) representing the majority of habitat types found in the Amazon. We used a total of 13 acoustic indices to describe the soundscapes. Our goals were to test if acoustic indices can predict habitat type, and to test how vegetation structure relates to acoustic indices. We then discuss the implications of our findings for biodiversity assessments.

2. METHODS

2.1. Study sites

We conducted this research in and around Viruá National Park (VNP), Roraima, Brazil, in the north of the Brazilian Amazon (Fig. S2.1). VNP was established in 1998 and is 240,000 ha (ICMBio, 2014). The climate in VNP is warm and wet with mean annual temperature of 26 °C and mean annual precipitation of ~2,000 mm (ICMBio, 2014). Rainfall is mostly concentrated from May to September (ICMBio, 2014). VNP is regulated by floods that create a vegetation mosaic ranging from dense forests to grasslands, and representing most major habitats found across the Amazon biome (ICMBio, 2014). These habitats share common species, but also harbor unique fauna and flora (Table S2.1). Based on vegetation characteristics and flooding regime, the eight habitats surveyed can be grouped as open habitats (burned campina, campina, and pastures), flooded forests (igapó, islands, and várzea), and non-flooded forests (campinarana and terra-firme). In summary, open habitats have lower species richness than forested habitats, and campiranana, igapó, and island forests are not as diverse as terra-firme and várzea forests (see Table S2.2 for additional information on habitats and Figs. S2.2 to S2.9 for photos).

2.2. Passive acoustic monitoring and index extraction

We used ARBIMON acoustic recorders (Campos-Cerqueira & Aide, 2016) to collect acoustic data from November 2016 to February 2017 in the eight habitat types. We deployed recorders at 20 replicate sites in each habitat, with the exception of pastures, which were limited to six replicates because they comprised a small area in the

VNP; and terra-firme, which had 17 replicates because of recorder malfunctions. This resulted in a total of 143 sites surveyed. Recorders were spaced over 500 m apart to minimize overlap in detections across recorders. Previous field tests conducted with ARBIMON recorders indicate that calls of the majority of bird and frog species can be detected up to ~100 m (Campos-Cerqueira et al., 2019). We attached recorders to trees or fixed poles at the height of 1.5 m. Acoustic devices were programmed to record 1 min of audio every 10 min for six days in each sampling site (sample rate = 44.1 kHz; resolution = 16 bit; format = WAV). After six days, the 20 recorders were moved to a different habitat type and the method repeated (see Table S2.2 for sampling periods).

Acoustic data collection resulted in 96,726 one-minute recordings (1,612 hours). For each one-minute recording, we calculated 13 acoustic indices to summarize the soundscapes of the eight habitats studied (Table 2.2). Two broad types of indices were used: indices that rely on statistical features of recordings (as described in Bormpoudakis et al., 2013; Mitrović et al., 2010); and signal complexity indices specifically developed for biodiversity assessments and landscape investigation (Sueur et al., 2014). We selected indices that were previously used to describe habitats in peer-reviewed publications (Bormpoudakis et al., 2013; Bradfer-Lawrence et al., 2019) and that could be calculated through open-source software.

Calculations were performed in the R Environment (R Core Team, 2019). The function “specprop” from Seewave package (Sueur et al., 2008b) was used to calculate the centroid (CENT), dominant frequency (DF), first quartile (FQ), kurtosis (KURT), skewness (SKEW), spectral flatness (FLAT), standard deviation (SD), and the third quartile (TQ) with default parameters of the package. The Soundecology package

(Villanueva-Rivera & Pijanowski, 2018) was used to calculate the acoustic complexity index (ACI; Pieretti et al., 2011), acoustic evenness index (AEI; Villanueva-Rivera et al., 2011), bioacoustic index (BIO; Boelman et al., 2007), total entropy (H; Sueur et al., 2008a), and the normalized difference soundscape index (NDSI; Kasten et al., 2012). Minimum frequency for ACI calculation was set to 500 Hz and maximum frequency to 12 kHz because the package did not have default values for this index, and this range encompasses most of birds', amphibians', and non-flying-mammals' sounds while also reducing possible microphone self-noise interference (Bradfer-Lawrence et al., 2019). All other parameters used in the indices' calculations were set to default values provided in the package.

We inspected index calculations for outliers that could be linked to file corruption, rain, or wind, and removed recordings containing these anomalies because they affected indices values disproportionately as observed in other studies (Bradfer-Lawrence et al. 2019; Depraetere et al., 2012; Pieretti et al., 2015). This removal of 4,443 files resulted in 92,283 one-minute recordings (1,538 hours) for subsequent analysis. Our sites lacked significant anthropogenic sounds due their remoteness, but studies in more urbanized locations should inspect recordings for this source of sound because they may also affect indices values disproportionately (Fairbrass et al., 2017).

2.3. Vegetation structure survey

Vegetation structure data were collected within a 20-m radius plot from each acoustic recorder location (143 sites) after the devices were moved to a different habitat type to avoid interference with the recordings (similar to Rankin and Axel, 2017). We

took two measurements of percent canopy cover facing north and then south with a densiometer at two points (5 m and 10 m from recorder's original location) in each cardinal direction for a total of eight locations and 16 measurements per plot. We measured canopy height by visually estimating the height of the two largest trees in each plot. Two field assistants along one of the researchers took independent measurements of tree height to reduce possible bias in the field. We took two measurements (spaced 1 m apart) of litter depth at two points (5 m and 10 m from recorder original location) in each cardinal direction for a total of eight locations and 16 measurements per site. We measured diameter at breast height (DBH) of trees in four subplots 4 m from the recorder location and stretching for 10 m in length and 8 m wide in each cardinal direction. We counted all trees with $DBH > 1$ cm and divided them in small ($DBH > 1$ cm to < 10 cm) and large ($DBH > 10$ cm) classes for subsequent analysis. Finally, we used a 20-m tape to take two measurements of shrub cover per site (along north and south directions from the recorder location) using the line-intercept method (Floyd & Anderson, 1987). For each vegetation structure variable, we used the mean value per site for subsequent analysis. Similar methods have been used in other studies to determine vegetation structure (Hill et al., 2019; Rankin and Axel, 2017; Smith et al., 2018).

2.4. Statistics

All statistical tests and model diagnostics were run in the R environment (R Core Team, 2019). To test for the existence of habitat-specific soundscapes, we used a random forest (RF) modeling approach (Cutler et al., 2007) with the 13 acoustic indices calculated from the recordings. We used RF because this approach allowed us to

summarize the importance of individual indices in the classification (as in Bormpoudakis et al., 2013; Bradfer-Lawrence et al., 2019). We built two RF models, one classifying soundscapes of the eight different habitats and another classifying soundscapes of the three broader habitat types (open habitats, flooded forests, and non-flooded forests). In the first RF model, we separated the data into training (80%) and testing (20%) datasets. With the training dataset and the R package `RandomForestSRC` (Ishwaran et al., 2008), we built a random forest classifier with default values. We used the 13 acoustic indices from each one-minute recording to build the classifier to tentatively assign each one-minute recording to one of the eight habitats. We then used the “predict” function in the `Caret` package (Kuhn, 2008) to measure the accuracy of our model to predict the testing dataset. In the second RF model, we used the same approach as the first RF model; the only difference was that recordings were assigned to the three broader habitats instead of the eight finer-scale habitat types.

After visualizing the soundscapes and noticing distinct diel patterns among the different habitats for each index (Fig. 2.1), we decided to separate day and night data to better understand the RF outputs. We averaged each one-minute recording made in the same time across all replicates within a habitat and assigned each recording to day (0600-1800 h) or night (1800-0600 h). We ran a permutational multivariate analysis of variance (PERMANOVA) with the `Vegan` package (Oksanen et al., 2019) to test if diurnal soundscapes were different from nocturnal soundscapes across habitats.

To determine whether acoustic indices relate to vegetation structure, we first calculated the mean value of each acoustic index per site (143 total), averaged across the six days of data collection (as in Fuller et al., 2015; Ng et al., 2018). Using the package

nlme (Pinheiro et al., 2019), we built linear mixed models (LMMs) with each of the 13 acoustic indices as dependent variables, six vegetation structure variables (canopy cover, canopy height, litter depth, number of large trees, number of small trees, and shrub cover) as independent fixed effects, and habitat type as a random effect. Independent fixed effects were scaled (“scale” function in base R) to make their parameter estimates comparable within models. We performed model selection using the corrected Akaike information criteria (AICc; Burnham and Anderson, 2004). We selected the top four performing models based on ΔAICc and considered models to be similar if $\Delta\text{AICc} < 2$ (Burnham and Anderson, 2004). With the package car (Fox and Weisberg, 2019), we checked for multicollinearity of predictors and removed canopy height from the analysis. Residuals of the models were checked for linearity, homoscedasticity, independence, and normality with the package SjPlot (Lüdecke, 2020). We consider a fixed effect to be significant at an alpha level of < 0.05 . With the package R2glmm (Jaeger, 2017), we calculated the marginal and conditional R^2 values (Nakagawa & Schielzeth, 2013) to estimate the proportion of variance explained by fixed and random effects.

3. RESULTS

3.1. Habitat-specific soundscape patterns

The first RF classifier separated all 13 acoustic indices in the training dataset into the eight habitat classes. Internal error of the classifier was 26% and the model accuracy when predicting on the testing dataset was 74%. The habitat with the lowest internal error was igapó (18%), and the habitat with the highest internal error was the pasture (55%). Soundscapes from pastures were usually misclassified as the other two

open habitats (Table 2.3). The most important acoustic indices to distinguish habitat types were TQ and CENT; if these variables were removed, the accuracy of the model would proportionally drop 0.15 and 0.14, respectively. SD, NDSI, ACI, KURT, and SKEW also performed well in the classification. The least important acoustic indices were DF and FLAT (Fig. 2.2).

The second RF classifier built to distinguish the soundscapes of three broader habitat types performed better than the first one. It had a lower internal error, 13%, than the first classifier and accuracy of the model to predict habitat types within the testing dataset increased to 87% (Table 2.3). TQ and CENT were again the most important acoustic indices, reflecting a proportional drop of 0.11 in the classification accuracy if either was removed. The least important indices were ACI and BIO (Fig. 2.2).

The PERMANOVA revealed that diurnal and nocturnal soundscapes were different across all habitats ($F_{1,3447} = 2493.7$, $p < 0.001$; Table S2.3) and supported the RF classification because it showed a significant effect of habitat type on acoustic indices ($F_{7,3447} = 421.6$, $p < 0.001$). FLAT, H, SD, and TQ values were lower during the day and higher at night for open habitats, while forested habitats had the opposite pattern. BIO, DF, FQ, and NDSI were the only indices with consistent diel patterns across habitats; they were lower during the day and higher at night. Diel patterns of ACI, AEI, CENT, KURT, and SKEW were more marked for open than forested habitats; values in open habitats were higher during day than night, except for CENT that had the opposite pattern (Fig. 2.1).

3.2. Relationships between vegetation structure and soundscapes

Across the top performing models (Table S2.4), 11 of 13 acoustic indices were significantly associated with percent canopy cover (Fig. 2.3). We found a positive relationship between canopy cover and BIO, CENT, DF, FQ, H, NDSI, and TQ, and a negative relationship between canopy cover and ACI, AEI, KURT, and SKEW (Fig. 2.3). Other vegetation variables appeared in some top performing models, but their effect was not significant (Fig. 2.3). The only exception was the significant negative association of ACI with the number of large trees, but this effect was smaller than canopy cover (Fig. 2.3). A null model appeared between the two top performing models for the index FLAT, therefore we did not consider this index to be significantly related with vegetation structure (Table S2.4). Conditional R² of models with significant vegetation effects ranged from 19% to 81% while marginal R² of canopy cover ranged from 5% to 81% (Table S2.5).

4. DISCUSSION

Determining the ability of soundscapes to discriminate habitat types and the response of acoustic indices to changes in vegetation structure is critical for improving ecological monitoring using ecoacoustic methods. In the present study, we found that eight habitat types in the Amazon biome have unique and predictable soundscapes. We found that, in general, acoustic indices that rely on statistical features of recordings (Bormpoudakis et al., 2013; Mitrović et al., 2010) were better at identifying habitat-specific soundscapes than acoustic indices based on signal complexity (Sueur et al., 2014). We also found that canopy cover was the primary vegetation variable explaining

variance in acoustic indices. These results expand our knowledge regarding which acoustic indices best link changes in habitats to changes in soundscapes. These findings are particularly important for diverse ecosystems, like the Amazon, which are known to have complex soundscapes with sound producing animals that are difficult to detect with traditional survey methods (e.g., visual transects).

4.1. Habitat-specific soundscape patterns

We evaluated the ability of 13 acoustic indices to distinguish soundscapes of eight habitat types in the Amazon. In our study, TQ and CENT were the best indices at distinguishing habitat-specific soundscapes (Fig. 2.2), similar to results reported from Greece (Bormpoudakis et al., 2013). In both our first classification of eight habitat types and second classification of three habitat groups, the top indices for variable importance were statistical in nature and the majority of the indices based on signal complexity were in the bottom half of variables. Acoustic indices that rely on statistical features, like the TQ and CENT, indicate at which frequency the majority of species are producing sounds, while signal complexity indices, like the AEI and H, measure overall acoustic diversity over a pre-defined range (e.g., 0 – 1). While one type of index performed better than the other, they all contributed to the predictive power of the RF classifications, and because they reflect different aspects of soundscapes (Table 2.2), they can facilitate the interpretation of patterns when analyzed together (Bradfer-Lawrence et al., 2019; Bradfer-Lawrence et al., 2020).

One of the main soundscape features that separated habitat types was the diel patterns of the indices (Fig. 2.1). Based on our field observations and listening to the

original recordings, open habitats have insect activity at nighttime but during the day were mostly devoid of animal sounds. In contrast, the soundscapes of forested habitats have a lot of animal sounds over 24-h periods, leading to the subtler differences between day and night. In addition, each of the habitats is known to have a unique composition of bird species which can further help explain the differences in soundscapes observed among the habitats (Laranjeiras et al., 2014). To the best of our knowledge, indices based on statistical features of recordings (DF, FQ, KURT, FLAT, SD, SKEW, and TQ) have not had their diel patterns described (with the exception of CENT; Eldridge et al., 2018), but they all showed differences between nocturnal and diurnal soundscapes in our study. This feature likely increased the ability of these indices to identify habitat-specific soundscapes, and could make these indices useful in identifying habitats in other regions. For the signal complexity indices that have had their diel patterns previously described (ACI, AEI, BIO, H, and NDSI), it seems that differences between the day and night are dependent on the region, habitat type, and components of soundscapes due to inconsistent reports in the literature (Bradfer-Lawrence et al., 2019; Fuller et al., 2015; Gage et al., 2017; Ng et al., 2018; Pieretti et al., 2015).

4.2. Vegetation structure and acoustic indices

In general, we found that the amount of tree cover, represented by percent canopy cover and large trees, were the most important variables explaining soundscapes. For some acoustic indices, such as AEI and SKEW, canopy cover substantially explained their variances (81% and 52%, respectively), but for other indices, such as DF, even though there was a significant relationship with canopy cover, only a small percent of the

variance was explained (5%). Besides canopy cover, the other vegetation variables we measured did not appear important in explaining acoustic indices despite their appearance in some top performing models (Fig. 2.3).

Soundscapes rich in frequencies were linked to high canopy cover, while soundscapes poor in frequencies were linked to low canopy cover. This is similar to the effects of canopy cover on species richness across different animal taxa (reviewed in Stein et al., 2014). In our study sites, habitats with greater layer complexity (forested habitats) have greater avian and insect richness than less complex habitats (open habitats) (Table S2.1). These differences in species richness can help explain the soundscape patterns observed, especially if we consider that insects are a major driver of acoustic diversity in the tropical region (Aide et al., 2017). The direction of the relationship between canopy cover and acoustic indices was positive for seven indices and negative for four indices (Fig. 2.3). Two indices, FLAT and SD, were not significant related to any vegetative structure variable (Table S5). The four indices with negative relationships reflect the way the indices are calculated and not lower acoustic diversity in forested sites. For example, AEI is expected to have lower values in sites with rich soundscapes (i.e., forested) because sound intensity does not vary greatly between frequency bands in such sites (Bradfer-Lawrence et al., 2019).

Our findings partially agree with past research on this topic (Table 2.1). For example, two independent studies in Australia found that AEI, H, and NDSI were associated with vegetation structure (Fuller et al., 2015; Ng et al., 2018), but unlike our study, they found no relationship with ACI or BIO. But, in Madagascar, it was found that the BIO was associated with vegetation structure (Rankin and Axel 2017). Also,

vegetation structure seems to be an important predictor of ACI in Greece, Italy, and Panama, similar to our study (Bradfer-Lawrence et al., 2020; Farina and Pieretti 2014; Myers et al., 2019). Our results expand the number of acoustic indices linking vegetation to soundscapes and ultimately contribute to the body of research suggesting that relationships between vegetation and soundscapes may be a widespread phenomenon across regions and ecosystems. These results further indicate that vegetation structural characteristics (especially canopy cover) may be used with acoustic indices to predict changes in habitats across large spatial scales (see Pekin et al., 2012, for a first spatial forecast attempt).

4.3. Limitations

One limitation of this study was that we could not sample all habitats simultaneously due to logistical constraints. However, we do not think this significantly biased our data because we collected data within a short period of time (~2 months) (Table S2.2), in similar weather conditions (dry season), and with many replications in each habitat type that showed minimal variability. In addition, by performing the RF classification that grouped the eight habitats into broader classes, this temporal constraint was addressed because habitats were sampled randomly (Table S2.2). Similarly, by using habitat type as a random effect in the LMMs this issue is partially addressed. Another limitation is that we did not identify individual species in the recordings, which limits our interpretation of specific soundscape components. However, this was beyond the scope of this research.

5. IMPLICATIONS FOR BIODIVERSITY ASSESSMENTS

Acoustic indices have been proposed as proxies to monitor biodiversity and environmental change (Buxton et al., 2018; Krause and Farina, 2016; Sueur et al., 2014). Our findings suggest that scientists and practitioners can differentiate and predict soundscapes of different habitats by using acoustic indices. In particular, our study highlights that acoustic indices (especially TQ and CENT) are able to classify habitats, even among those that are structurally similar or share similar fauna (Table S2.2). For example, soundscapes of grasslands burned nine months prior to data collection were different than those of intact grasslands (campina), suggesting that acoustic indices can be used to track the impacts of wildfire, an increasing threat to tropical ecosystems (Staver et al., 2020). Similarly, soundscapes of várzea forests were different than islands forests, and because islands have species in jeopardy due plans of dam construction (Naka et al., 2020), acoustic indices could serve as a cost-effective way to monitor such species.

A second important implication of our findings for biodiversity assessments is the ability to build predictive models linking fine-scale changes in vegetation structure to acoustic diversity. While TQ and CENT indices worked best to differentiate habitats, the acoustic indices that were best linked to changes in vegetation structure (canopy cover) were AEI and SKEW. The reason that some indices might be better at some tasks than others should be explored in future studies. AEI and SKEW could be used together with vegetation remote sensing tools, such as LiDAR, to predict how habitat degradation (e.g., canopy loss due to deforestation) affects animal diversity. This synergetic approach between two scalable remote sensing methods, ecoacoustic and airborne surveys, may

offer an alternative for multi-taxa animal surveys at policy-relevant extents (Bush et al., 2017; Pekin et al., 2012; Rappaport et al., 2020).

REFERENCES

- Aide, T. M., Hernández-Serna, A., Campos-Cerqueira, M., Acevedo-Charry, O., & Deichmann, J. L. (2017). Species richness (of insects) drives the use of acoustic space in the tropics. *Remote Sensing*, 9(11), 1096.
<https://doi.org/10.3390/rs9111096>
- Bormpoudakis, D., Sueur, J., & Pantis, J. D. (2013). Spatial heterogeneity of ambient sound at the habitat type level: ecological implications and applications. *Landscape Ecology*, 28(3), 495-506. <https://doi.org/10.1007/s10980-013-9849-1>
- Bradfer-Lawrence, T., Gardner, N., Bunnefeld, L., Bunnefeld, N., Willis, S. G., & Dent, D. H. (2019). Guidelines for the use of acoustic indices in environmental research. *Methods in Ecology and Evolution*, 10(10), 1796-1807.
<https://doi.org/10.1111/2041-210X.13254>
- Bradfer-Lawrence, T., Bunnefeld, N., Gardner, N., Willis, S. G., & Dent, D. H. (2020). Rapid assessment of avian species richness and abundance using acoustic indices. *Ecological Indicators*, 115, 106400.
<https://doi.org/10.1016/j.ecolind.2020.106400>
- Burivalova, Z., Wahyudi, B., Boucher, T. M., Ellis, P., Truskinger, A., Towsey, M., Roe, P., Marthinus, D., Griscom, B., & Game, E. T. (2019). Using soundscapes to investigate homogenization of tropical forest diversity in selectively logged

forests. *Journal of Applied Ecology*, 56(11), 2493-2504.

<https://doi.org/10.1111/1365-2664.13481>

Burnham, K. P., & Anderson, D. R. (2004). Multimodel inference: understanding AIC and BIC in model selection. *Sociological methods & research*, 33(2), 261-304.

<https://doi.org/10.1177/0049124104268644>

Bush, A., Sollmann, R., Wilting, A., Bohmann, K., Cole, B., Balzter, H., ... & Dawson, T. P. (2017). Connecting Earth observation to high-throughput biodiversity data.

Nature ecology & evolution, 1(7), 0176. <https://doi.org/10.1038/s41559-017-0176>

Buxton, R. T., McKenna, M. F., Clapp, M., Meyer, E., Stabenau, E., Angeloni, L. M., ...

& Wittemyer, G. (2018). Efficacy of extracting indices from large-scale acoustic recordings to monitor biodiversity. *Conservation Biology*, 32(5), 1174-1184. <https://doi.org/10.1111/cobi.13119>

Campos-Cerqueira, M., & Aide, T. M. (2016). Improving distribution data of threatened species by combining acoustic monitoring and occupancy modelling. *Methods in Ecology and Evolution*, 7(11), 1340-1348. <https://doi.org/10.1111/2041-210X.12599>

Campos-Cerqueira, M., Mena, J. L., Tejeda-Gómez, V., Aguilar-Amuchastegui, N.,

Gutierrez, N., & Aide, T. M. (2019). How does FSC forest certification affect the acoustically active fauna in Madre de Dios, Peru?. *Remote Sensing in Ecology and Conservation*. <https://doi.org/10.1002/rse2.120>

Colonna, J. G., Carvalho, J. R. H., & Rosso, O. A. (2020). The Amazon rainforest

soundscape characterized through Information Theory quantifiers. *bioRxiv*.

<https://doi.org/10.1101/2020.02.09.940916>

Cutler, D. R., Edwards Jr, T. C., Beard, K. H., Cutler, A., Hess, K. T., Gibson, J., & Lawler, J. J. (2007). Random forests for classification in ecology. *Ecology*, 88(11), 2783-2792. <https://doi.org/10.1890/07-0539.1>

Depraetere, M., Pavoine, S., Jiguet, F., Gasc, A., Duvail, S., & Sueur, J. (2012). Monitoring animal diversity using acoustic indices: implementation in a temperate woodland. *Ecological Indicators*, 13(1), 46-54. <https://doi.org/10.1016/j.ecolind.2011.05.006>

Duarte, M. H. L., Sousa-Lima, R. S., Young, R. J., Farina, A., Vasconcelos, M., Rodrigues, M., & Pieretti, N. (2015). The impact of noise from open-cast mining on Atlantic forest biophony. *Biological Conservation*, 191, 623-631. <https://doi.org/10.1016/j.biocon.2015.08.006>

Eldridge, A., Guyot, P., Moscoso, P., Johnston, A., Eyre-Walker, Y., & Peck, M. (2018). Sounding out ecoacoustic metrics: Avian species richness is predicted by acoustic indices in temperate but not tropical habitats. *Ecological Indicators*, 95, 939-952. <https://doi.org/10.1016/j.ecolind.2018.06.012>

Fairbrass, A. J., Rennert, P., Williams, C., Titheridge, H., & Jones, K. E. (2017). Biases of acoustic indices measuring biodiversity in urban areas. *Ecological Indicators*, 83, 169-177. <https://doi.org/10.1016/j.ecolind.2017.07.064>

Farina, A., & Pieretti, N. (2014). Sonic environment and vegetation structure: a methodological approach for a soundscape analysis of a Mediterranean maqui. *Ecological Informatics*, 21, 120-132. <https://doi.org/10.1016/j.ecoinf.2013.10.008>

- Farina, A., & Gage, S. H. (Eds.). (2017). *Ecoacoustics: The ecological role of sounds*. Wiley. <https://doi.org/10.1002/9781119230724>
- Floyd, D. A., & Anderson, J. E. (1987). A comparison of three methods for estimating plant cover. *The Journal of Ecology*, 75, 221-228.
<https://doi.org/10.2307/2260547>
- Fox, J., & Weisberg, S. (2019). *An R companion to applied regression*. Sage.
- Fuller, S., Axel, A. C., Tucker, D., & Gage, S. H. (2015). Connecting soundscape to landscape: Which acoustic index best describes landscape configuration?. *Ecological Indicators*, 58, 207-215.
<https://doi.org/10.1016/j.ecolind.2015.05.057>
- Gage, S. H., Napoletano, B. M., & Cooper, M. C. (2001). Assessment of ecosystem biodiversity by acoustic diversity indices. *The Journal of the Acoustical Society of America*, 109(5), 2430-2430. <https://doi.org/10.1121/1.4744597>
- Gage, S. H., Wimmer, J., Tarrant, T., & Grace, P. (2017). Acoustic patterns at the Samford Ecological Research Facility in South East Queensland, Australia: The Peri-Urban SuperSite of the Terrestrial Ecosystem Research Network. *Ecological Informatics*, 38, 62-75. <https://doi.org/10.1016/j.ecoinf.2017.01.002>
- Gasc, A., Pavoine, S., Lellouch, L., Grandcolas, P., & Sueur, J. (2015). Acoustic indices for biodiversity assessments: Analyses of bias based on simulated bird assemblages and recommendations for field surveys. *Biological Conservation*, 191, 306-312. <https://doi.org/10.1016/j.biocon.2015.06.018>
- Gasc, A., Gottesman, B. L., Francomano, D., Jung, J., Durham, M., Mateljak, J., & Pijanowski, B. C. (2018). Soundscapes reveal disturbance impacts: Biophonic

response to wildfire in the Sonoran Desert Sky Islands. *Landscape Ecology*, 33(8), 1399-1415. <https://doi.org/10.1007/s10980-018-0675-3>

Gibb, R., Browning, E., Glover-Kapfer, P., & Jones, K. E. (2019). Emerging opportunities and challenges for passive acoustics in ecological assessment and monitoring. *Methods in Ecology and Evolution*, 10(2), 169-185. <https://doi.org/10.1111/2041-210X.13101>

Gómez, W. E., Isaza, C. V., & Daza, J. M. (2018). Identifying disturbed habitats: A new method from acoustic indices. *Ecological informatics*, 45, 16-25. <https://doi.org/10.1016/j.ecoinf.2018.03.001>

Harris, S. A., Shears, N. T., & Radford, C. A. (2016). Ecoacoustic indices as proxies for biodiversity on temperate reefs. *Methods in Ecology and Evolution*, 7(6), 713-724. <https://doi.org/10.1111/2041-210X.12527>

Hill, S. A., Beard, K. H., Siers, S. R., & Shiels, A. B. (2019). Invasive coqui frogs are associated with differences in mongoose and rat abundances and diets in Hawaii. *Biological Invasions*, 21(6), 2177-2190. <https://doi.org/10.1007/s10530-019-01965-3>

ICMBio. Instituto Chico Mendes de Conservação da Biodiversidade (2014). Plano de Manejo do Parque Nacional do Viruá. ICMBio, Boa Vista, Roraima.

Ishwaran, H., Kogalur, U. B., Blackstone, E. H., & Lauer, M. S. (2008). Random survival forests. *The annals of applied statistics*, 2(3), 841-860. <https://doi.org/10.1214/08-AOAS169>

Jaeger, B. (2017). R2glmm: computes R squared for mixed (multilevel) models. R package version, 0.1.2.

- Kasten, E. P., Gage, S. H., Fox, J., & Joo, W. (2012). The remote environmental assessment laboratory's acoustic library: An archive for studying soundscape ecology. *Ecological Informatics*, 12, 50-67.
- Krause, B., & Farina, A. (2016). Using ecoacoustic methods to survey the impacts of climate change on biodiversity. *Biological conservation*, 195, 245-254.
<https://doi.org/10.1016/j.biocon.2016.01.013>
- Kuhn, M. (2008). Building predictive models in R using the caret package. *Journal of statistical software*, 28(5), 1-26. <https://doi.org/10.18637/jss.v028.i05>
- Laranjeiras, T. O., Naka, L. N., Bechtoldt, C. L., da Costa, T. V. V., Andretti, C. B., Cerqueira, M. C., ... & Pacheco, A. M. F. (2014). The avifauna of Virua National Park, Roraima, reveals megadiversity in northern Amazonia. *Ornithology Research*, 22(2), 138-171. <https://doi.org/10.1007/BF03544242>
- Lüdecke, D. (2020). sjPlot: Data visualization for statistics in social science. R package version, 2.8.3.
- Mitrović, D., Zeppelzauer, M., & Breiteneder, C. (2010). Features for content-based audio retrieval. In M. V. Zelkowitz (Ed.), *Advances in computers* (Vol. 78, pp. 71-150). Elsevier. [https://doi.org/10.1016/S0065-2458\(10\)78003-7](https://doi.org/10.1016/S0065-2458(10)78003-7)
- Moreno-Gómez, F. N., Bartheld, J., Silva-Escobar, A. A., Briones, R., Márquez, R., & Penna, M. (2019). Evaluating acoustic indices in the Valdivian rainforest, a biodiversity hotspot in South America. *Ecological Indicators*, 103, 1-8.
<https://doi.org/10.1016/j.ecolind.2019.03.024>
- Myers, D., Berg, H., & Maneas, G. (2019). Comparing the soundscapes of organic and conventional olive groves: A potential method for bird diversity monitoring.

Ecological indicators, 103, 642-649.

<https://doi.org/10.1016/j.ecolind.2019.04.030>

- Naka, L. N., Laranjeiras, T. O., Lima, G. R., Plaskiewicz, A. C., Mariz, D., Da Costa, B. M., ... & Cohn-Haft, M. (2020). The Avifauna of the Rio Branco, an Amazonian evolutionary and ecological hotspot in peril. *Bird Conservation International*, 30(1), 21-39. <https://doi.org/10.1017/S0959270919000133>
- Nakagawa, S., & Schielzeth, H. (2013). A general and simple method for obtaining R² from generalized linear mixed-effects models. *Methods in ecology and evolution*, 4(2), 133-142. <https://doi.org/10.1111/j.2041-210x.2012.00261.x>
- Ng, M. L., Butler, N., & Woods, N. (2018). Soundscapes as a surrogate measure of vegetation condition for biodiversity values: A pilot study. *Ecological Indicators*, 93, 1070-1080. <https://doi.org/10.1016/j.ecolind.2018.06.003>
- Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., O'hara, R. B., Simpson, G. L., ... & Wagner, H. (2019). *Vegan: community ecology package*. R package version, 2.5-6.
- Pekin, B. K., Jung, J., Villanueva-Rivera, L. J., Pijanowski, B. C., & Ahumada, J. A. (2012). Modeling acoustic diversity using soundscape recordings and LIDAR-derived metrics of vertical forest structure in a neotropical rainforest. *Landscape ecology*, 27(10), 1513-1522. <https://doi.org/10.1007/s10980-012-9806-4>
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & Team, R. C. (2019). *nlme: Linear and nonlinear mixed effects models*. R package version, 3.1-141.
- Pieretti, N., Farina, A., & Morri, D. (2011). A new methodology to infer the singing activity of an avian community: The Acoustic Complexity Index (ACI).

Ecological Indicators, 11(3), 868-873.

<https://doi.org/10.1016/j.ecolind.2010.11.005>

Pieretti, N., Duarte, M. H. L., Sousa-Lima, R. S., Rodrigues, M., Young, R. J., & Farina, A. (2015). Determining temporal sampling schemes for passive acoustic studies in different tropical ecosystems. *Tropical Conservation Science*, 8(1), 215-234.

<https://doi.org/10.1177/194008291500800117>

Pijanowski, B. C., Villanueva-Rivera, L. J., Dumyahn, S. L., Farina, A., Krause, B. L., Napoletano, B. M., ... & Pieretti, N. (2011a). Soundscape ecology: the science of sound in the landscape. *BioScience*, 61(3), 203-216.

<https://doi.org/10.1525/bio.2011.61.3.6>

Pijanowski, B. C., Farina, A., Gage, S. H., Dumyahn, S. L., & Krause, B. L. (2011b). What is soundscape ecology? An introduction and overview of an emerging new science. *Landscape ecology*, 26(9), 1213-1232.

<https://doi.org/10.1007/s10980-011-9600-8>

Rankin, L., & Axel, A. C. (2017). Biodiversity assessment in tropical biomes using ecoacoustics: linking soundscape to forest structure in a human-dominated tropical dry forest in southern Madagascar. In A. Farina, & S. H. Gage (Eds.), *Ecoacoustics: the ecological role of sounds* (pp. 129-144). Wiley.

<https://doi.org/10.1002/9781119230724.ch8>

Rappaport, D. I., Royle, J. A., & Morton, D. C. (2020). Acoustic space occupancy: Combining ecoacoustics and lidar to model biodiversity variation and detection bias across heterogeneous landscapes. *Ecological Indicators*, 113, 106172.

<https://doi.org/10.1016/j.ecolind.2020.106172>

- R Core Team (2019). A language and environment for statistical computing. Vienna, Austria. R Foundation for Statistical Computing.
- Santiago, J., Uranga, J., Quincoces, I., Orue, B., Grande, M., Murua, H., Merino, G., Urtizberea, A., Pascual, P., & Boyra, G. (2020). A novel index of abundance of juvenile yellowfin tuna in the Atlantic Ocean derived from echosounder buoys. *Collect. Vol. Sci. Pap. ICCAT*, 76(6), 321-343.
- Scarpelli, M. D., Ribeiro, M. C., Teixeira, F. Z., Young, R. J., & Teixeira, C. P. (2020). Gaps in terrestrial soundscape research: it's time to focus on tropical wildlife. *Science of The Total Environment*, 707, 135403.
<https://doi.org/10.1016/j.scitotenv.2019.135403>
- Smith, R. L., Beard, K. H., & Koons, D. N. (2018). Invasive coqui frogs are associated with greater abundances of nonnative birds in Hawaii, USA. *The Condor: Ornithological Applications*, 120(1), 16-29. <https://doi.org/10.1650/CONDOR-17-109.1>
- Staver, A. C., Brando, P. M., Barlow, J., Morton, D. C., Paine, C. T., Malhi, Y., ... & del Aguila Pasquel, J. (2020). Thinner bark increases sensitivity of wetter Amazonian tropical forests to fire. *Ecology letters*, 23(1), 99-106.
<https://doi.org/10.1111/ele.13409>
- Stein, A., Gerstner, K., & Kreft, H. (2014). Environmental heterogeneity as a universal driver of species richness across taxa, biomes and spatial scales. *Ecology letters*, 17(7), 866-880. <https://doi.org/10.1111/ele.12277>
- Sueur, J., Pavoine, S., Hamerlynck, O., & Duvail, S. (2008a). Rapid acoustic survey for biodiversity appraisal. *PloS one*, 3(12).

<https://doi.org/10.1371/journal.pone.0004065>

Sueur, J., Aubin, T., & Simonis, C. (2008b). Seewave, a free modular tool for sound analysis and synthesis. *Bioacoustics*, 18(2), 213-226.

<https://doi.org/10.1080/09524622.2008.9753600>

Sueur, J., Farina, A., Gasc, A., Pieretti, N., & Pavoine, S. (2014). Acoustic indices for biodiversity assessment and landscape investigation. *Acta Acustica united with Acustica*, 100(4), 772-781. <https://doi.org/10.3813/AAA.918757>

Tucker, D., Gage, S. H., Williamson, I., & Fuller, S. (2014). Linking ecological condition and the soundscape in fragmented Australian forests. *Landscape Ecology*, 29(4), 745-758. <https://doi.org/10.1007/s10980-014-0015-1>

Tuneu-Corral, C., Puig-Montserrat, X., Flaquer, C., Mas, M., Budinski, I., & López-Baucells, A. (2020). Ecological indices in long-term acoustic bat surveys for assessing and monitoring bats' responses to climatic and land-cover changes. *Ecological Indicators*, 110, 105849.

<https://doi.org/10.1016/j.ecolind.2019.105849>

Villanueva-Rivera, L. J., Pijanowski, B. C., Doucette, J., & Pekin, B. (2011). A primer of acoustic analysis for landscape ecologists. *Landscape ecology*, 26(9), 1233.

<https://doi.org/10.1007/s10980-011-9636-9>

Villanueva-Rivera, L. J., & Pijanowski, B. C. (2018). Soundecology: soundscape ecology. R package version, 1.3.3.

Zhao, Z., Xu, Z. Y., Bellisario, K., Zeng, R. W., Li, N., Zhou, W. Y., & Pijanowski, B. C. (2019). How well do acoustic indices measure biodiversity? Computational experiments to determine effect of sound unit shape, vocalization intensity, and

frequency of vocalization occurrence on performance of acoustic indices.

Ecological Indicators, 107, 105588.

<https://doi.org/10.1016/j.ecolind.2019.105588>

TABLES AND FIGURES

Table 2.1. Acoustic indices used to investigate habitat-specific soundscapes and the effect of vegetation structure on indices at different countries in comparison to this study that investigated both topics. Total recording hours and the number of sites surveyed are indicated. Abbreviations: ACI = Acoustic complexity index, ADI = Acoustic diversity index, AEI = Acoustic evenness index, BIO = Bioacoustics index, CENT = Centroid, D = Acoustic dissimilarity index, DF = Dominant frequency, ESM = Entropy spectral maxima, ESV = Entropy spectral variance, FLAT = Spectral flatness, FQ = First quartile, H = Total entropy, KURT = Kurtosis, M = Mean amplitude, MID = mid-band activity, NDSI = Normalized difference soundscape index, NP = Number of peaks, RSP = Relative soundscape power, SD = Standard deviation, SKEW = Skewness, TQ = Third quartile, ZCR = Zero-crossing Rate, 1/F = Spearman correlation to 1/f noise.

Topic	Study	Acoustic indices	Country	Hours	Sites
Habitat-specific soundscapes	This study	ACI, AEI, BIO, CENT, DF, FLAT, FQ, H, KURT, NDSI, SD, SKEW, TQ	Brazil	1,538	143
	Bormpoudakis et al., (2013)	CENT, FLAT, H, KURT, SD, SKEW, ZCR, 1/F	Greece	2	32
	Bradfer-Lawrence et al., (2019)	ACI, ADI, AEI, BIO, H, M, NDSI	Panama	26,000	117
	Gómez et al., (2018)	ACI, ADI, AEI, BIO, ESM, ESV, H, M, MID, NDSI, NP	Colombia	905	8
Vegetation structure effects	Bradfer-Lawrence et al., (2020)	ACI, ADI, AEI, BIO, H, M, NDSI	Panama	84	43
	Farina and Pieretti (2014)	ACI	Italy	520	20
	Fuller et al., (2015)	ACI, ADI, AEI, BIO, H, NDSI	Australia	465	19
	Myers et al., (2019)	ACI, ADI, BIO	Greece	132	22
	Ng et al., (2018)	ACI, ADI, AEI, BIO, D, H, NDSI, RSP	Australia	378	9
	Pekin et al., (2012)	ADI	Costa Rica	14	14

Rankin and Axel (2017)	BIO	Madagascar	3,504	6
Tucker et al., (2014)	RSP	Australia	272	10

Table 2.2. Description of the eight statistical indices and five complexity indices used in this study.

Index type and name	Description
Statistical indices	
Centroid (CENT)	Mean frequency of the spectrum.
Dominant frequency (DF)	The frequency with the most energy in the spectrum.
First quartile (FQ)	Median frequency of the lower half of the spectrum.
Kurtosis (KURT)	Measures tailedness of signals in the spectrum. High values indicate outliers.
Skewness (SKEW)	Measures symmetry of signals in the spectrum. High values indicate that signals are skewed towards the high or low end of the spectrum.
Spectral flatness (FLAT)	Ratio between geometric and arithmetic mean amplitudes. Noisy signals will tend towards one and pure tones to zero.
Standard deviation (SD)	Spectral distribution of sounds.
Third quartile (TQ)	Median frequency of the upper half of the spectrum.
Complexity indices	
Acoustic Complexity Index (ACI)	Based on differences in amplitude between one time step and the next within a frequency band. Filters out constant sounds (e.g., insect chorus), this may lead to low values in rich soundscapes.
Acoustic Evenness Index (AEI)	Based on applying the Gini index to a specific number of frequency bands with signals above an amplitude threshold. High values indicate sound intensity is restricted to few frequencies.
Bioacoustic Index (BIO)	Based on the amplitude of a signal relative to the quietest frequency band within the 2-8 kHz range. High values indicate a great difference between loudest and quietest bands.
Total entropy (H)	Based on applying the Shannon index to a specific number of frequency bands and time steps. High values indicate sound intensity is distributed through many frequencies and time steps.
Normalized Difference Soundscape Index (NDSI)	Ratio between anthropogenic (1-2 kHz) and biological (2-11 kHz) sounds. High values indicate more biological sounds in the upper frequencies.

Table 2.3. Confusion matrices from random forest classification of eight different habitats (first classification) and three broad habitat types (second classification) using 13 acoustic indices calculated from 73,827 one-minute recordings made in the Viruá National Park, Roraima, Brazil. Accuracy was measured by predicting the testing dataset (18,456 one-minute recordings). Abbreviations: B = burned campina, C = campina, Ca = campinarana, Ig = igapó, Is = island, P = pasture, T = terra-firme, V = várzea, O = open habitats (B + C + P), F = flooded forests (Ig + Is + V), NF = non-flooded forests (Ca + T), Total = total error rate across all habitats.

		Actual										
Predicted	First classification			B	C	Ca	Ig	Is	P	T	V	Error
	B	7724	1782	641	35	118	171	74	66	0.27		
	C	2108	7341	571	135	106	251	106	168	0.31		
	Ca	537	549	7408	272	297	20	194	219	0.21		
	Ig	65	220	466	7813	313	27	263	423	0.18		
	Is	175	265	450	498	7413	16	342	1394	0.29		
	P	505	637	172	154	98	1486	153	124	0.55		
	T	112	220	222	404	459	16	6790	635	0.23		
	V	70	120	241	453	1230	10	530	7953	0.24		
										Total = 0.26 Accuracy = 0.74		
Second classification			O	F	NF					Error		
O	22207	1318	1200					0.10				
F	940	28299	1510					0.07				
NF	1879	3087	13387					0.26				
								Total = 0.13 Accuracy = 0.87				

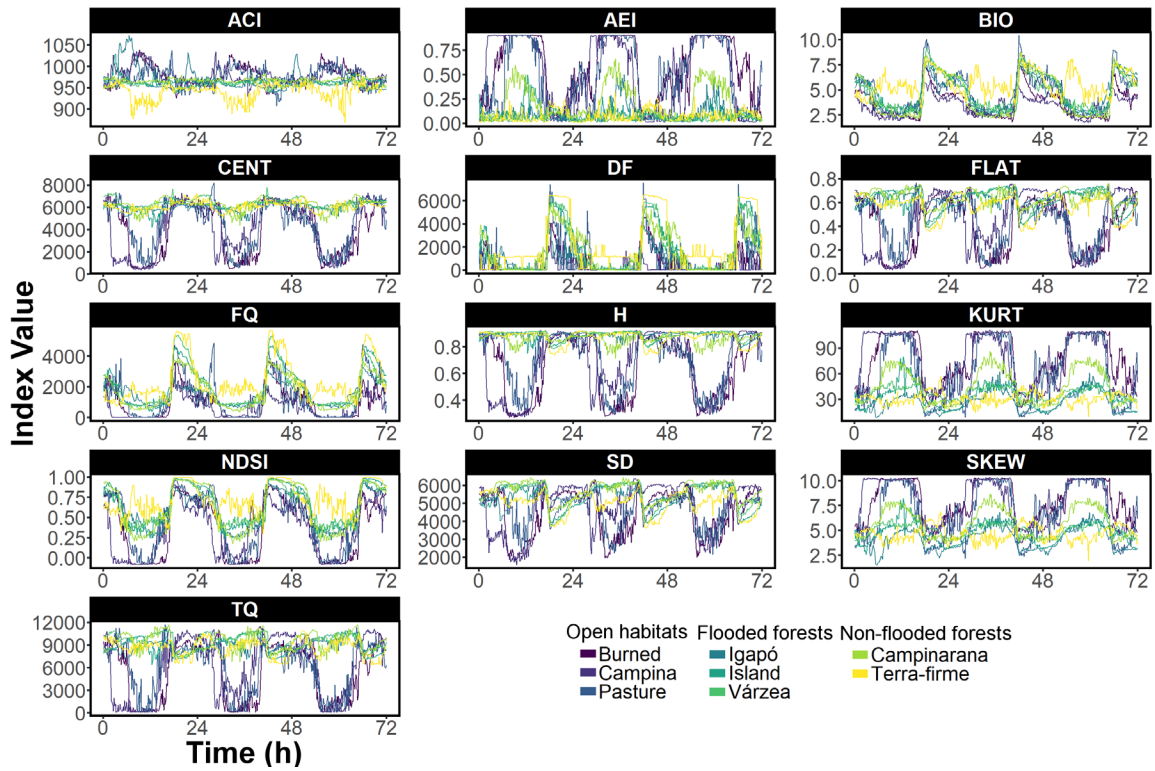


Fig. 2.1. Temporal trends of 13 acoustic indices across the eight habitats studied. Values of indices are the mean values calculated across all replicates within a habitat for each one-minute recording. Graph starts at 0100 h. For simplicity only three days of data are shown because patterns were consistent across the six-day sampling period.

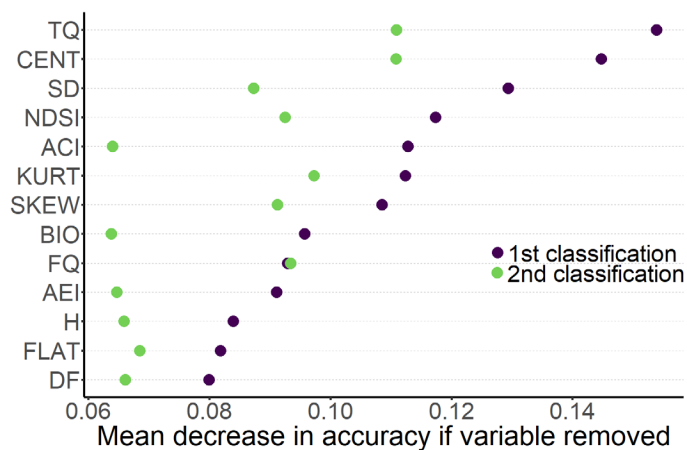


Fig. 2.2. Importance of acoustic indices at classifying eight habitat types (1st classification) and three broader habitat types (2nd classification) grouping the eight habitats. Graph shows the mean decline in accuracy of the models if a variable is removed. Accuracy of the first classification was 74% while in the second classification increased to 87%.

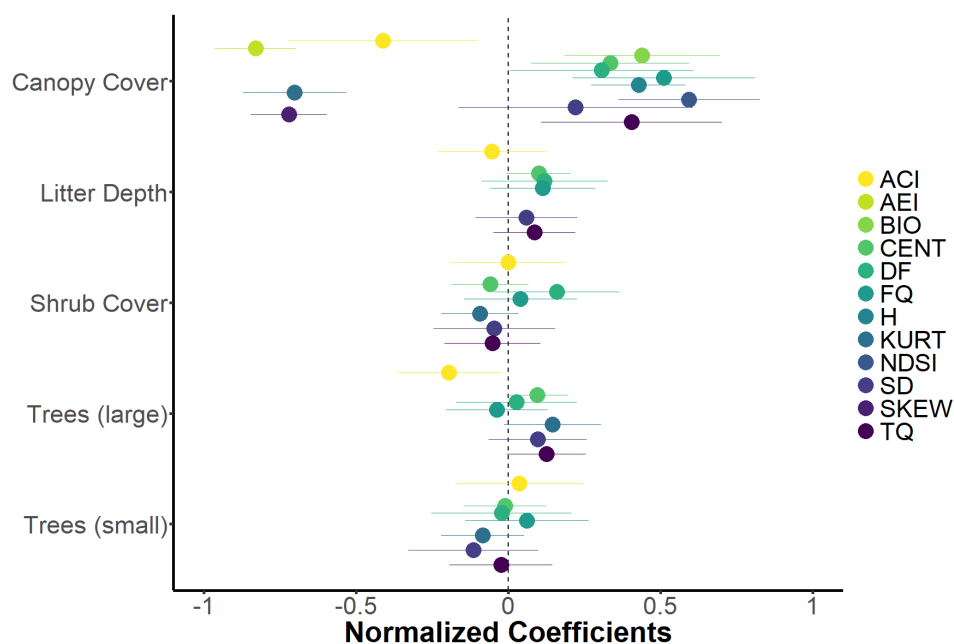


Fig. 2.3. Results of linear mixed models for 12 acoustic indices showing the effects of five vegetation variables on the indices. Dots are the normalized coefficients' values and lines represent the 95% confidence intervals. Coefficients were normalized by subtracting raw values by the mean and dividing by the standard deviation. Index FLAT is not shown because top performing model included a null model.

CHAPTER 3

ACOUSTIC DIFFERENCES BETWEEN DIURNAL AND NOCTURNAL LOUD
CALLS OF THE GUIANAN RED HOWLER MONKEY²**ABSTRACT**

Nighttime studies are greatly underrepresented in ecological research. Even well-known behaviors, such as the remarkably loud calls of howler monkeys, are rarely studied at night. Our goal was to fill this gap in knowledge by studying the 24-hour vocal behavior of the Guianan red howler (*Alouatta macconnelli*), specifically, we aimed to determine if howling bouts made during the day have a different acoustic structure than bouts made at night. We used 12 passive recording devices deployed in the home ranges of three groups of howlers to collect acoustic data over three months in the Viruá National Park, Roraima, Brazil. Our results show that during the day howling bouts were longer and had lower harmonic-to-noise ratio, lower frequencies, and more symmetric energy distributions than bouts at night. A pilot playback experiment with four alpha males showed that the species responds in different ways to bouts made during the day versus night. For example, they fled the playback area more often in response to diurnal than nocturnal bouts. Taken together, these results show that howler monkeys modify the structure of their howling bouts over 24-hour periods. We speculate that the differences found between diurnal and nocturnal bouts may be related to more exaggerated vocal

² Do Nascimento, L. A., Beard, K. H. *In review*. Acoustic differences between diurnal and nocturnal loud calls of the Guianan red howler monkey. *Primates*.

displays during the day because most intergroup encounters happen during daylight hours. This study highlights the importance of studying animals throughout their entire period of activity to uncover the full spectrum of their behavioral ecology.

INTRODUCTION

Howler monkeys emit the most powerful primate vocalization in the Neotropics, which may outperform all animals worldwide in both call duration and amplitude per body size (da Cunha et al. 2015). These loud calls are thought to have multiple functions (reviewed in Kitchen et al. 2015). For example, loud calls may reduce predation risk (Gilda-Costa et al. 2003), facilitate group cohesion (Steinmetz 2005), mediate sexual selection by male-male competition or female choice (Kitchen et al. 2015), and regulate intergroup use of space (Kitchen et al. 2015). Despite the large interest in their remarkable loud calls, several species of howler monkeys still lack an acoustic description of their calls (da Cunha et al. 2015; Bergman et al. 2016). Even less is known about loud calls made at night because most studies focus on diurnal vocal behavior (da Cunha et al. 2015; Kitchen et al. 2015).

The fact that howler's loud calls are rarely studied at night is no surprise because nighttime studies are underrepresented in ecological research (reviewed in Gaston 2019). For example, calls from birds and primates, two of the most studied animal taxa, are mostly studied during the day because it is easier to collect data during this time and researchers often assume that diurnal animals are not active during the night, despite the lack of empirical support for such assumption (Ankel-Simons and Rasmussen 2008; Parga 2011; La 2012; Tan et al. 2013; Piel 2018; Gaston 2019). For example,

anthropoids, except the genus *Aotus*, are considered diurnal. However, there are reports of mantled howler monkeys (*Alouatta palliata*), red-tailed monkeys (*Cercopithecus ascanius*), chimpanzees (*Pan troglodytes*), and Ugandan red colobus monkeys (*Procolobus rufomitratus tephrosceles*) all showing nocturnal activity (Ankel-Simons and Rasmussen 2008; Piel 2018; Tagg et al. 2018). Guianan red howler monkeys (*Alouatta macconnelli*) are another diurnal anthropoid that is active at night (Vercauteren Drubbel and Gautier 1993). In fact, it has been suggested that Guianan red howlers vocalize more at night than during the day (Vercauteren Drubbel and Gautier 1993). Emerging new technologies, like autonomous audio recorders, greatly facilitate studying soniferous animals at night and have the potential to advance both behavioral ecology and conservation practices (Deichman et al. 2018; Darras et al. 2019; Gaston 2019).

Even though the loud calls of Guianan red howler monkeys were described almost three decades ago in French Guiana (Vercauteren Drubbel and Gautier 1993), constraints with software and recording equipment limited both spectrogram analysis and the number of calls analyzed. In addition, this species has a wide distribution in South America and therefore it is reasonable to expect variation in their loud calls across disjointed populations, although to date this was not tested (da Cunha et al. 2015). Like other howler species, Guianan red howler monkeys engage in howling bouts (Fig. 3.1) that consist of a series of continuous roars, a type of loud call described as low-pitched sounds with a mean duration of 3 min and 28 s and a range of 1 to 10 minutes (Vercauteren Drubbel and Gautier 1993). Guianan red howlers produce another type of loud call, referred to as a bark, that usually is not mixed with roars during howling bouts in South American howlers' species (da Cunha et al. 2015), and soft calls (low-amplitude

vocalizations) that have not been studied in detail, but are thought to be produced in a variety of situations and may be a good candidate for contact calls (Kitchen et al. 2015).

Recently, the number of acoustic features and number of howling bouts analyzed for black howlers (*Alouatta pigra*) and mantled howlers have expanded (Bergman et al. 2016). Here we expand the number of acoustic features and howling bouts analyzed for the Guianan red howler monkey. Previous research on this species analyzed the acoustic structure of 20 howling bouts from a population in French Guiana (Vercauteren Drubbel and Gautier 1993). We analyze 102 howling bouts from a population disconnected to those in French Guiana in the northern Brazilian Amazon. Our goal was to determine if the acoustic structure of howling bouts made during the day were different than those made at night. We also performed a pilot playback experiment in which we tested this species response to nocturnal and diurnal howling bouts playbacks. This study is important because it elucidates the vocal behavior of a poorly studied Neotropical primate species while also providing insight about differences in nocturnal and diurnal loud calls, an understudied topic in primatology.

METHODS

Study area and groups

We conducted this research in the Viruá National Park (VNP), Roraima, Brazil (Fig. S3.1). The park was established in 1998 and is 240,000 ha (ICMBio 2014). VNP is regulated by flood pulses that create a rich habitat mosaic ranging from dense forest to grassland (ICMBio 2014). The climate in this region is warm and wet with mean annual temperatures of 26° C and mean annual precipitation of ~2,000 mm (ICMBio 2014). The

wet season is typically from May to September and the dry season from October to April (ICMBio 2014). We conducted this study primarily in terra-firme forest, which is a habitat located at elevations higher than surrounding lands (ca. 100 m a.s.l.) and, therefore, not susceptible to intense flooding during the rainy season (ICMBio 2014).

From February to April 2018, we followed four groups of Guianan red howler monkeys with roughly six individuals each (see Table S3.1 for specific composition). We followed them daily from 430 h to 1800 h (occasionally until 2200 h) so they would become more habituated to our presence before we conducted playback experiments. This totaled 300 contact hours for each group (total of 1200 hours). In the beginning, they displayed defensive behavior, such as hiding in the presence of the researchers, but after one month of following the groups, they did not do this as often. During this period, we also collected their position with a handheld GPS so we could estimate their home ranges.

Passive acoustic monitoring protocol

We used 12 ARBIMON recorders (Campos-Cerqueira and Aide 2016) to collect acoustic data from February to April 2018. We deployed four devices in the home range of three groups of howlers (Pequi, Viruá, and Calados) we followed. In each home range (Fig. S3.1), we placed two recorders where the animals were most often seen eating and resting (core area) and two ~100 m apart where they were occasionally seen moving or eating (periphery of home range). Preliminary data collected in 2017 along with local knowledge of field assistants aided in the placement of the recorders. We placed recorders, programed to record 24-hours per day in 20-minute segments (Sample rate

44.1 kHz, resolution 16 bit, WAV format), on trees 1.5 m off the forest floor. We synchronized all 12 recorders to make simultaneous recordings. We checked recorders every 1 to 2 days, retrieved the audio files with a laptop, and then re-attached them to the trees. During the study period, we never observed an invasion of the home range by a neighbor group and, by comparing the time of simultaneous recording in the different areas and field observations, we are confident that the recordings used belonged to the focal groups studied.

Acoustic parameter extraction protocol

We separated nocturnal (1800 h – 0600 h) and diurnal recordings (0600 h – 1800 h). We randomly selected an even number of high quality diurnal and nocturnal howling bouts (see Fig. S3.2 for spectrogram examples). Each group contributed 34 howling bouts to the analysis for a total of 51 nocturnal and 51 diurnal bouts (Pequi: 20 nocturnal and 14 diurnal; Viruá: 20 nocturnal and 14 diurnal; Calados: 11 nocturnal and 23 diurnal). We used a maximum of two bouts from each group each night or day (separated by more than one hour when in the same day) to minimize dependence among the samples (see Fig. S3.3 for temporal distribution of selected howling bouts).

For consistency, we followed Bergman et al. (2016) for the extraction of nine acoustic features from howling bouts (Table 1). We drew spectrograms with a Fast Fourier Transformation (FFT), Hanning window type, and a time window of 512 points to measure the mean frequency, median frequency, dominant frequency, skewness, and kurtosis of howling bouts; this was completed in the package *Seewave* (Sueur et al. 2008) within the R environment (R Core Team 2019). We also drew spectrograms with a FFT,

Gaussian window type, 0.1 sec window length, a 50 dB dynamic range, a maximum formant of 4000 Hz, and resolutions of 1500 time steps and 250 frequency steps to measure the first formant, the highest frequency (sixth formant), and the harmonic-to-noise ratio (a measure of deterministic chaos; Tokuda et al. 2002); this was done using Praat software (Boersma and Weenink 2013). Individual roar syllables were used in Praat from the middle section of the full howling bout sequence for consistency across all recordings. Finally, the duration of the bouts was measured manually in seconds using the Audacity software (Audacity Team 2019).

Pilot playback experiment protocol

From March to April 2018 (more than one month after the groups were followed), we tested alpha male responses to nocturnal and diurnal howling bouts in all four groups. Only the responses of alpha males were recorded because they always vocalized during howling bouts, whereas participation of other members of the group seemed facultative (Kitchen 2004). We followed a playback protocol similar to one used in past research with howler monkeys (Kitchen 2004) and best practices to conduct playbacks with non-human primates (Zuberbühler and Wittig 2011). We ran a total of eight experimental trials. To avoid habituation and stress, we tested each group only twice and waited a minimum of 7 days (mean=8 \pm SD 1 days) between consecutive trials. Each of the four groups was tested once for the diurnal and nocturnal howling bout stimulus. Order of the playbacks (diurnal or nocturnal) was randomized across groups.

Diurnal howling bouts were on average longer than nocturnal bouts (Table 3.2), therefore, a playback trial consisted of either one howling bout made during the day

selected for its long duration (mean duration= $300 \pm$ SD 10 seconds) or one bout made at night selected for its short duration (mean duration= $200 \pm$ SD 10 seconds). Post-hoc comparisons of the recordings used revealed that the diurnal bouts used in the trials also had lower frequencies and harmonic-to-noise ratio than the nocturnal bouts, similar to our overall description of the 102 howling bouts (Table 3.2). To simulate an intruder in the home range of the tested subjects, bouts from group Calado were played back to Tanque and Pequi; bouts from group Tanque were played back to Calado and Viruá; bouts from group Pequi were played back to Calado and Viruá; bouts from group Viruá were played back to Tanque and Pequi. We used recordings only once in the trials and the bouts we played back to a specific group were never from the same alpha male.

We normalized all files used in the playbacks to similar amplitude levels using the Audacity software (Audacity Team 2019). The speaker (model UE ROLL 2; audio output = 15 Watt; frequency Range = 108 Hz – 20 kHz) was set to maximum volume across all trials and the output stimulus emulated natural vocalization levels (measured in the field with a sound level meter Extech HD600). To remove any background noise, we applied a low pass filter of 3000 Hz and a high pass filter of 50 Hz in the recordings using the Audacity software (Audacity Team 2019). We conducted the playbacks in the core area of the group and in the morning, around 900 h (\pm 15 min), and in similar weather conditions, sunny and not windy.

Once a group was located, we raised a wireless speaker on an 8-m collapsible pole concealed in vegetation at an approximate distance of 30 m from test subjects. The speaker was pointed toward the alpha male. Densely vegetated hills that separated the home ranges of the studied groups (Figure 1) greatly attenuate their vocalizations,

therefore, it is unlikely that non-focal groups heard the playback stimulus (if it was heard, it would have been a greatly degraded signal). Observers remained concealed in vegetation during all trials and started the trial if the monkeys seemed unaware of our presence (feeding or resting) and no loud call was heard for one hour from the targeted group or neighbors to make sure the responses we observed were due to our playback stimulus. A trial lasted for 70 minutes (from the onset of the playback) and during this period an observer (LAN, aided by a field assistant) collected behavioral data on the alpha male and noted all occurrences of vocalizations and movements toward or away from the speaker. Specifically, we consider an approach to be when the alpha male left his original tree and went towards the speaker and a retreat to be when he moved in the opposite direction. All the responses reported occurred within less than one hour of playback onset and no neighboring groups were heard during this period. Alpha males and other individuals who approached the speaker paid no attention to the equipment and continued to move past or around it with clearly vigilant posture and often vocalized (soft or loud calls) while searching for the source of the sound (simulated intruder).

Statistics

All statistical analyses were performed in the R environment (R Core Team 2019). We explored possible differences between diurnal and nocturnal howling bouts in two ways. First, we separated the data into training (80%) and testing (20%) datasets. We ran a linear discriminant analysis (LDA) to assign tentatively all nine acoustic parameters to day or night using the training dataset and the *Caret* package (Kuhn, 2008). We then used the “predict” function in the *Caret* package to measure the accuracy of the LDA

model to predict the testing dataset. Second, we used linear mixed models (LMM) to test for differences in diurnal and nocturnal bouts while controlling for group identity. In each LMM model, the dependent variable was one of nine acoustic parameters, the fixed effect was the period of the bout (diurnal or nocturnal) and the random effect was group identity. We fit nine individual models (restricted maximum likelihood) with the package *nlme* (Pinheiro et al. 2019). Residuals of the models were checked for linearity, homoscedasticity, independence, and normality with the package *SjPlot* (Lüdtke 2020). We considered a fixed effect to be significant at an alpha level of < 0.05 . To avoid a Type I error due to multiple comparisons (testing the howling bouts multiple times), we lowered our alpha using a sequential Bonferroni correction (Holm 1979).

We tabulated the number of the playback experiments that elicited approaches and retreats from the speaker area, and loud and soft calls, and used a Fisher's exact test to determine if the differences in responses to diurnal and nocturnal playbacks were statistically significant. Four response variables extracted from the playback experiment (approach latency, retreat latency, loud call latency, soft call latency) were summarized by their means and standard deviations, and used in a survival analysis (Kaplan-Meier method and log-rank test) to determine if the differences in responses to diurnal and nocturnal playbacks were statistically significant. We used the package *Survival* (Therneau 2015) to run the analysis. We used this non-parametric approach (Kaplan-Meier method and log-rank test) because our sample size made it difficult to test for normality and also because of the nature of our data, which included time to an event (time to focal males reaction to the playback stimulus) and censored data (data collection ceased by the end of a trial and events observed may or may not occur in the future).

When no reaction was observed during the playbacks (e.g. no loud calls from focal alpha males), we used the total time of the trial (70 min) as the response variable (as in Kitchen 2004).

Ethics statement

Research complied with protocols approved by The Utah State University's Animal Care and Use Committee (IACUC #2690) and all Brazilian legal requirements.

RESULTS

Differences between diurnal and nocturnal howling bouts

The LDA revealed a separation between most of the acoustic parameters depending on the time of the day the howling bouts were made (Fig. 3.2). The most discriminant acoustic parameter between diurnal and nocturnal bouts was the harmonic-to-noise ratio (Table S3.2) and the LDA model accuracy to predict the testing dataset was 95%. As implied by the LDA, the LMM revealed that diurnal and nocturnal howling bouts differed on six of 9 acoustic parameters (Fig. 3.3). Diurnal bouts were significantly longer than nocturnal bouts. The harmonic-to-noise ratio, skewness, kurtosis, first formant, and highest frequency parameters were all significantly lower in diurnal than nocturnal bouts (Table 3.2).

Pilot playback experiment

Our pilot experiment revealed that when presented with nocturnal howling bout playbacks, alpha males always approached the source of the sound (simulated intruder) and gave soft calls. They also gave a loud call in response to three out of the four

nocturnal trials (roars and barks). This contrasts with responses to diurnal bouts, where the alpha males mostly fled (three trials) the speaker area by running in the opposite direction. An alpha male only approached the speaker and gave loud (only roars) and soft calls during one diurnal trial (Table 3.3; pairwise comparisons of responses were not statistically significant). Time to flee the playback area was higher in response to nocturnal than diurnal howling bouts. Time to approach the speaker and produce soft calls were higher in response to diurnal than nocturnal howling bouts, but differences were only marginally significant. Differences in the time to produce loud calls in response to diurnal or nocturnal bouts were not significant (Table 3.4).

DISCUSSION

Nocturnal vocalizations from diurnal primates have rarely been studied (Ankel-Simons and Rasmussen 2008; Piel 2018; Gaston 2019). While previous research on the Guianan red howler monkey described different types of calls in their repertoire (Vercauteren Drubbel and Gautier 1993), a detailed description of their diurnal and nocturnal howling bouts was lacking. We found that howling bouts made during the day differed in structure from those made at night primarily because they were, on average, 26% longer; had, on average, 73% lower harmonic-to-noise ratio; and had, on average, 5% lower frequencies (first formant and highest frequency), and more symmetric energy distributions (skewness and kurtosis). In addition, Guianan red howlers appeared to respond differently to diurnal versus nocturnal howling bouts. Diurnal bouts have acoustic characteristics that are linked to more exaggerated vocal displays that likely require more energy to produce and may appear more intimidating than nocturnal bouts.

Duration of vocalizations is indicative of effort, with longer vocalizations requiring more energy to produce (Fischer et al. 2004; Vannoni and McElligott 2009). We propose that howlers invest more energy in longer diurnal howling bouts than nocturnal bouts because they experience more intergroup encounters during the day when they forage than at night when they typically stay at sleeping sites (Vercauteren Drubbel and Gautier 1993; L. Do Nascimento pers. obs.). These longer vocal displays could be used to settle disputes for limiting resources, such as fruit, and better defend the group from potential invaders (Kitchen et al. 2015; Van Belle and Estrada 2019). A similar behavior, longer loud calls during contests, was observed in black howlers (Kitchen 2004) and baboons (*Papio cynocephalus ursinus*; Kitchen et al. 2003).

Guianan red howlers increased the amount of deterministic chaos (as measured by the harmonic-to-noise ratio) during the day (Table 3.2). This acoustic characteristic leads to harsher calls that are more intimidating than tonal calls (Morton 1977; Bergman et al. 2016; Demartsev et al. 2016). Across many mammals, vocalizations with more chaos have been shown to either elicit a reluctance to escalate a contest with a threatening male (Garcia et al. 2014) or make them respond more strongly to a conspecific (Townsend and Manser 2011). The mechanism through which these responses may occur is unknown, but in chimpanzees, it is believed that the presence of non-linear phenomena, such as deterministic chaos in their loud calls, may be used to assess the physical condition of the caller (Riede et al. 2007). Harsher howling bouts of the Guianan red howler monkey may also serve to more efficiently intimidate in other groups, increase the chance of winning contests, and thus avoid the more energetic costs of chases and fights.

The lower frequency of howling bouts during the day than at night may also reflect a greater motivation to intimidate other groups (Morton 1977; Reby and McComb 2003; Ordóñez-Gómez et al. 2015; Mercier et al. 2019). Because animals may modify the frequency of their calls to signal competitive abilities (Whitehead 1992; Fischer et al. 2004), lower frequency calls may be better at intimidating other groups during contests (Morton 1977). However, it is important to note that changes in frequencies between diurnal and nocturnal howling bouts were of a smaller magnitude than the duration and harmonic-to-noise ratio, suggesting that they may play a smaller role in intergroup competition or that they are harder to modify due morphological constraints (Kitchen et al. 2019).

Our findings provide the first report of a population of Guianan red howlers not studied before and expanded the number of howling bouts and acoustic parameters described for this species. The harmonic-to-noise ratio, kurtosis, skewness, first formant, mean frequency, and median frequency acoustic parameters were not described before for this species while the parameters previously described, such as dominant frequency, highest frequency, and duration were similar between this population from Brazil and another from French Guiana (Vercauteren Drubbel and Gautier 1993).

While the results from the playback experiment should be taken with caution because of the small sample size and our inability to conduct playbacks at night without special equipment, they suggest that howlers respond to the differences between diurnal and nocturnal vocalizations. Future studies could expand the number of groups studied, conduct playbacks at night and day, and isolate what aspect of the bout (e.g. duration, harmonic-to-noise ratio, and frequency) may drive the apparent difference in responses to

diurnal versus nocturnal bouts. These further tests could elucidate if diurnal and nocturnal howling bouts have different functions or if they simply represent different levels of sequential and cumulative assessment, as suggested in Kitchen et al. (2015).

In summary, in this study we provide a detailed analysis of the Guianan red howler vocalizations focusing on unexplored differences between diurnal and nocturnal howling bouts. We show that diurnal and nocturnal bouts possess significant structural differences that appear to elicit different behavioral responses. We speculate that the differences found between diurnal and nocturnal bouts may be related to more exaggerated vocal displays during the day because most intergroup encounters happen during daylight hours. This ability of howler monkeys to modify the acoustic structure of their howling bouts over the diel cycle is novel, and highlights the importance of studying animals throughout their entire period of activity. This is now facilitated through remote sensing methods, such as camera trapping and passive acoustic monitoring (Deichman et al. 2018), which hold great potential to tackle the difficulties associated with studying nocturnal patterns in ecology (Gaston et al. 2019).

REFERENCES

Audacity Team (2019). Audacity software. Pittsburg, PA.

Ankel-Simons F, Rasmussen DT (2008) Diurnality, nocturnality, and the evolution of primate visual systems. *American Journal of Physical Anthropology*, 137(S47), 100-117. <https://doi.org/10.1002/ajpa.20957>

Bergman TJ, Cortés-Ortiz L, Dias PA, Ho L, Adams D, Canales-Espinosa D, Kitchen DM (2016) Striking differences in the loud calls of howler monkey sister species

(*Alouatta pigra* and *A. palliata*). *American Journal of Primatology*, 78(7), 755-766. <https://doi.org/10.1002/ajp.22539>

Boersma, P., & Weenink, D. (2013). Praat software. Amsterdam, Netherlands.

Campos-Cerqueira M, Aide TM (2016) Improving distribution data of threatened species by combining acoustic monitoring and occupancy modelling. *Methods in Ecology and Evolution*, 7(11), 1340-1348. <https://doi.org/10.1111/2041-210X.12599>

da Cunha RGT, de Oliveira DAG, Holzmann I, Kitchen DM (2015) Production of loud and quiet calls in howler monkeys. In *Howler Monkeys* (pp. 337-368). Springer, New York, NY. https://doi.org/10.1007/978-1-4939-1957-4_13

Darras, K, Batáry P, Furnas BJ, Grass I, Mulyani YA, Tschardt T (2019) Autonomous sound recording outperforms human observation for sampling birds: a systematic map and user guide. *Ecological Applications*, 29(6), e01954. <https://doi.org/10.1002/eap.1954>

Deichmann JL, Acevedo-Charry O, Barclay L, Burivalova Z, Campos-Cerqueira M, d'Horta F, Game ET, Gottesman BL, Hart PJ, Kalan AK, Linke S, Do Nascimento LA, Pijanowski B, Staatterman E, Aide MT (2018) It's time to listen: there is much to be learned from the sounds of tropical ecosystems. *Biotropica*, 50(5), 713-718. <https://doi.org/10.1111/btp.12593>

Demartsev V, Bar Ziv E, Shani U, Goll Y, Koren L, Geffen E (2016) Harsh vocal elements affect counter-singing dynamics in male rock hyrax. *Behavioral ecology*, 27(5), 1397-1404. <https://doi.org/10.1093/beheco/arw063>

- Fischer J, Kitchen DM, Seyfarth RM, Cheney DL (2004) Baboon loud calls advertise male quality: acoustic features and their relation to rank, age, and exhaustion. *Behavioral Ecology and Sociobiology*, 56(2), 140-148.
<https://doi.org/10.1007/s00265-003-0739-4>
- Garcia M, Wyman MT, Charlton BD, Fitch WT, Reby D (2014) Response of red deer stags (*Cervus elaphus*) to playback of harsh versus common roars. *Naturwissenschaften*, 101(10), 851-854. <https://doi.org/10.1007/s00114-014-1217-8>
- Gaston KJ (2019) Nighttime ecology: the “nocturnal problem” revisited. *The American Naturalist*, 193(4), 481-502. <https://doi.org/10.1086/702250>
- Gil-da-Costa R, Palleroni A, Hauser MD, Touchton J, Kelley JP (2003) Rapid acquisition of an alarm response by a neotropical primate to a newly introduced avian predator. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270(1515), 605-610. <https://doi.org/10.1098/rspb.2002.2281>
- Holm S (1979) A simple sequentially rejective multiple test procedure. *Scandinavian journal of statistics*, 6, 65-70. <https://www.jstor.org/stable/4615733>
- ICMBio. Instituto Chico Mendes de Conservação da Biodiversidade (2014) Plano de Manejo do Parque Nacional do Viruá. ICMBio, Boa Vista, Roraima.
http://www.icmbio.gov.br/portal/images/stories/docs-planos-de-manejo/parna_virua_pm_diag1.pdf Assessed 10 January 2020
- Kitchen DM, Seyfarth RM, Fischer J, Cheney DL (2003) Loud calls as indicators of dominance in male baboons (*Papio cynocephalus ursinus*). *Behavioral Ecology and Sociobiology*, 53(6), 374-384. <https://doi.org/10.1007/s00265-003-0588-1>

- Kitchen DM (2004) Alpha male black howler monkey responses to loud calls: effect of numeric odds, male companion behaviour and reproductive investment. *Animal Behaviour*, 67(1), 125-139. <https://doi.org/10.1016/j.anbehav.2003.03.007>
- Kitchen DM, da Cunha RGT, Holzmann I, de Oliveira DAG (2015) Function of loud calls in howler monkeys. In *Howler Monkeys* (pp. 369-399). Springer, New York, NY. https://doi.org/10.1007/978-1-4939-1957-4_14
- Kitchen DM, Bergman TJ, Dias PA, Ho L, Canales-Espinosa D, Cortés-Ortiz L (2019) Temporal but not acoustic plasticity in hybrid howler monkey (*Alouatta palliata* × *A. pigra*) loud calls. *International Journal of Primatology*, 40(1), 132-152. <https://doi.org/10.1007/s10764-017-0004-8>
- Kuhn M (2008) Building predictive models in R using the caret package. *Journal of statistical software*, 28(5), 1-26. <https://doi.org/10.18637/jss.v028.i05>
- La VT (2012) Diurnal and nocturnal birds vocalize at night: a review. *The Condor*, 114(2), 245-257. <https://doi.org/10.1525/cond.2012.100193>
- Lüdecke D (2020) sjPlot: Data visualization for statistics in social science. R package version, 2.8.3.
- Mercier S, Déaux EC, Van de Waal E, Bono AE, Zuberbühler K (2019) Correlates of social role and conflict severity in wild vervet monkey agonistic screams. *PloS one*, 14(5), e0214640. <https://doi.org/10.1371/journal.pone.0214640>
- Morton ES (1977) On the occurrence and significance of motivation-structural rules in some bird and mammal sounds. *The American Naturalist*, 111(981), 855-869. <https://doi.org/10.1086/283219>

- Ordóñez-Gómez JD, Dunn JC, Arroyo-Rodríguez V, Méndez-Cárdenas MG, Márquez-Arias A, Santillán-Doherty AM (2015) Role of emitter and severity of aggression influence the agonistic vocalizations of Geoffroy's spider monkeys (*Ateles geoffroyi*). *International Journal of Primatology*, 36(2), 429-440.
<https://doi.org/10.1007/s10764-015-9833-5>
- Parga JA (2011) Nocturnal ranging by a diurnal primate: are ring-tailed lemurs (*Lemur catta*) cathemeral?. *Primates*, 52(3), 201-205. <https://doi.org/10.1007/s10329-011-0257-3>
- Piel AK (2018) Temporal patterns of chimpanzee loud calls in the Issa Valley, Tanzania: Evidence of nocturnal acoustic behavior in wild chimpanzees. *American journal of physical anthropology*, 166(3), 530-540.
- Pinheiro J, Bates D, DebRoy S, Sarkar D, Team RC (2019) nlme: Linear and nonlinear mixed effects models. R package version, 3.1-141.
- R Core Team (2019) A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.
- Reby D, McComb K (2003) Anatomical constraints generate honesty: acoustic cues to age and weight in the roars of red deer stags. *Animal Behaviour*, 65(3), 519-530.
<https://doi.org/10.1006/anbe.2003.2078>
- Riede T, Arcadi AC, Owren MJ (2007) Nonlinear acoustics in the pant hoots of common chimpanzees (*Pan troglodytes*): vocalizing at the edge. *The Journal of the Acoustical Society of America*, 121(3), 1758-1767.
<https://doi.org/10.1121/1.2427115>

- Steinmetz S (2005) Vocalizações de longo alcance como comunicação intra-grupal nos bugios (*Alouatta guariba*). *Neotropical Primates*, 13(2), 11-16.
<https://doi.org/10.1896/1413-4705.13.2.11>
- Sueur J, Aubin T, Simonis C (2008) Seewave, a free modular tool for sound analysis and synthesis. *Bioacoustics*, 18(2), 213-226.
<https://doi.org/10.1080/09524622.2008.9753600>
- Tagg N et al (2018) Nocturnal activity in wild chimpanzees (*Pan troglodytes*): Evidence for flexible sleeping patterns and insights into human evolution. *American Journal of Physical Anthropology*, 166(3), 510-529.
<https://doi.org/10.1002/ajpa.23478>
- Tan CL, Yang Y, Niu K (2013) Into the night: camera traps reveal nocturnal activity in a presumptive diurnal primate, *Rhinopithecus brelichi*. *Primates*, 54(1), 1-6.
<https://doi.org/10.1007/s10329-012-0318-2>
- Therneau T (2015) A Package for Survival Analysis in R. R package version, 3.1-12
- Tokuda I, Riede T, Neubauer J, Owren MJ, Herzog H (2002) Nonlinear analysis of irregular animal vocalizations. *The Journal of the Acoustical Society of America*, 111(6), 2908-2919. <https://doi.org/10.1121/1.1474440>
- Townsend SW, Manser MB (2011) The function of nonlinear phenomena in meerkat alarm calls. *Biology Letters*, 7(1), 47-49. <https://doi.org/10.1098/rsbl.2010.0537>
- Van Belle S, Estrada A (2019) The influence of loud calls on intergroup spacing mechanism in black howler monkeys (*Alouatta pigra*). *International Journal of Primatology*, 1-22. <https://doi.org/10.1007/s10764-019-00121-x>

- Vannoni E, McElligott AG (2009) Fallow bucks get hoarse: vocal fatigue as a possible signal to conspecifics. *Animal Behaviour*, 78(1), 3-10.
<https://doi.org/10.1016/j.anbehav.2009.03.015>
- Vercauteren Drubbel V, Gautier JP (1993) On the occurrence of nocturnal and diurnal loud calls, differing in structure and duration, in red howlers (*Alouatta seniculus*) of French Guyana. *Folia Primatologica*, 60(4), 195-209.
<https://doi.org/10.1159/000156693>
- Whitehead JM (1992) Acoustic correlates of social contexts and inferred internal states in howling monkeys (*Alouatta palliata*). *The Journal of the Acoustical Society of America*, 91(4), 2465-2465. <https://doi.org/10.1121/1.403017>
- Zuberbühler K, Wittig RM (2011) Field experiments with non-human primates: a tutorial. In *Field and laboratory methods in primatology: a practical guide* (pp. 207-224). Cambridge Univ. Pr.
<https://doi.org/10.1017/CBO9780511921643.013>

TABLES AND FIGURES

Table 3.1. Acoustic parameters measured from 102 howling bouts of Guianan red howler monkeys at the Viruá National Park, Roraima, Brazil.

Acoustic parameter	Description
Mean frequency (Hz)	Mean spectral frequency of call.
Median frequency (Hz)	Median spectral frequency of call.
Dominant frequency (Hz)	Frequency with highest energy in the call.
Skewness	Spectral symmetry of call.
Kurtosis	Spectral tailedness of call.
First formant (Hz)	First peak of energy in the call spectrum.
Highest frequency (Hz)	Upper frequency bound of the call.
Harmonic-to-noise ratio (dB)	Relative energy given to tonal versus atonal noise.
Duration (s)	Duration of continuous loud calling.

Table 3.2. Acoustic parameters (mean and \pm SD) for nocturnal and diurnal howling bouts of Guianan red howlers and results for linear mixed models (t statistics and p values are indicated).

Acoustic parameter	Diurnal	Nocturnal	N	t	p^a	α^b
Mean frequency (Hz)	1063 \pm 126	1023 \pm 89	102	-1.77	0.070	0.630
Median frequency (Hz)	920 \pm 125	895 \pm 89	102	-1.03	0.300	1
Dominant frequency (Hz)	696 \pm 264	730 \pm 277	102	0.11	0.900	1
Skewness	4.0 \pm 0.4	4.3 \pm 0.4	102	3.05	0.002	0.018
Kurtosis	20 \pm 4.5	23 \pm 5	102	3.06	0.003	0.027
First formant (Hz)	457 \pm 27	494 \pm 19	102	7.57	<0.001	<0.001
Highest frequency (Hz)	2495 \pm 68	2569 \pm 37	102	6.82	<0.001	<0.001
Harmonic-to-noise ratio (dB)	1.16 \pm 0.3	2.01 \pm 0.4	102	11.2	<0.001	<0.001
Duration (s)	327 \pm 93	258 \pm 83	102	-3.81	<0.001	<0.001

^aStatistically significant results ($p < 0.05$) are in bold.

^bAlpha values adjustments using Bonferroni correction.

Table 3.3. Percentage of playback experiments that elicited approach responses, retreat responses, loud calls, and soft calls by four alpha male Guianan red howlers. *P*-values were retrieved from Fisher's exact test for all pairwise comparisons.

Playback	# of trials	% Approach	% Retreat	% Loud call^a	% Soft call
Diurnal	4	25	75	25	25
Nocturnal	4	100	0	75	100
		<i>p</i> = 0.14	<i>p</i> = 0.14	<i>p</i> = 0.48	<i>p</i> = 0.14

^aIncludes roars and barks in response to nocturnal playbacks

Table 3.4. Responses (mean and \pm SD) of four alpha male Guianan red howlers to nocturnal and diurnal howling bouts playbacks and results of a survival analysis testing if the latency to respond to diurnal and nocturnal loud call playbacks are different for each response variable (survival probability and associated p -values are indicated).

Response variable	Playback	N	mean	SD	survival	p^a
Approach latency (min)	Diurnal	4	53.12	33.75	0.75	0.07
	Nocturnal	4	4.18	2.29	0	
Retreat latency (min)	Diurnal	4	27.38	30.80	0.25	0.04
	Nocturnal	4	70	0	1	
Loud call latency (min)	Diurnal	4	61.75	16.5	0.75	0.24
	Nocturnal	4	39.67	26.48	0.25	
Soft call latency (min)	Diurnal	4	53.64	32.7	0.75	0.07
	Nocturnal	4	5.46	1.31	0	

^aStatistically significant results ($p < 0.05$) are in bold.

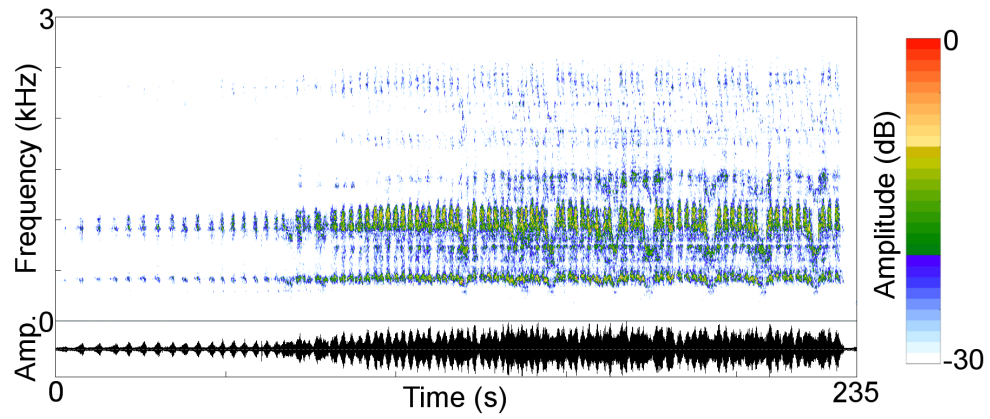


Fig. 3.1. Example of a howling bout of the Guianan red howler monkey recorded in the Viruá National Park, Roraima, Brazil.

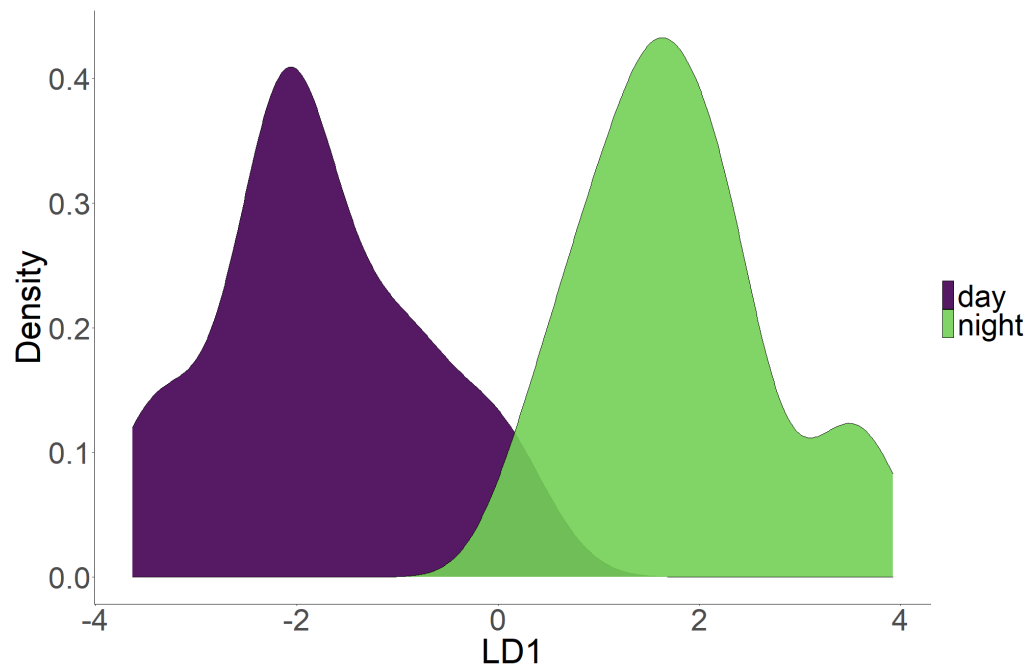


Fig. 3.2. Density plot of coefficients of linear discriminants built with nine acoustic parameters from 41 diurnal and 41 nocturnal howling bouts of Guianan red howler monkeys. Accuracy of the model to predict the testing dataset (20 howling bouts) was 95%.

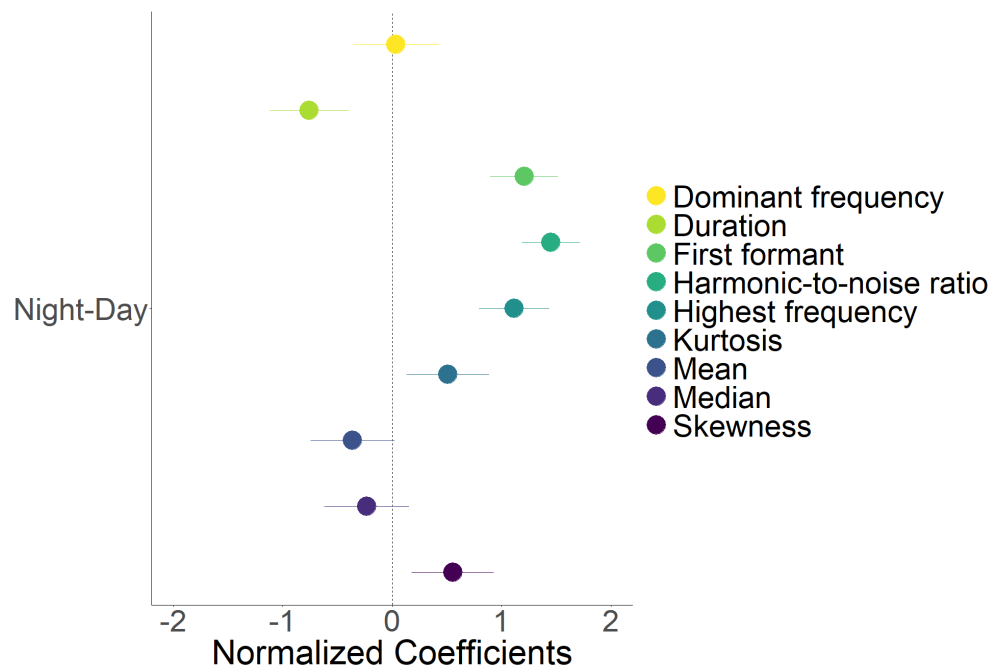


Fig. 3.3. Results of linear mixed models showing the effect of the time howling bouts were made (night or day) on nine acoustic parameters. The reference level for the models (i.e., the intercept) was “day”. Dots are the normalized coefficients values and lines represent the 95% confidence intervals. Coefficients were normalized by subtracting raw values by the mean and dividing by the standard deviation.

CHAPTER 4
MONITORING THREATENED SPECIES USING PASSIVE ACOUSTIC
RECORDERS AND AUTOMATIC CLASSIFIERS³

ABSTRACT

As soundscape recordings are accumulating around the world, it is essential to develop better analytical tools to extract information from these large audio datasets. Presence or absence of species in the recordings is essential information that could help with species management and conservation, but the availability of free and open-source software to retrieve this information is still scarce. Here we tested a promising and free alternative to build automatic detectors of animal sounds. Specifically, we tested the potential of a cross-correlation template matching technique to identify the calls of two bird species of conservation concern across 60 sites in an ecological and evolutionary hotspot in the Brazilian Amazon. We found that despite an extremely noisy background (e.g., over 500 bird species), the automatic detectors performed surprisingly well and could potentially be extended to the detection of other species in the Amazon. The overall recall rate of the classifiers was 100% while the precision was 28% for the Rio Branco antbird and 25% for the festive parrot. Future work should focus at converting the detections to encounter histories to fit statistical models that can account for imperfect detection.

³Do Nascimento, L. A., Beard, K. H. *In preparation*. Monitoring threatened species using passive acoustic recorders and automatic classifiers. *Target journal: Biological Conservation*.

1. INTRODUCTION

Passive acoustic monitoring (PAM) is revolutionizing the way we understand natural and human modified ecosystems (Sugai et al., 2019). PAM is able to generate large datasets quickly that allow scientists to better understand natural dynamics that went mostly unnoticed for decades (Deichman et al., 2018). For example, PAM allowed for a better understanding of 24 hours activity cycles of species (Pérez-Granados et al., 2020), changes in species behavior between day and night (Piel et al., 2018), and more broadly, better assessment of human disturbances on ecosystems (Burivalova et al., 2019). This revolution in the way that ecologists are collecting data to answer a multitude of questions is facilitated by the substantial decrease in prices of acoustic recorders (Hill et al., 2018) and better analytical tools to analyze large streams of data (Zhong et al., 2020).

One method that is receiving growing attention to analyze large audio datasets is the implementation of automatic classifiers of animal sounds (Aide et al., 2013). This method provides information about the presence or absence of target species in the recordings, which allows among other features (Deichman et al., 2018), allows the implementation of occupancy models that account for imperfect detection (Campos-Cerqueira and Aide, 2016). However, most of the methods available require expensive software and considerable coding experience, which limit its usage by researchers and potentially by users outside of academia, which would probably benefit the most from this technology (Ducrettet et al., 2020). Therefore, there is an urgent need to develop and test alternatives that are free, open source, and user-friendly for automatic acoustic classification of animal sounds (Balantic and Donovan, 2020).

Here we test a promising alternative to build automatic detectors of animal sounds. Specifically, we tested the potential of a cross-correlation template matching technique to identify the calls of two bird species of conservation concern across 60 sites in an ecological and evolutionary hotspot in the Brazilian Amazon. The habitats surveyed are threatened by dam construction, among other infrastructure developments (Naka et al. 2020), which makes it urgent to develop efficient, reliable, and verifiable animal monitoring methods (Ribeiro et al., 2017; Ritter et al., 2017).

2. METHODS

2.1. Study sites

We conducted this research in and around Viruá National Park (VNP), Roraima, Brazil, in the north of the Brazilian Amazon (Figure 4.1). VNP was established in 1998 and is 240,000 ha (ICMBio, 2014). The climate in VNP is warm and wet with mean annual temperature of 26 °C and mean annual precipitation of ~2,000 mm (ICMBio, 2014). Rainfall is mostly concentrated from May to September (ICMBio, 2014). VNP is regulated by floods that create a vegetation mosaic ranging from dense forests to grasslands, and representing most major habitats found across the Amazon biome (Do Nascimento et al., 2020). We focused our surveys on flooded forests which are comprised of three riverine habitats (igapó, riverine islands, and várzea) because they will be likely the most impacted by dam construction in the future (Naka et al. 2020). In our study area, riverine islands and várzea forests are drained by the Rio Branco (white river in Portuguese) at the west portion of the park. This river is located entirely in the state of Roraima (Naka et al. 2020) and is ranked 12th in discharge volume in the Amazon basin

(Ferreira et al., 2017). Igapó, in the other hand, is drained by a small black water river named Iruá, the main river inside the limits of VNP.

2.2. Study species

We selected species from a list of 50 birds that were recommended for surveys in the Rio Branco basin (Naka et al., 2020). From this list, we focused on two species that are known to occur in the flooded forests and are of conservation concern. The Rio Branco anbird (*Cercomacra carbonaria*) is a critically endangered bird (BirdLife International 2018) that is range restricted and near-endemic to Rio Branco basin (Laranjeiras et al. 2014). The festive parrot (*Amazona festiva*) is a near-threatened bird (BirdLife International 2016) with a wide distribution throughout flooded forests in the Amazon basin. Both species possesses loud, highly repeated, and unique calls, which should facilitate their automatic classification through template matching techniques (Figure 4.2).

2.3. Acoustic data collection

We used ARBIMON acoustic recorders (Campos-Cerqueira and Aide, 2016) to collect acoustic data during the dry season from November to January 2017. We deployed recorders at 20 replicate sites in each habitat. Recorders were spaced over 500 m apart to minimize overlap in detections across recorders. Previous field tests have demonstrated that the detection range of ARBIMON recorders for several bird species in the Amazon is ~100 m (Campos-Cerqueira et al. 2019). We attached recorders to trees at the height of 1.5 m. Acoustic devices were programmed to record 1 min of audio every 10 min for six days in each sampling site (sample rate = 44.1 kHz; resolution = 16 bit;

format = WAV). Acoustic data collection resulted in 38,400 one-minute recordings (640 hours).

2.4. Manual validation dataset

We manually annotated all calls of the two studied species from one recorder (i.e., site) for each habitat type. The recorder chosen was selected randomly from the 60 sites available for each species. This resulted in a total of 2,044 recordings where the calls of the Rio Branco antbird were manually classified and 2,002 the festive parrot were manually classified. We used the Audacity software (Audacity Team, 2019) to visualize spectrograms and listen to the recordings to build this dataset. We compared the calls and spectrograms of our manual validation dataset with the recordings and spectrograms available and annotated at the Xeno-Canto database to reduce possible errors in the manual classification.

2.5. Template selection

The most critical part in building template-based automatic detectors is the construction of representative templates for the automatic classification process (Ducretet et al., 2020). Templates should be representative of the call of interest and also the soundscapes in which they are embedded (Katz et al., 2016). A good template maximizes detections and minimizes false positives. We selected high quality recordings of both species to build the templates. For the Rio Branco antbird, we chose male calls described as “hitch-coks” notes while for the festive parrot individual “screeches” notes were selected that are commonly given when this animal is perched. We built a total of five templates for each species (Figure 4.3). Templates were created with the R package

MonitoR (Hafner and Katz, 2018).

2.6. Template matching

The automatic detection consisted of a comparison of the spectral and temporal features of the template (Table 4.1 and 4.2) with the recording at different time lags (t) through a non-overlapping moving window (S). The comparison between template (k) and recording was achieved with a cross-correlation at each time lag where both templates and recordings were converted to a short-term Fourier transform with a Hanning window size of 512 samples. The implementation of this workflow in the MonitoR package is based on the following equation (Mellinger and Clark, 1997):

$$d(t) = \sum_{t1} \sum_f S(t + t1, f)k(t1, f)$$

Where $d(t)$ is the detection score at each time t , S is the spectrogram in which signals will be detected through each time interval $t + t1$ and frequencies f , and k is the template kernel used for the detection.

Detection scores may vary from -1 to 1, where 1 indicates a perfect correlation between templates and sound events (peaks). A threshold of 0.4 was selected to filter detections with low similarities with the templates (false positives).

2.7. Evaluation of automatic detection system

We evaluated the automatic detection systems by matching the ground truth dataset (manual classification) with the predictions (automatic classification). We obtained a confusion matrix with four categories of detections: true positive (TP), false

negative (FN), false positive (FP) and true negative (TN). We calculated two metrics to evaluate the detectors, the recall ($TP / (TP+FN)$) and precision ($TP / (TP+FP)$). The recall rate indicates how well the segmentation algorithm detects sounds of interest, and the precision indicates how reliable the detector is. Recall and precision are inverse related to each other, thus is possible to gain recall at the cost of losing precision, and vice versa (Priyadarshani et al., 2018).

3. RESULTS

3.1. Performance of classifiers

The first automatic system built to detect calls of the Rio Branco antbird correctly classified 32 recordings with calls (TP), correctly classified 1930 recordings as sounds produced by other sound sources (TN), misclassified 82 recordings as containing the call (FP), and did not miss any calls in the validation dataset (FN). The automatic system therefore detected 100% of the labeled vocalizations, with a recall of 1 and overall precision of 28% (Table 4.1). Precision was much higher though if we consider only the island habitats where this species is believed to be more common. In fact, in our study sites, we have not detected any call of this species in the other two habitats, which reinforces the high degree of specialization of this species to riverine islands in the lower Rio Branco basin. In addition, if we consider only the diurnal recordings (the time that this species is expected to call), the number of false positives will be likely much smaller and therefore the precision of the detector would also increase. Common false positives were due to other species calling at the same frequency and tree branches breaking, which produces an acoustic signature with a wide frequency range.

The second automatic system built to detect calls of the festive parrot correctly classified 45 recordings with calls (TP), correctly classified 1827 recordings as sounds produced by other sound sources (TN), misclassified 130 recordings as containing the call (FP), and did not miss any calls in the validation dataset (FN). The automatic system therefore detected 100% of the labeled vocalizations, with a recall of 1 and overall precision of 25% (Table 4.2). Similar to the first classifier, precision was much higher if we consider only the island habitats where this species seems to be more common likely due closer river proximity. In addition, also similar to the first classifier, if we consider only the diurnal recordings (the time that this species is expected to call), the number of false positives will be likely much smaller and therefore the precision of the detector would also increase. Common false positives were due to other species calling at the same frequency range.

3.2. Total number of detections

Across the whole dataset, the classifier of the Rio Branco antbird detected a total of 1787 recordings with at least one call while for the festive parrot a total of 3210 recordings with at least one call were detected. One recording could and often contained more than one call (detections) of the targeted species, but we only considered the most salient calls (highest cross-correlation score) in each recording.

4. CONCLUSIONS

The cross-correlation template matching technique to identify automatically the calls of two species of conservation concern performed quite well in our study sites. The habitats surveyed have a high diversity of species (e.g., over 500 birds species) that

introduces substantial background noise and makes automatic detection of calls a challenging task. A large effort has been made to develop automatic detectors using multiple approaches (LeBien et al., 2020); however, most of these approaches are not accessible through open source software and they require considerable coding experience (Ducrettet et al., 2020). In addition, most of these automatic detectors were tested in controlled settings and species-poor sites (Priyadarshani et al., 2018). Here, we demonstrated the potential of a simpler template matching technique that does not require as much expertise to use and is able to achieve results similar to other more complicated and expensive methods (Campos-Cerqueira and Aide, 2016). These are exciting results because it opens more opportunities for collaboration between scientists and the organizations (private and public sectors) trying to implement biodiversity monitoring techniques in the Amazon and other tropical areas.

We will expand this methodology for automatic detection to several other species of conservation concern in the Rio Branco basin. Our ultimate goal is to have high performing automatic detectors able to identify threatened, cryptic, and indicator species enabling their efficient acoustic monitoring in flooded forests habitats of Rio Branco basin. In addition, because more than 300 dams are planned for the Amazon basin that will likely disrupt many ecosystems (Gerlak et al., 2020), these methods can be potentially expanded to other Amazonian rivers in jeopardy. When our dataset with several species is finalized, we will convert false positives to true negatives and fit occupancy models for each of the species studied that can account for imperfect detection (Campos-Cerqueira and Aide, 2016). We will use detailed site-level vegetative covariates collected during the acoustic surveys with the models to better understand the drivers of

occupancy for the studied species, which is currently unknown. For example, it is unknown what the habitat preferences of the two species here studied are, and this information is critical for species management (Vickery et al., 2001). Ultimately, our work hopes to enable efficient and reliable monitoring of animals in an ecological and evolutionary hotspot that is high danger by infra-structure development (Naka et al. 2020).

REFERENCES

- Aide, T. M., Corrada-Bravo, C., Campos-Cerqueira, M., Milan, C., Vega, G., & Alvarez, R. (2013). Real-time bioacoustics monitoring and automated species identification. *PeerJ*, *1*, e103.
- Audacity Team (2019). Audacity software. Pittsburg, PA.
- Balantic, C., & Donovan, T. (2020). AMMonitor: Remote monitoring of biodiversity in an adaptive framework with r. *Methods in Ecology and Evolution*.
- BirdLife International. 2016. *Amazona festiva*. *The IUCN Red List of Threatened Species* 2016: e.T22727626A94954576. <https://dx.doi.org/10.2305/IUCN.UK.2016-3.RLTS.T22727626A94954576.en>. Assessed 20 January 2020
- BirdLife International. 2018. *Cercomacra carbonaria* (amended version of 2017 assessment). *The IUCN Red List of Threatened Species* 2018: e.T22701686A128257225. <https://dx.doi.org/10.2305/IUCN.UK.2017-1.RLTS.T22701686A128257225.en>. Assessed 20 January 2020
- Burivalova, Z., Wahyudi, B., Boucher, T. M., Ellis, P., Truskinger, A., Towsey, M., ... & Game, E. T. (2019). Using soundscapes to investigate homogenization of

- tropical forest diversity in selectively logged forests. *Journal of Applied Ecology*, 56(11), 2493-2504.
- Campos-Cerqueira, M., & Aide, T. M. (2016). Improving distribution data of threatened species by combining acoustic monitoring and occupancy modelling. *Methods in Ecology and Evolution*, 7(11), 1340-1348.
- Campos-Cerqueira, M., Mena, J. L., Tejeda-Gómez, V., Aguilar-Amuchastegui, N., Gutierrez, N., & Aide, T. M. (2019). How does FSC forest certification affect the acoustically active fauna in Madre de Dios, Peru?. *Remote Sensing in Ecology and Conservation*.
- Deichmann, J. L., Acevedo-Charry, O., Barclay, L., Burivalova, Z., Campos-Cerqueira, M., d'Horta, F., ... & Linke, S. (2018). It's time to listen: there is much to be learned from the sounds of tropical ecosystems. *Biotropica*, 50(5), 713-718.
- Ducrettet, M., Forget, P. M., Ulloa, J. S., Yguel, B., Gaucher, P., Princé, K., ... & Sueur, J. (2020). Monitoring canopy bird activity in disturbed landscapes with automatic recorders: A case study in the tropics. *Biological Conservation*, 245, 108574.
- ICMBio. Instituto Chico Mendes de Conservação da Biodiversidade (2014) Plano de Manejo do Parque Nacional do Viruá. ICMBio, Boa Vista, Roraima.
http://www.icmbio.gov.br/portal/images/stories/docs-planos-de-manejo/parna_virua_pm_diag1.pdf Assessed 10 January 2020
- Hafner, S. D., & Katz, J. (2018). A short introduction to acoustic template matching with monitoR.
- Hill, A. P., Prince, P., Piña Covarrubias, E., Doncaster, C. P., Snaddon, J. L., & Rogers,

- A. (2018). AudioMoth: Evaluation of a smart open acoustic device for monitoring biodiversity and the environment. *Methods in Ecology and Evolution*, 9(5), 1199-1211.
- Ferreira, E., Zuanon, J., Forsberg, B., Goulding, M., & Briglia-Ferreira, S. R. (2007). *Rio Branco: peixes, ecologia e conservação de Roraima*. Amazon Conservation Association.
- Gerlak, A. K., Saguier, M., Mills-Novoa, M., Fearnside, P. M., & Albrecht, T. R. (2020). Dams, Chinese investments, and EIAs: A race to the bottom in South America?. *Ambio*, 49(1), 156-164.
- Naka, L. N., Laranjeiras, T. O., Lima, G. R., Plaskiewicz, A. C., Mariz, D., Da Costa, B. M., ... & Cohn-Haft, M. (2020). The Avifauna of the Rio Branco, an Amazonian evolutionary and ecological hotspot in peril. *Bird Conservation International*, 30(1), 21-39.
- Katz, J., Hafner, S. D., & Donovan, T. (2016). Tools for automated acoustic monitoring within the R package monitoR. *Bioacoustics*, 25(2), 197-210.
- Laranjeiras, T. O., Naka, L. N., Bechtoldt, C. L., da Costa, T. V. V., Andretti, C. B., Cerqueira, M. C., ... & Pacheco, A. M. F. (2014). The avifauna of Virua National Park, Roraima, reveals megadiversity in northern Amazonia. *Ornithology Research*, 22(2), 138-171.
- LeBien J, Zhong M, Campos-Cerqueira M, Velez JP, Dodhia R, Ferres JL, Aide TM. A pipeline for identification of bird and frog species in tropical soundscape recordings using a convolutional neural network. *Ecological Informatics*. 2020 Jun 8:101113.

- Mellinger, D. K., & Clark, C. W. (1997). Methods for automatic detection of mysticete sounds. *Marine & Freshwater Behaviour & Phy*, 29(1-4), 163-181.
- Pérez-Granados, C., Schuchmann, K. L., & Marques, M. I. (2020). Vocal behavior of the Undulated Tinamou (*Crypturellus undulatus*) over an annual cycle in the Brazilian Pantanal: New ecological information. *Biotropica*, 52(1), 165-171.
- Priyadarshani, N., Marsland, S., & Castro, I. (2018). Automated birdsong recognition in complex acoustic environments: a review. *Journal of Avian Biology*, 49(5), jav-01447.
- Piel, A. K. (2018). Temporal patterns of chimpanzee loud calls in the Issa Valley, Tanzania: Evidence of nocturnal acoustic behavior in wild chimpanzees. *American Journal of Physical Anthropology*, 166(3), 530-540.
- Ribeiro, J. W., Sugai, L. S. M., & Campos-Cerqueira, M. (2017). Passive acoustic monitoring as a complementary strategy to assess biodiversity in the Brazilian Amazonia. *Biodiversity and Conservation*, 26(12), 2999-3002.
- Ritter, C. D., McCrate, G., Nilsson, R. H., Fearnside, P. M., Palme, U., & Antonelli, A. (2017). Environmental impact assessment in Brazilian Amazonia: Challenges and prospects to assess biodiversity. *Biological Conservation*, 206, 161-168.
- Sugai, L. S. M., Silva, T. S. F., Ribeiro Jr, J. W., & Llusia, D. (2019). Terrestrial passive acoustic monitoring: review and perspectives. *BioScience*, 69(1), 15-25.
- Vickery, J. A., Tallwin, J. R., Feber, R. E., Asteraki, E. J., Atkinson, P. W., Fuller, R. J., & Brown, V. K. (2001). The management of lowland neutral grasslands in Britain: effects of agricultural practices on birds and their food resources. *Journal of Applied Ecology*, 38(3), 647-664.

Zhong, M., LeBien, J., Campos-Cerqueira, M., Dodhia, R., Ferres, J. L., Velez, J. P., & Aide, T. M. (2020). Multispecies bioacoustic classification using transfer learning of deep convolutional neural networks with pseudo-labeling. *Applied Acoustics*, 166, 107375.

TABLES AND FIGURES

Table 4.1. Temporal and spectral characteristics of templates from Rio Branco antbird used in the classification.

Template	Lower frequency	Upper frequency	Lower amp	Upper amp	duration	points
1	0.947	2.584	-62.61	-8.88	0.441	780
2	1.206	2.584	-52.28	-3.66	0.325	493
3	1.12	2.584	-58.9	-1.24	0.186	306
4	1.034	2.498	-52.79	-2.21	0.151	252
5	1.034	3.445	-73.94	-0.23	0.325	841

Table 4.2. Temporal and spectral characteristics of templates of festive parrot used in the classification.

Template	Lower frequency	Upper frequency	Lower amp	Upper amp	Duration	Points
1	1.034	4.479	-67.79	-4.55	0.267	984
2	0.861	4.737	-72.76	-6.74	0.488	1978
3	0.947	4.996	-74.66	-12.39	0.418	1776
4	1.034	4.91	-52.72	-2.01	0.36	1472
5	0.861	4.479	-69.19	-11.07	0.267	1032

Table 4.3. Performance of Rio Branco antbird classifier obtained by comparing the manual validation dataset with the predictions from the classifier.

Habitat	TP	TN	FP	FN	Recall	Precision
Igapó	0	662	18	0	0	0
Island	32	580	46	0	1	0.41
Várzea	0	688	18	0	0	0
Total	32	1930	82	0	1	0.28

Table 4.4. Performance of Festive parrot classifier obtained by comparing the manual validation dataset with the predictions from the classifier.

Habitat	TP	TN	FP	FN	Recall	Precision
Igapó	3	639	29	0	1	0.09
Island	41	577	46	0	1	0.47
Várzea	1	611	55	0	1	0.01
Total	45	1827	130	0	1	0.25

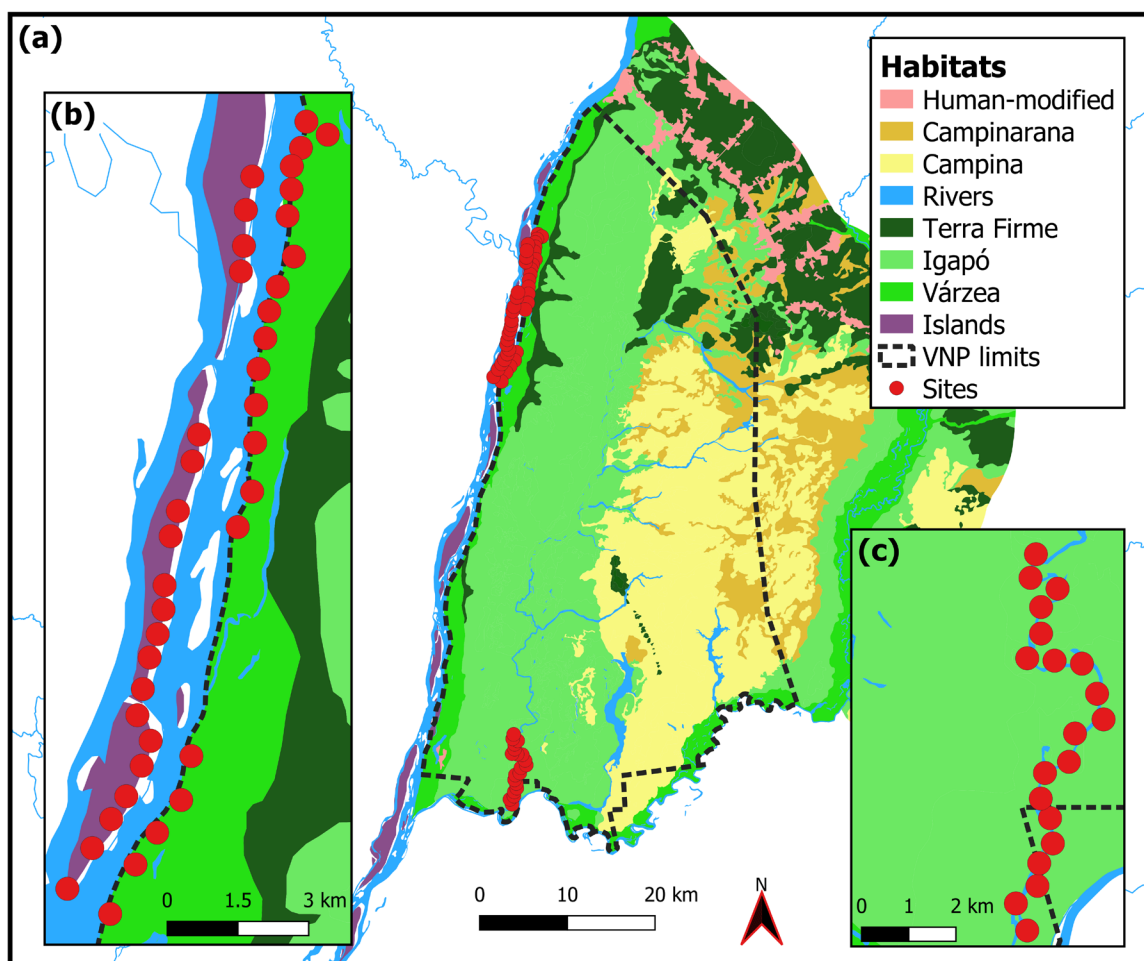


Fig. 4.1. Habitat types at Viruá National Park (a), expanded view to show details of islands and várzea sites, and expanded view on igapós sites (c).

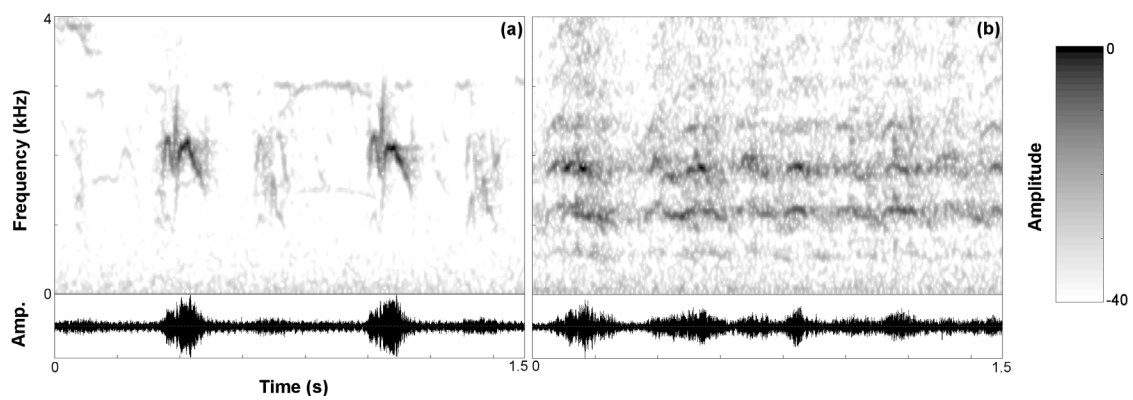


Fig. 4.2. Spectrograms of Rio Branco antbird calls (a) and festive parrot calls (b).

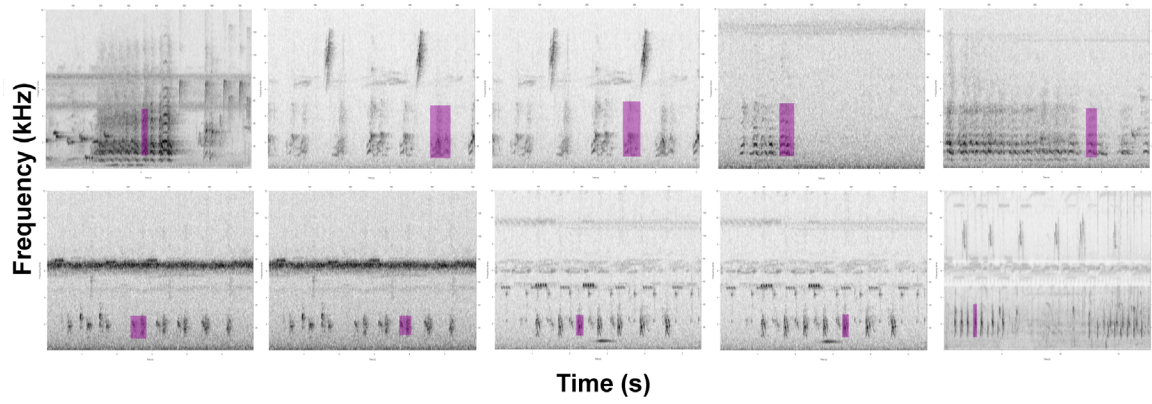


Fig. 4.3. Templates used for the automatic classification of festive parrot calls (top spectrograms) and Rio Branco antbird calls (bottom spectrograms). Purple color shows the selected part of the calls used to build the templates for classification.

CHAPTER 5

CONCLUSIONS

Acoustic metrics and habitat changes

In Chapter 2, I show that acoustic metrics can predict habitat types and are strongly related to changes in vegetation structure. This was the most comprehensive tests to date of two major assumptions of the ecoacoustics field, that habitats have unique acoustic signatures and that soundscapes are intrinsically linked to vegetation structure. Our findings help advance the field by providing unequivocal evidence that soundscapes are strongly connected to habitat changes. More importantly, our findings seem to follow the general relationship between species richness and habitat heterogeneity in ecology. In other words, soundscapes rich in frequencies and calling species were linked to high layer complexity while soundscapes poor in frequencies and calling species were linked to degraded and less complex habitats. These findings highlight that soundscapes and acoustic indices are effective methods for multi-taxa animal surveys in the Amazon and likely beyond. Future studies could focus at potential synergies among different remote sensing methods. For example, while satellite imagery provides us with a richness of information about vegetation cover, they are unable to survey the fauna directly. Satellite imagery and airborne surveys could be used to retrieve essential vegetation variables and ecoacoustic surveys reliable fauna estimates which then could be combined to build rigorous and verifiable models for more effective and routine biodiversity assessments.

Passive acoustic monitoring and the nocturnal ecology

In Chapter 3, I show that the loud calls of howler monkeys, a key component of

neotropical soundscapes vary in structure accordingly to the time of day. Our findings points for a possible different function between nocturnal and diurnal loud calls in howler monkeys, but more tests should be conducted. Nocturnal ecology is understudied but likely greatly differs from diurnal ecology; this may be simply because ecological conditions and pressures greatly differ between these periods. There is a need for more studies exploring the nocturnal ecology of all animal taxa and in this chapter we show that passive acoustic monitoring coupled with aural identification of calls are an efficient methodology to retrieve information about vocalizing fauna during 24 h periods and is able to advance our understanding of their behavioral ecology. Future studies could employ passive acoustic monitoring and aural identification to unveil the nocturnal ecology and 24-h cycles of activity of other animals. These methods coupled with innovative playback experiments, such as the one reported Chapter 3, could greatly advance our understanding of animal behavioral ecology. In addition, nocturnal soundscapes are increasingly threatened by noise, light, and other human disturbances (Gaston, 2019); therefore the study of the nocturnal activity of animals could also help with their conservation in changing environments.

Automatic classification of threatened species

In Chapter 4, I show that passive acoustic monitoring and an automatic classification technique is able to produce reliable information about the presence or absence of calling species. The method presented can be easily expanded to other sound-producing species in the Amazon region that are poorly studied and are threatened by a myriad of development projects. More importantly, the output data of species presence or

absence can be used with statistical techniques that account for imperfect detection. This would potentially allow for more effective management of threatened and cryptic species that are difficult to detect by other traditional survey methods (Robinson et al., 2018). Ecoacoustic surveys, like any other animal surveys, suffer from imperfect detection. But only recently has a method to account for imperfect detection in environmental recordings been proposed (see Rappaport et al., 2020). Future work will be focused on expanding the automatic detector implemented in Chapter 4 to other species of conservation concern to model their occupancy in the Rio Branco basin. This will likely help guide their conservation in the face of dam construction and other human threats to their survival.

REFERENCES

- Gaston, K. J. (2019). Nighttime ecology: the “nocturnal problem” revisited. *The American Naturalist*, 193(4), 481-502. <https://doi.org/10.1086/702250>
- Rappaport, D. I., Royle, J. A., & Morton, D. C. (2020). Acoustic space occupancy: Combining ecoacoustics and lidar to model biodiversity variation and detection bias across heterogeneous landscapes. *Ecological Indicators*, 113, 106172. <https://doi.org/10.1016/j.ecolind.2020.106172>
- Robinson, W. D., Lees, A. C., & Blake, J. G. (2018). Surveying tropical birds is much harder than you think: a primer of best practices. *Biotropica*, 50(6), 846-849. <https://doi.org/10.1111/btp.12608>

APPENDICES

APPENDIX A

CHAPTER 2 SUPPLEMENTAL INFORMATION

Table S2.1. Species richness of different taxa associated with each habitat type in the Viruá National Park, Roraima, Brazil. Expected richness (low, medium, high) are based on information about each taxon found in the management plan of the park.

Habitat	Trees	Birds	Amphibians/Insects/Mammals
Burned campina	0	Low	Low
Campina	0	88	Low
Campinarana	60	130	Medium
Igapó	69	144	Medium
Island	Medium	Medium	Medium
Pasture	0	89	Low
Terra-firme	98	240	High
Várzea	69	276	High

ICMBio. Instituto Chico Mendes de Conservação da Biodiversidade (2014). Plano de Manejo do Parque Nacional do Viruá. ICMBio, Boa Vista, Roraima.

Laranjeiras, T. O., Naka, L. N., Bechtoldt, C. L., da Costa, T. V. V., Andretti, C. B., Cerqueira, M. C., ... & Pacheco, A. M. F. (2014). The avifauna of Viruá National Park, Roraima, reveals megadiversity in northern Amazonia. *Ornithology Research*, 22(2), 138-171.

Table S2.2. Dates that the acoustic surveys were conducted and a description of the eight habitats studied in the Viruá National Park, Roraima, Brazil.

Habitat	Survey period	Description
Burned campina	12/08/16 – 12/13/16	Grassland that was first affected by wildfire outbreaks nine months prior we conducted this study. It was showing signs of recovering (re-sprout) when we surveyed it.
Campina	12/29/16 – 01/03/17	Grassland located in white sand soils that are poorly drained. Possess low species richness but high endemism rates and mostly no trees or shrubs (ICMbio, 2014; Laranjeiras et al., 2014).
Campinarana	01/09/17 – 01/14/17	Located in white sand soils that are poorly drained and with a thick leaf litter layer often exceeding 20 cm. Possess small to medium trees and moderate species richness with high rates of endemism (ICMbio, 2014; Laranjeiras et al., 2014).
Igapó	01/29/17 – 02/03/17	Seasonally flooded swamp forest drained by a nutrient poor black-water river (“Rio Iruá”) with medium to large trees and moderate species richness (Laranjeiras et al., 2014; Montero et al., 2014).
Island	01/17/17 – 01/22/17	Isolated by riverways of “Rio Branco” in the west portion of the park, this habitat is characterized by patches of várzea forests along the river and with large trees present (ICMbio, 2014).
Pasture	02/07/17 – 02/12/17	Terra-firme forests that were cleared for cattle ranching (ICMbio, 2014), comprises the smallest habitat within the park.
Terra-firme	11/22/16 – 11/27/16	Moist broadleaf forest located in the north section of the park on higher elevations than surrounding lands with large trees and high species richness (De Oliveira and Mori, 1999; ICMbio, 2014).
Várzea	11/29/16 – 12/04/16	Floodplain forest drained by a nutrient rich white-water river (“Rio Branco”) in the west portion of the park, possess large trees and high species richness (ICMbio, 2014; Wittmann et al., 2004).

ICMbio. Instituto Chico Mendes de Conservação da Biodiversidade (2014). Plano de Manejo do Parque Nacional do Viruá. ICMbio, Boa Vista, Roraima.

De Oliveira, A. A., & Mori, S. A. (1999). A central Amazonian terra firme forest. I. High tree species richness on poor soils. *Biodiversity & Conservation*, 8(9), 1219-1244.

Laranjeiras, T. O., Naka, L. N., Bechtoldt, C. L., da Costa, T. V. V., Andretti, C. B., Cerqueira, M. C., ... & Pacheco, A. M. F. (2014). The avifauna of Viruá National Park, Roraima, reveals megadiversity in northern Amazonia. *Ornithology Research*, 22(2), 138-171.

Montero, J. C., Piedade, M. T. F., & Wittmann, F. (2014). Floristic variation across 600 km of inundation forests (Igapó) along the Negro River, Central Amazonia. *Hydrobiologia*, 729(1), 229-246.

Wittmann, F., Junk, W. J., & Piedade, M. T. (2004). The várzea forests in Amazonia: flooding and the highly dynamic geomorphology interact with natural forest succession. *Forest ecology and Management*, 196(2-3), 199-212.

Table S2.3. Results of a permutational multivariate analysis of variance (PERMANOVA) testing if nocturnal soundscapes were different than diurnal soundscapes and the influence of habitat type on mean values of 13 acoustic indices.

Effect	d.f.	Sum of sq.	R^2	F-value	p-value
Period	1	13.50	0.28	2493.7	<0.001
Habitat	7	15.98	0.33	421.6	<0.001
Residual	3447	18.67	0.38		
Total	3455	48.16	1.00		

Table S2.4. Top four performing models for acoustic indices response variables based on AICc model selection in the Viruá National Park, Roraima, Brazil.

Index	Model^a	logLik	AICc	ΔAICc	d.f.	Weight
ACI	Full Model	-534.1	1085.3	0.0	8	0.160
	Canopy Cover + Litter Depth + Trees (large) + Trees (small)	-535.4	1085.7	0.3	7	0.135
	Canopy Cover + Shrub Cover + Trees (large) + Trees (small)	-535.5	1085.9	0.5	7	0.122
	Canopy Cover + Litter Depth + Shrub Cover + Trees (large)	-535.5	1086.0	0.6	7	0.116
AEI	Canopy Cover	172.3	-336.3	0.0	4	0.918
	Canopy Cover + Shrub Cover	170.4	-330.5	5.8	5	0.050
	Canopy Cover + Litter Depth	169.1	-327.8	8.4	5	0.013
	Canopy Cover + Trees (small)	168.8	-327.3	8.9	5	0.010
BIO	Canopy Cover	-184.9	378.1	0.0	4	0.513
	Canopy Cover + Shrub Cover	-185.5	381.6	3.5	5	0.088
	Canopy Cover + Trees (small)	-185.8	382.1	4.0	5	0.069
	Null Model	-188.0	382.3	4.1	3	0.064
CENT	Full Model	-1051.0	2119.3	0.0	8	0.964
	Canopy Cover + Litter Depth + Shrub Cover + Trees (large)	-1056.3	2127.5	8.2	7	0.016
	Canopy Cover + Litter Depth + Trees (large) + Trees (small)	-1056.6	2128.2	8.9	7	0.011
	Canopy Cover + Litter Depth + Shrub Cover + Trees	-1057.7	2130.3	11.0	7	0.004

	(small)					
DF	Full Model	-1112.8	2242.7	0.0	8	0.962
	Canopy Cover + Litter Depth + Shrub Cover + Trees (small)	-1118.2	2251.3	8.6	7	0.013
	Canopy Cover + Litter Depth + Shrub Cover + Trees (large)	-1118.3	2251.6	8.8	7	0.011
	Canopy Cover + Shrub Cover + Trees (large) + Trees (small)	-1118.9	2252.6	9.9	7	0.007
FLAT	Null Model	209.7	-413.4	0.0	3	0.592
	Canopy Cover	210.3	-412.4	0.9	4	0.363
	Trees (large)	206.8	-405.4	8.0	4	0.011
	Litter Depth	206.4	-404.6	8.7	4	0.007
FQ	Full Model	-1059.6	2136.4	0.0	8	0.944
	Canopy Cover + Litter Depth + Shrub Cover + Trees (small)	-1064.7	2144.4	8.0	7	0.017
	Canopy Cover + Litter Depth + Trees (large) + Trees (small)	-1064.8	2144.6	8.2	7	0.016
	Canopy Cover + Litter Depth + Shrub Cover + Trees (large)	-1065.0	2144.9	8.5	7	0.013
H	Canopy Cover	284.3	-560.4	0.0	4	0.981
	Canopy Cover + Shrub Cover	280.4	-550.5	9.9	5	0.007
	Canopy Cover + Trees (small)	279.9	-549.5	10.9	5	0.004
	Canopy Cover + Litter Depth	279.8	-549.3	11.1	5	0.004
KURT	Canopy Cover + Shrub	-616.0	1246.9	0.0	7	0.549

	Cover + Trees (large) + Trees (small)					
	Canopy Cover + Shrub Cover + Trees (large)	-618.2	1249.2	2.2	6	0.174
	Canopy Cover + Trees (large) + Trees (small)	-618.4	1249.6	2.7	6	0.141
	Canopy Cover + Shrub Cover + Trees (small)	-619.3	1251.2	4.3	6	0.063
NDSI	Canopy Cover	125.0	-241.9	0.0	4	0.757
	Null Model	122.3	-238.5	3.3	3	0.142
	Canopy Cover + Litter Depth	122.9	-235.5	6.4	5	0.031
	Trees (small)	121.5	-234.7	7.1	4	0.021
SD	Full Model	-1024.7	2066.7	0.0	8	0.933
	Canopy Cover + Shrub Cover + Trees (large) + Trees (small)	-1029.8	2074.5	7.8	7	0.019
	Canopy Cover + Litter Depth + Trees (large) + Trees (small)	-1029.8	2074.6	7.8	7	0.018
	Canopy Cover + Litter Depth + Shrub Cover + Trees (small)	-1030.2	2075.3	8.6	7	0.012
SKEW	Canopy Cover	-233.9	476.2	0.0	4	0.435
	Canopy Cover + Trees (large)	-233.7	477.9	1.6	5	0.187
	Canopy Cover + Trees (small)	-233.8	478.1	1.8	5	0.169
	Canopy Cover + Shrub Cover	-234.4	479.3	3.1	5	0.090

TQ	Full Model	-1159.6	2336.3	0.0	8	0.980
	Canopy Cover + Litter Depth + Shrub Cover + Trees (large)	-1165.6	2346.1	9.8	7	0.007
	Canopy Cover + Litter Depth + Trees (large) + Trees (small)	-1165.7	2346.3	10.0	7	0.006
	Canopy Cover + Shrub Cover + Trees (large) + Trees (small)	-1166.1	2347.1	10.8	7	0.004

^aBolding indicate top models with $\Delta AIC < 2$.

Table S2.5. Fixed effects of the top-performing models with $\Delta\text{AICc} < 2$ on acoustic indices.

Model	Effect	Estimate	SE	d.f.	t-value	p-value ^a	R ²
ACI Model 1	Intercept	968.53	2.32	130	415.94	< 0.001	0.33
	Canopy Cover	-6.17	2.36	130	-2.60	0.010	0.11
	Litter Depth	-0.79	1.36	130	-0.58	0.560	0.00
	Shrub Cover	-0.00	1.46	130	-0.00	0.998	0.00
	Trees (large)	-2.91	1.31	130	-2.21	0.028	0.03
	Trees (small)	0.54	1.60	130	0.33	0.736	0.00
ACI Model 2	Intercept	968.53	2.27	131	426.40	< 0.001	0.33
	Canopy Cover	-6.23	2.31	131	-2.70	0.007	0.12
	Litter Depth	-0.80	1.35	131	-0.59	0.553	0.00
	Trees (large)	-2.91	1.30	131	-2.22	0.028	0.03
	Trees (small)	0.57	1.59	131	0.36	0.718	0.00
	ACI Model 3	Intercept	968.53	2.34	131	412.80	< 0.001
Canopy Cover		-6.29	2.35	131	-2.66	0.008	0.12
Shrub Cover		-0.04	1.46	131	-0.03	0.973	0.00
Trees (large)		-2.99	1.30	131	-2.28	0.023	0.03
Trees (small)		0.27	1.54	131	0.17	0.860	0.00
ACI Model 4		Intercept	968.52	2.35	131	411.04	< 0.001
	Canopy Cover	-5.82	2.18	131	-2.66	0.008	0.10
	Litter Depth	-0.67	1.30	131	-0.51	0.607	0.00
	Shrub Cover	0.00	1.46	131	0.00	0.999	0.00
	Trees (large)	-3.01	1.28	131	-2.35	0.019	0.03
	AEI Model 1	Intercept	0.25	0.01	134	13.74	< 0.001
Canopy		-0.17	0.01	134	-12.17	< 0.001	0.81

	Cover						
BIO Model 1	Intercept	4.42	0.14	134	30.59	< 0.001	0.19
	Canopy	0.44	0.13	134	3.37	0.001	0.19
	Cover						
CENT Model 1	Intercept	5489.98	236.48	130	23.21	< 0.001	0.26
	Canopy	366.15	144.43	130	2.53	0.012	0.09
	Cover						
	Litter Depth	108.98	58.54	130	1.86	0.064	0.01
	Shrub	-65.28	70.56	130	-0.92	0.356	0.00
	Cover						
	Trees (large)	103.76	56.29	130	1.84	0.067	0.01
	Trees (small)	-11.97	75.37	130	-0.15	0.874	0.00
DF Model 1	Intercept	1221.14	118.67	130	10.29	< 0.001	0.21
	Canopy	269.75	135.39	130	1.99	0.048	0.05
	Cover						
	Litter Depth	104.65	92.72	130	1.12	0.261	0.00
	Shrub	140.35	92.08	130	1.52	0.129	0.02
	Cover						
	Trees (large)	23.68	89.03	130	0.26	0.790	0.00
	Trees (small)	-19.06	103.06	130	-0.18	0.853	0.00
FLAT Model 1	Intercept	0.55	0.03	135	14.31	< 0.001	-
FLAT Model 2	Intercept	0.55	0.02	134	23.14	< 0.001	0.24
	Canopy	0.04	0.01	134	3.50	< 0.001	0.24
	Cover						
FQ Model 1	Intercept	1530.47	108.10	130	14.15	< 0.001	0.36
	Canopy	366.90	109.76	130	3.34	0.001	0.17
	Cover						
	Litter Depth	81.36	63.10	130	1.28	0.199	0.01
	Shrub	28.83	68.07	130	0.42	0.672	0.00
	Cover						
	Trees (large)	-27.19	60.96	130	-0.44	0.656	0.00
	Trees (small)	44.06	74.50	130	0.59	0.555	0.00
H Model 1	Intercept	0.80	0.02	134	32.13	< 0.001	0.28
	Canopy	0.04	0.00	134	5.41	< 0.001	0.28
	Cover						
KURT Model 1	Intercept	49.90	1.61	131	30.98	< 0.001	0.46
	Canopy	-18.29	2.25	131	-8.09	< 0.001	0.31
	Cover						
	Shrub	-2.41	1.69	131	-1.42	0.156	0.01

	Cover						
	Trees	3.78	2.11	131	1.79	0.075	0.02
	(large)						
	Trees	-2.20	1.80	131	-1.21	0.225	0.01
	(small)						
NDSI Model 1	Intercept	0.54	0.02	134	23.06	<0.001	0.42
	Canopy	0.09	0.01	134	5.01	<0.001	0.42
	Cover						
SD Model 1	Intercept	5157.27	152.16	130	33.89	<0.001	0.07
	Canopy	125.50	111.40	130	1.12	0.262	0.02
	Cover						
	Litter Depth	34.07	48.66	130	0.70	0.485	0.00
	Shrub	-25.94	57.58	130	-0.45	0.653	0.00
	Cover						
	Trees	54.93	46.88	130	1.17	0.243	0.00
(large)							
SKEW Model 1	Trees	-65.28	61.67	130	-1.05	0.291	0.00
	(small)						
	Intercept	5.95	0.11	134	52.17	<0.001	0.52
SKEW Model 1	Canopy	-1.28	0.11	134	-11.28	<0.001	0.52
	Cover						
SKEW Model 2	Intercept	5.95	0.12	133	48.08	<0.001	0.52
	Canopy	-1.41	0.14	133	-9.44	<0.001	0.44
	Cover						
SKEW Model 2	Trees	0.22	0.13	133	1.63	0.104	0.02
	(large)						
	Intercept	5.96	0.10	133	58.46	<0.001	0.53
SKEW Model 3	Canopy	-1.21	0.11	133	-10.91	<0.001	0.45
	Cover						
SKEW Model 3	Trees	-0.19	0.11	133	-1.71	0.087	0.02
	(small)						
	Intercept	8056.86	367.47	130	21.92	<0.001	0.32
TQ Model 1	Canopy	769.16	287.60	130	2.67	0.008	0.13
	Cover						
TQ Model 1	Litter Depth	162.86	130.44	130	1.24	0.214	0.00
	Shrub	-97.97	152.80	130	-0.64	0.522	0.00
	Cover						
TQ Model 1	Trees	237.66	125.78	130	1.88	0.061	0.01
	(large)						
	Trees	-45.46	163.96	130	-0.27	0.782	0.00
	(small)						

^aStatistically significant effects are in bold.

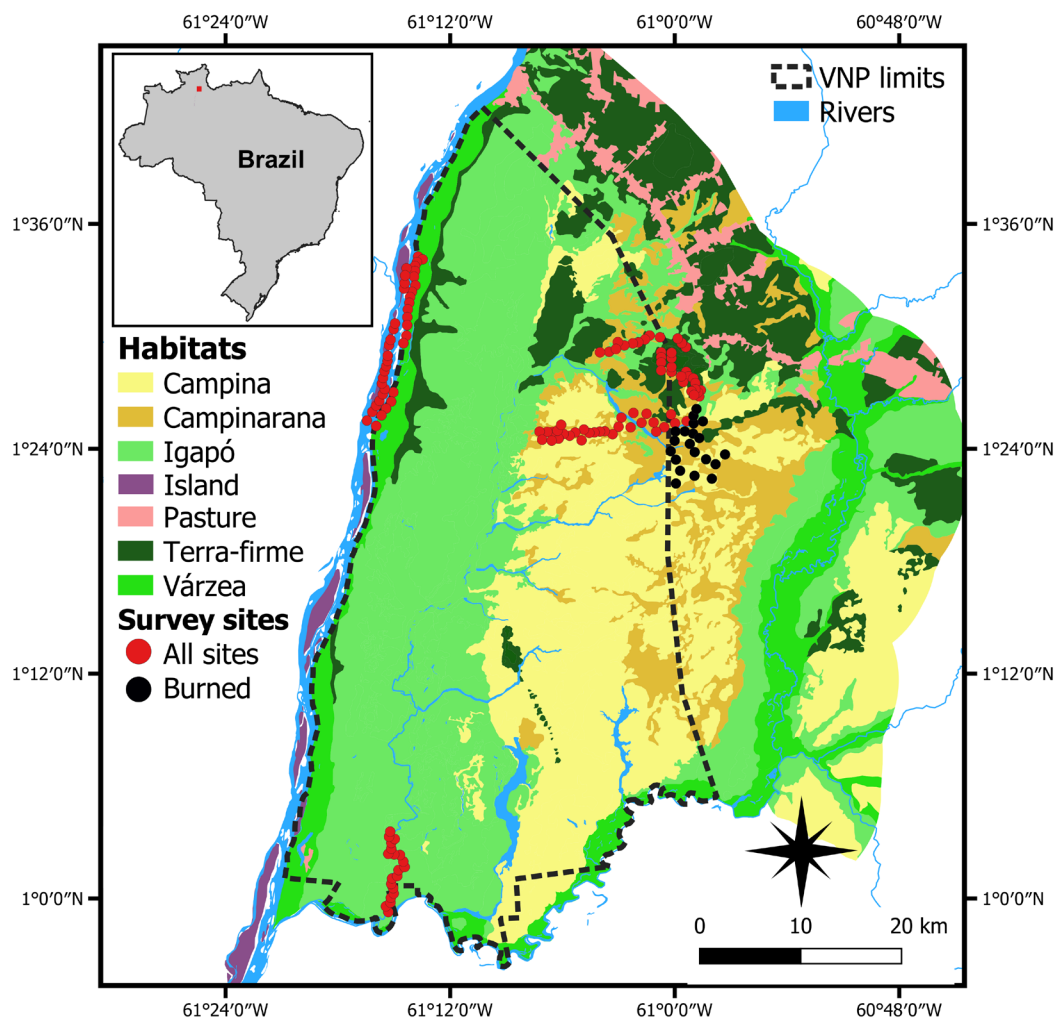


Fig. S2.1. Location of the surveyed sites (143) across the eight habitats studied in the Viruá National (VNP), northern Brazilian Amazon.



Fig. S2.2. Burned campina habitat with scorched shrubs and a *Ciconia maguari*. Photo by Leandro A. Do Nascimento.



Fig. S2.3. Campina habitat. Photo by Leandro A. Do Nascimento.



Fig. S2.4. Campinarana habitat profile in the background. In the front, two *Jabiru mycteria* and the campina transitioning to a campinarana forest formation. Photo by Leandro A. Do Nascimento.



Fig. S2.5. Igapó habitat at “Rio Iruá”. Photo by Leandro A. Do Nascimento.



Fig. S2.6. Island habitat in the “Rio Branco” with an ARBIMON recorder. Photo by Leandro A. Do Nascimento.



Fig. S2.7. Pasture habitat with a *Caracara cheriway*. Photo by Leandro A. Do Nascimento.



Fig. S2.8. Terra-firme habitat. Photo by Leandro A. Do Nascimento.



Fig. S2.9. Várzea habitat profile in the “Rio Branco”. Photo by Leandro A. Do Nascimento.

APPENDIX B

CHAPTER 3 SUPPLEMENTAL INFORMATION

Table S3.1. Composition of the four studied groups of Guianan red howler monkey at Viruá National Park, Roraima, Brazil.

Group name	Male	Female	Juvenile	Infant	Total
Calados	1	4	1	1	7
Pequi	1	3	1	1	6
Tanque	1	3	2	1	7
Viruá	1	3	1	1	6

Table S3.2. Coefficients of linear discriminants of nine acoustic parameters extracted from 51 diurnal and 51 nocturnal howling bouts of Guianan red howlers at Viruá National Park, Roraima, Brazil.

Acoustic parameter	Function 1 coefficient
Dominant frequency	-0.12
Duration	-0.38
First formant	0.47
Harmonic-to-noise ratio	1.20
Highest frequency	0.82
Kurtosis	-1.15
Mean	0.47
Median	0.29
Skewness	1.02

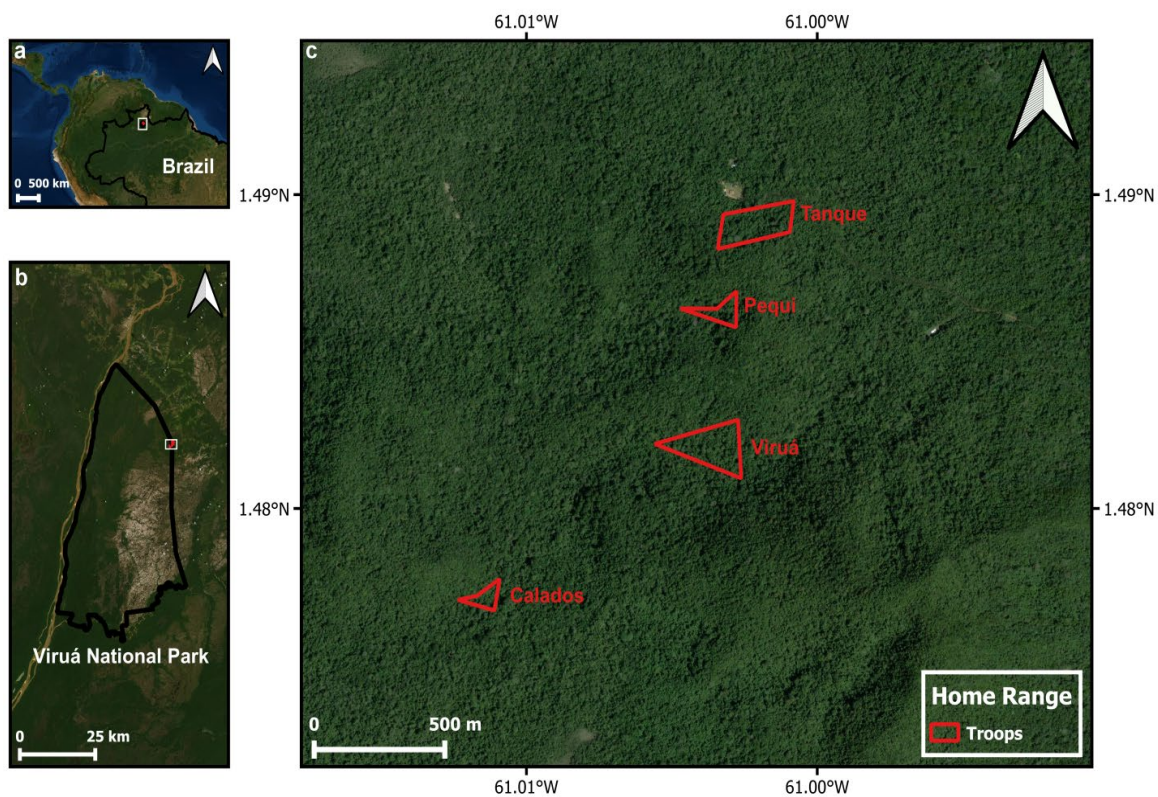


Fig. S3.1. Study area (a) in Brazil and (b) at Viruá National Park and (c) location of the Guianan red howler groups surveyed. Home range was estimated by the locations where the animals were observed moving during the study period.

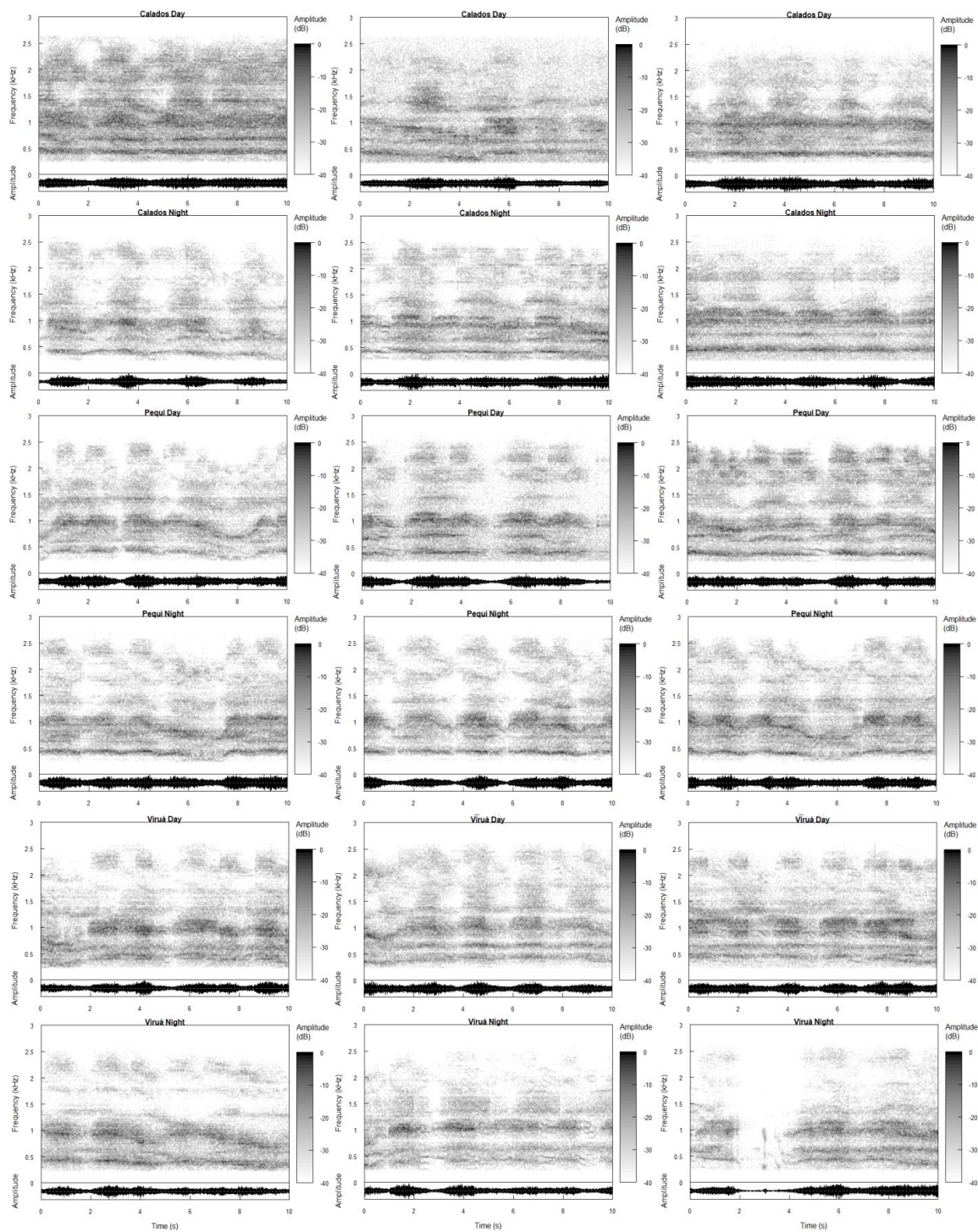


Fig. S3.2. Examples of nocturnal and diurnal howling bouts from each studied group. 10 s clips of different recordings are showed with a windows length of 4012 points.

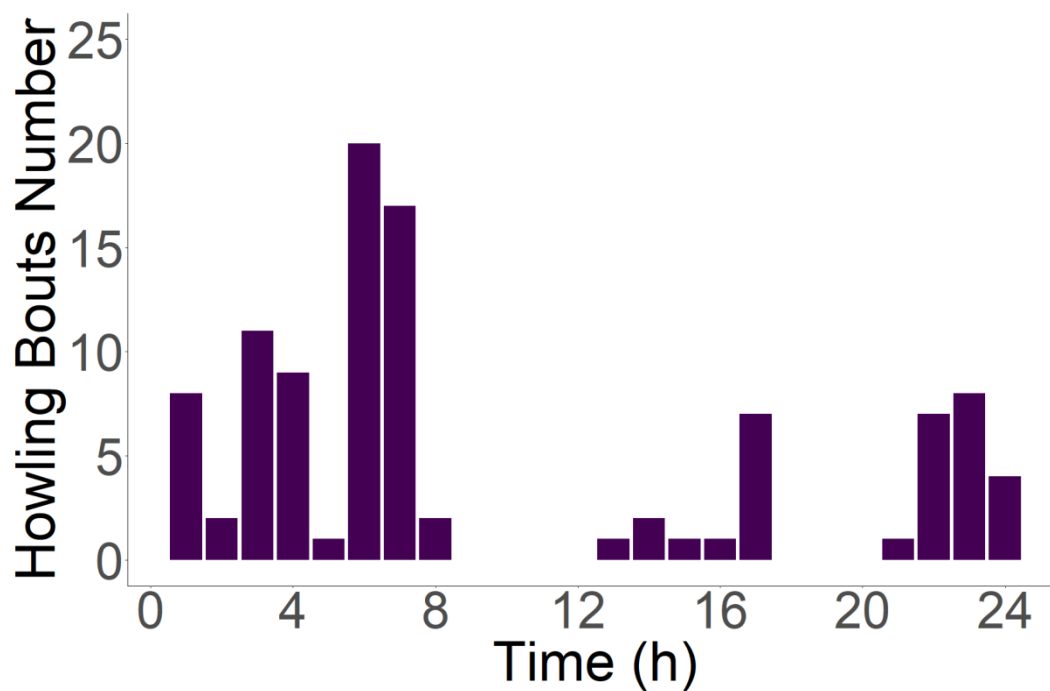


Fig. S3.3. Temporal distribution of the 102 howling bouts used in our analysis. Data were collected from February to April in 2018. Diurnal bouts are graphed from 0600 h to 1800 h while nocturnal bouts from 1800 h to 0600 h. The graph starts at 0100 h and ends at 2400 h.

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- Do Nascimento, L. A., Campos-Cerqueira, M., & Beard, K. H. (2020). Acoustic metrics predict habitat type and vegetation structure in the Amazon. *Ecological Indicators*, 117, 106679. <https://doi.org/10.1016/j.ecolind.2020.106679>
- Do Nascimento, L. A., & Beard, K. (2019). Alpha male Guianan red howler monkey responses to nocturnal and diurnal loud calls. *The Journal of the Acoustical Society of America*, 146(4), 2769-2769. <https://doi.org/10.1121/1.5136591>
- Deichmann, J. L., Acevedo-Charry, O., Barclay, L., Burivalova, Z., Campos-Cerqueira, M., d'Horta, F., ... & Linke, S. (2018). It's time to listen: there is much to be

learned from the sounds of tropical ecosystems. *Biotropica*, 50(5), 713-718.
<https://doi.org/10.1111/btp.12593>

- Lunardi, V. O., Oliveira-Silva, C. C., Nascimento, L. A. D., & Lunardi, D. G. (2013). Synanthropic behavior of the Neotropical palm swift *Tachornis squamata* (Apodiformes: Apodidae) in the Brazilian Caatinga. *Zoologia (Curitiba)*, 30(6), 697-700. <https://doi.org/10.1590/S1984-46702013005000012>

Talks at professional conferences

- Do Nascimento LA, Beard KH. 2020. Vegetation and habitat type are key drivers of soundscape heterogeneity in the Amazon. In: *105th Annual Meeting of the Ecological Society of America*. Salt Lake City-USA.
- Do Nascimento LA, Beard KH. 2019. Alpha male Guianan red howler monkey responses to diurnal and nocturnal loud calls. In: *178th Meeting of the Acoustical Society of America*. San Diego-USA.
- Do Nascimento LA, Beard KH. 2018. Passive acoustic monitoring unveils howler monkeys' vocal repertoire and call functions. In: *LV Annual Meeting of the Association of Tropical Biology and Conservation*. Kuching-Malaysia.
- Do Nascimento LA, Beard KH. 2017. Linking soundscapes patterns to the vegetation structure in the Amazon Rainforest. In: *LIV Annual Meeting of the Association of Tropical Biology and Conservation*. Mérida-México. ***Invited presentation for symposium***
- Do Nascimento LA, Assis AA, Baldauf C. 2015. Socioecological aspects related to the establishment of Furna Feia National Park in Rio Grande do Norte state. In: *XXI Scientific Initiation Seminar*, Mossoró-RN, Federal Rural University of the Semi-Arid (UFERSA).
- Do Nascimento LA, Souza HO, Cavalheiro DO, Wachlevski M. 2014. Diversity and disturbances in the soundscape of Guaribas Biological Reserve, Paraíba state, Brazil. In: *XXXII Ethology Annual Meeting e V Latin American Ethology Symposium*. Mossoró-RN.
- Do Nascimento LA, Loveless EJ, Lima FGF. 2014. Land use change in the Furna Feia National Park. In: *II Ecology Regional Meeting*. Rio Tinto-PB. II Ecology Regional Meeting. Rio Tinto: UFPB, 2014. v. 01. p. 1-4.
- Nascimento LA, Silva EM, Lunardi VO, Lunardi DG. 2012. Roost behavior of the neotropical palm swift *Tachornis squamata* (Aves, Apodidae). In: *XVIII Scientific Initiation Seminar*, Mossoró-RN, Federal Rural University of the Semi-Arid (UFERSA).

Conference proceedings

- Lunardi VO, Oliveira-Silva CC, Nascimento LA, Silva EM, Lunardi DG. 2012. Sinanthropy of neotropical palm swift (*Tachornis squamata*) in the Brazilian semiarid. In: *XXX Ethology Annual Meeting e III Latin American Ethology Symposium*. Ribeirão Preto-SP. *Ethology Journal*, São Paulo, SBE, 11:161-161.

INVITED LECTURES

Bioacoustics methods for data collection and analysis (Guest lecture for Dr. Eric Lamalfa class at Utah State University, 2019)

What is conservation biology? (Guest lecture for Dr. Ekaterina Arshavskaya class at Utah State University, 2019).

Conservation and soundscape ecology in Brazil (Guest lecture for Dr. Ekaterina Arshavskaya class at Utah State University, 2018).

AWARDS / SCHOLARSHIPS / RESEARCH GRANTS

TOTAL: \$271,322

2020 - Utah State University: Teaching Assistant Scholarship (Biology II BIOL 1625) \$8,797

2019 - Seely-Hinckley Scholarship (only eight awards per year for outstanding students) \$12,000

2019 - Robins Award Finalist (most prestigious award at USU) for Graduate Student Teacher of the Year Award

2019 - Quinney College of Natural Resources Graduate Student Teacher of the Year Award

2019 - Elizabeth Bulluck Haderlie Scholarship \$3,500

2019 - Utah State University: Teaching Assistant Scholarship (Biology II BIOL 1625) \$8,625

2019 - Utah State University: Ecology Center Travel Award \$500

2019 - Utah State University: Graduate Student Travel Award \$300

2018 - Utah State University: Teaching Assistant Scholarship (Biology I BIOL 1615) \$8,625

2018 - Rufford Foundation Research Grant \$7,000

2018 - Utah State University: Teaching Assistant Scholarship (Conservation Biology WILD 4600) \$1,760

2018 - Utah State University: Wildland Resources Travel Award \$800

2018 - Utah State University: Ecology Center Travel Award \$1,400

2018 - Utah State University: Graduate Student Travel Award \$400

2016 - Utah State University: GRCO research award \$1,000

2016 - Utah State University: Ecology Center Research Award \$5,000

2016 - Utah State University: Jeb Stuart Scholarship \$1,000

2016 - Utah State University: Teaching Assistant Scholarship (Conservation Biology WILD 4600) \$1,760

2015 - Brazil's National Council for Scientific and Technological Development (CNPq) doctoral's fellowship \$200,000

2014 - Brazil's National Council for Scientific and Technological Development (CNPq): Undergraduate Research Scholarship (PIBIC) \$2,200

2013 - Universidade Federal Rural do Semiárido: Undergraduate Teaching Assistant Scholarship (Landscape Ecology ANI 0655) \$800

2012 - Brazil's Agency for Support and Evaluation of Graduate Education (CAPES): Science Without Borders fellowship (ecology coursework and internship at University of Arizona) \$10,008 plus tuition and living expenses

2011 - Brazil's National Council for Scientific and Technological Development (CNPq): Undergraduate Research Scholarship \$1,400

2011 - Universidade Federal Rural do Semiárido: Undergraduate Research Scholarship \$200

2010 - Brazil's Ministry of Education: Undergraduate Outreach Scholarship (Knowledge Connections Program, Dialogs Between the University and the Local Communities) \$1,980

CONFERENCE ORGANIZATION

“XXXII Ethology Annual Meeting / V Latin American Ethology Symposium”
Responsible mainly for logistics of the Bioacoustics workshop, opening talk by Daniel Blumstein, two sections of oral talks. 2014.
(<http://www.etologiabrasil.org.br/xxxiiiae/index.html>)
Contact: Professor Michael Hrcir; email: michaelufersa@ufersa.edu.br

“Universidade Federal Rural do Semiárido: Rio+20 and environmental movements”

Head of the organizer seminar given by professor Vera Lucia Imperatriz Fonseca. 2012.
Contact: Professor Cristina Baldauf; email: crisbaldauf@ufersa.edu.br

“I Ecology Workshop: Experiences and future research plans in the Brazilian semi-arid”
Head of the organizer workshop committee. 2011.
Contact: Professor Gustavo Henry-Silva; email: gustavo@ufersa.edu.br

COMPUTER SKILLS

Programming Languages: R and Python

Platforms: Linux, Windows

Applications: ArcGIS, QGIS, Audacity, GIMP, R studio, Spyder

LANGUAGES

Portuguese: native language

English: fluent

Spanish: fluent

Japanese: learning

SERVICE AND ADDITIONAL EXPERIENCE

“Food Recovery Network Program at Utah State University”

Volunteer for 4 years. This program helps preventing food waste across the campus and improves food security of students. The food recovered is redistributed to students for free at our food pantry. In 2019 we won a Robins Award at Utah State University; this is the most prestigious award at USU. We also prevented the waste of over 100 thousand pounds of food.

<https://servicecenter.usu.edu/programs/snac>

“Capacity building of local villagers in the Amazon”

As part of my doctoral research, I trained people living at or below the poverty line to guide researchers and tourists in the Viruá National Park, Roraima, Brazil. Some of these people also received a substantial stipend to help with my PhD fieldwork from 2016 to 2018. This work was funded by Rufford Foundation, UK.

https://www.rufford.org/projects/leandro_do_nascimento

“Musical Conservatoire of Salto (Maestro Henrique Castellari), Sao Paulo, Brazil”

Classical Guitar orchestra, municipal choir, Italian choir, classical music choir, violin classes. 2007 – 2010.

PROFESSIONAL SOCIETIES

Acoustical Society of America (ASA)