UNIVERSIDADE DE LISBOA

FACULDADE DE CIÊNCIAS

# Assessment of the effects of forest fragmentation on aerial insectivorous bats in the Amazonian rainforest

*“Documento Definitivo”*

**Doutoramento em Biodiversidade, Genética e Evolução**

Adrià López-Baucells

Tese orientada por:

Doutor Christoph Friedrich Johannes Meyer

Professor Doutor Jorge Manuel Mestre Marques Palmeirim

Documento especialmente elaborado para a obtenção do grau de doutor

**2018**

UNIVERSIDADE DE LISBOA

FACULDADE DE CIÊNCIAS

# Assessment of the effects of forest fragmentation on aerial insectivorous bats in the Amazonian rainforest

Adrià López-Baucells

Tese orientada por:

Doutor Christoph Friedrich Johannes Meyer

Professor Doutor Jorge Manuel Mestre Marques Palmeirim

Júri:

Presidente:

- Doutora Maria Manuela Gomes Coelho de Noronha Trancoso, Professora Catedrática e Presidente do Departamento de Biologia Animal da Faculdade de Ciências da Universidade de Lisboa.

Vogais:

- Doutor Christoph Friedrich Johannes Meyer, Lecturer, School of Environment & Life Sciences da University of Salford, Reino Unido (Orientador).
- Doutor Hugo Emanuel Vitorino Rebelo, Investigador Auxiliar, Centro de Investigação em Biodiversidade e Recursos Genéticos (CIBIO – InBIO Laboratório associado) da Universidade do Porto.
- Doutor António Paulo Pereira de Mira, Professor auxiliar, Departamento de Biologia da Universidade de Évora.
- Doutora Maria Margarida de Mello dos Santos Reis Guterres da Fonseca, Professora Associada com Agregação, Faculdade de Ciências da Universidade de Lisboa.
- Doutor Rui Miguel Borges Sampaio e Rebelo, Professor Auxiliar, Faculdade de Ciências da Universidade de Lisboa.
- Doutora Ana Margarida Torres Rainho, Bolseira Pós-doutoramento, Faculdade de Ciências da Universidade de Lisboa.

Documento especialmente elaborado para a obtenção do grau de doutor

**2018**

**Notas prévia:** A presente tese apresenta artigos científicos já publicados ou submetidos para publicação (capítulos 2, 3, 6 & 7), de acordo com o previsto no nº 2 do artigo 25º do Regulamento de Estudos de Pós-Graduação da Universidade de Lisboa, publicado no Diário da República, 2.ª série — N.º 60 — 26 de março de 2018. Uma vez que estes trabalhos foram realizados em colaboração o candidato esclarece que participou integralmente na conceção dos trabalhos, obtenção dos dados, análise e discussão dos resultados, bem como na redação dos manuscritos.

Lisboa, novembro de 2018

**Adrià López-Baucells**

*“A great silence is spreading over the natural world even*

*as the sound of man is becoming deafening.”*

**Bernie Krause**

*“Do. Or do not. There is no try.”*

**Grand MSc Yoda**

# ACKNOWLEDGMENTS

This may be the last part of the thesis writing, but it is the first to be without references, p-values, tables and passive voices. This space is where you can speak freely, with no fear of potential reviewers' criticism, and you can acknowledge all people and institutions that have made this piece of work possible but are rarely properly listed in scientific papers. By listing and acknowledging all these friends, family and colleagues that have joined me during this 4-year-long endeavour named 'PhD', I realize how much I will miss it, how much I have learned, and how much (much more!) I still need to learn. But it also reminds me a bunch of extraordinary experiences and a tremendous adventure that I would never change for anything.

When I now find myself discussing my experiences with early PhD students and they ask me for advice, I always say the same: Do not focus too much on the topic, or the research field, there are too many interesting questions and problems to solve on this planet. But try to find the right partner, try to find the right person to share a PhD project with, try to find someone with whom you will fully enjoy four years of your life and beyond, someone with whom you will dream about new projects and will give life to the craziest ideas, someone to share trips to congresses worldwide, or maybe random biological expeditions in remote sites in Asia and Africa, someone with whom to share the excitement of finding new species in the field, someone that would cover your back whilst doing the craziest (usually not-recommended) activities into the wild, someone to whom you'd trust your life to with your eyes blindfolded, someone with a huge heart with whom you could talk with about anything. There are so many research topics, but just a few people like that. This has been the first and most noteworthy lesson of my four year PhD experience. Try to find what I found in **Ricardo Rocha**. I cannot imagine this PhD without him by my side. He would deserve a whole chapter if I was to express everything he has taught and helped me with during the last years.

First, I would like to thank my two supervisors, **Christoph Meyer** and **Jorge Palmeirim**, without whom this thesis would have never been possible. You both have boosted my professional career in an unimaginable way and have allowed me to accomplish one of the most important dreams in my life. I want to thank you deeply for all your teaching and inspiration, but also for the extraordinary trust you bestowed upon me, by offering me the most incredible present in a PhD students' life: freedom to manage and develop my own ideas, my time and freedom to carry out my side-projects. This flexibility has undoubtedly allowed me to enjoy the PhD experience to its fullest and has facilitated the future direction of my life. And because of that, my PhD experience has acquired another full dimension I could never have imagined. Your continuous help and guidance during the whole PhD process have been essential. However, I feel I also have to formally apologise to you, as you have had to deal with my usual disorder and messy working method and mindset, that led me to get involved in probably too-many PhD-side adventures, which have probably slowed down the whole work.

Now that I mention the side-projects, what to say about the amazing adventures we have had together **Mar Cabeza**, **Daniel Burgas**, **Álvaro Fernández-Llamazares**, **Sara Fraixedas**, **Miquel Torrents-Ticó**, **Joni Uusitalo**, **Joan de la Malla**, **James Kemp** and **Irene Conenna**, from the GCC research group. My first steps in Africa were driven by this crazy team of excellent colleagues from the University of Helsinki, that has dragged me to the deepest deserts in Turkana, the original land of humankind, the hilly landscape of Taita, the remaining tropical rainforests and the impressive caves with massive colonies of bats in the south of Madagascar. I have the feeling I have grown with you and your madness, and so you have shaped how me and my research are now. But mostly, you have shown me what a team is, you have shown me how to build a collaborative relationship and how to enjoy science in its fullness. I honestly hope to continue exploring the secrets of nature with you wherever it brings us. So, what is the next destination?

And what to say about my dear friend **Oriol Massana**? You deserve a monument! If a PhD is something that will mark my life, I am sure that a photographic camera will be my lifelong friend. And this is all your fault. You made this hobby grow inside me since we first met at the university and then, during the most memorable of photographic experiences, during three months in the heart of the Amazonian rainforest. Do you remember how we started taking pictures of bats in Montjuïc using our worst home-made devices to attract them? We then tried it in more “natural conditions” in the countryside, then with our *cuca fera* and many other inventions… We spent entire nights trying to get our perfect shots. And we failed again and again and again… until we finally got something decent enough, so we could go and try again. Every single shot is built on top of so many stories and anecdotes that makes them unforgettable. You taught me everything I know about photography, but more than that, you showed me what a good friend is, always being there, during difficult times, independent of where you are or whatever happens.

I have always been admired by the motivation and resolution of my friends **Joan de la Malla**, **Madalena Boto** and **Blanca Martí**. Your strong determination in pursuing your own professional career has been a living lesson. I have always been interested on natural photography and scientific illustration for years, but the three of you provided me with an unexpected conception of these fields and the real power of an image. I have been dazed by the power of your shots, stories and drawings and your art of going deeper and deeper into the planet problems using the right images. I am glad we have had the chance to collaborate during these years, and I wish we will find new opportunities to continue working together in the near future! Joan, our common projects seem to start just now. I am delivering my PhD thesis the same week we are enjoying a tremendous fieldwork together in the north of Madagascar which, I wish, will be the start of a long list of collaborations.

I am extremely thankful to **Tally Yoh**, **Laura Torrent**, **Sílvia Fraixedas** and **Peter Syme** for their support in the most difficult part of my thesis, the sound analyses in which we worked quite intensively during my stage in the United Kingdom. It is obvious that the whole thesis would have never ended on time without your help analysing bat calls and your profound and great contributions. You are not only co-authors of several papers within this thesis, you are part of the family of the whole project. You gave me strength to continue when the end seemed unreachable and the purpose lost. You taught me how better we are when we join forces, when we split the grief. And I hope this will not end now that the thesis is finished. We have more than a million sounds to be used, to be squeezed for further analyses and side-projects. It is now time to play and enjoy the results of such a hard effort.

A 3-year-long fieldwork could have never been done without the precious help and company in the field of **Fabio Farneda**, **Diogo Ferreira**, **Marta Acácio**, **Madalena Boto**, **Gilberto Fernández**, **Milou Groenenberg**, **Julia Treitler**, **Ubirajara Capaverde**, **Maria Mas**, **Eva Sánchez**, **Iolanda Guerra**, **Solange Farias**, **Leonardo Oliveira**, **Inês Silva**, **Joana Carvalho**, **Ileana Mayes**, **Kevina Vulinec** and many others that contributed in one way or another to make the experience in the Amazon an unforgettable life adventure.

Apart of the multiple aspects in which I have received support by my Brazilian friends and colleagues from INPA, the acoustic part of this thesis would have been unfeasible without the close collaboration with **Paulo Bobrowiec** and his team, **Bill Magnusson**, **Giuliana Appel** and **Rodrigo Marciente**, who provided me with the largest set of detectors that I have managed so far, which hung in the rainforest for two entire years. Thank you so much for your trust on the project. The collaboration with this team allowed us to start upcoming projects and collaborations embedded on a research line focused on bat acoustics ecology and conservation in the Neotropics.

As important as the continuous help from the academy, **José Luis Camargo**, **Rosely Hipólito**, **Ary Jorge Ferreira**, **Alaércio dos Reis**, **Luiz de Queiroz**, **Josimar Menezes**, **Osmaildo da Silva** and **José Tenaçol** were key elements for the success of the project. I cannot be more grateful for all your support in logistics, your organization skills both in the offices and in the field sites, and the constant help from the **Biological Dynamics of Forest Fragments Project**. For three years you took care of a bunch of European kids in the most impressive biodiverse ecosystem in Earth, you showed us the beauty of the Amazonian rainforest, hidden in many tiny details and remote corners of the woods, the dangers that can surprise you in the middle of the night, and how to survive in it; something that cannot be forgotten.

Apart of the Biological Dynamics of Forest Fragments Project, I am also extremely thankful to all other institutions that have devoted energy and support to the project, starting by the **Centre in Ecology, Evolution and Environmental Changes** (Portugal), the **National Institute for Amazonian Research** (Brazil), **Bat Conservation International** (United States of America), the **Smithsonian Tropical Research Institute** (United States of America) and my second home, the **Natural Sciences Museum of Granollers** (Catalonia).

In Portugal I found my second home, embraced and surrounded by an extremely nice group of researchers and friends. Thanks **Federica**, **Jorge**, **Adriana**, **Paula**, **Flavio**, **Sara**, **Joana**, **Mario** and **Anas** and all the excellent frisbee players for all the experiences we had in Lisbon. Amongst them all, I will never forget **Luisa Rodrigues**, who introduced me to the speleology world while travelling all around Portugal.

And then, I also want to thank those that provided me with the answer to the eternal question… Why bats? I have been asked this thousands and thousands of times. And the answer is clear and unambiguous: **Xevi Puig** and **Carles Flaquer**. Our first contact was in Barcelona, 2005, within the context of a volunteering bat project. There, I met Xevi, an extraordinary chiropterologist with no limits on his imagination, no barriers to his dreams and infinite knowledge of natural history. We bonded very easily, and after a year, he brought me to my first *Bat Research Technique Course* in the Montseny Natural Park. When I met Carles in the workshop we forged a friendship in a flash. And after all, it was he who sent me the PhD offer with Dr. Christoph Meyer, from the Elisabeth Kalko research team. It was he who pushed me forward and on from his group, so I could pursue a PhD on tropical conservation. And at the end of the day, after more than a decade since we all met, they all showed me what a family is... what taking care of your people and colleagues really is. And last, but not least, they taught me to keep my feet on the real life, to fight the real conservation problems through science, but without falling into the academic maelstrom.

What to say about my dear office/field old colleague and friend, with whom I have shared many stories on several bat research projects. It would never be the same without the role you played in my life. We first met holding flashes in Ebro's Delta for long hours (what a nightmare!), and diving, and visiting caves, and listening to our particular music in the car… We spent entire nights looking for the best spots to capture *Tadarida teniotis*, we lived the adventure of opening an 8 km long trail in the middle of the Amazon with a single *machete*, and we decided to join efforts as editors of the new Journal of Bat Research & Conservation against all circumstances. I need to thank **Maria Mas** for her endless patience bearing my odd request and behaviour, my working obsessions or my common, grumpy monologues in the Museum.

Needless to say, I am so grateful for the long-lasting friendships that stand over years and remain trustful and deep in our minds. Friendships that persist despite being separated by oceans and cultures and that remain as they have always been. I have never felt alone or lost, and this is thanks to all of you team. I could not be happier every time I received any of your mails, letters, calls, and brief encounters, **Arnau Sardà**, **David Boteller** and **Mònica Utjés**.

My last two years in the Manchester could not have been better without you **Steve Parker**, **Fiona Parker** and **Baptiste Chadeyron**. Arriving in a new country (cold and rainy!) and find SLBG and all its friendly members, has been more than a gift… Your intensity, full-dedication and craziness for bat conservation is well-known by almost any bat researcher in Europe and beyond. I am not going to insist on that. But I wanted to thank you again for everything you kindly did to make my arrival easy and smooth. Your heart and kindness are so huge I felt adopted since the beginning. I have learned so much from you and your society and somehow, and maybe unintentionally you built the basis in my head of what I expect will be my next life project in Catalonia. I hope we find a way to share fieldwork again very soon!

And when I left UK, the next destination was Kuching! The last part of my PhD drove me to take my first steps in Asia, for which I wanted to thank **Faisal Ali**, **Joe Chun-Chia Huang**,

**Krizler Tanalgo**, **Tuanjit Sritongchuay**, **Ellen Mc Arthur** and **Alice Hughes** for being so supportive during my visit in Borneo and introducing me to this amazing region in the planet.

I òbviament, no puc estar més agraït a tota la meva família, però en especial a **pares** i **germana** que m'han donat suport i energia continuadament per poder portar endavant no només un projecte de doctorat, sinó una vida sencera que ara gira al voltant de la conservació del planeta. Sent com soc un urbanita de naixement, que he crescut al bell mig d'una de les grans urbs capitals del país, van ser ells els que van propiciar els meus primers passos al camp i en el contacte amb la natura, els que van foragitar les pors a tot allò desconegut i als animals. Han estat des de casa que han cregut en les meves decisions en tot moment, que m'han impulsat a tirar endavant els meus somnis independentment d'on la societat actual ens empeny, que m'han ensenyat a creure en els meus somnis, però sobretot, a lluitar per fer-los realitat.

And what is the contribution of an artist in a biological thesis about bats and forest fragmentation? One could imagine that the main input would be the amazing drawings that **Eva Sánchez Gómez** did for the Field Guide to the Bats of the Amazon, but that would be somehow false. And do not take me wrong… The drawings are extraordinary! I am not sure how, but Eva managed to bring my eyes and mind back on Earth to realize how diverse is the beauty of the world (apparently there are pretty things apart of bats!), to appreciate the role of the art in our society and the power of the imagination. Eva showed me how the world, in all its dimensions, is driven by people's perception, and how art shapes this from the shadows of life. If we want to conserve wildlife and biodiversity in this planet, we need more art, more sensitivity and more mind freedom. Since that 28th of December, the PhD has kept us separated for long periods of time, but has also been our night-time reading, our lunch and dinner main topic discussion, our car trip-dialogue, our most used words in e-mails. We will have to find a substitute now! And obviously, her vital support, patience and continuous motivation have allowed this thesis to reach its end. I am so tremendously grateful to count with you in my life.

Landscape and fieldwork images included in this thesis: © Oriol Massana
Bat images included in this thesis: © Oriol Massana & Adrià López-Baucells
All illustrations included in this thesis: © Eva Sánchez Gómez

This work was supported by the Portuguese Foundation for Science and Technology under grants [PTDC/BIABIC/111184/2009] (Christoph F. J. Meyer), [SFRH/BD/80488/2011] (Ricardo Rocha), [PD/BD/52597/2014] (Adrià López Baucells) by the Foundation for Research Support of the State of Amazonas [FAPEAM 062.01173/2015] (Paulo E. D. Bobrowiec) and by the CNPq fellowship [160049/2013-0] (Paulo E. D. Bobrowiec). Additional funding was provided by two student research fellowship from Bat Conservation International.

# TABLE OF CONTENTS

# ABSTRACT

Land use change and habitat fragmentation are among the most severe threats to biodiversity, especially in the tropics. In the Amazon, the abandonment of formerly deforested areas allowed the expansion of secondary regrowth, a type of habitat where bats are known to provide important ecosystem services. Amongst them, aerial insectivorous bats have been neglected in most Neotropical studies and remain poorly studied. However, the current upsurge in acoustic technology makes them easy targets to be monitored using ultrasound detectors. The aim of this thesis was to reveal the diversity of aerial insectivorous bats and quantify the effects of forest fragmentation on this ensemble within the Biological Dynamics Forest Fragments Project, a whole ecosystem experiment in the Amazon, currently composed of a mosaic of unflooded rainforest with continuous forest, and forest fragments embedded in a matrix of secondary regrowth.

As part of this thesis, the first "Field Guide to the Bats of the Amazon" was published. A custom-built classifier was developed which was able to identify a large proportion of files to sonotype level (with > 90% accuracy), leaving the rest (<25%) to be manually classified. I also tested 20 different recording schemes and provided guidelines to optimize protocols for acoustic studies. In forest fragments and their adjoining secondary forests, taxonomic, phylogenetic and functional α diversity became gradually poorer with decreasing fragment size. In terms of β diversity, bat assemblage composition in secondary forests after ~30 years of recovery was still significantly different from that in continuous forest. However, forest edges harboured highly diverse bat assemblages due to the opening of cluttered areas, and the increase of less-sensitive species. Responses towards fragmentation were species-specific and strongly related to their functional traits. The results of this thesis highlight the irreplaceable value of tropical primary forests due to the long time required to recover fragmented ecosystems.

**Keywords:** Amazon, Bioacoustics, Fragmentation, Aerial Insectivorous Bats, Secondary forests.

# RESUMO

As alterações do uso do solo e a fragmentação dos habitats estão entre as mais sérias ameaças à biodiversidade, particularmente nas regiões tropicais. Na Amazónia, graças a melhorias na rede de áreas protegidas e na aplicação de leis ambientais, durante as últimas décadas as taxas de desflorestação, causadas principalmente pela expansão da agricultura e redes estradas, tem vindo a diminuir de forma substancial. Simultaneamente, o abandono de áreas previamente desflorestadas tem permitido uma parcial recuperação da vegetação. No entanto, a expansão de plantações para a produção de soja e óleo de palma, assim como o aumento na construção de barragens, estão agora a ameaçar este processo de regeneração parcial.

Os morcegos providenciam importantes serviços dos ecossistemas como polinização, dispersão de sementes e controle de populações de insetos. Os morcegos insetívoros aéreos têm sido particularmente pouco estudados nos Neotrópicos, principalmente devido a limitações inerentes à amostragem realizada com redes de neblina que são pouco eficazes na captura desses animais. Felizmente, o recente desenvolvimento de métodos de amostragem acústica permite agora monitorizar estas espécies com o auxílio de gravadores de ultrassons.

O objetivo geral desta tese foi estudar os efeitos da fragmentação da floresta nas comunidades de morcegos insetívoros aéreos. O trabalho foi desenvolvido na área de implantação do Projeto Dinâmica Biológica de Fragmentos Florestais (BDFFP), uma experiência de manipulação do habitat de grande escala na Amazônia brasileira que criou um mosaico de floresta primária continua e de fragmentos de floresta primária, incluídos numa matriz de vegetação secundária. Esta tese foi baseada num conjunto de dados acústicos recolhidos durante três anos e que gerou um total de 1,088,940 gravações analisadas.

## Capítulo 2: Guia de campo dos morcegos da Amazónia

O primeiro guia de morcegos da Amazónia foi publicado no âmbito do desenvolvimento desta tese. O livro foi primeiro publicado em 2016 em formato digital pelo Instituto Nacional de Pesquisas Amazónicas (INPA) e reeditado em versão impressa pela Pelagic Publishing em 2018. Foi pensado como um guia para satisfazer as necessidades de quem realiza trabalho de campo com morcegos na Amazónia. É em grande parte baseado em chaves de identificação previamente publicadas, modificadas com base em descrições das espécies na literatura e em observações pessoais. Incluí a primeira chave de identificação acústica de morcegos da Amazónia e é ilustrada com espectrogramas dos sons emitidos pela maior parte das espécies

da região. Representa um passo importante no sentido de facilitar a tarefa difícil de identificar as muitas espécies de morcegos insetívoros que ocorrem na Amazónia, com base na sua ecolocalização.

### Capítulo 3: Uma combinação de classificadores automáticos com validação manual otimiza o balanço entre o esforço despendido e a precisão das identificações em inventários acústicos de morcegos.

Graças a avanços tecnológicos recentes, a bioacústica transformou-se numa importante área de investigação ecológica. No entanto, a escassez de bibliotecas de sons completas ainda dificulta a sua utilização em comunidades tropicais muito diversas. Avaliei primeiro se gravações pré-identificadas de morcegos em voo livre e logo após serem libertados. Podem ser utilizados para treinar um algoritmo de classificação automática. Utilizando modelos “random forest” foi possível confirmar esta possibilidade utilizando ambos os tipos de gravações. Avaliei também como otimizar os protocolos de identificação acústica combinando classificação automática com validação visual. Para atingir uma precisão de ~85%, os modelos têm de ser treinados com pelo menos 500 pulsos por espécie/sonótipo. Para sete dos vinte sonótipos, os mais abundantes nos nossos dados, obtive classificações corretas em 90% dos casos. Adotando como limiar de precisão 95%, foi possível reduzir a quantidade de pulsos que requerem validação manual em até 75%, uma poupança significativa de esforço na análise deste tipo de dados.

### Capítulo 4: Otimização de inventários com base em acústica em ambientes tropicais modificados pelo Homem.

Há ainda uma importante falta de protocolos para a realização de inventários acústicos de morcegos em regiões tropicais. A determinação do esforço mínimo necessário para detetar alterações ecologicamente relevantes em comunidades de morcegos é importante para minimizar o custo da obtenção de dados. Assim, avaliei o esforço necessário para a realização de inventários de espécies e para quantificar os efeitos da fragmentação da floresta em morcegos insetívoros aéreos. Foi testada uma combinação de 20 esquemas de amostragem diferindo no número de horas amostradas por noite, no número de noites por local, e ainda amostrando apenas na época seca ou das chuvas ou em ambas. Isto foi avaliado em dois tipos de paisagens: em fragmentos de floresta primária incluídos numa matriz de floresta secundária e nestes mesmos fragmentos após terem sido re-isolados pelo corte da floresta secundária. Com base nos resultados obtidos são formuladas recomendações que poderão ajudar a otimizar protocolos de amostragem acústica de morcegos nos neotrópicos.

### Capítulo 5: Recuperação da diversidade taxonómica, funcional e filogenética de morcegos insetívoros em antigas florestas secundárias neotropicais.

Foi modelada a resposta dos morcegos insetívoros aéreos à fragmentação e à recuperação da vegetação, em termos de atividade e de diversidade taxonómica, funcional e filogenética no mosaico de floresta continua e fragmentos florestais (de diferentes tamanhos), inseridos numa matriz com floresta secundária de 30 anos. A atividade variou independentemente do tamanho dos fragmentos e do habitat, mas a diversidade foi fortemente afetada pela fragmentação. As comunidades de morcegos da floresta secundária tiveram em geral diversidade taxonómica funcional e filogenética mais baixa do que a floresta continua. Em geral, as orlas dos fragmentos apresentaram um pico de diversidade específica, mais elevada do que o interior da floresta, provavelmente porque as orlas incluem uma maior diversidade de micro habitats. As alterações na biodiversidade β estavam principalmente relacionadas com a perda de espécies e de traços funcionais o que possivelmente resulta num empobrecimento dos serviços de ecossistemas que os morcegos disponibilizam. As comunidades de morcegos nos fragmentos não são muito distintas das do interior da floresta continua, mas um período de trinta anos foi insuficiente para a total recuperação da comunidade das florestas secundárias.

### Capítulo 6: Ecolocalização e morfologia da asa: correlações de traços funcionais com a vulnerabilidade de morcegos insetívoros em florestas tropicais.

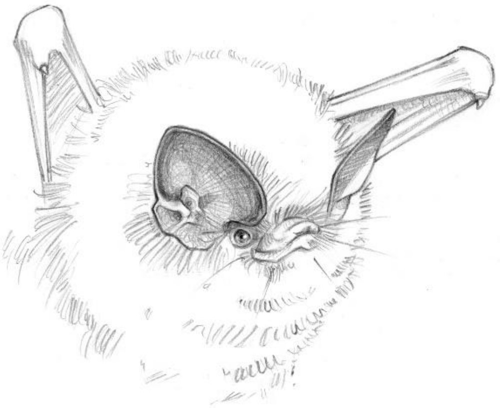
A resposta à fragmentação do habitat varia entre espécies e está fortemente associada aos seus traços funcionais. A interação entre os traços funcionais, as características do ambiente e a distribuição das espécies foi investigada. Os resultados mostram que a estrutura dos pulsos de ecolocalização, a utilização vertical do espaço e a forma da asa são os melhores preditores da sensibilidade à fragmentação da floresta. A frequência de máxima energia, a massa corporal e a carga alar não mostraram qualquer correlação com características do meio. No entanto, as espécies com pulsos de frequência constante estão associadas a vegetação densa, sendo possivelmente mais sensíveis à fragmentação florestal do que espécies com pulsos de frequência modulada. O traço funcional "utilização vertical do espaço" está também correlacionado com a estrutura da vegetação, indicando que espécies do sub-bosque são mais sensíveis à perda de floresta do que espécies das copas. Finalmente, as espécies com asas

largas estão associadas às orlas e clareiras das florestas. Os resultados sugerem que os traços funcionais das espécies de morcegos insetívoros aéreos influenciam a sua sensibilidade à fragmentação do habitat.

**Capítulo 7: A importância dos lagos para a conservação de morcegos na Amazónia: uma avaliação utilizando estações de gravação.**

Estudos recentes preveem uma diminuição na precipitação em grande parte das regiões tropicais, particularmente na Amazónia, podendo ter possíveis consequências negativas nos ambientes aquáticos, como lagos. Ponderando essa possibilidade, foi avaliada a importância sazonal dos lagos para a conservação de morcegos insetívoros aéreos na Amazónia central. Foi comparada a riqueza específica e atividade de alimentação sobre lagos e na floresta adjacente. De um total de 21 espécies/sonótipos registados em ambos os habitats, todas foram detetadas nos lagos e 18 foram significativamente mais ativas nos lagos do que na floresta. Apenas duas espécies tiveram níveis de atividade significativamente mais elevados na floresta do que nos lagos. Os níveis de atividade e a riqueza em espécies nos lagos foi mais alta na época seca do que na época das chuvas. A atividade de alimentação foi também mais elevada nos lagos do que na floresta em ambas as estações.

**Palavras chave:** Amazônia, Bioacústica, Fragmentação, Morcegos aéreos insetívoros, Floresta secundaria.



# CHAPTER 1

## General introduction

Top: *Furipterus horrens;* Bottom: *Lasiurus egregius*

# CHAPTER 1

## General introduction

### Tropical deforestation

Land use change and forest fragmentation are some of the most severe threats that alter the Earth's carbon cycle and imperil biodiversity and the delivery of important ecosystem services worldwide (Ewers and Didham 2006a, Foley et al. 2011, Haddad et al. 2015). Due to land use change, many natural habitats are now converted to mosaic landscapes composed of patches of human-modified land (Numata and Cochrane 2012), with limited habitat connectivity. Habitat loss and fragmentation are currently identified as one of the main causes of massive biodiversity loss in what is already known as the sixth mass extinction (Dirzo et al. 2014).

Contrary to what was generally expected, the latest global forest evaluation highlights that global forest area has increased by 2.24 million km$^2$ since 1982 (Song et al. 2018). However, this overall increase is a clear consequence of the difference between the high net gain of tree cover in subtropical, temperate and boreal regions, compared to the net loss in the tropics (Hansen et al. 2013, Keenan et al. 2015, Song et al. 2018). Sixty percent of the global net loss in forest area are a direct consequence of human activity including deforestation, agricultural expansion and urbanization. Due to the increase in human population and the continuing overexploitation of natural resources, the effects of habitat loss and modification are predicted to increase in the near future, especially in the tropics, where most of the biodiversity resides (Laurance et al. 2014b, Wilson et al. 2016).

Due to the crucial role of the tropics for global biodiversity conservation, several authors have tried to quantify the magnitude of tropical forest loss, which has been estimated to be about 5.5 M ha per year (Keenan et al. 2015). Forest loss differs geographically between the Neotropics and the Paleotropics. While the reduction in deforestation rate in Brazil has been widely recognised, increasing deforestation in Southeast Asia and Africa outweigh any subtle positive trends in the Neotropics (Hansen et al. 2013). The three countries in the Neotropics with the greatest loss of forest cover between 1982-2016 are Brazil (-385,000 km$^2$, -8%), Argentina (-113,000 km$^2$, -25%) and Paraguay (-79,000 km$^2$, -34%) (Song et al. 2018). However, the biggest hotspots of deforestation are currently in Queensland (Australia), Myanmar, Vietnam, Cambodia and Indonesia (Asia), Congo and Tanzania (Africa), and the causes driving these changes range from multiple smallholder's agriculture to extensive crop monocultures managed and directed by large companies.

In the Amazon basin, the greatest deforestation rates are commonly found in the south-eastern edge, an area that is commonly known as the "arc of deforestation". During the last decades, deforestation rates have dropped from 30,000 $km^2$/year in the 1980s, mostly caused by road and agricultural expansion (Rosa et al. 2017), to 5,843 $km^2$/year in 2013. This reduction is due to increased law enforcement and the improvement of the protected area network (Davidson et al. 2012, Nepstad et al. 2014). Meanwhile, the abandonment of formerly deforested areas has allowed the expansion of secondary regrowth, and between 1978 and 2002 the area covered by secondary forest increased from 29,000 $km^2$ to 161,000 $km^2$. However, new palm oil (Butler and Laurance 2009) and soya plantations (Rosa et al. 2017), as well as dam construction (Lees et al. 2016), are now threatening the positive trends of forest regeneration in the Amazon.

Rainforest in Central Amazon

## Tropical forest fragmentation

Due to pervasive deforestation and loss of natural habitat, continuous undisturbed habitat eventually becomes broken up into small, isolated patches embedded in a matrix of modified habitat, a process generally referred to as habitat fragmentation (Fahrig 2003). Numerous definitions of habitat fragmentation can be found in the literature, but there is some conceptual confusion because habitat loss and habitat fragmentation are sometimes not properly distinguished (Fahrig 2003, Didham et al. 2012, Fahrig 2017).

The consequences of habitat fragmentation upon wildlife are strongly influenced by edge effects, patch area, landscape composition and configuration or level of disturbance of the remaining habitat. The cumulative consequence of all these factors involves long-lasting impacts on the forest structure and ecological function (Ewers and Didham 2006a, b, Haddad et al. 2015), as well as on its intrinsic biodiversity. Research on fragmentation has followed a variety of approaches and addressed distinct issues. Studies have focused on patch area (Fahrig 1997), patch shape, isolation and landscape composition (Arroyo-Rodríguez et al. 2016), edge effects (Ewers and Didham 2006b, Lenz et al. 2014), influence of different matrix types (Powell et al. 2015b) and connectivity (Powell et al. 2015a).

Forest fragmentation research has evolved under the umbrella of island biogeography theory (IBT) (MacArthur and Wilson 1967), which links higher species diversity to larger and less isolated patches (islands or forest fragments). The simplicity of IBT turned it a crucial tool for the scientific community working on forest fragmentation for several decades. However, its binary view of the landscape (with patches that are either habitat or non-habitat) turns this framework incapable of assessing the impacts of fragmentation in terrestrial systems where patches are usually embedded in a matrix of modified habitats that are to a greater or lesser extent usable by species. Thus, IBT also cannot properly address temporal changes in ecosystems where the modification of matrix habitats allows a progressive ecosystem recolonization and species turnover. These limitations led to the recent development of alternative frameworks such as countryside biogeography theory (Daily 1997, Laurance et al. 2007, Mendenhall et al. 2013). This approach integrates the importance of the nature of the matrix and how animals perceive it (Kupfer et al. 2006) and evaluates species responses acknowledging the dynamics of the landscapes and the permeability of the matrix (Mendenhall et al. 2014, Wolfe et al. 2015). It is well-known that matrix habitat quality and permeability strongly influence species responses to habitat fragmentation (Laurance et al. 2007, Mendenhall et al. 2014). In a dynamic landscape, the succession of matrix habitats over time affects connectivity, gene flow, species distribution, and therefore long-term persistence of populations or recolonizations (Struebig et al. 2011, Powell et al. 2013).

In the Neotropics, and especially in the Amazon, continuous forest becomes fragmented as a result of high deforestation pressure driven by the expansion of agricultural areas (which still is the dominant driver of deforestation), urban cover increase or wildfires, amongst many other sources (Chazdon 2014). Secondary forest regrowth in abandoned agricultural areas reduces patch-matrix contrast, minimizes edge effects, and decreases the isolation of the remaining natural habitat patches, thus potentially minimizing the consequences of habitat fragmentation upon species diversity (Chazdon 2014). It has been estimated that between

2001 and 2010 in Latin America there has been an increase of >360,000 km$^2$ of woody vegetation, which corresponds to approximately 66% of the deforested areas (Aide et al. 2013). Thus, in human-modified tropical landscapes, it is common to find forest fragments embedded in a matrix of secondary forest. Secondary forests act as important reservoirs of tropical diversity (Barlow et al. 2007, Chazdon et al. 2009, Chazdon 2014, Edwards et al. 2017) and understanding the interaction between both habitats in the context of habitat fragmentation is crucial for conservation and to better comprehend the diversity of responses to forest fragmentation (Haddad et al. 2015).

Fragmentation experiment in the Biological Dynamics of Forest Fragments Project, in Central Amazon.

Acknowledging the widespread fragmentation pressure and threat, one of the key challenges that conservationists face is developing more efficient ways to protect natural habitats and determine priority conservation areas (White and Strittholt 2014). In order to improve land management, planning and policies, it is crucial that we are able to identify i) the species that are more vulnerable to fragmentation, ii) the landscape attributes that contribute to ecosystem and species protection and resilience, and iii) the methods to improve habitat structure and composition to minimize fragmentation consequences. However, countering habitat fragmentation and designing efficient wildlife conservation plans can only be achieved by interdisciplinary research, sharing experiences and knowledge from the most distant branches of science, from sociology and anthropology, to economics, politics or zoology. It is therefore crucial to gather the most comprehensive picture of the natural processes and communities, including information from invertebrates and plants or algae, to vertebrates, in which we find the mammal group this thesis focuses on; aerial insectivorous bats.

## Neotropical aerial insectivorous bats

### General introduction of the species ensemble

A total of 1,386 species are currently recognised within the order Chiroptera (Burgin et al. (2018), making it the second most diverse mammal group after rodents. The highest density of species occurs in the Neotropics, with more than 300 species and 80 genera (Mickleburgh et al. 2002), and single locations that harbour more than 100 sympatric species (Rex et al. 2008). The Amazon basin, as the most biodiverse terrestrial ecosystem on Earth, also represents one of the hotspots of global bat diversity, with one tenth of all extant species occurring there. However, the available information on bat assemblages in the Neotropics is clearly biased towards phyllostomid bats, because data have been commonly gathered with ground mistnets and through roost searches (MacSwiney et al. 2008). It is widely acknowledged that mist nets are inappropriate for assessing assemblages of aerial insectivorous bats, underestimating their richness and abundance (O'Farrell and Gannon 1999, Kalko et al. 2008). Even if mist nets are placed in the canopy, where most aerial insectivorous bats forage, the species in this ensemble can usually avoid the mist nets as they detect them very efficiently through echolocation (Bernard 2001, Marques et al. 2013, Marques et al. 2015, Marques 2016). As this ensemble is characterized by using strong echolocation calls during commuting and foraging, they are much more efficiently surveyed with ultrasound detectors than using mistnets (MacSwiney et al. 2008, Arias-Aguilar et al. 2018).

Brazil is the country with the second-highest bat species richness in the world, with more than 170 species currently reported (Arias-Aguilar et al. 2018). According to the last checklist published by Nogueira et al. (2014), Brazil has a total of 92 recognised phyllostomid bat species and 86 belonging to the remaining eight families (Emballonuridae, Furipteridae, Molossidae, Mormoopidae, Thyropteridae, Vespertilionidae, Noctilionidae and Natalidae). For most non-phyllostomid bats, information about their natural history, behaviour, conservation status and echolocation descriptions are scarce (Cunto and Bernard 2012).

Bats are well-known for providing several important ecosystem services in tropical habitats such as pollination or seed dispersal (Kunz et al. 2011). In addition, insectivorous bats play an important role in the regulation of insect populations (including some pest species or potential vectors of human diseases), an ecosystem service that has received increased attention worldwide during the last decades (e.g. Kalka et al. 2008, Kunz et al. 2011, Maas et al. 2013, Wanger et al. 2014, Puig-Montserrat et al. 2015).

The ensemble of aerial insectivorous bats includes all those species that use mostly echolocation for orientation and hunting. They hunt insects from the most cluttered forest interior to open areas such grasslands (Estrada-Villegas et al. 2010). Its species are commonly split into three different functional groups according to the habitat type where they commonly forage: a) highly cluttered space, b) background cluttered space and 3) uncluttered space (Schnitzler and Kalko 2001, Denzinger et al. 2016). The type of echolocation of each group has evolved specifically to optimize their foraging activity in the different habitats (e.g. low vs high frequencies, high vs low duty cycle echolocation or high plasticity in the shape of the pulses). They have adapted to pursuing insects in forest edges or high above the canopy, or trawling insects from water surfaces, for instance.

During these last decades, probably due to the increase in usage of ultrasound detectors and the expansion of the field of bioacoustics, the distribution ranges of many species of this ensemble have been updated, enlarged or modified (López-Baucells et al. 2014, Falcão et al. 2015, Moratelli et al. 2015).

## Neotropical aerial insectivorous bat families in a nutshell

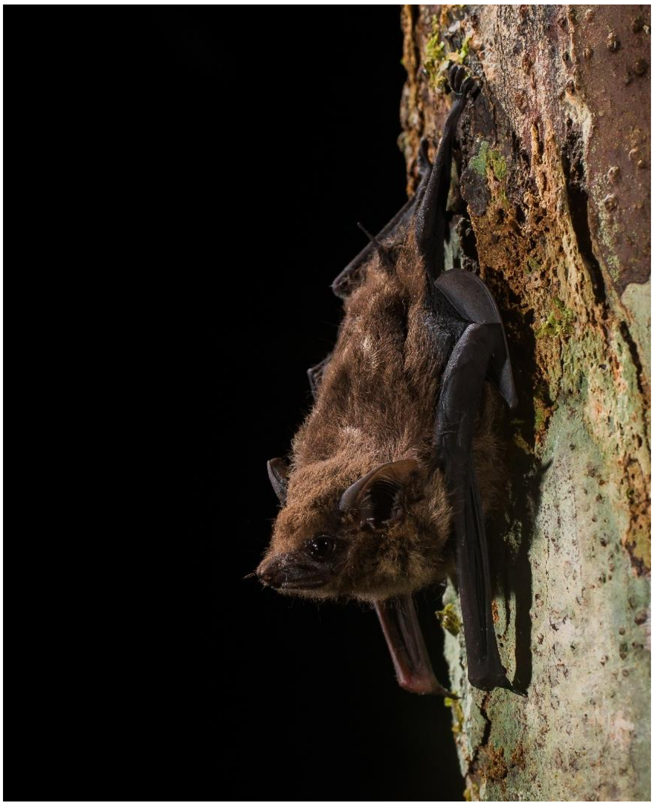
The family Thyropteridae is composed of five species of small-sized aerial insectivorous bats. The common name, disc-winged bats, derives from the characteristic fleshy pads (suckers) present at the base of the thumbs and ankles that are used to cling to the smooth walls of unfurling leaves of *Heliconia* and related bananalike plants in which they roost. Several cryptic species have been recently described. This turned most of the previously collected distribution information of the family rather uncertain (Solari et al. 2004, Bezerra et al. 2005, Gregorin et al. 2006, Velazco et al. 2014).

The Furipteridae, known as smoky bats, is one of the smallest bat families and only contains two species, including the thumbless bat *Furipterus horrens* that occurs in the Amazon. The family's characteristic feature is the minute and functionless thumb, which is partly enveloped by the wing membrane. Although they are reported to live in many kinds of environments and roost in many types of structures (e.g. hollow trees, buildings and caves), due to their very precise echolocation which increases their ability to avoid mistnets it is still one of the least known bat families of the Neotropics (Falcão et al. 2015).

The family Mormoopidae is composed of two genera, the mustached or nakedbacked (*Pteronotus*) and the ghost-faced (*Mormoops*) bats. Mormoopids are found from humid tropical to semiarid and arid sub-tropical habitats below 3,000 m throughout the New World, from the southwestern USA to south-eastern Brazil. The common names of *Pteronotus* species are due to a peculiar fringe of long hairs around the mouth. This family includes some aerial insectivorous bats that are easily trapped by mistnets (Rocha 2017) due to their very low altitude flight. *P. parnellii*, one of the most common species within the family has been recently split into eight species (Pavan and Marroig 2017), two of them sympatric in the Central Amazon. In fact, during this project I described geographic patterns of variation in their echolocation and ecology (López-Baucells et al. 2017), with individuals spanning from Central Amazon to French Guiana.

The Emballonuridae is a pantropical family that in the New World is found from northern Mexico to southern Brazil. Some Neotropical species of this family possess sac-shaped glands near their shoulders, which explains the family's common name (sac-winged bats). These glands are usually more prominent in males and are used to produce pheromones. Emballonurids are small aerial insectivorous bats, with relatively large eyes and long, narrow wings, and are assumed to be edge-specialists. Studies conducted in Panama (e.g. Jung et al. 2007, Jung and Kalko 2011) and elsewhere (e.g. Castano and Corrales 2007, Lim et al. 2010, Hintze et al. 2016, Novaes et al. 2017) make it one of the better-known families of aerial insectivorous bats in the Neotropics.

Vespertilionids, commonly known as vesper or evening bats, are the largest bat family. This near cosmopolitan family comprises more than 300 species and is



*Saccopteryx leptura*

present on all continents except Antarctica. Four vesper bat genera are known to occur in the Amazon. They thrive in a wide range of habitats and exploit virtually all types of available roost sites. While some species are relatively well-studied such as *Myotis riparius or M. nigricans* (Siemers et al. 2001a, Vicente et al. 2005, Aires 2008), others such as *Rhogeessa io* or *Lasiurus egregius*, represent marked gaps of knowledge.

The Molossidae is a near cosmopolitan family that, like the Vespertilionidae, is present on all continents. Molossids have relatively long narrow wings and are adapted to rapid flight in open spaces. They are strong fliers and can cover large distances every night in search of food. They tend to forage well above the canopy, making their study difficult, and therefore, with some exceptions, to date have only infrequently been included in scientific research projects (Jung and Kalko 2010, 2011, Jung et al. 2014, Jung and Threlfall 2016).

The family Noctilionidae comprises just one genus and two species, including the so-called fishing bats, which have large distributions, from Mexico to Argentina, and roost in hollow trees and caves (Brooke 1997). Characterized by their orangish fur, relatively large body mass, strong claws and marked smell, these bats are adapted to hunt insects (small beetles, moths and other insects) and one of them fish (Lewis-Oritt et al. 2001). *Noctilio leporinus* uses its echolocation to detect ripples in the water surface that reveal the location of potential prey (Brooke 1994). They are one of the few dimorphic bat species in the Neotropics (Brooke 1997).

Natalidae is a monotypic family of small insectivorous bats that roost in caves and have greyish, blackish and reddish fur (Taddei and Uieda 2001). Some of the diagnostic traits of these species are their funnel-shaped ears or their unusually long tails (Davalos 2005). This family is closely related to the Furipteridae and Thyropteridae (Dalquest 1950, Davalos 2005). It is found from Mexico to Brazil, and in the islands of West Indies (Dalquest 1950). However, some species have completely disappeared from several Caribbean islands due to habitat alteration (Davalos 2005).

## Bioacoustics and bat echolocation

Bats have some impressive adaptations, such as the ability to fly or the capacity of laryngeal echolocation. Echolocating bats use an ultrasound-based biological sonar to navigate and hunt in the dark. Due to that, some of the bat families occurring in the Neotropics can be monitored non-invasively and very efficiently using ultrasound detectors. The discovery of this sensory capacity was about three centuries by Lazaro Spallanzani (1729-1799) a professor at the

University of Pavia (Italy) who had a naturalist spirit and endless curiosity. This led him to investigate why bats could orientate themselves in complete darkness, but owls could not (Grinnell et al. 2016). After several experiments artificially blinding and deafening captive bats, he reached the conclusion that bats use the ears for their orientation, although he never discovered how. Unfortunately, his discoveries were never accepted by contemporary scientists. More than a century later, another scientist, Don Griffin, together with two of his classmates (Talbot Waterman and Jim Fisk) used a new salt-crystal microphone (developed by a Harvard physicist to hear high frequency insect sounds) to record bat sounds for the first time. Soon Griffin coined the term "echolocation" to refer to the sonar system used by bats. This was the first of many advances in understanding bat echolocation and a turning point in our understanding of animal perception (Grinnell et al. 2016).

It was not until 1953 that Griffin started his investigations on Neotropical bats, recording the echolocation calls of several species in Panama. It was then that, after several failed attempts to record *Carollia perspicillata* (a common phyllostomid bat), he labelled them as "*whispering bats*". He continued his studies recording some rhinolophid and vespertilionid species, thus realising the extreme variability amongst bat calls and the shape of their pulses. These discoveries and the publication of his book "Listening in the dark", may be identified as the starting point of bat echolocation studies worldwide (Grinnell et al. 2016).

Subsequently, numerous scientists have devoted their professional careers to addressing questions about bat echolocation and improving knowledge about this sensorimotor system. It

Setting up the automatic detectors in one of the field sites.

was not until the mid-1970s with the establishment of some international high-quality labs (Grinnell, Simmons, Suga, Pollak, Neuweiler and Schnitzler) and the availability of commercially available bat detectors that the study of echolocation reached its most notable achievements (Grinnell et al. 2016). A combination of various technical advances (e.g. hand-held detectors, sound filters, software to analyse and visualize the recordings and more sensitive microphones) allowed a great increase in knowledge about bat echolocation (Fenton et al. 2016).

Echolocation is used by most bats as a primary sense for spatial perception for: i) tracking target trajectories, ii) locating targets, iii) identifying targets by their traits, iv) intercepting or avoiding targets, v) remembering locations and general orientation through the environment (Schnitzler and Kalko 2001). Echolocating bats can be divided into those that emit high-intensity echolocation calls and are therefore easy to survey with ultrasound detectors (e.g. Vespertilionidae, Emballonuridae, Molossidae) and those that use much less intense calls such as Phyllostomidae or Megadermatidae (whispering bats) (Kalko 2004). In this thesis I focused on the former group. There are also bats that produce calls at different duty cycles: low duty cycle echolocators which separate pulse and echo in time (e.g. Vespertilionidae and Emballonuridae), and high duty cycle species, which do not separate pulse and echo in time, but by frequency due to the Doppler effect (e.g. Rhinolophidae, Hipposideridae and some Mormoopidae) (Fenton 1999, Fenton et al. 2012).

Bat echolocation pulses are highly variable, especially regarding duration, shape and frequency. This within-species variation mostly depends on activity or behaviour (commuting, foraging, hunting or interacting through social calls), environment (cluttered vs open areas) and atmospheric conditions (humidity and temperature) (Kalko and Schnitzler 1998, Schnitzler and Kalko 2001, Siemers et al. 2001a, Siemers et al. 2001b, Schnitzler et al. 2003). Some species even alternate different types of pulses (e.g. *Saccopteryx bilineata*, Jakobsen et al. 2012) or dramatically change the shape and frequency of their pulses during feeding buzzes (e.g. *Molossus rufus*, López-Baucells et al. 2016).

The identification of bat species by their echolocation calls started in the 1980s (e.g. Ahlén 1981, Fenton and Bell 1981), although variability in the acoustic parameters has only been more extensively investigated more recently (e.g. Surlykke and Kalko 2008, Brinkløv et al. 2009, Mora and Macias 2011, Frick 2013, Rodríguez-San Pedro and Simonetti 2013, Clement et al. 2014, Jung et al. 2014, Hackett et al. 2016, López-Baucells et al. 2017, Rydell et al. 2017, Vassilios et al. 2017, Russo et al. 2018). Monitoring bats by passively recording them using ultrasound detectors has opened many opportunities for research and conservation (Frick 2013, Russo and Voigt 2016, Vassilios et al. 2017). Acoustic monitoring allows researchers to collect massive datasets using a non-invasive sampling method that provide excellent baselines for studies on spatio-temporal activity patterns and habitat use (e.g. Razafimanahaka et al. 2016), foraging behaviour (e.g. Scott et al. 2010), species distribution (e.g. Hughes et al. 2010), and even the discovery of cryptic diversity (Horta et al. 2015, López-Baucells et al. 2017).

Owing to major technological advances, bioacoustics has now become a burgeoning field in ecological research worldwide (Adams et al. 2012, Law et al. 2015). Autonomous detectors are becoming widely used worldwide and automatic classifiers have emerged to aid in the daunting task of analysing the resulting massive acoustic datasets (Russo and Voigt 2016, Zamora-Gutiérrez et al. 2016). However, the scarcity of comprehensive reference call libraries still hampers their wider application in highly biodiverse countries. Comprehensive reference call libraries, which adequately capture variability in bat echolocation calls, are crucial for the success of acoustic bat studies. The fact that many species are very plastic and can adjust their calls to different environmental conditions and behaviours often results in interspecific overlap of call characteristics, greatly complicating species identification (Russo and Voigt 2016). Many algorithms and commercial software for bat species classification have recently been released (Russo and Jones 2002, Armitage and Ober 2010, Walters et al. 2012, Zamora-Gutiérrez et al. 2016). However, there is still controversy around the trade-off between automatic classifiers and manual species identification (Russo and Voigt 2016), and the former are especially weak for tropical countries.

In temperate regions, acoustics has been widely used for bat research (Kunz and Parsons 2009) and we have comprehensive information about the echolocation calls and their variability for most temperate bats. In the Neotropics, although bat acoustic studies have been conducted since the mid-1960s (Griffin and Novick 1955, Griffin 1958), the use of bioacoustics only recently started to increase (Jung et al. 2007, Jung and Kalko 2011, Jung et al. 2014, Marques et al. 2015, Arias-Aguilar et al. 2018). A substantial rise in bat echolocation descriptions has taken place in the last two decades (Russ and Bennett 2001, Jones et al. 2006, Teixeira and Jesus 2009, Barataud et al. 2013, López-Baucells et al. 2014, Falcão et al. 2015, Hackett et al. 2016, López-Baucells et al. 2016, Arias-Aguilar et al. 2018), but there is still a pressing need to improve local reference call libraries, especially in understudied countries in mega-diverse tropical regions such as Brazil.

## Neotropical bats and fragmentation

Bats have been used as model organisms to study the effects of habitat fragmentation and are widely regarded as good bioindicators (Jones et al. 2009). Their ability to fly, their large home ranges, the diversity of feeding habits and roost types make them excellent candidates to quantify the health status of disturbed habitats (Gorresen and Willig 2004, Meyer et al. 2008, Estrada-Villegas et al. 2010). These traits make them very sensitive to environmental changes as they can easily switch foraging or roosting sites if the environment becomes unsuitable to sustain their populations.

Quite a lot of research on forest fragmentation and bats has been carried out in the Neotropics over the past decades, although much more intensively in Central America than in South America (Meyer et al. 2016). In the Amazon, the topic remained rather unexplored until the work done by Sampaio (2001) and Sampaio et al. (2003) in the late 1990s. A follow-up project started about 15 years later (Farneda et al. 2015, Ferreira et al. 2017, Rocha 2017, Rocha et al. 2017a, Rocha et al. 2017b, Rocha et al. 2017c, Farneda et al. 2018a, Farneda et al. 2018b, Rocha et al. 2018), and this thesis is integrated in this project.

The accumulated evidence shows that Neotropical frugivorous and nectarivorous bats tend to increase their abundance in secondary regrowth due to the higher fruit availability from pioneer plant species such as *Vismia* or *Cecropia* (Delaval and Charles-Dominique 2006, Bobrowiec and Gribel 2010, Rocha 2017). On the other side animalivorous gleaners are more sensitive to forest fragmentation due to the resulting lower prey and roost availability (Gorresen and Willig 2004, Meyer and Kalko 2008). However, information on the impact of fragmentation on aerial insectivorous bats is much scarcer (Estrada-Villegas et al. 2010, Jung and Kalko 2010, Taylor et al. 2013, Bader et al. 2015, Rodríguez-San Pedro and Simonetti 2015).

Several studies addressed the consequences of fragmentation on Neotropical insectivorous birds and reported local extinctions or decreases in abundance for the most sensitive species (Willis 1974, Stouffer and Bierregaard 1995, Şekercioğlu et al. 2002, Powell et al. 2015a, Stratford and Stouffer 2015, Powell et al. 2016). Aerial insectivorous bats are highly heterogeneous in their response to deforestation. The consequences of habitat fragmentation upon them are eventually linked to canopy cover (Bader et al. 2015) and forest edges (Grindal and Brigham 1999), where activity tends to be higher than in forest fragment interiors. In fact, small disturbances have also been associated to local increases in bat activity and richness

(Grindal and Brigham 1998). Estrada-Villegas et al. (2010) found that aerial insectivorous bat species richness was higher on islands (fragments) than mainland sites, and that most differences in ensemble composition were linked to island isolation and vegetation structure. However, abundance tended to be similar between islands and continuous forest sites. Similar research undertaken in Southeast Asia (Struebig et al. 2008) found that forest fragment area was positively related with abundance and species richness of cavity/foliage-roosting bats, but not in the case of cave-dwelling bats and edge/open- space foraging species. In general, open-space bats (with long and narrow wings) do not commonly reveal negative responses to fragmentation, in contrast to edge- and forest- dwelling bat species (with shorter and broader wings) that are much more sensitive (Estrada-Villegas et al. 2010, Bader et al. 2015). All studies underline the importance of large forest fragments for maintaining high bat diversity and suggest that small patches are important in highly degraded habitats (Struebig et al. 2008, Estrada-Villegas et al. 2010).

Few researchers have explored the extent to which forest fragment assemblages recover with matrix regrowth (Rocha et al. 2018). These authors demonstrated that the development of the matrix from pasture to secondary forest may contribute to the recovery of certain fragmentation-sensitive species. Because of the importance of the remaining natural habitat patches for wildlife conservation, many studies have focused on the bat assemblages within the fragments, but very few have looked at bat assemblages in the secondary forests (but see Avila-Cabadilla et al. 2012, Avila-Cabadilla et al. 2014, Falcão et al. 2014). Across the tropics, secondary forests are increasingly being recognised for their important role in biodiversity conservation (Chazdon et al. 2009). Some frugivorous bat species increase in abundance in secondary habitats due to the predominance of pioneering plants. However, the required time for secondary forests to support full ecosystem recovery is barely explored and depend on a wide range of environmental variables (e.g. latitude, forest type, vegetation structural complexity, landscape configuration, matrix structure, edge effects or the presence of natural corridors; Bowen et al. 2007). For Neotropical insectivorous bats this is almost completely unknown. After a long process of vegetation regeneration, old secondary forests might offer some species habitat conditions similar to those of continuous primary forest, but in general, there is no consensus on the required period of ecosystem recovery.

## Biological Dynamics of Forest Fragments Project

There are very few experimental long-term studies of habitat loss and fragmentation (e.g. SAFE Project: http://www.safeproject.org, the Savannah River Site Corridor Experiment: http://nickhaddadlab.com/landscape-corridors/srs-corridor-project, the Biological Dynamics of Forest Fragments Project (BDFFP): http://pdbff.inpa.gov.br). The BDFFP is the largest and longest-running such project and is located ~80 Km north of Manaus (S 2º30', W 60º), in the heart of the Brazilian Amazon (Lovejoy and Bierregaard 1990, Laurance et al. 2002, Laurance et al. 2011, Laurance et al. 2018). The project was created in 1979 by Thomas E. Lovejoy with the aim to address the SLOSS (Single Large or Several Small) debate about the role of forest fragments in biodiversity conservation in relation to their size (Gascon and Bierregaard Jr 2001). It resulted in some of the most influential and highly cited publications about long-term effects of forest fragmentation worldwide (e.g. Gardner et al. 2009, Peres et al. 2010).

In the late 70s, the Brazilian government tried to incentivize the economy of the central regions of the country by subsidizing cattle ranching. Back then, the legislation required that 50% of the owner's land had to be preserved as natural habitat, which created an excellent opportunity for a project on fragmentation. T. Lovejoy seized the moment to convince the landowners and coordinated the start of the BDFFP project. The aim was to isolate forest patches of different sizes, surrounded by pastures, instead of leaving the intact forest only at the edges of the properties. As a result, in the early 80s, three large cattle ranches (~5000 ha) were created by clearing and burning vegetation but 11 forest fragments (five of 1 ha, four of 10 ha and two of 100 ha) isolated from the nearby continuous forest were preserved. However, after a decade, from 1988 onwards, the ranches were generally abandoned due to the low productivity of the soils and the process of vegetation regeneration started. Secondary regrowth conquered the land and covered the cleared areas. This regrowth is still clearly dominated by *Vismia* spp. (in areas that were cleared and burned) and *Cecropia* spp. (in areas that were cleared, but not burned).

In order to maintain fragment isolation, the fragments have been regularly (4-5 times) re-isolated by clearing 100 m-wide strips of surrounding vegetation (Laurance et al. 2018). During the whole period, the project has been managed by the National Institute of Amazonian Research (INPA) in collaboration with the Smithsonian Tropical Research Institute (STRI) (Laurance et al. 2018). The matrix surrounding the fragments that were targeted during this thesis project was ~30-year-old secondary forest. Therefore, all research that has been carried out in the context of the overall project since 2011 has taken landscape features into consideration (e.g. Farneda 2013, Farneda et al. 2015, Ferreira et al. 2017, Rocha et al. 2017a, Rocha et al. 2017b, Rocha et al. 2017c, Farneda et al. 2018a, Farneda et al. 2018b, Rocha et al. 2018).

*Recently re-isolated forest fragments in the Biological Dynamics of Forest Fragments Project*

The experimental landscape occupies more than 1000 km$^2$, has a relatively flat topography (80-160 m), and well-drained and nutrient-poor soil covered by unflooded *terra firme* rainforest with an average canopy height of 30-37 m (although some emergent trees can reach up to 55 m) (Laurance et al. 2011). The temperature ranges from 19 to 39 $^{o}$C, with an average of 26 $^{o}$C (de Oliveira and Mori 1999). Annual rainfall oscillates between 1900 and 3500 mm with a dry season between June-July and September-October and a wet season between October-November and April-June (Lovejoy and Bierregaard 1990, de Oliveira and Mori 1999). The hydrological system is characterized by a network of narrow streams and low hills that cover the whole area and isolated lakes that accumulate rain water, especially during the wet season (Torrent et al. 2018).

Inventories of many groups of animals (e.g. trees, birds, frogs) were undertaken before the forest clearing in the early 1980s, which served as excellent baseline datasets to investigate the long-term effects of deforestation and habitat fragmentation on several taxa. Over the last decades, projects on a multitude of topics have been carried out at the BDFFP (e.g. restoration, migration, landscape dynamics, climatology). There are now good datasets for plants (Ter Steege et al. 2013, Laurance et al. 2014a, Santos et al. 2014, Ribeiro et al. 2016) and several faunal groups such as birds (Stouffer et al. 2009, Stouffer et al. 2011, Powell et al. 2015a, Powell et al. 2016), amphibians (Tocher et al. 2001, Rocha et al. 2014, Rocha and López-Baucells 2014b), primates (Gilbert 2003, Boyle 2008) and various invertebrate taxa (Quintero and Roslin 2005, Vasconcelos and Bruna 2012).

No bat data were collected before the forest clearing and the first bat project was conducted in the late 90s by Erica Sampaio (Sampaio 2001, Sampaio et al. 2003) and Enrico Bernard (Bernard 2001). While E. Sampaio studied the effects of forest fragmentation and surveyed bats with ground mistnets in continuous forest and forest fragments, E. Bernard studied vertical stratification, diet, activity patterns and phenology in continuous forest using ground- and canopy- mistnets (Bernard 2002, Bernard and Fenton 2002). Bobrowiec and Gribel (2010) later studied the effects of secondary regrowth on phyllostomid bat assemblages and Wieland et al. (2011) focused on seed dispersal, using passive seed rain traps. However, none of these published studies in the BDFFP involved bioacoustics and its aerial insectivorous bat assemblages remained practically unknown.

In 2011, a new research project funded by the Portuguese Foundation for Science and Technology (Fundação para a Ciência e a Tecnologia, FCT) started at the BDFFP. It included field work carried out over the course of three years and greatly increased the regional knowledge of bat assemblages and of the consequences of forest fragmentation on bats. This project included three main PhD theses: one focused on phyllostomid bats led by R. Rocha (2017); another about bat functional diversity led by F. Farneda (in progress), and this one. Additionally, eight MSc theses were carried out during the same period, focusing on vertical stratification of bat assemblages (Silva 2012), matrix and area effects (Groenenberg 2012), trait-mediated fragmentation vulnerability in phyllostomid bats (Farneda 2013, Farneda et al. 2015), seasonal responses to fragmentation (Ferreira 2015, Ferreira et al. 2017), vertical stratification of aerial insectivorous bats (Mas 2014), the importance of temporary lakes for insectivorous bats (Torrent 2017, Torrent et al. 2018), trait-related vulnerability of insectivorous bats (Fraixedas 2017) and edge effects on aerial insectivorous bats (Yoh 2018). In parallel, several short notes describing echolocation calls and natural history notes have been published (e.g. López-Baucells et al. 2013, Treitler et al. 2013, López-Baucells et al. 2014, Rocha and López-Baucells 2014a, Rocha et al. 2016, Gonçálvez et al. 2017, López-

Baucells et al. 2017, López-Baucells et al. 2018). Additionally, the first Field Guide to the Bats of the Amazon - which provided the first echolocation keys for the bats of the Amazon - was released in digital format in 2016, published by the National Institute for Amazonian Research (INPA, Brazil) and re-edited by Pelagic Publishing (UK) in print in 2018.

This thesis focuses on aerial insectivorous bats as all sampling was undertaken using bioacoustics. Although some species might not be entirely considered "aerial", to simplify the flow of the thesis, I will hereafter refer to all non-phyllostomid bats suitable to be surveyed with bioacoustic methods as aerial insectivorous bats.

## Main aims of this thesis and outline

The overarching aim of this thesis was to reveal the diversity of aerial insectivorous bats and quantify the effects of forest fragmentation on this bat ensemble within the BDFFP landscape. Specifically, I aimed to i) explore spatial patterns in their foraging activity, ii) investigate how functional traits of species (echolocation, body mass and wing morphology) influence their fragmentation vulnerability, and iii) assess the importance of the lakes embedded in primary forest for bat conservation. However, in order to accomplish these objectives, it was first necessary to fill several knowledge gaps regarding insectivorous bats in the Amazon by iv) describing the echolocation calls of the species present in the study area, v) developing an echolocation key to classify them, and vi) testing automatic algorithms to aid in the task of classifying all the recordings. This thesis was based on extensive fieldwork carried out between August 2011 and June 2014, and an acoustic database comprised of >1,000,000 recordings. Each of the project's aims was addressed in the following separate publications that make up individual chapters of this dissertation:

1. Field Guide to the Bats of the Amazon
2. Stronger together: Combining automated classifiers with manual post-validation optimizes the workload vs reliability trade-off of species identification in bat acoustic surveys
3. Optimising bat bioacoustic surveys in human-modified tropical landscapes
4. Taxonomic, functional and phylogenetic diversity recovery of aerial insectivorous bats in old secondary Neotropical rainforest
5. Echolocation and wing morphology: key trait correlates of vulnerability of aerial insectivorous bats to tropical forest fragmentation
6. The importance of lakes for bat conservation in Amazonian rainforests: an assessment using autonomous recorders

## References

Adams, A. M., M. K. Jantzen, R. M. Hamilton, and M. B. Fenton. 2012. Do you hear what I hear? Implications of detector selection for acoustic monitoring of bats. Methods in Ecology and Evolution **3**:992-998.

Ahlén, I. 1981. Field identification of bats and survey methods based on sounds. Myotis **18**:128-136.

Aide, T. M., M. L. Clark, H. R. Grau, D. López-Carr, M. A. Levy, D. Redo, M. Bonilla-Moheno, G. Riner, M. J. Andrade-Núñez, and M. Muñiz. 2013. Deforestation and Reforestation of Latin America and the Caribbean (2001–2010). Biotropica **45**:262-271.

Aires, C. 2008. Caracterização das espécies brasileiras de *Myotis* Kaup, 1829 (Chiroptera: Vespertilionidae) e ensaio sobre filogeografia de *Myotis nigricans* (Schinz, 1821) e *Myotis riparius* Handley, 1960. Universidade de Sao Paulo, Sao Paulo Brazil.

Arias-Aguilar, A., F. Hintze, L. M. S. Aguiar, V. Rufray, E. Bernard, and M. J. R. Pereira. 2018. Who’s calling? Acoustic identification of Brazilian bats. Mammal Research **63**:231-253.

Armitage, D. W., and H. K. Ober. 2010. A comparison of supervised learning techniques in the classification of bat echolocation calls. Ecological Informatics **5**:465-473.

Arroyo-Rodríguez, V., C. Rojasa, R. A. Saldaña-Vázqueza, and K. E. Stonerb. 2016. Landscape composition is more important than landscape configuration for phyllostomid bat assemblages in a fragmented biodiversity hotspot. Biological Conservation **198**:84-92.

Avila-Cabadilla, L. D., G. A. Sanchez-Azofeifa, K. E. Stoner, M. Y. Alvarez-Añorve, M. Quesada, and C. A. Portillo-Quintero. 2012. Local and landscape factors determining occurrence of phyllostomid bats in tropical secondary forests. PLoS ONE **7**:e35228.

Avila-Cabadilla, L. D., K. E. Stoner, J. M. Nassar, M. M. Espírito-Santo, M. Y. Alvarez-Añorve, C. I. Aranguren, M. Henry, J. A. González-Carcacía, L. A. Dolabela Falcão, and G. A. Sanchez-Azofeifa. 2014. Phyllostomid bat occurrence in successional stages of Neotropical dry forests. PLoS ONE **9**:e84572.

Bader, E., K. Jung, E. K. V. Kalko, R. A. Page, R. Rodriguez, and T. Sattler. 2015. Mobility explains the response of aerial insectivorous bats to anthropogenic habitat change in the Neotropics. Biological Conservation **186**:97-106.

Barataud, M., S. Giosa, F. Leblanc, V. Rufray, T. Disca, L. Tillon, M. Delaval, A. Haquart, and M. Dewynter. 2013. Identification et écologie acoustique des chiroptères de Guyane française. Le Rhinolophe **19**:103–145.

Barlow, J., L. A. Mestre, T. A. Gardner, and C. A. Peres. 2007. The value of primary, secondary and plantation forests for Amazonian birds. Biological Conservation **136**:212-231.

Bernard, E. 2001. Vertical stratification of bat communities in primary forests of Central Amazon, Brazil. Journal of Tropical Ecology **17**:115-126.

Bernard, E. 2002. Diet, activity and reproduction of bat species (Mammalia, Chiroptera) in Central Amazonia, Brazil. Revista Brasileira De Zoologia **19**:173-188.

Bernard, E., and M. B. Fenton. 2002. Species diversity of bats (Mammalia : Chiroptera) in forest fragments, primary forests, and savannas in central Amazonia, Brazil. Canadian Journal of Zoology **80**:1124-1140.

Bezerra, A. M. R., F. Escarlate-Tavares, and J. Marinho. 2005. First record of *Thyroptera discifera* (Chiroptera : Thyropteridae) in the Cerrado of Central Brazil. Acta Chiropterologica **7**:165–170.

Bobrowiec, P., and R. Gribel. 2010. Effects of different secondary vegetation types on bat community composition in Central Amazonia, Brazil. Animal Conservation **13**:204-216.

Bowen, M. E., C. A. McAlpine, A. P. N. House, and G. C. Smith. 2007. Regrowth forests on abandoned agricultural land: A review of their habitat values for recovering forest fauna. Biological Conservation **140**:273-296.

Boyle, S. A. 2008. Human impacts on primate conservation in central Amazonia. Tropical Conservation Science **1**:6-17.

Brinkløv, S., E. K. Kalko, and A. Surlykke. 2009. Intense echolocation calls from two whispering bats, *Artibeus jamaicensis* and *Macrophyllum macrophyllum* (Phyllostomidae). Journal of Experimental Biology **212**:11-20.

Brooke, A. P. 1994. Diet of the fishing bat, *Noctilio leporinus* (Chiroptera: Noctilionidae). Journal of Mammalogy **75**:212-218.

Brooke, A. P. 1997. Social organization and foraging behaviour of the fishing bat, *Noctilio leporinus* (Chiroptera:Noctilionidae). Ethology **103**:421-436.

Burgin, C. J., J. P. Colella, P. L. Kahn, and N. S. Upham. 2018. How many species of mammals are there? Journal of Mammalogy **99**:1-14.

Butler, R. A., and W. F. Laurance. 2009. Is oil palm the next emerging threat to the Amazon? Tropical Conservation Science **2**:1-10.

Castano, J. H., and J. D. Corrales. 2007. First record of *Centronycteris centralis* (Chiroptera : Emballonuridae) for the Colombian Andes. Mastozoologia Neotropical **14**:69-72.

Clement, M. J., K. L. Murray, D. I. Solick, and J. C. Gruver. 2014. The effect of call libraries and acoustic filters on the identification of bat echolocation. Ecology and Evolution **4**:3482-3493.

Cunto, G. C., and E. Bernard. 2012. Neotropical bats as indicators of environmental disturbance: What is the emerging message? Acta Chiropterologica **14**:143-151.

Chazdon, R. L. 2014. Second growth: The promise of tropical forest regeneration in an age of deforestation. University of Chicago Press, Connecticut, United States of America.

Chazdon, R. L., C. A. Peres, D. Dent, D. Sheil, A. E. Lugo, D. Lamb, N. E. Stork, and S. E. Miller. 2009. The potential for species conservation in tropical secondary forests. Conservation Biology **23**:1406-1417.

Daily, G. C. 1997. Countryside biogeography and the provision of ecosystem services. Pages 104-113 *in* Raven, editor. Nature and human society: The quest for a sustainable world. National Academy Press, Washington, United States of America.

Dalquest, W. W. 1950. The genera of the chiropteran family Natalidae. Journal of Mammalogy **31**:436-443.

Davalos, L. M. 2005. Molecular phylogeny of funnel-eared bats (Chiroptera: Natalidae), with notes on biogeography and conservation. Molecular Phylogenetics and Evolution **37**:91-103.

Davidson, E. A., A. C. de Araujo, P. Artaxo, J. K. Balch, I. F. Brown, M. M. C. Bustamante, M. T. Coe, R. S. DeFries, M. Keller, M. Longo, J. W. Munger, W. Schroeder, B. S. Soares-Filho, C. M. Souza, and S. C. Wofsy. 2012. The Amazon basin in transition. Nature **481**:321-328.

de Oliveira, A. A., and S. A. Mori. 1999. A central Amazonian terra firme forest. I. High tree species richness on poor soils. Biodiversity and Conservation **8**:1219-1244.

Delaval, M., and P. Charles-Dominique. 2006. Edge effects on frugivorous and nectarivorous bat communities in a neotropical primary forest in French Guiana. Revue d'écologie **61**:343-352.

Denzinger, A., E. K. Kalko, M. Tschapka, A. D. Grinnell, and H. U. Schnitzler. 2016. Guild structure and niche differentiation in echolocating bats. Pages 141-166 *in* M. Brock Fenton, editor. Bat bioacoustics. Springer, New York, United States of America.

Didham, R. K., V. Kapos, and R. M. Ewers. 2012. Rethinking the conceptual foundations of habitat fragmentation research. Oikos **121**:161-170.

Dirzo, R., H. S. Young, M. Galetti, G. Ceballos, N. J. B. Isaac, and B. Collen. 2014. Defaunation in the Anthropocene. Science **345**:401-406.

Edwards, D. P., M. R. Massam, T. Haugaasen, and J. J. Gilroy. 2017. Tropical secondary forest regeneration conserves high levels of avian phylogenetic diversity. Biological Conservation **209**:432-439.

Estrada-Villegas, S., C. F. Meyer, and E. K. Kalko. 2010. Effects of tropical forest fragmentation on aerial insectivorous bats in a land-bridge island system. Biological Conservation **143**:597-608.

Ewers, R. M., and R. K. Didham. 2006a. Confounding factors in the detection of species responses to habitat fragmentation. Biological Reviews **81**:117-142.

Ewers, R. M., and R. K. Didham. 2006b. Continuous response functions for quantifying the strength of edge effects. Journal of Applied Ecology **43**:527-536.

Fahrig, L. 1997. Relative effects of habitat loss and fragmentation on population extinction. The Journal of Wildlife Management **61**:603-610.

Fahrig, L. 2003. Effects of habitat fragmentation on biodiversity. Annual Review of Ecology, Evolution, and Systematics **34**:487-515.

Fahrig, L. 2017. Forty years of bias in habitat fragmentation research. Pages 32-38 *in* P. Kareiva, editor. Effective Conservation Science: Data Not Dogma. Oxford University Press, United Kingdom.

Falcão, F., J. A. Ugarte-Núñez, D. Faria, and C. B. Caselli. 2015. Unravelling the calls of discrete hunters: acoustic structure of echolocation calls of furipterid bats (Chiroptera, Furipteridae). Bioacoustics **24**:175-183.

Falcão, L. A. D., M. M. do Espírito-Santo, L. O. Leite, R. N. S. L. Garro, L. D. Avila-Cabadilla, and K. E. Stoner. 2014. Spatiotemporal variation in phyllostomid bat assemblages over a successional gradient in a tropical dry forest in southeastern Brazil. Journal of Tropical Ecology **30**:123-132.

Farneda, F. Z. 2013. Bat species vulnerability to forest fragmentation in the Central Amazon. Universidade de Lisboa, Lisbon, Portugal.

Farneda, F. Z., R. Rocha, A. López-Baucells, M. Groenenberg, I. Silva, J. M. Palmeirim, P. E. D. Bobrowiec, C. F. J. Meyer, and M. Pocock. 2015. Trait-related responses to habitat fragmentation in Amazonian bats. Journal of Applied Ecology **52**:1381-1391.

Farneda, F. Z., R. Rocha, A. López-Baucells, E. M. Sampaio, J. M. Palmeirim, P. E. D. Bobrowiec, C. E. V. Grelle, and C. F. J. Meyer. 2018a. Functional recovery of Amazonian bat assemblages following secondary forest succession. Biological Conservation **218**:192-199.

Farneda, F. Z., R. Rocha, A. López-Baucells, E. M. Sampaio, J. M. Palmeirim, P. E. D. Bobrowiec, C. E. V. Grelle, and C. F. J. Meyer. 2018b. The road to functional recovery: temporal effects of matrix regeneration on Amazonian bats. Tropical Conservation Science **11**:1-4.

Fenton, M. B. 1999. Describing the echolocation calls and behaviour of bats. Acta Chiropterologica **1**:127-136.

Fenton, M. B., and G. P. Bell. 1981. Recognition of species of insectivorous bats by their echolocation calls. Journal of Mammalogy **62**:233-243.

Fenton, M. B., P. A. Faure, and J. M. Ratcliffe. 2012. Evolution of high duty cycle echolocation in bats. The Journal of Experimental Biology **215**:2935-2944.

Fenton, M. B., A. D. Grinnell, A. N. Popper, and R. R. Fay. 2016. Bat Bioacoustics. Springer, New York, United States of America.

Ferreira , D. 2015. Effects of seasonality on the responses of Neotropical bats to local- and landscape-scale attributes in a fragmented landscape. MSc Thesis. University of Lisbon, Lisbon, Portugal.

Ferreira, D. F., R. Rocha, A. López-Baucells, F. Z. Farneda, J. M. B. Carreiras, J. M. Palmeirim, and C. F. J. Meyer. 2017. Season-modulated responses of Neotropical bats to forest fragmentation. Ecology and Evolution **7**:4059-4071.

Foley, J. A., N. Ramankutty, K. A. Brauman, E. S. Cassidy, J. S. Gerber, M. Johnston, N. D. Mueller, C. O/'Connell, D. K. Ray, P. C. West, C. Balzer, E. M. Bennett, S. R. Carpenter, J. Hill, C. Monfreda, S. Polasky, J. Rockstrom, J. Sheehan, S. Siebert, D. Tilman, and D. P. M. Zaks. 2011. Solutions for a cultivated planet. Nature **478**:337-342.

Fraixedas, S. 2017. Which functional traits make Neotropical aerial insectivorous bats more vulnerable to forest fragmentation? MSc Thesis. Autonomous University of Barcelona, Barcelona, Spain.

Frick, W. F. 2013. Acoustic monitoring of bats, considerations of options for long-term monitoring. Therya **4**:69-78.

Gardner, T. A., J. Barlow, R. Chazdon, R. M. Ewers, C. A. Harvey, C. A. Peres, and N. S. Sodhi. 2009. Prospects for tropical forest biodiversity in a human-modified world. Ecology Letters **12**:561-582.

Gascon, C., and R. O. Bierregaard Jr. 2001. The biological dynamics of forest fragments project. Pages 31-42 *in* R. O. Bierregaard, editor. Lessons from Amazonia: The ecology and conservation of a fragmented forest. Yale University Press, Michigan, United States of America.

Gilbert, K. A. 2003. Primates and fragmentation of the amazon forest. Pages 145-157 *in* L. K. Marsh, editor. Primates in Fragments: Ecology and Conservation. Springer, Boston, United States of America.

Gonçálvez, C., A. López-Baucells, and R. Rocha. 2017. Opportunistic predation of a silky short-tailed bat (*Carollia brevicauda*) by a tawny-bellied screech-owl (*Megascops watsonii*), with a compilation of predation events upon bats entangled in mist-nets. Journal of Bat Research & Conservation **10**:41-46.

Gorresen, P. M., and M. R. Willig. 2004. Landscape responses of bats to habitat fragmentation in Atlantic forest of Paraguay. Journal of Mammalogy **85**:688-697.

Gregorin, R., E. Gonçalves, B. K. Lim, and M. D. Engstrom. 2006. New species of disk-winged bat *Thyroptera* and range expansion for *T. discifera*. Journal of Mammalogy **87**:238-246.

Griffin, D. R. 1958. Listening in the dark. New Haven Yale University Press, Connecticut, United States of America.

Griffin, D. R., and A. Novick. 1955. Acoustic orientation of neotropical bats. Journal of Experimental Zoology **130**:251-299.

Grindal, S. D., and R. M. Brigham. 1998. Short-term effects of small-scale habitat disturbance on activity by insectivorous bats. The Journal of Wildlife Management **62**:996-1003.

Grindal, S. D., and R. M. Brigham. 1999. Impacts of forest harvesting on habitat use by foraging insectivorous bats at different spatial scales. Ecoscience **6**:25-34.

Grinnell, A. D., E. Gould, and M. B. Fenton. 2016. A history of the study of echolocation. Pages 1-24 *in* M. B. Fenton, editor. Bat bioacoustics. Springer, New York, United States of America.

Groenenberg, M. 2012. Matrix-mitigated edge and area effects on Neotropical bats in a fragmented landscape. MSc Thesis. Imperial College London, London, United Kingdom.

Hackett, T. D., M. W. Holderied, and C. Korine. 2016. Echolocation call description of 15 species of Middle-Eastern desert dwelling insectivorous bats. Bioacoustics Journal **26**:217-235.

Haddad, N. M., L. A. Brudvig, J. Clobert, K. F. Davies, A. Gonzalez, R. D. Holt, T. E. Lovejoy, J. O. Sexton, M. P. Austin, and C. D. Collins. 2015. Habitat fragmentation and its lasting impact on Earth’s ecosystems. Science advances **1**:e1500052.

Hansen, M. C., P. V. Potapov, R. Moore, M. Hancher, S. A. Turubanova, A. Tyukavina, D. Thau, S. V. Stehman, S. J. Goetz, T. R. Loveland, A. Kommareddy, A. Egorov, L.

Chini, C. O. Justice, and J. R. G. Townshend. 2013. High-resolution global maps of 21st-century forest cover change. Science **342**:850-853.

Hintze, F., E. Barbier, and E. Bernard. 2016. Emballonuridae Gervais, 1855 (Chiroptera) of Reserva Biológica de Saltinho (Atlantic Forest), in Brazil, revealed by echolocation. Check List **12**:1925.

Horta, P., H. Raposeira, H. Santos, P. Alves, J. Palmeirim, R. Godinho, G. Jones, and H. Rebelo. 2015. Bats' echolocation call characteristics of cryptic Iberian *Eptesicus* species. European Journal of Wildlife Research **61**:813-818.

Hughes, A. C., C. Satasook, P. J. J. Bates, P. Soisook, T. Sritongchuay, G. Jones, and S. Bumrungsri. 2010. Echolocation call analysis and presence-only modelling as conservation monitoring tools for rhinolophoid bats in Thailand. Acta Chiropterologica **12**:311-327.

Jakobsen, L., E. K. V. Kalko, and A. Surlykke. 2012. Echolocation beam shape in emballonurid bats, *Saccopteryx bilineata* and *Cormura brevirostris*. Behavioral Ecology and Sociobiology **66**:1493-1502.

Jones, G., D. S. Jacobs, T. Kunz, M. R. Willig, and P. A. Racey. 2009. Carpe noctem: the importance of bats as bioindicators. Endangered Species Research **8**:93-115.

Jones, G., S. Parsons, S. Y. Zhang, B. Stadelmann, P. Benda, and M. Ruedi. 2006. Echolocation calls, wing shape, diet and phylogenetic diagnosis of the endemic Chinese bat *Myotis pequinius*. Acta Chiropterologica **8**:451-463.

Jung, K., E. K. Kalko, and O. Von Helversen. 2007. Echolocation calls in Central American emballonurid bats: signal design and call frequency alternation. Journal of Zoology **272**:125-137.

Jung, K., and E. K. V. Kalko. 2010. Where forest meets urbanization: foraging plasticity of aerial insectivorous bats in an anthropogenically altered environment. Journal of Mammalogy **91**:144-153.

Jung, K., and E. K. V. Kalko. 2011. Adaptability and vulnerability of high flying Neotropical aerial insectivorous bats to urbanization. Diversity and distributions **17**:262-274.

Jung, K., J. Molinari, and E. K. V. Kalko. 2014. Driving factors for the evolution of species-specific echolocation call design in New World free-tailed bats (Molossidae). PLoS ONE **9**:e85279.

Jung, K., and C. G. Threlfall. 2016. Urbanisation and its effects on bats - A global meta-analysis. Pages 13-33 *in* C. Voigt, editor. Bats in the Anthropocene: Conservation of Bats in a Changing World Springer, New York, United States of America.

Kalka, M. B., A. R. Smith, and E. K. V. Kalko. 2008. Bats limit arthropods and herbivory in a tropical forest. Science **320**:71-71.

Kalko, E. 2004. Neotropical leaf-nosed bats (Phyllostomidae): Whispering bats as candidated for acoustic surveys? Bat Echolocation Research **203**:63.

Kalko, E. K., S. Estrada Villegas, M. Schmidt, M. Wegmann, and C. F. Meyer. 2008. Flying high - assessing the use of the aerosphere by bats. Integrative and Comparative Biology **48**:60-73.

Kalko, E. K., and H. Schnitzler. 1998. How echolocating bats approach and acquire food. Pages 197-204 *in* K. T. H. and R. P. A., editors. Bat biology and conservation. Smithsonian Institution Press, Washington, United States of America.

Keenan, R. J., G. A. Reams, F. Achard, J. V. de Freitas, A. Grainger, and E. Lindquist. 2015. Dynamics of global forest area: Results from the FAO Global forest resources assessment 2015. Forest Ecology and Management **352**:9-20.

Kunz, T. H., E. B. de Torrez, D. Bauer, T. Lobova, and T. H. Fleming. 2011. Ecosystem services provided by bats. Annals of the New York Academy of Sciences **1223**:1-38.

Kunz, T. H., and S. Parsons. 2009. Ecological and behavioral methods for the study of bats. 2nd edition. Johns Hopkins University Press, Baltimore, United States of America.

Kupfer, J. A., G. P. Malanson, and S. B. Franklin. 2006. Not seeing the ocean for the islands: the mediating influence of matrix-based processes on forest fragmentation effects. Global Ecology and Biogeography **15**:8-20.

Laurance, W. F., A. S. Andrade, A. Magrach, J. L. C. Camargo, J. J. Valsko, M. Campbell, P. M. Fearnside, W. Edwards, T. E. Lovejoy, and S. G. Laurance. 2014a. Long-term changes in liana abundance and forest dynamics in undisturbed Amazonian forests. Ecology **95**:1604-1611.

Laurance, W. F., J. L. C. Camargo, P. M. Fearnside, T. E. Lovejoy, G. B. Williamson, R. C. G. Mesquita, C. F. J. Meyer, P. E. D. Bobrowiec, and S. G. W. Laurance. 2018. An Amazonian rainforest and its fragments as a laboratory of global change. Biological Reviews of the Cambridge Philosophical Society **93**:223-247.

Laurance, W. F., J. L. C. Camargo, R. C. C. Luizao, S. G. Laurance, S. L. Pimm, E. M. Bruna, P. C. Stouffer, G. B. Williamson, J. Benitez-Malvido, H. L. Vasconcelos, K. S. Van Houtan, C. E. Zartman, S. A. Boyle, R. K. Didham, A. Andrade, and T. E. Lovejoy. 2011. The fate of Amazonian forest fragments: A 32-year investigation. Biological Conservation **144**:56–67.

Laurance, W. F., T. E. Lovejoy, H. L. Vasconcelos, E. M. Bruna, R. K. Didham, P. C. Stouffer, C. Gascon, R. O. Bierregaard, S. G. Laurance, and E. Sampaio. 2002. Ecosystem decay of Amazonian forest fragments: a 22-year investigation. Conservation Biology **16**:605-618.

Laurance, W. F., H. E. M. Nascimento, S. G. Laurance, A. Andrade, R. M. Ewers, K. E. Harms, R. C. C. Luizão, and J. E. Ribeiro. 2007. Habitat fragmentation, variable edge effects and the landscape - Divergence hypothesis. PLoS ONE **2**:e1017.

Laurance, W. F., J. Sayer, and K. G. Cassman. 2014b. Agricultural expansion and its impacts on tropical nature. Trends in Ecology & Evolution **29**:107-116.

Law, B., L. Gonsalves, P. Tap, T. Penman, and M. Chidel. 2015. Optimizing ultrasonic sampling effort for monitoring forest bats. Austral Ecology **40**:886–897.

Lees, A. C., C. A. Peres, P. M. Fearnside, M. Schneider, and J. A. Zuanon. 2016. Hydropower and the future of Amazonian biodiversity. Biodiversity and Conservation **25**:451-466.

Lenz, B. B., K. M. Jack, and W. R. Spironello. 2014. Edge effects in the primate community of the biological dynamics of forest fragments project, Amazonas, Brazil. American Journal of Physical Anthropology **155**:436-446.

Lewis-Oritt, N., R. A. Van Den Bussche, and R. J. Baker. 2001. Molecular evidence for evolution of piscivory in *Noctilio* (Chiroptera: Noctilionidae). Journal of Mammalogy **82**:748-759.

Lim, B. K., M. D. Engstrom, F. A. Reid, N. B. Simmons, R. S. Voss, and D. W. Fleck. 2010. A new species of *Peropteryx* (Chiroptera: Emballonuridae) from western Amazonia with comments on phylogenetic relationships within the genus. American Museum Novitates **3686**:1-20.

López-Baucells, A., R. Rocha, P. E. D. Bobrowiec, E. Bernard, J. Palmeirim, and C. Meyer. 2016. Field Guide to Amazonian Bats. INPA, Manaus, Brazil.

López-Baucells, A., R. Rocha, G. Fernández-Arellano, P. E. D. Bobrowiec, J. M. Palmeirim, and C. F. J. Meyer. 2014. Echolocation of the big red bat *Lasiurus egregius* (Chiroptera: Vespertilionidae) and first record from the Central Brazilian Amazon. Studies on Neotropical Fauna and Environment **49**:18-25.

López-Baucells, A., R. Rocha, I. García-Mayes, K. Vulinec, and C. F. J. Meyer. 2013. First record of *Micronycteris sanborni* (Chiroptera: Phyllostomidae) from Central Amazonia, Brazil: range expansion and description of its echolocation. Mammalia **78**:127–132.

López-Baucells, A., R. Rocha, V. C. Tavares, L. Mora, P. E. D. Bobrowiec, S. E. Silva, and C. Meyer. 2018. Molecular, morphological and acoustic identification of *Eumops maurus* and *E. hansae* (Chiroptera: Molossidae) with new reports from Central Amazonia. Tropical Zoology **31**:1-20.

López-Baucells, A., L. Torrent, R. Rocha, A. C. Pavan, P. E. D. Bobrowiec, and C. F. J. Meyer. 2017. Geographical variation in the high-duty cycle echolocation of the cryptic common mustached bat *Pteronotus* cf. *rubiginosus* (Mormoopidae). Bioacoustics Journal **27**:341-357.

Lovejoy, T. E., and R. Bierregaard. 1990. Central Amazonian forests and the minimum critical size of ecosystems project. Pages 60–71 *in* A. H. Gentry, editor. Four neotropical rainforests. Yale University Press, London, unityed Kingdo.

Maas, B., Y. Clough, and T. Tscharntke. 2013. Bats and birds increase crop yield in tropical agroforestry landscapes. Ecology Letters **16**:1480-1487.

MacArthur, R. H., and E. O. Wilson. 1967. Theory of Island Biogeography. Princeton University Press, Princeton, United States of America.

MacSwiney, G. C., F. M. Clarke, and P. A. Racey. 2008. What you see is not what you get: the role of ultrasonic detectors in increasing inventory completeness in Neotropical bat assemblages. Journal of Applied Ecology **45**:1364-1371.

Marques, J. T. 2016. Structuring Amazonian bat assemblages: importance of horizontal and vertical dimensions of habitat. University of Lisbon, Lisbon, Portugal.

Marques, J. T., M. J. Ramos Pereira, T. A. Marques, C. D. Santos, J. Santana, P. Beja, and J. M. Palmeirim. 2013. Optimizing sampling design to deal with mist-net avoidance in Amazonian birds and bats. PLoS ONE **8**:e74505.

Marques, J. T., M. J. Ramos Pereira, and J. M. Palmeirim. 2015. Patterns in the use of rainforest vertical space by Neotropical aerial insectivorous bats: all the action is up in the canopy. Ecography **39**:476-486.

Mas, M. 2014. Vertical stratification on insectivorous bats ensembles in Central Amazon. MSc Thesis. Universitat de Barcelona, Barcelona, Spain.

Mendenhall, C. D., C. V. Kappel, and P. R. Ehrlich. 2013. Countryside Biogeography. Pages 347-360 *in* S. A. Levin, editor. Encyclopedia of Biodiversity. Academic Press, Waltham, United States of America.

Mendenhall, C. D., D. S. Karp, C. F. J. Meyer, E. A. Hadly, and G. C. Daily. 2014. Predicting biodiversity change and averting collapse in agricultural landscapes. Nature **509**:213-217.

Meyer, C. F. J., J. Fruend, W. P. Lizano, and E. K. V. Kalko. 2008. Ecological correlates of vulnerability to fragmentation in Neotropical bats. Journal of Applied Ecology **45**:381-391.

Meyer, C. F. J., and E. K. V. Kalko. 2008. Bat assemblages on Neotropical land-bridge islands: nested subsets and null model analyses of species co-occurrence patterns. Diversity and distributions **14**:644-654.

Meyer, C. F. J., M. J. Struebig, and M. R. Willig. 2016. Responses of tropical bats to habitat fragmentation, logging, and deforestation. Pages 63-103 *in* C. C. Voigt and T. Kingston, editors. Bats in the Anthropocene: Conservation of bats in a changing world. Springer, New York, United States of America.

Mickleburgh, S. P., A. M. Hutson, and P. A. Racey. 2002. A review of the global conservation status of bats. Oryx **36**:18-34.

Mora, E. C., and S. Macias. 2011. Short CF-FM and FM-short CF calls in the echolocation behavior of Pteronotus macleayii (Chiroptera: Mormoopidae). Acta Chiropterologica **13**:457-463.

Moratelli, R., L. Idárraga, and D. Wilson. 2015. Range extension of *Myotis midastactus* (Chiroptera, Vespertilionidae) to Paraguay. Biodiversity Data Journal **3**:e5708.

Nepstad, D., D. McGrath, C. Stickler, A. Alencar, A. Azevedo, B. Swette, T. Bezerra, M. DiGiano, J. Shimada, R. Seroa da Motta, E. Armijo, L. Castello, P. Brando, M. C. Hansen, M. McGrath-Horn, O. Carvalho, and L. Hess. 2014. Slowing Amazon deforestation through public policy and interventions in beef and soy supply chains. Science **344**:1118-1123.

Nogueira, M. R., I. P. de Lima, R. Moratelli, V. C. Tavares, R. Gregorin, and A. L. Peracchi. 2014. Checklist of Brazilian bats, with comments on original records. Check List **10**:808-821.

Novaes, R. L. M., V. C. Cláudio, and A. A. Kuniy. 2017. Range extension of *Diclidurus scutatus* (Chiroptera, Emballonuridae) to southwestern Amazonia. Acta Amazonica **47**:359-364.

Numata, I., and M. A. Cochrane. 2012. Forest fragmentation and its potential implications in the Brazilian Amazon between 2001 and 2010. Open Journal of Forestry **2**:265-271.

O'Farrell, M. J., and W. L. Gannon. 1999. A comparison of acoustic versus capture techniques for the inventory of bats. Journal of Mammalogy **80**:24-30.

Pavan, A. C., and G. Marroig. 2017. Timing and patterns of diversification in the Neotropical bat genus *Pteronotus* (Mormoopidae). Molecular Phylogenetics and Evolution **108**:61-69.

Peres, C. A., T. A. Gardner, J. Barlow, J. Zuanon, F. Michalski, A. C. Lees, I. C. Vieira, F. M. Moreira, and K. J. Feeley. 2010. Biodiversity conservation in human-modified Amazonian forest landscapes. Biological Conservation **143**:2314-2327.

Powell, L. L., P. C. Stouffer, and E. I. Johnson. 2013. Recovery of understory bird movement across the interface of primary and secondary amazon rainforest. The Auk **130**:459-468.

Powell, L. L., J. D. Wolfe, E. I. Johnson, J. E. Hines, J. D. Nichols, and P. C. Stouffer. 2015a. Heterogeneous movement of insectivorous Amazonian birds through primary and secondary forest: A case study using multistate models with radiotelemetry data. Biological Conservation.

Powell, L. L., J. D. Wolfe, E. I. Johnson, and P. C. Stouffer. 2016. Forest recovery in post-pasture Amazonia: Testing a conceptual model of space use by insectivorous understory birds. Biological Conservation **194**:22-30.

Powell, L. L., G. Zurita, J. D. Wolfe, E. I. Johnson, and P. C. Stouffer. 2015b. Changes in habitat use at rain forest edges through succession: a case study of understory birds in the Brazilian Amazon. Biotropica **47**:723-732.

Puig-Montserrat, X., I. Torre, A. López-Baucells, E. Guerrieri, M. M. Monti, R. Rafols-Garcia, X. Ferrer, D. Gisbert, and C. Flaquer. 2015. Pest control service provided by bats in Mediterranean rice paddies: linking agroecosystems structure to ecological functions. Mammalian Biology **80**:237-245.

Quintero, I., and T. Roslin. 2005. Rapid recovery of dung beetle communities following habitat fragmentation in Central Amazonia. Ecology **86**:3303-3311.

Razafimanahaka, J. H., M. Ralisata, F. Randrianandrianina, R. K. B. Jenkins, J. Ratsirarson, and P. A. Racey. 2016. Habitat use by the endemic Malagasy bat *Hipposideros commersoni* in a littoral forest. Acta Chiropterologica **18**:423-431.

Rex, K., D. H. Kelm, K. Wiesner, T. H. Kunz, and C. C. Voigt. 2008. Species richness and structure of three Neotropical bat assemblages. Biological Journal of the Linnean Society **94**:617-629.

Ribeiro, E. M. S., B. A. Santos, V. Arroyo-Rodríguez, M. Tabarelli, G. Souza, and I. R. Leal. 2016. Phylogenetic impoverishment of plant communities following chronic human disturbances in the Brazilian Caatinga. Ecology **97**:1583-1592.

Rocha, R. 2017. Tropical forest fragmentation: effects on the spatio-temporal dynamics of its bat communities. PhD Thesis. University of Lisbon, Lisbon, Portugal.

Rocha, R., T. Almeida, and A. López-Baucells. 2014. Field observation of an adult Lesser treefrog *Dendropsophus minutus* (Anura: Hylidae) being consumed by a neotropical *Lethocerus* sp.(Hemiptera: Belostomatidae) nymph. Alytes **31**:37-39.

Rocha, R., D. F. Ferreira, A. López-Baucells, F. Z. Farneda, J. Carreiras, J. M. Palmeirim, and C. F. Meyer. 2017a. Does sex matter? Gender-specific responses to forest fragmentation in Neotropical bats. Biotropica **49**:881-890.

Rocha, R., M. Gordo, and A. López-Baucells. 2016. Completing the menu: addition of *Scinax cruentommus* and *Scinax* cf. *garbei* (Anura: Hylidae) to the diet of *Trachops cirrhosus* (Chiroptera: Phyllostomidae) in Central Amazon. North-Western Journal of Zoology **12**:199-201.

Rocha, R., and A. López-Baucells. 2014a. Opportunistic predation by Crested owl *Lophostrix cristata* upon Seba's Short-tailed Bat *Carollia perspicillata*. Revista Brasileira de Ornitologia-Brazilian Journal of Ornithology **22**:35-37.

Rocha, R., and A. López-Baucells. 2014b. Predation attempt of *Hypsiboas boans* (Anura: Hylidae) by *Helicops angulatus* (Squamata: Dipsadidae) with notes on defensive behavior. Alytes **30**:78-81.

Rocha, R., A. López-Baucells, F. Z. Farneda, M. Groenenberg, P. E. D. Bobrowiec, M. Cabeza, J. M. Palmeirim, and C. F. J. Meyer. 2017b. Consequences of a large-scale fragmentation experiment for Neotropical bats: disentangling the relative importance of local and landscape-scale effects. Landscape Ecology **32**:31–45.

Rocha, R., O. Ovaskainen, A. López-Baucells, F. Z. Farneda, D. F. Ferreira, P. E. D. Bobrowiec, M. Cabeza, J. M. Palmeirim, and C. F. J. Meyer. 2017c. Design matters: An evaluation of the impact of small man-made forest clearings on tropical bats using a before-after-control-impact design. Forest Ecology and Management **401**:8-16.

Rocha, R., O. Ovaskainen, A. Lopez-Baucells, F. Z. Farneda, E. M. Sampaio, P. E. D. Bobrowiec, M. Cabeza, J. M. Palmeirim, and C. F. J. Meyer. 2018. Secondary forest regeneration benefits old-growth specialist bats in a fragmented tropical landscape. Scientific Reports **8**:3819.

Rodríguez-San Pedro, A., and J. A. Simonetti. 2013. Acoustic identification of four species of bats (Order Chiroptera) in central Chile. Bioacoustics Journal **22**:165-172.

Rodríguez-San Pedro, A., and J. A. Simonetti. 2015. The relative influence of forest loss and fragmentation on insectivorous bats: does the type of matrix matter? Landscape Ecology **30**:1561-1572.

Rosa, I. M. D., C. Gabriel, and J. M. B. Carreiras. 2017. Spatial and temporal dimensions of landscape fragmentation across the Brazilian Amazon. Regional Environmental Change **17**:1687-1699.

Russ, J., and D. Bennett. 2001. The bats of Madagascar: a field guide with descriptions of echolocation calls. Viper press, Glossop, United Kingdom.

Russo, D., L. Ancillotto, and G. Jones. 2018. Bats are still not birds in the digital era: echolocation call variation and why it matters for bat species identification. Canadian Journal of Zoology **96**:63-78.

Russo, D., and G. Jones. 2002. Identification of twenty-two bat species (Mammalia: Chiroptera) from Italy by analysis of time-expanded recordings of echolocation calls. Journal of Zoology **258**:91–103.

Russo, D., and C. C. Voigt. 2016. The use of automated identification of bat echolocation calls in acoustic monitoring: A cautionary note for a sound analysis. Ecological Indicators **66**:598-602.

Rydell, J., S. Nyman, J. Eklöf, G. Jones, and D. Russo. 2017. Testing the performances of automated identification of bat echolocation calls: A request for prudence. Ecological Indicators **78**:416-420.

Sampaio, E. 2001. Effects of the forest fragmentation on the diversity and abundance patterns of central Amazonian bats. PhD Thesis. University of Tübingen, Berlin, Germany.

Sampaio, E. M., E. K. V. Kalko, E. Bernard, B. Rodríguez-Herrera, and C. O. Handley. 2003. A biodiversity assessment of bats (Chiroptera) in a tropical lowland rainforest of Central Amazonia, including methodological and conservation considerations. Studies on Neotropical Fauna and Environment **38**:17-31.

Santos, B. A., M. Tabarelli, F. P. L. Melo, J. L. C. Camargo, A. Andrade, S. G. Laurance, and W. F. Laurance. 2014. Phylogenetic impoverishment of Amazonian tree communities in an experimentally fragmented forest landscape. PLoS ONE **9**:e113109.

Scott, S. J., G. McLaren, G. Jones, and S. Harris. 2010. The impact of riparian habitat quality on the foraging and activity of pipistrelle bats (*Pipistrellus* spp.). Journal of Zoology **280**:371-378.

Schnitzler, H. U., and E. K. V. Kalko. 2001. Echolocation by insect-eating bats: We define four distinct functional groups of bats and find differences in signal structure that correlate with the typical echolocation tasks faced by each group Bioscience **51**:557-569.

Schnitzler, H. U., C. F. Moss, and A. Denzinger. 2003. From spatial orientation to food acquisition in echolocating bats. Trends in Ecology & Evolution **18**:386-394.

Şekercioǧlu, Ç. H., P. R. Ehrlich, G. C. Daily, D. Aygen, D. Goehring, and R. F. Sandí. 2002. Disappearance of insectivorous birds from tropical forest fragments. Proceedings of the National Academy of Sciences **99**:263-267.

Siemers, B., E. Kalko, and H. U. Schnitzler. 2001a. Echolocation behavior and signal plasticity in the Neotropical bat *Myotis nigricans* (Schinz, 1821) (Vespertilionidae): a convergent case with European species of *Pipistrellus*? Behavioral Ecology and Sociobiology **50**:317-328.

Siemers, B. M., P. Stilz, and H. U. Schnitzler. 2001b. The acoustic advantage of hunting at low heights above water: behavioural experiments on the European 'trawling' bats *Myotis capaccinii*, *M. dasycneme* and *M. daubentonii*. Journal of Experimental Biology **204**:3843-3854.

Silva, I. 2012. Estratificação vertical e efeito da fragmentação numa comunidade de morcegos (Chiroptera, Mammalia) na Amazónia Central. MSc Thesis. University of Lisbon, Lisbon, Portugal.

Solari, S., R. A. Van den Bussche, S. R. Hoofer, and B. D. Patterson. 2004. Geographic distribution, ecology, and phylogenetic affinities of *Thyroptera lavali* Pine 1993. Acta Chiropterologica **6**:293-302.

Song, X. P., M. C. Hansen, S. V. Stehman, P. V. Potapov, A. Tyukavina, E. F. Vermote, and J. R. Townshend. 2018. Global land change from 1982 to 2016. Nature **560**:639-643.

Stouffer, P. C., and R. O. Bierregaard. 1995. Use of Amazonian forest fragments by understory insectivorous birds. Ecology **76**:2429-2445.

Stouffer, P. C., E. I. Johnson, R. O. Bierregaard, Jr., and T. E. Lovejoy. 2011. Understory bird communities in Amazonian rainforest fragments: species turnover through 25 years post-isolation in recovering landscapes. PLoS ONE **6**:e20543.

Stouffer, P. C., C. Strong, and L. N. Naka. 2009. Twenty years of understorey bird extinctions from Amazonian rain forest fragments: consistent trends and landscape-mediated dynamics. Diversity and distributions **15**:88-97.

Stratford, J. A., and P. C. Stouffer. 2015. Forest fragmentation alters microhabitat availability for Neotropical terrestrial insectivorous birds. Biological Conservation **188**:109-115.

Struebig, M. J., T. Kingston, E. J. Petit, S. C. Le Comber, A. Zubaid, A. Mohd-Adnan, and S. J. Rossiter. 2011. Parallel declines in species and genetic diversity in tropical forest fragments. Ecology Letters **14**:582-590.

Struebig, M. J., T. Kingston, A. Zubaid, A. Mohd-Adnan, and S. J. Rossiter. 2008. Conservation value of forest fragments to Palaeotropical bats. Biological Conservation **141**:2112-2126.

Surlykke, A., and E. K. V. Kalko. 2008. Echolocating bats cry out loud to detect their prey. PLoS ONE **3**:e2036.

Taddei, V. A., and W. Uieda. 2001. Distribution and morphometrics of *Natalus stramineus* from South America (Chiroptera, Natalidae). Iheringia Serie Zoologia **91**:123-132.

Taylor, P. J., A. Monadjem, and J. N. Steyn. 2013. Seasonal patterns of habitat use by insectivorous bats in a subtropical African agro-ecosystem dominated by macadamia orchards. African Journal of Ecology **51**:552-561.

Teixeira, S., and J. Jesus. 2009. Echolocation calls of bats from Madeira Island: acoustic characterization and implications for surveys. Acta Chiropterologica **11**:183-190.

Ter Steege, H., N. C. Pitman, D. Sabatier, C. Baraloto, R. P. Salomão, J. E. Guevara, O. L. Phillips, C. V. Castilho, W. E. Magnusson, and J. F. Molino. 2013. Hyperdominance in the Amazonian tree flora. Science **342**:1243092.

Tocher, M. D., C. Gascon, and J. Meyer. 2001. Community composition and breeding success of Amazonian frogs in continuous forest and matrix habitat aquatic sites. Pages 235-247 Lessons from Amazonia: The ecology and conservation of a fragmented forest. Yale University Press, Michigan, United States of America.

Torrent, L. 2017. The role of temporary lakes and ponds for bat conservation in the Neotropical rainforests. MSc Thesis. Czech University of Life Sciences, Prague, Czech Republic.

Torrent, L., A. López-Baucells, R. Rocha, P. E. D. Bobrowiec, and C. F. J. Meyer. 2018. The importance of lakes for bat conservation in Amazonian rainforests: an assessment using autonomous recorders. Remote Sensing in Ecology and Conservation:(in press).

Treitler, J. T., A. López-Baucells, S. Gomes Farias, J. F. Tenaçol Jr, and R. Rocha. 2013. First record of a leucistic piebald *Phyllostomus discolor* (Chiroptera: Phyllostomidae). Chiroptera Neotropical **19**:1179-1181.

Vasconcelos, H. L., and E. M. Bruna. 2012. Arthropod responses to the experimental isolation of Amazonian forest fragments. Zoologia **29**:515-530.

Vassilios, S., Z.-G. Veronica, J. K. E., and G. Mark. 2017. Bat echolocation call identification for biodiversity monitoring: a probabilistic approach. Journal of the Royal Statistical Society: Series C (Applied Statistics) **67**:165-183.

Velazco, P. M., R. Gregorin, R. S. Voss, and N. B. Simmons. 2014. Extraordinary local diversity of disk-winged bats (Thyropteridae, Thyroptera) in northeastern Peru, with the description of a new species and comments on roosting behaior. American Museum Novitates **3795**:1-28.

Vicente, E. C., J. Jim, and V. A. Taddei. 2005. Características morfológicas externas distintivas de *Myotis albescens*, *M. nigricans*, *M. simus e M. riparius* (Chiroptera; Vespertilionidae). Ensaios e Ciência **9**:293-304.

Walters, C. L., R. Freeman, A. Collen, C. Dietz, M. B. Fenton, G. Jones, M. K. Obrist, S. J. Puechmaille, T. Sattler, B. M. Siemers, S. Parsons, and K. E. Jones. 2012. A continental-scale tool for acoustic identification of European bats. Journal of Applied Ecology **49**:1064-1074.

Wanger, T. C., K. Darras, S. Bumrungsri, T. Tscharntke, and A. M. Klein. 2014. Bat pest control contributes to food security in Thailand. Biological Conservation **171**:220-223.

White, M. D., and J. R. Strittholt. 2014. Forest conservation planning. Pages 205-226 *in* K. M. Reynolds, P. F. Hessburg, and P. S. Bourgeron, editors. Making Transparent Environmental Management Decisions. Springer, New York, United States of America.

Wieland, L. M., R. C. Mesquita, P. E. D. Bobrowiec, T. V. Bentos, and G. B. Williamson. 2011. Seed rain and advance regeneration in secondary succession in the Brazilian Amazon. Tropical Conservation Science **4**:300-316.

Wilson, M. C., X.-Y. Chen, R. T. Corlett, R. K. Didham, P. Ding, R. D. Holt, M. Holyoak, G. Hu, A. C. Hughes, L. Jiang, W. F. Laurance, J. Liu, S. L. Pimm, S. K. Robinson, S. E. Russo, X. Si, D. S. Wilcove, J. Wu, and M. Yu. 2016. Habitat fragmentation and biodiversity conservation: key findings and future challenges. Landscape Ecology **31**:219-227.

Willis, E. O. 1974. Populations and local extinctions of birds on Barro Colorado Island, Panama. Ecological Monographs **44**:153-169.

Wolfe, J. D., P. C. Stouffer, K. Mokross, L. L. Powell, and M. M. Anciães. 2015. Island vs. countryside biogeography: an examination of how Amazonian birds respond to forest clearing and fragmentation. Ecosphere **6**:1-14.

Zamora-Gutiérrez, V., C. López-Gonzalez, M. C. MacSwiney González, B. Fenton, G. Jones, E. K. V. Kalko, S. J. Puechmaille, V. Stathopoulos, K. E. Jones, and R. Freckleton. 2016. Acoustic identification of Mexican bats based on taxonomic and ecological constraints on call design. Methods in Ecology and Evolution **7**:1082-1091.

# CHAPTER 2

## Field Guide to the Bats of the Amazon

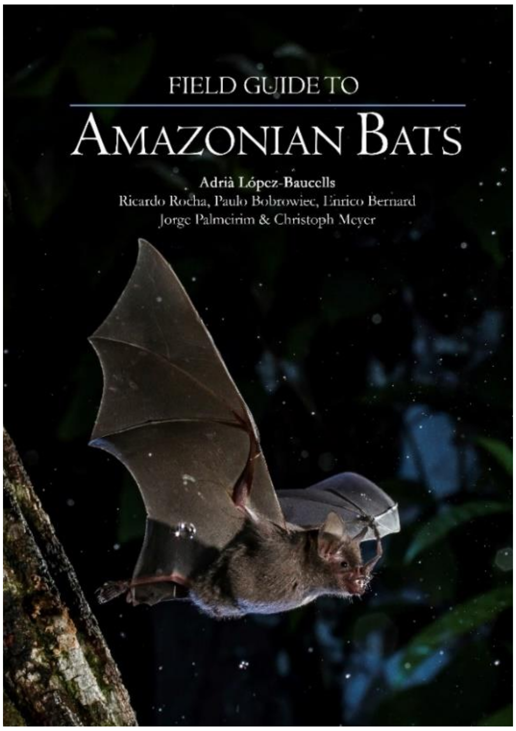
**López-Baucells, A.**, Rocha, R., Bobrowiec, P.E.D., Bernard, E., Palmeirim, J.M., Meyer, C.F.J., 2018. Pelagic Publishing, ISBN: 978-1-78427-165-7 (Pbk)

Top: *Lophostoma silvicolum;* Bottom: *Cynomops planirostris*

# CHAPTER 2

## Field Guide to the Bats of the Amazon

### Preface


FIELD GUIDE TO
AMAZONIAN BATS
Adrià López-Baucells
Ricardo Rocha, Paulo Bobrowiec, Enrico Bernard
Jorge Palmeirim & Christoph Meyer

The Amazon is home to the most diverse bat communities on the planet with more than 160 species currently described. Local species richness often exceeds 100 and for many, their identification in the field is, to say the least, challenging. To make this task easier, in the context of this thesis, I published the *Field Guide to Amazonian Bats*, a landmark handbook aimed at facilitating species identification in the field.

The book, first published as an online version by the National Institute of Amazonian Research (INPA) in 2016 and re-edited by Pelagic Publishing (UK) in 2018, is designed as a comprehensive guide aimed at satisfying the needs of those conducting field work on bats in the Amazon. It is largely based on previous published keys with modifications derived from both personal observations and years of field experience in the Brazilian Amazon at the Biological Dynamics of Forest Fragments Project (BDFFP) as well as a thorough revision of available bat keys and species descriptions for the region. The aim was to produce an easy-to-use guide that would be both practical and visually appealing. The guide is available in digital format (www.tropicalconservation.net) and can thus be readily consulted on tablets and even smartphones. All steps of the identification keys are hyperlinked making it easy to navigate across the book and for each species direct links to the IUCN Red List webpage are also included where more information can be found. The field guide also features the first acoustic key for Amazonian bats, illustrated with the echolocation spectrogram of most species. This represents a major step towards alleviating the daunting task of identifying the numerous species of aerial insectivorous bats that occur in the Amazon based on their echolocation calls. It further constitutes an important tool to improving the knowledge and optimizing surveys of aerial insectivorous Neotropical bats, a group which remains largely understudied.

The field guide provides an essential tool, not only for researchers, but also for bat conservationists, consultancies and anyone interested in Amazonian bats. As one of the first interactive online field guides for bats, it was purposely designed to be continuously updated and improved. The book was published online as an e-book, downloadable free of charge, on the 5th of September 2016, and included a foreword from Dr. Merlin Tuttle.

Given the book´s large page count (167), in this thesis I have only partially included its content: the general introduction and the acoustic section with the echolocation keys. To consult the whole book or download it in pdf, it is freely accessible at www.tropicalconservation.net or from the ResearchGate repository.

*Sampling bats in a lake embedded in primary continuous forest in Central Amazon*

# Introduction

Although elusive due to their mostly nocturnal behaviour, bats (order Chiroptera, from the Greek *cheir* 'hand' and *pteron* 'wing') are undoubtedly one of the most fascinating faunal groups in the world. Only outnumbered by rodents, they constitute the second most numerous mammalian order, but are arguably the most diverse and demonstrate just how ecologically adaptive mammals can be.

At present, over 1,300 species of bats are known to science. Nevertheless, this number is growing steadily, mostly due to the splitting of taxa based on new genetic evidence and the discovery of hitherto truly unknown species in remote corners of the planet. Bats range in size from one of the smallest of all mammals, the bumblebee bat *Craseonycteris thonglongyai* (1.5–2 g), to the large *Pteropus* flying foxes, which possess a wide array of shapes and colours; in some cases, they weigh over 1 kg and have wingspans exceeding 1.5 m. Bats have been around for some 50 million years and have taken advantage of two exceptional aspects of their biology – echolocation and powered flight – to conquer the night skies in nearly all of the available ecosystems across the globe, the exception being the Arctic, Antarctic and a few isolated oceanic islands.

No other mammalian order exploits such a broad diversity of food resources. Although most bat species have evolved as highly specialized hunters of aerial insects, a number have developed a taste for vertebrates (ranging from fish to amphibians, reptiles, birds and even small mammals, including other bats), plant matter (chiefly fruit, but also nectar, pollen, and occasionally leaves and seeds) and blood. Certain species are omnivorous, but many bats have highly specialized diets and are involved in complex co-evolutionary interactions. A good example of this is the relationship between the South American plant *Centropogon nigricans* and its (probably) only pollinator, the recently discovered tube-lipped nectar bat *Anoura fistulata*, holder of the record for longest tongue (8.5 cm) in relation to body size in any mammal (its tongue measures 150% of the size of its overall body length!). Predator–prey interactions are equally intricate and reach their evolutionary climax in the 'arms race' between aerial insectivorous bats and their prey.

Roost selection is another example of the enormous plasticity displayed by bats. Caves are probably the best-known bat roost sites; indeed, many species are mostly cave-dwellers and some caves harbour millions of conspecific bats, as in the case of the Brazilian free-tailed bats *Tadarida brasiliensis* in Central America and the southern USA. Apart from caves however, bats make use of a myriad of natural and man-made structures for roosting. Some species of neotropical stenodermatinae fruit-eating bats make tents by biting the central rib of palms and *Heliconia* leaves. In an interesting case of convergent evolution, *Thyroptera* bats from Central and South America and *Myzopoda* from Madagascar have both evolved suction cups or suckers on the base of their thumbs and ankles that allow them to cling to smooth surfaces and roost inside curled leaves. Some species, such as the hoary bat *Lasiurus cinereus,* are solitary tree dwellers, whilst others including many Old World fruit bats roost in large tree colonies numbering several thousands. Man-made structures such as mines, bridges and roof cavities are used by many species, while others (e.g. several neotropical Emballonuridae) simply take advantage of their camouflage to roost on lichen-covered tree bark or rocks. A few species roost in underground cavities, while the South and Central American white-throated round-eared bat *Lophostoma silvicolum* even roosts colonially inside the nests of arboreal termites.

True powered flight and echolocation undoubtedly lie at the heart of this group’s evolutionary success. Flying is much less energy-consuming than running and, given that it removes the need to touch ground, it reduces potentially deadly encounters with terrestrial predators. Echolocation probably evolved hand-in-hand with flight and, by allowing early bats to analyse the echoes of emitted sound pulses and so negotiate obstacles, served as an entrance to an ecological niche that was inaccessible to most other groups: the night sky.

Although other animal groups, including cetaceans, use sound in this way, none does so in such a complex manner. Echolocation has reached its evolutionary peak in bats and, for most species, is key to their ability to avoid physical obstacles and find food. Bats tend to have good auditory sensitivity and therefore can listen to sounds made by moving prey or, as in the case of the neotropical fringe-lipped bat *Trachops cirrhosus*, can even identify edible frogs from their calls. Good night vision and a well-developed sense of smell are also of utmost importance and enable many species to find food; this is especially true for the Old-World fruit bats.

Bats have unfortunately been the subject of disdain and persecution by many and are frequently portrayed as blood-sucking demons and associated with dark practices. On the other hand, some cultures such as the Middle-to-Late Qing Dynasty (1644–1911) in China have regarded bats as symbols of good fortune, a much more faithful reflection of their importance to the planet’s ecological health and to our own well-being. Bats are key providers of many ecosystem services such as seed dispersal, pollination and pest suppression. Their disappearance can lead to enormous economic losses (e.g. the economic value of bats to North American agriculture alone has been estimated at around $23 billion per year) and probable wide-scale ecosystem collapse.

Over the last 500 years the planet has faced a human-generated wave of extinctions that is comparable to the Earth's five previous mass extinctions. Despite their uniqueness, bats face the same threats as many other species on the planet and are consequently being severely affected by the ongoing 'sixth mass extinction'. Currently, approximately one-quarter of all bat species are globally threatened. Increasing rates of habitat loss and fragmentation, overexploitation, misguided persecution, climate change, and epidemic diseases (such as white-nose syndrome, a fungal infection that has killed millions of bats throughout North America in recent years) mean that many more species are likely to become extinct in the near future.

Fortunately, not all is gloom. As we come to better understand bats, their importance for ecosystem well-being and functioning, and ultimately, how they benefit humankind, attitudes towards them are slowly starting to change. Across the globe multiple grass-roots conservation projects are braving their way to try to reverse ongoing population declines and the image of bats in books, movies and the general media is starting to reflect some elements of truth. Conservation of the planet's unique biological richness will ultimately depend on how much we treasure the natural world. I hope that by revealing some of the tremendous richness of the Amazonian bat fauna this book will aid in a better understanding of their natural history, our impacts on them and, consequently, how we can combine our efforts to better contribute to their conservation, because as the Senegalese conservationist Baba Dioum once said:

*"In the end we will conserve only what we love. We will love only what we understand. We will understand only what we are taught."*

*Field work in the Biological Dynamics of Forest Fragments Project*

## Bats in the Amazon

The increase in species richness with increasing proximity to the Equator is a major biogeographic pattern to which bats are no exception. Bat diversity peaks in tropical regions, and the neotropics of South and Central America constitute the epicenter of this diversity, harbouring more than 200 currently recognized species. The Amazon basin holds over half of the world's remaining rainforests and represents the largest and most biodiverse expanse of tropical rainforest on the planet. Roughly one in ten known bat species occurs in the Amazon basin and in some Central Amazonian localities more than 100 species live in sympatry.

Bats are divided into 17 families (or 18, depending on the acceptance of Miniopteridae as a separate family), of which nine (Phyllostomidae, Thyropteridae, Furipteridae, Noctilionidae, Mormoopidae, Emballonuridae, Vespertilionidae, Molossidae, and Natalidae) are present in the Amazon. The distribution of the species across the Amazonian bat families is rather uneven: the bulk of species belongs to the family of New World leaf-nosed bats (Phyllostomidae), the ecologically most diverse family within the order (nearly 200 species throughout Central and South America). On the other hand, the Furipteridae are represented in the Amazon by just one of the two members of its family, the thumbless bat *Furipterus horrens*.

Bats are key elements in the Amazon's intricate ecological networks and, through countless links to other animal and plant groups, help support and sustain the biome in all its complexity and magnificence. Many Amazonian bats such as the Phyllostomidae subfamilies Stenodermatinae and Carolliinae feed almost exclusively on fruit and act as 'forest gardeners' by dispersing seeds far and wide. They often introduce seeds into previously disturbed habitats and consequently help the forest reclaim some of its lost domains. Some other species such as the Glossophaginae hover like hummingbirds in front of flowers and with their long muzzles and tongues probe flowers to extract their nectar, effectively acting as pollinators, thereby helping to maintain the genetic diversity of flowering plants. However, most Amazonian bats are either obligate or facultative insect-eaters and glean insects and other arthropods directly from the vegetation in the forest understory or capture prey in open spaces above or below the forest canopy. By doing so, they greatly reduce arthropod-related herbivory and redistribute nutrients via their guano, thereby helping to maintain terrestrial and aquatic ecosystems throughout the Amazon. Four species of Phyllostomidae, namely the greater spear-nosed bat *Phyllostomus hastatus*, the fringe-lipped bat *Trachops cirrhosus*, the big-eared woolly bat *Chrotopterus auritus*, and the spectral bat *Vampyrum spectrum*, are confirmed carnivores, while the two *Noctilio* species are both fish-eaters. On the other hand, bats regularly form part of the diet of several faunal groups including spiders, giant centipedes, frogs, marsupials, other bats, birds, and snakes.

In recent years several new species have been described and new records have extended the geographic range of some species by hundreds of kilometers. However, knowledge of Amazonian bats is still limited and extremely biased towards certain relatively well-studied localities such as the Biological Dynamics of Forest Fragments Project (BDFFP) and Alter do Chão, in the heart of the Brazilian Amazon. As bat researchers venture into the last unknown Amazonian frontiers we are learning more about the fascinating diversity of the bats of this region, knowledge that is vital for both bat conservation and the conservation of the Amazon biome as a whole.

## Echolocation Keys

Across most of the Neotropics, aerial insectivorous bats remain poorly studied. Aerial-hawking insectivorous bats are usually difficult to capture by mistnetting and the best technique for studying them is the use of ultrasound recording devices. However, the echolocation calls of many Neotropical aerial insectivorous bats are still inadequately described. Thus, intensified research efforts are urgently required to fill gaps in knowledge so that acoustic sampling can be used to its full potential in environmental impact assessments and monitoring programs.

In terms of acoustic sampling techniques, the advent of automatic and fully autonomous recording stations has opened up new avenues for studying Neotropical aerial insectivorous bats. However, reliable analysis of the data generated by acoustic surveys and monitoring studies requires the creation of a good call reference library for the bats of the study region. Currently, this kind of information is largely lacking for areas such as the Amazon.

It is well known that some species' echolocation calls are often similar and have considerable overlap in frequencies, which can complicate identification and even render findings unreliable. In addition, factors such as weather conditions, geographic location, habitat structure, flight height, and various other physiological and environmental factors can give rise to great variation in call structure within a particular species. Sex, age and reproductive status are other sources of variation, as has been found for several species. Thus, it is essential to quantify differences in echolocation call structure within and among tropical species to allow accurate acoustic assessments. It is also well known that handling and processing bats after capture can alter call properties due to the stress caused to individuals, and this is one of the main problems that arises when attempting to obtain high-quality recordings for reference libraries.

Several techniques such as discriminant function analysis, as well as, more recently, the use of synergetic pattern recognition algorithms in real time and artificial neural networks, have been employed in species identification based on echolocation call data. However, in order to develop and successfully use these techniques, an accurate description of the characteristics of the echolocation calls of all species known to occur in the study area is paramount. In the end, even with the development of new algorithms and techniques for automatic call identification, manual cross-checking and revision of results by experts remain essential.

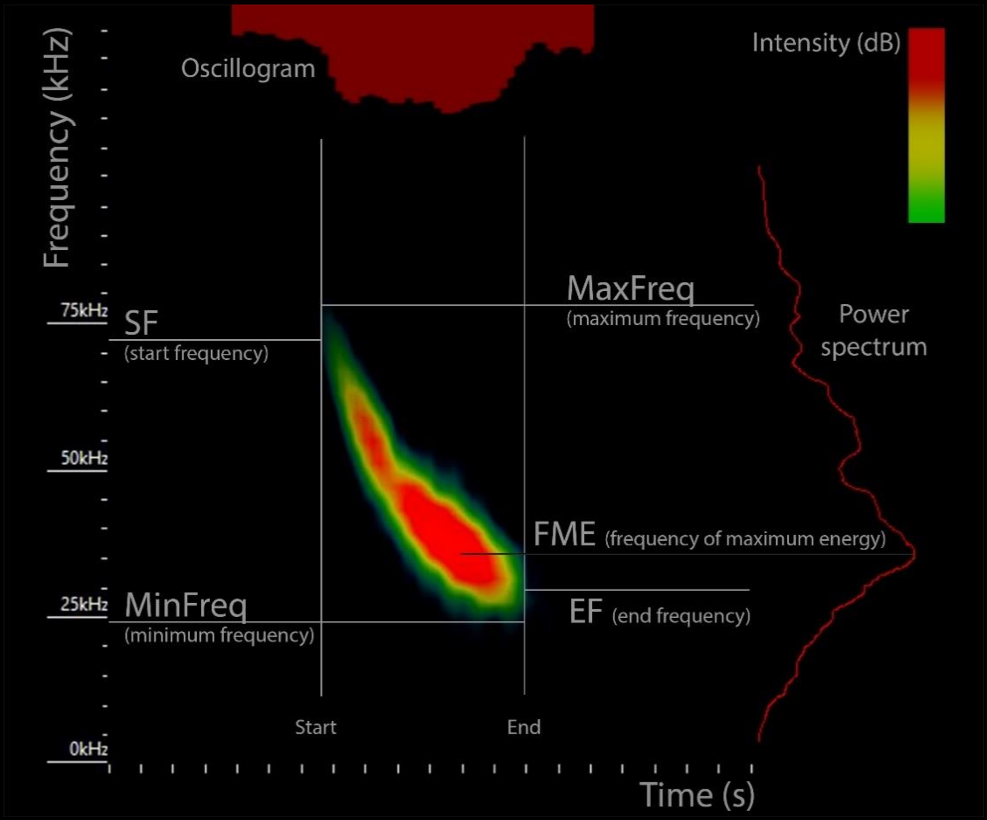
*Rhynchonycteris naso*

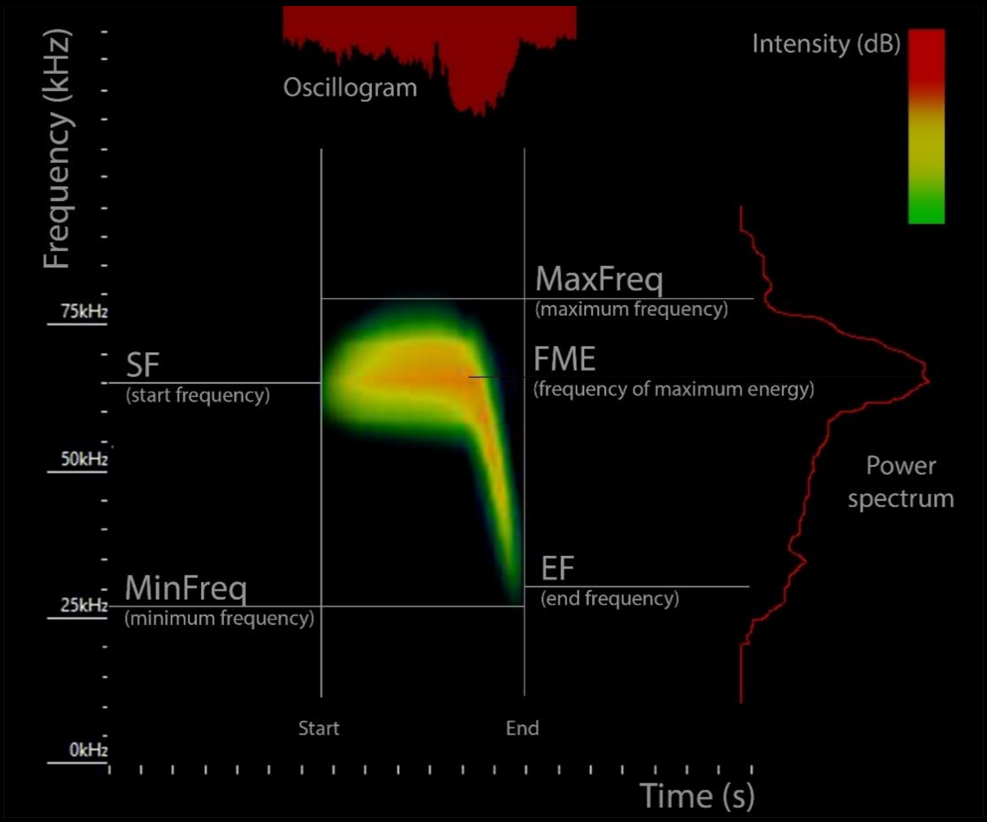
**Bat calls are highly variable due to numerous factors such as the type of activity and surrounding environmental clutter.** This variation often exacerbates overlap in the characteristics of the calls of certain species that can complicate the use of identification keys.

## How should measurements be taken?

In order to use this key properly, it is essential to understand and standardize how measurements of calls are taken. The most relevant parameters for bat species identification in bioacoustics are: frequency of maximum energy (FME), minimum and maximum frequency (MinFreq and MaxFreq), start and end frequency (SF and EF) and pulse duration and shape (constant, quasi-constant and modulated frequency). All measurements must be taken from the harmonic that concentrates most energy, which, although varying from family to family, is usually the first or the second. All harmonics will be integer multiples of the “fundamental” frequency (first harmonic). FME is extracted from the power spectrum as the frequency that is recorded at the moment of greatest call intensity. Maximum and minimum frequencies can be measured on the power spectrum or on the spectrogram at the moment that the pulse differs most from the background noise. Thus, bandwidth should be calculated as the difference between the maximum and the minimum frequencies. Start and end frequencies must be measured at the point where the amplitude of the oscillogram begins to consistently rise or decrease above the background noise. This can be obtained from the spectrogram when the intensity of the call is 20dB above the background noise. Accordingly, the call duration is measured between the start and the end point of the pulse. In order to describe the pulses the pulses can be classified as upward, if the frequency increases by time, or downward if it decreases. Although not commonly referred to in other available keys, pulse intervals may be of interest and are defined as the time between the start of one pulse and the start of the subsequent one.

| | |
|---|---|
| **CF**: | Constant frequency |
| **QCF**: | Quasi-constant frequency |
| **FM**: | Frequency modulated |
| **FME**: | Frequency of maximum energy |
| **EF/SF**: | End frequency / Start frequency |
| **MinFreq**: | Minimum frequency |
| **MaxFreq**: | Maximum frequency |
| **u**: | Upward modulated |
| **d**: | Downward modulated |
| **BW**: | Bandwidth |


Frequency (kHz)
Oscillogram
Intensity (dB)
75kHz
50kHz
25kHz
0kHz
SF
(start frequency)
MaxFreq
(maximum frequency)
Power
spectrum
FME (frequency of maximum energy)
EF (end frequency)
MinFreq
(minimum frequency)
Start
End
Time (s)


Frequency (kHz)
Oscillogram
Intensity (dB)
75kHz
50kHz
25kHz
0kHz
MaxFreq
(maximum frequency)
SF
(start frequency)
FME
(frequency of maximum energy)
Power
spectrum
EF
(end frequency)
MinFreq
(minimum frequency)
Start
End
Time (s)

## Some important issues to consider before deciding to work with echolocation data

Identification of Neotropical bat species by their echolocation calls is a challenging task. As stated at the beginning of this key, calls are very plastic. Some species have distinctive calls that are easy to identify, while others substantially overlap with those of other taxa, thereby making reliable species identification difficult, if not impossible. It is thus essential that anyone aiming to analyze bat acoustic data has appropriate training to minimize data misinterpretation. This is true for both scientific studies and environmental impact assessments carried out by local consultants. Bat acoustic assessments heavily depend on the quality of the recordings since poor recordings can negatively affect identification success and the reliability of results. Thus, it is vital to understand not only how to analyze acoustic recordings but also how to properly set up detectors, calibrate microphones, and use specific recording settings (e.g. background filtering and frequency triggers).

Due to the rapid increase in the number of people using acoustics as a tool for surveying and monitoring bats, several automatic algorithms are now available that can speed up classification work. The positive aspect of these algorithms is that they can generate standardized results from massive datasets with little time commitment by the researcher. On the other hand, even though call analysis by experienced researchers is subjective and much more time consuming, manual call classification can give more accurate results in terms of identifying rare species, quantifying true diversity, and the presence of feeding buzzes and social calls, which are neglected in all available automatic identification software. The best processing method will clearly depend on the type of data that is hoped to be extracted from recordings and the objectives of the study. Remember that the amount of bat activity is fairly well correlated with the true number of bats flying in the area. However, bat activity is rarely comparable between species due to differences in the detectability of their calls and dissimilarities in the structure of their calls. In conclusion:

1. Understand, prepare and place correctly your equipment in the field (attend training sessions if necessary).

2. Store your data adequately (labeled, georeferenced, and including a description of the relevant metadata).

3. State the details of the specific detector settings that were used and calibrate the microphones.

4. If you aim to quantify relative abundance, specify how exactly you will quantify it.

5. Decide which species or species-group categories will be used to classify the recordings.

6. If you combine automatic and manual classifications, explain in detail how the manual verification was undertaken and the reasoning behind your choice of specific species-groups and the limitations of your analysis.

8. Understand the limitations of your equipment, take special care when analyzing the data and exercise caution when interpreting your results.

9. Due to substantial variations in species detectability (e.g. quieter vs. louder calls), activity levels between species are rarely comparable.

## Some notes on identification at family level

The following pages contain two acoustic keys, one for when harmonics are clearly recorded and the other for when they are not. If the harmonics cannot be distinguished in the sonograms, try to adjust the gain and filters on your analysis software in order to detect weaker harmonics and thus be able to use the first key (much simpler and more reliable). If you cannot find the harmonics, follow the second key step-by-step, but be very careful with confusing or faint pulses. Do not worry about leaving many recordings as either "unidentified" or classified in "phonic groups" (including multiple species). This is preferable to ending up with a large number of incorrect species identifications.

Misidentifications can lead to bad management decisions and therefore it is always better to rely on fewer but good-quality data rather than a massive amount of low-quality data. Take into account the shape of pulses and the type of environment in which bats are recorded. Bats in highly cluttered habitats tend to greatly modulate their pulses. On the other hand, in open habitats calls tend to lose their modulated component and pulses may resemble emballonurid or molossid calls due to their almost constant-frequency components. The calls of the Molossidae and Vespertilionidae families are the most variable and can easily lead to misidentifications.

Setting detectors in the rainforest

## Main phonic-group selection (if you DO have harmonics recorded)

1a. FME located in the first harmonic.
  2a. Pulses with a short CF section and a long FMd tail (FME < 70kHz).
*Noctilionidae*

  2b. Mostly QCF (at least in one of the pulse types, when call sequences include alternating pulse types); sometimes with small FM tails.
*Molossidae* *

  2c. FM with final QCF part (very variable proportions of each type).
*Vespertilionidae - Thyropteridae* *

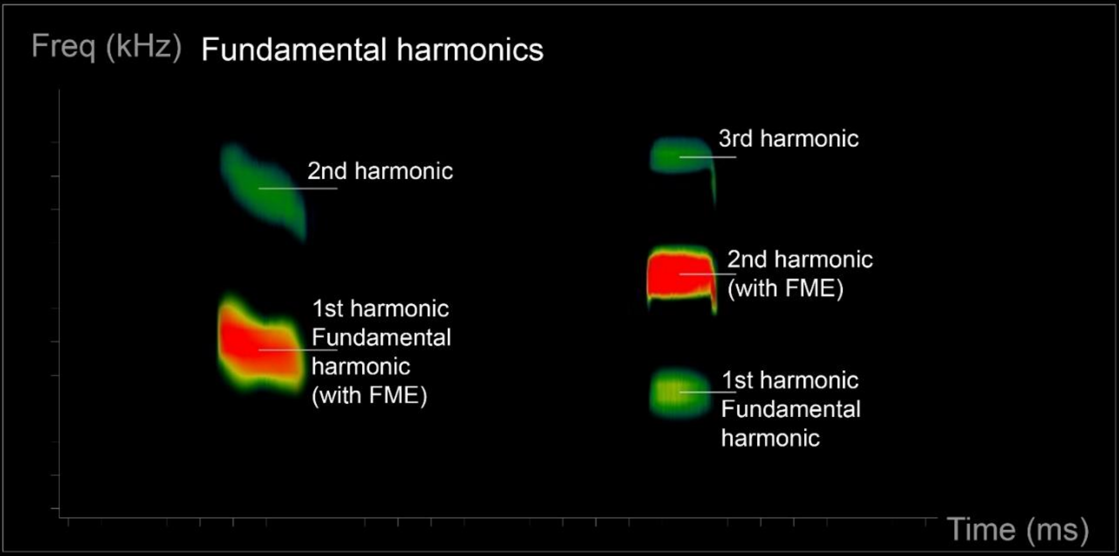
1b. FME located in any other harmonic.
  2a. Pulses with at least one CF section.
*Mormoopidae*

  2b. Mostly QCF, sometimes with small FMd tails.
*Emballonuridae*

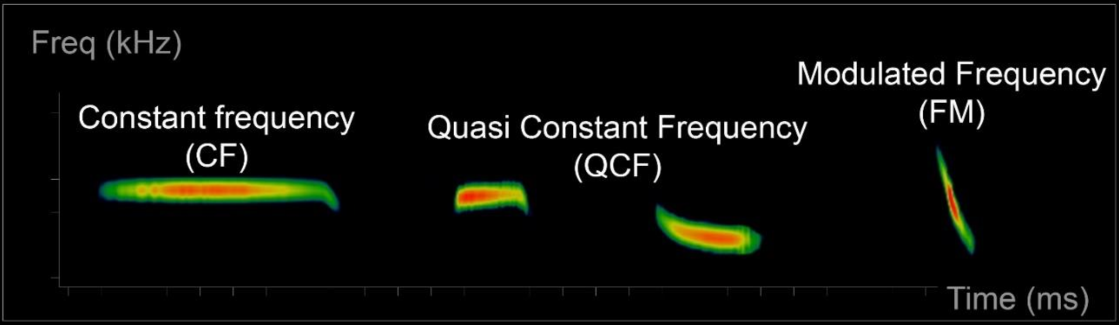
  2c. FM with final QCF section, FME > 110kHz.
*Natalidae*

  2d. Only FM (extremely modulated pulses).
    3a. FME ≈ 130-170kHz.
*Furipteridae*

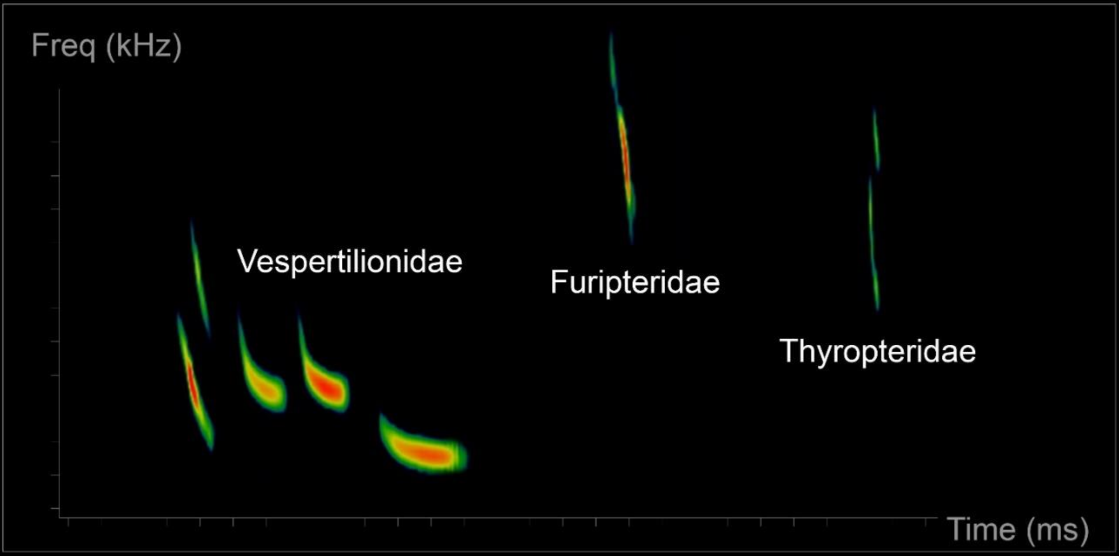
* Be aware of the great variability found in this group.



Position of fundamental harmonics in different bat species.



Different shapes of bat echolocation pulses.



Range of different shapes of frequency modulated pulses within different bat families.

## Main phonic-group selection (if you DO NOT have harmonics recorded)

1a. Pulses with one CF section and a long FMd tail (FME < 70kHz).

*Noctilionidae*

1b. Pulses with at least one CF section.

*Mormoopidae*

1c. Mostly QCF (at least in one of the pulse types, when call sequences include alternating pulse types); sometimes with small FM tails.

2a. QCF/ FMd (FME > 80kHz)

*Emballonuridae A*

2b. Convex QCFu with FMd tails at the beginning of the pulses (1 or 2 types of pulses).

*Emballonuridae B*

2c. Convex QCFd with FMd tails at the end of the pulses (1 or 2 types of pulses).

*Emballonuridae C*

2d. Convex QCFd with a small FMu tail (two or three types of pulses). *

*Molossidae A*

2e. Convex QCFu and concave QCFd.

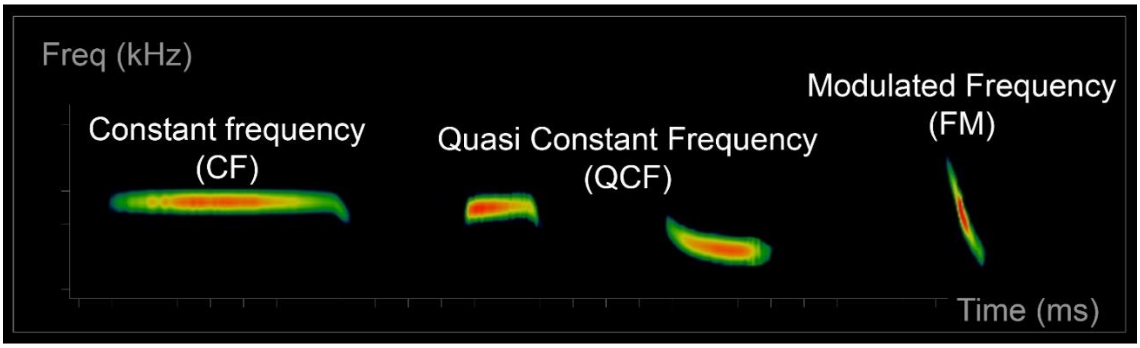
*Molossidae B*

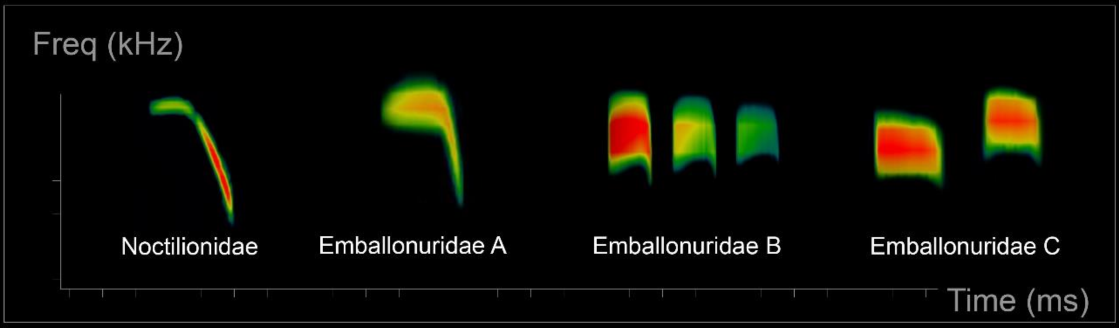
2f. Convex QCFd and concave QCFd.

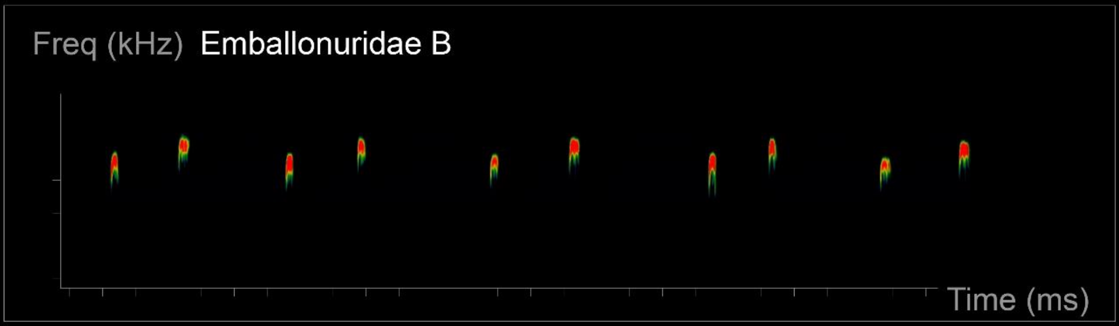
*Molossidae C*

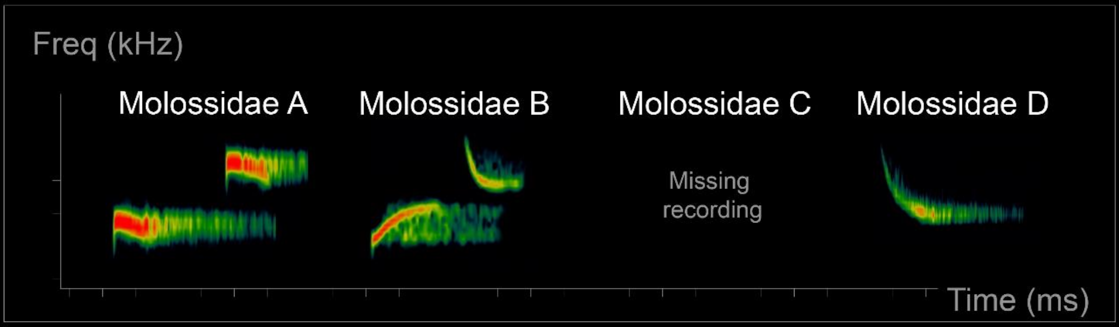
2g. Concave QCF (FME < 30kHz) (one or two types of pulses).

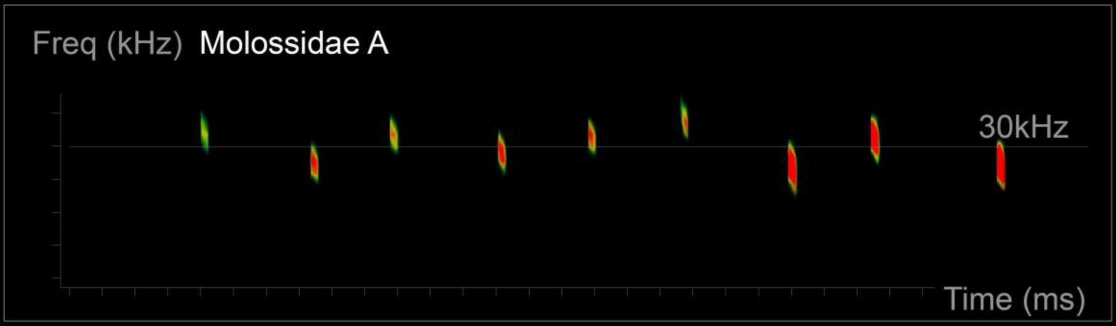
*Molossidae D*

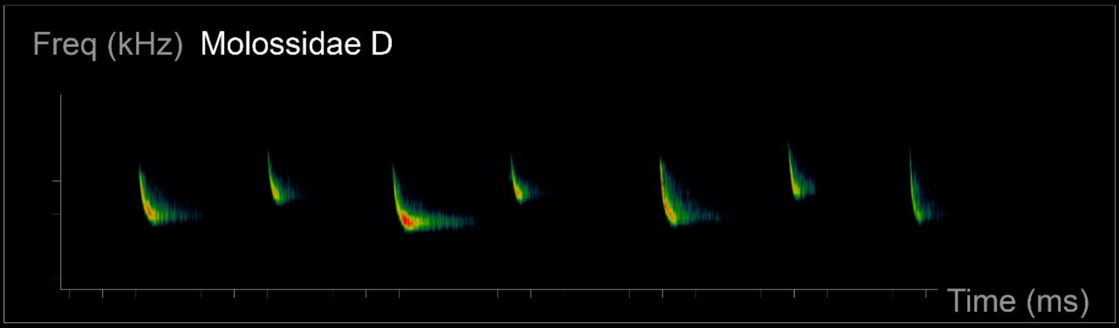



Freq (kHz)
Noctilionidae
Emballonuridae A
Emballonuridae B
Emballonuridae C
Time (ms)


Freq (kHz)
Emballonuridae B
Time (ms)


Freq (kHz)
Molossidae A
Molossidae B
Molossidae C
Molossidae D
Missing recording
Time (ms)


Freq (kHz)
Molossidae A
30kHz
Time (ms)


Freq (kHz)
Molossidae D
Time (ms)

1c. FM with final QCF (very variable proportions of each type). FME
≈ 30 - 100kHz.

*Vespertilionidae - Thyropteridae*

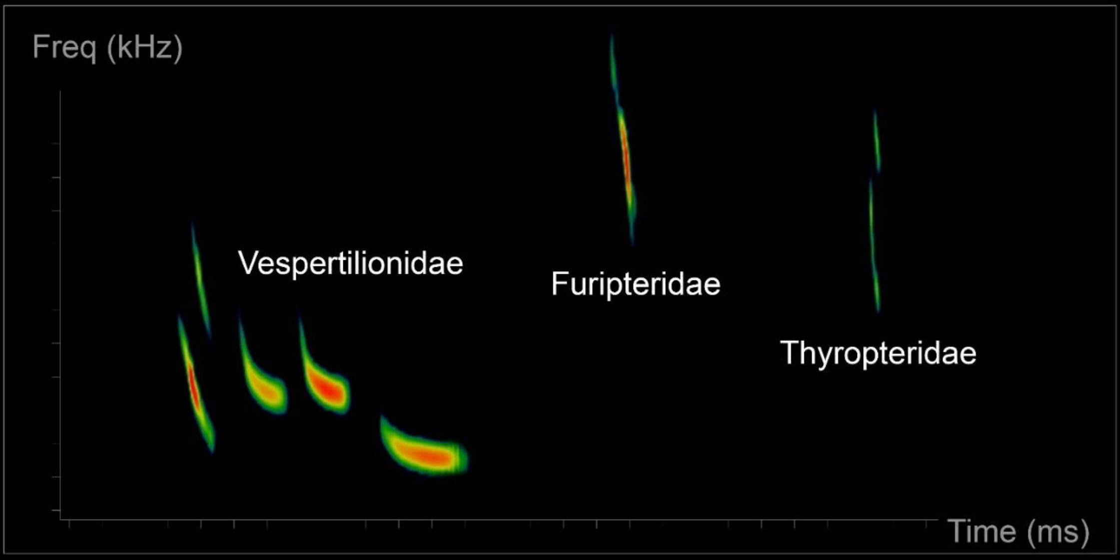
1d. FM with final QCF with FME > 110kHz.

*Natalidae*

1e. Only FM (extremely modulated pulses).
2a. FME ≈ 130-170kHz.

*Furipteridae*

Hanging detectors in the canopy



*Cynomops planirostris*

## *Noctilionidae*

1a. CF / FMd, sometimes alternating with QCF.
SF(CF) ≈ 68-76kHz
*Noctilio albiventris*

1b. CF / FMd, sometimes alternating with QCF.
SF(CF) ≈ 53-61kHz
*Noctilio leporinus*

## Some notes on the identification of *Mormoopidae*

Some genera of mormoopid bats can contain several cryptic species, and geographic variation may turn out to be greater in mormoopid bats than in other families. Specifically, *Pteronotus parnellii* seems to be a complex, comprising more than two sympatric species in the Amazon that can be easily separated by non-overlapping peak frequencies.

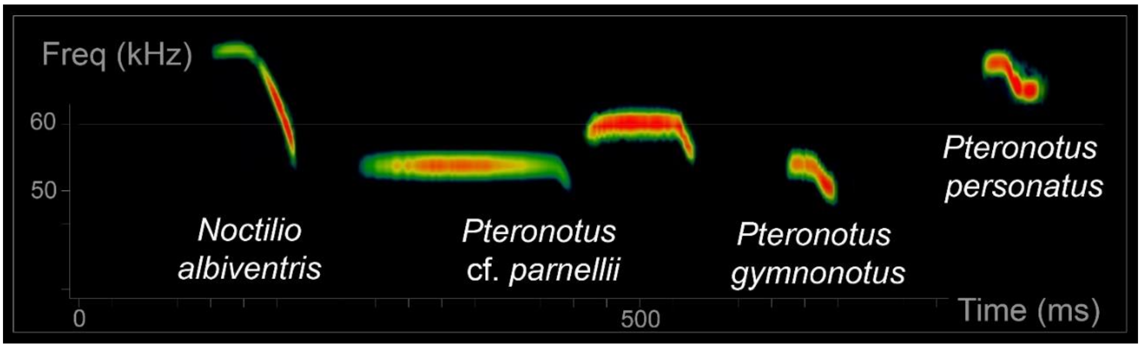
## *Mormoopidae*

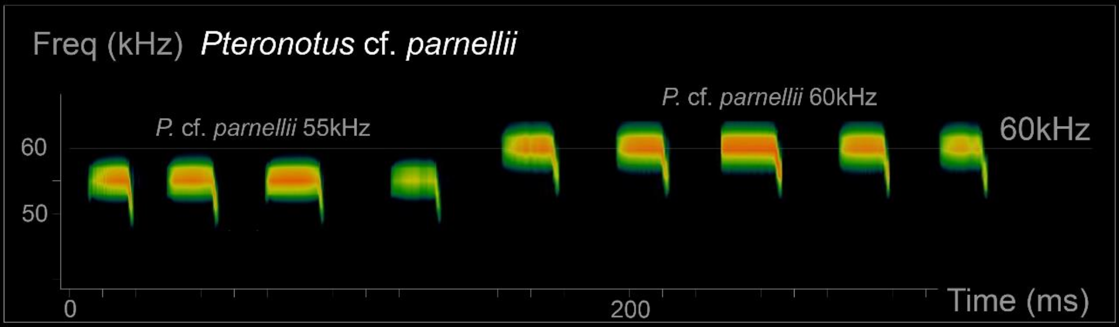
1a. CF / FMd (or small FMu / CF / FMd)
  2a. CF ≈ 55 kHz
*Pteronotus cf parnellii* 55kHz

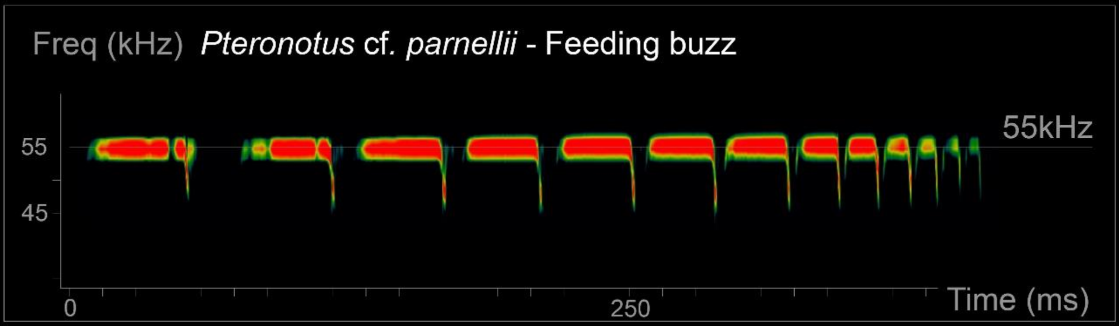
  2b. CF ≈ 60 kHz
*Pteronotus cf parnellii* 60kHz

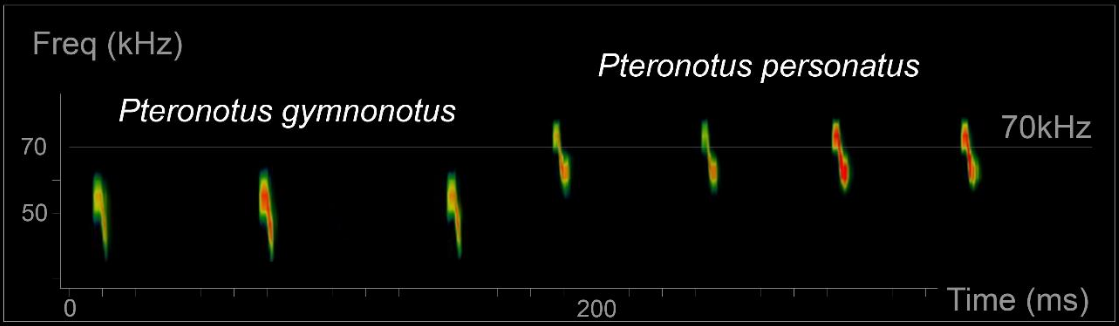
1b. CF / FMd / CF; SF(CF) ≈ 55 kHz
*Pteronotus gymnonotus*

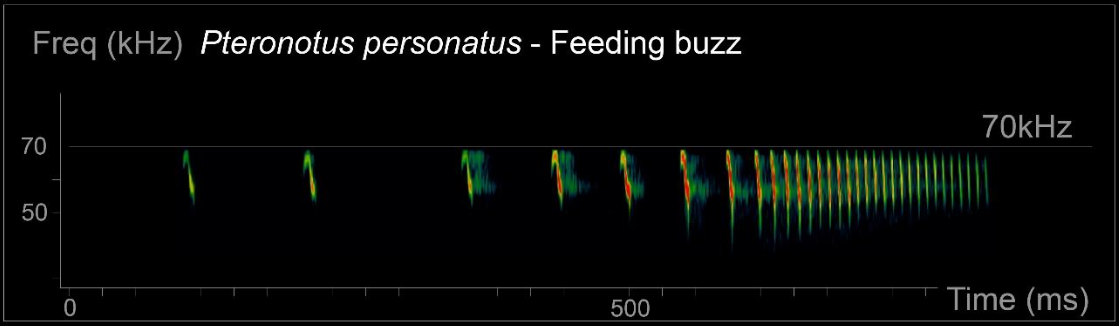
1c. CF / FMd / CF; SF(CF) ≈ 68-69kHz
*Pteronotus personatus*

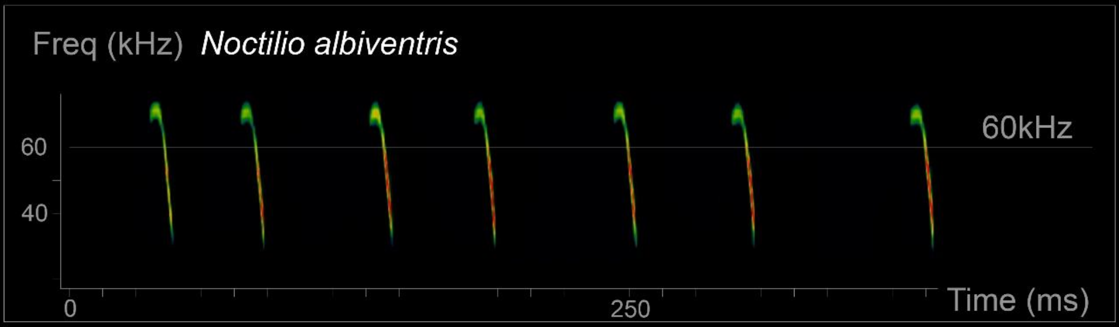



Freq (kHz) *Pteronotus* cf. *parnellii*
*P.* cf. *parnellii* 55kHz
*P.* cf. *parnellii* 60kHz
60kHz
60
50
0
200
Time (ms)


Freq (kHz) *Pteronotus* cf. *parnellii* - Feeding buzz
55kHz
55
45
0
250
Time (ms)


Freq (kHz)
*Pteronotus personatus*
*Pteronotus gymnonotus*
70kHz
70
50
0
200
Time (ms)


Freq (kHz) *Pteronotus personatus* - Feeding buzz
70kHz
70
50
0
500
Time (ms)


Freq (kHz) *Noctilio albiventris*
60kHz
60
40
0
250
Time (ms)

## Some notes on the identification of *Emballonuridae*

One of the most useful features for separating emballonurid species and phonic groups is the alternation of different call frequency types. However, this can be a source of misidentification. The problem lies in the fact that the last upper pulse is sometimes not recorded due to its low intensity or simply because some bats might not emit it under certain conditions. It is thus recommended to adjust the gain to try to highlight these faint pulses. If one fails to take this into account, the activity of the genus *Centronycteris* or of species such as *Saccopteryx gymnura*/*canescens* could be greatly overestimated, whereas that of *Saccopteryx leptura* or *Saccopteryx bilineata* could be underestimated.

Another point to bear in mind is how to determine the slope angle when separating the groups *Centronycteris*/*Saccopteryx* from *Diclidurus*/*Peropteryx* spp. Low-quality recordings with a lot of confusing background noise and faint calls are common and to avoid this it is sometimes a good idea to switch your full spectrum sonograms to a zero-crossing representation to improve the detection of the angle of the pulses.

### *Emballonuridae*

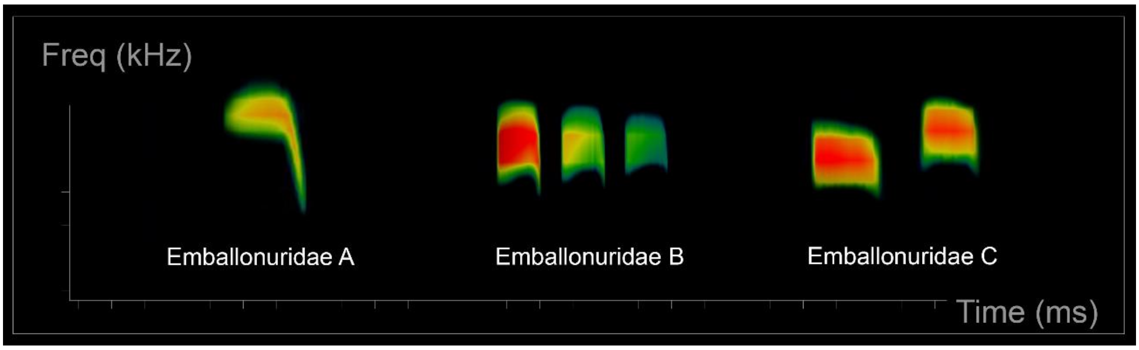
1a. QCF/ FMd; QCF ≈ 100 kHz.
*Emballonuridae* A

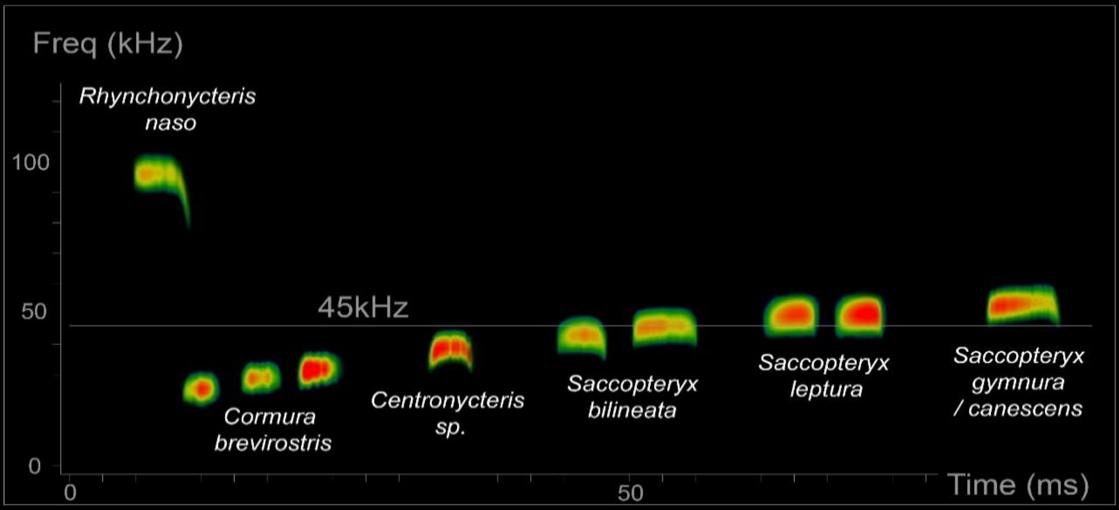
1b. Convex QCFu with FMd tails at the beginning of the pulses.
*Emballonuridae* B

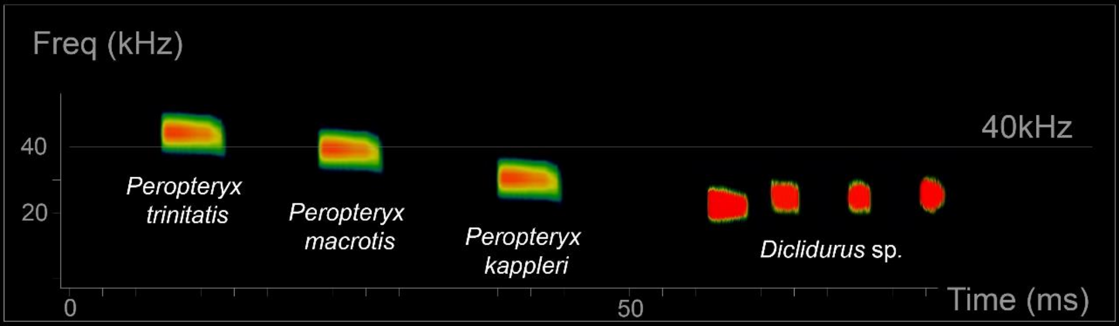
1c. Convex QCFd with FMd tails at the end of the pulses.
*Emballonuridae* C

#### *Emballonuridae A*

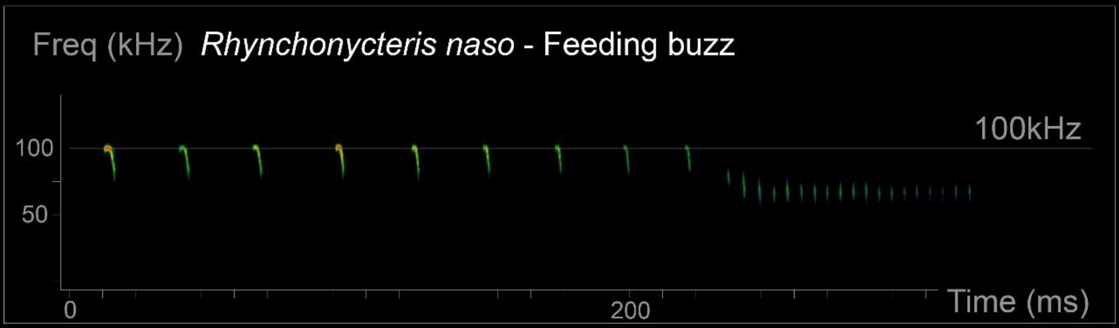
1a. Only one species with this type of pulse.
*Rhynchonycteris naso*




Freq (kHz)
100
50
0
Rhynchonycteris naso
45kHz
Cormura brevirostris
Centronycteris sp.
Saccopteryx bilineata
Saccopteryx leptura
Saccopteryx gymnura / canescens
0
50
Time (ms)


Freq (kHz)
40
20
40kHz
Peropteryx trinitatis
Peropteryx macrotis
Peropteryx kappleri
Diclidurus sp.
0
50
Time (ms)


Freq (kHz) Rhynchonycteris naso
100kHz
100
80
0
200
Time (ms)


Freq (kHz) Rhynchonycteris naso - Feeding buzz
100kHz
100
50
0
200
Time (ms)

### *Emballonuridae B*

1a. One single pulse type.
    2a. FME ≈ 54 kHz.
        *Emballonuridae* I
(*Saccopteryx gymnura* / *canescens*)

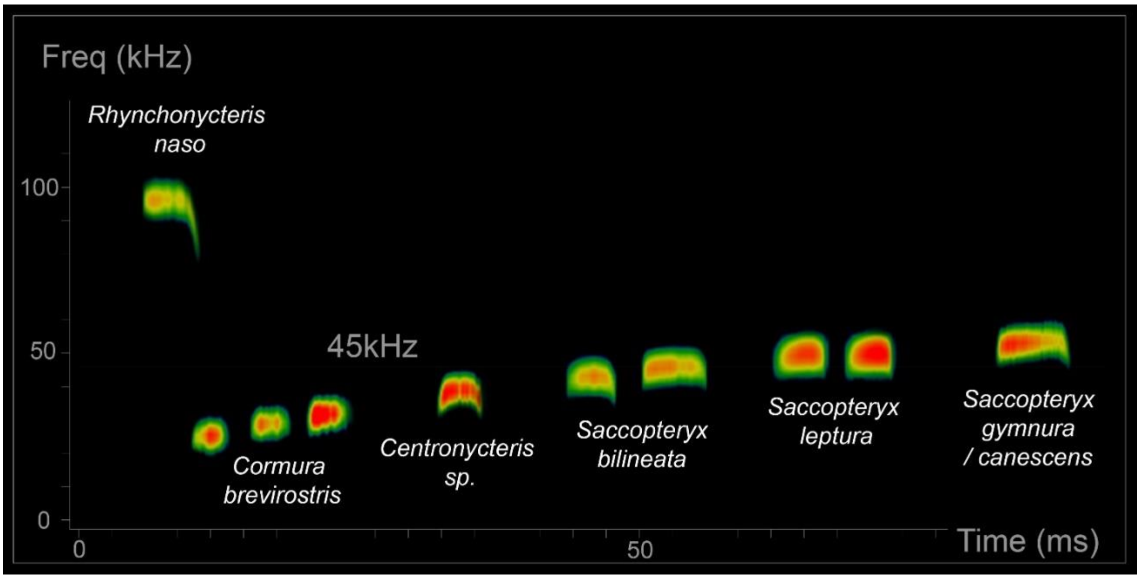
    2b. FME ≈ 40 kHz.
    *Emballonuridae* II
(*Centronycteris centralis* / *maximiliani*)

    2c. FME ≈ 35 kHz.
*Cyttarops alecto*

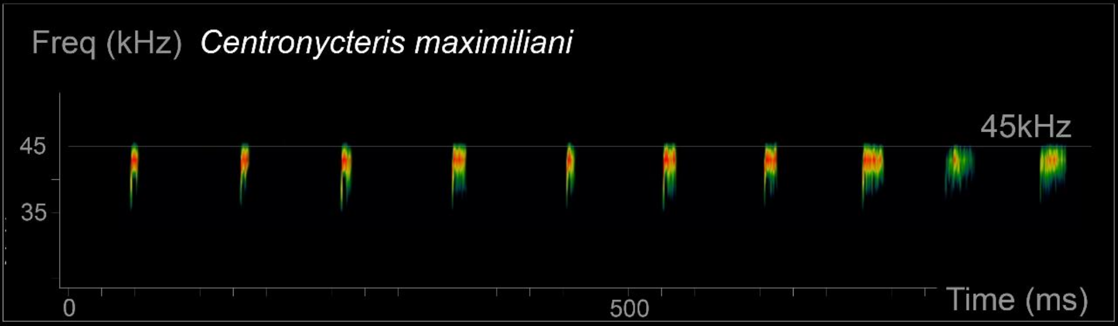
1b. Two alternating types of pulses.
    2a. Lower pulse FME ≈ 48 kHz.
        Higher pulse FME ≈ 55 kHz.
*Saccopteryx leptura*

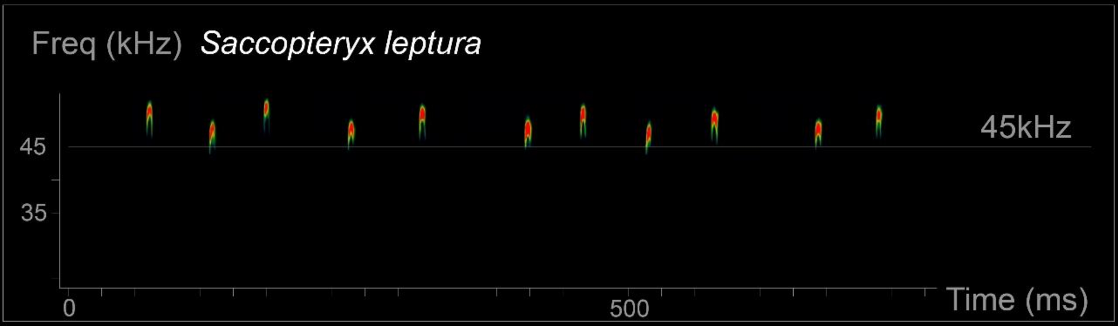
    2b. Lower pulse FME ≈ 42 kHz.
        Higher pulse FME ≈ 45 kHz.
*Saccopteryx bilineata*

1c. Three alternating types of pulses.
    2a. Lower pulse FME ≈ 25 kHz.
        Intermediate pulse FME ≈ 28 kHz.
        Higher pulse FME ≈ 30 kHz.
*Cormura brevirostris*

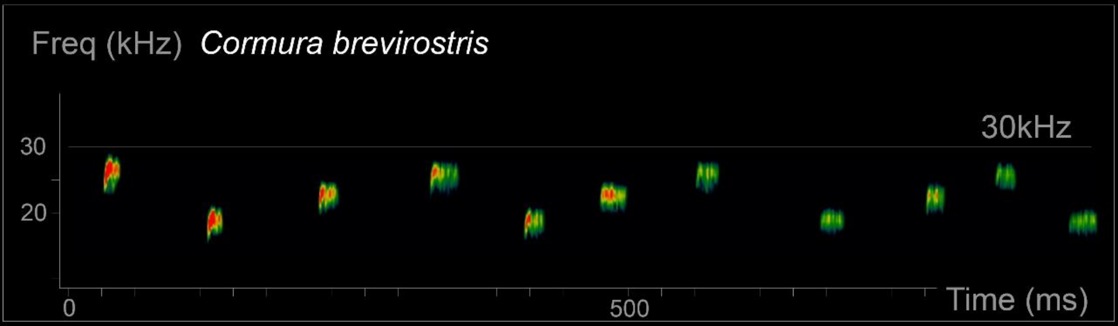



Freq (kHz) *Saccopteryx gymnura*
50kHz
50
40
0
500
Time (ms)


Freq (kHz) *Centronycteris maximiliani*
45kHz
45
35
0
500
Time (ms)


Freq (kHz) *Saccopteryx leptura*
45kHz
45
35
0
500
Time (ms)


Freq (kHz) *Saccopteryx bilineata*
45kHz
45
35
0
500
Time (ms)


Freq (kHz) *Cormura brevirostris*
30kHz
30
20
0
500
Time (ms)

***Emballonuridae C***

| | |
|---|---|
| 1a. One type of pulse. | |
| 2a. FME ≈ 42-44 kHz. | *Peropteryx trinitatis* |
| 2b. FME ≈ 37-39 kHz. | *Peropteryx macrotis* |
| 2c. FME ≈ 29-33 kHz. | *Peropteryx kappleri* |
| 1b. Two alternating types of pulses * | |
| 2a. Lower pulse FME ≈ 26 kHz<br>Higher pulse FME ≈ 30 kHz | *Diclidurus albus* / *scutatus* |
| 2b. Lower pulse FME ≈ 19 kHz<br>Higher pulse FME ≈ 22 kHz | *Diclidurus ingens* |

* These groups can sometimes overlap. Then we recommend classification as *Diclidurus* spp.

## How to separate *Diclidurus* and *Peropteryx* from molossid calls

Identification of species emitting low-frequency calls is challenging as calls are highly variable even within a single sequence. Due to the great overlap between the calls of some emballonurids (*Diclidurus* and *Peropteryx*) and molossid bats it is sometimes difficult to separate them.

We suggest following these steps:

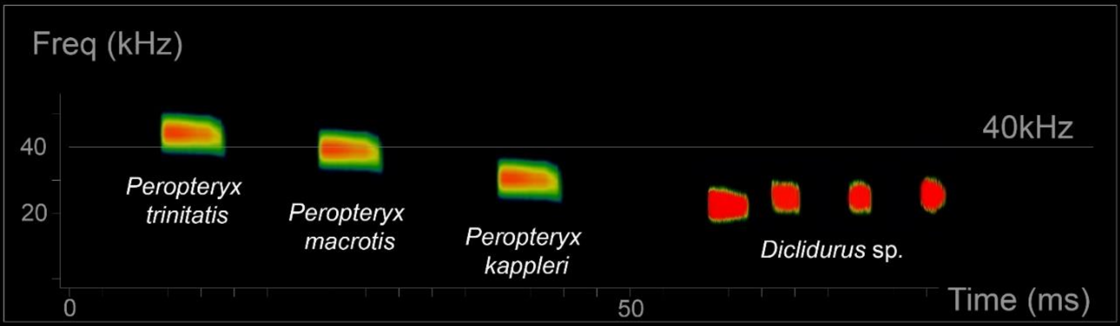
1st. Try to find the fundamental harmonic by adjusting the gain. If successful, genus separation is straightforward and clear.

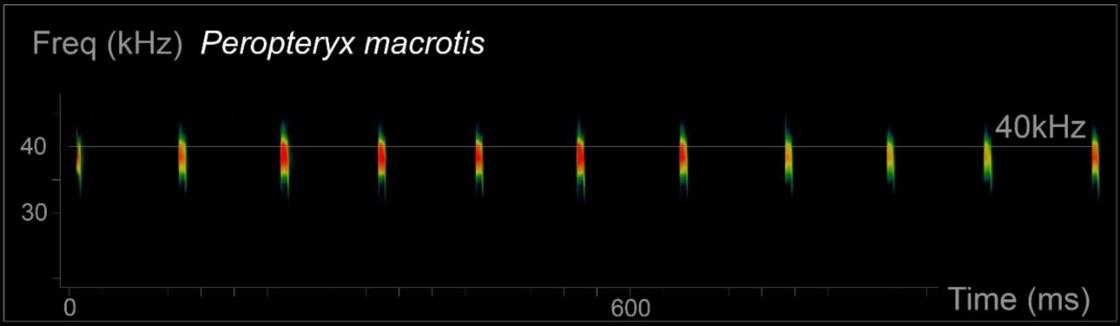
2nd. Try to identify an obvious downturn at the end of the pulses, which is different from those in emballonurid species.

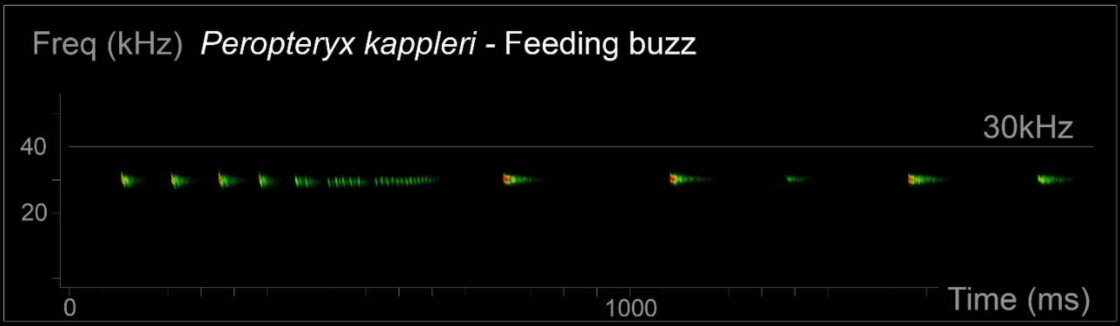
3rd. If it is impossible to see any harmonic, check the shape, angle and alternation.

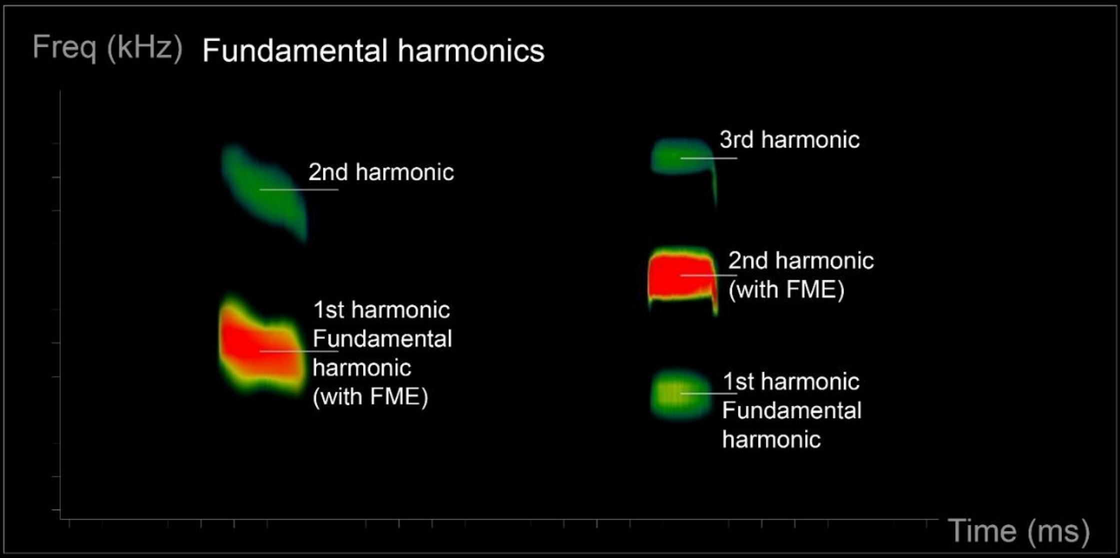
4th. If the calls overlap or show no clear patterns, it is recommended to classify them as “unidentified” which is the most conservative way of processing your data.

5th. If you are not completely certain about an identification, consult a more experienced specialist.


Freq (kHz)
40
20
40kHz
Peropteryx trinitatis
Peropteryx macrotis
Peropteryx kappleri
Diclidurus sp.
0
50
Time (ms)


Freq (kHz) Peropteryx macrotis
40
30
40kHz
0
600
Time (ms)


Freq (kHz) Peropteryx kappleri - Feeding buzz
40
20
30kHz
0
1000
Time (ms)


Freq (kHz) Fundamental harmonics
2nd harmonic
1st harmonic Fundamental harmonic (with FME)
3rd harmonic
2nd harmonic (with FME)
1st harmonic Fundamental harmonic
Time (ms)

## *Molossidae*

1b. Convex QCFd with one initial FMu (three types of pulses). *

*Molossidae A*

1c. Convex QCFu and concave QCFd.

*Molossidae B*

1d. Convex QCFd and concave QCFd.

*Molossidae C*

1e. Concave QCF (FME < 30kHz).

*Molossidae D*

***Molossidae A***

1a. Lower pulse FME ≈ 33-35 kHz.
Intermediate pulse FME ≈ 35-40 kHz.
Higher pulse FME ≈ 40-45 kHz.

***Molossus* I ***

*Molossus molossus*

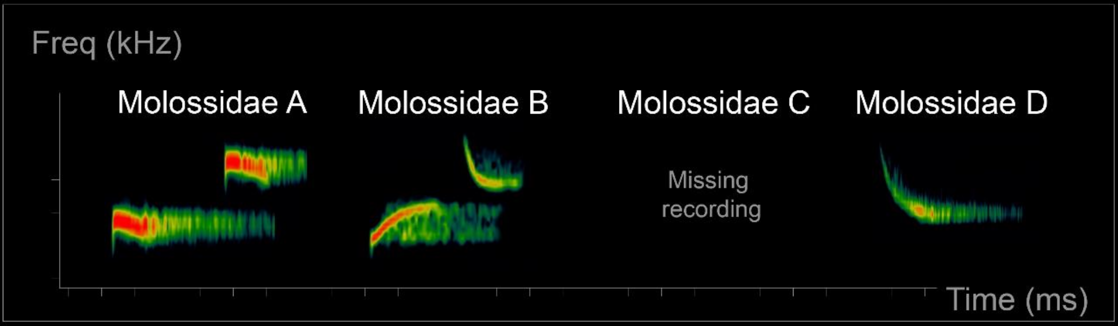
*1b*. Lower pulse FME ≈ 25-30kHz.
Intermediate pulse FME ≈ 30-35 kHz.
Higher pulse FME ≈ 35-40 kHz.

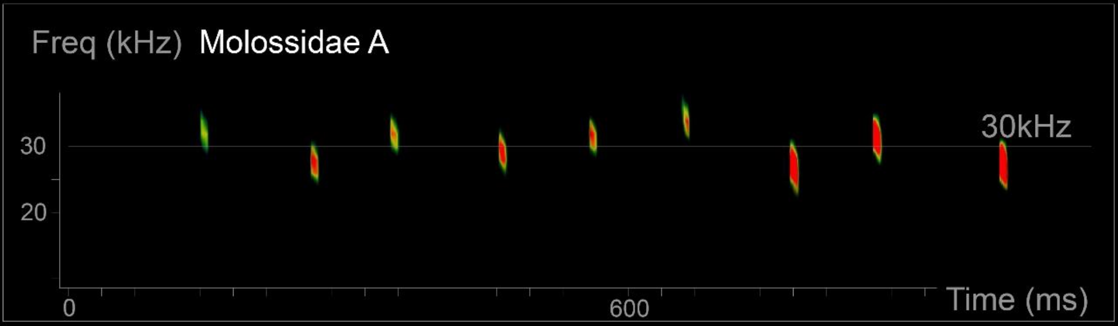
***Molossus* II ***

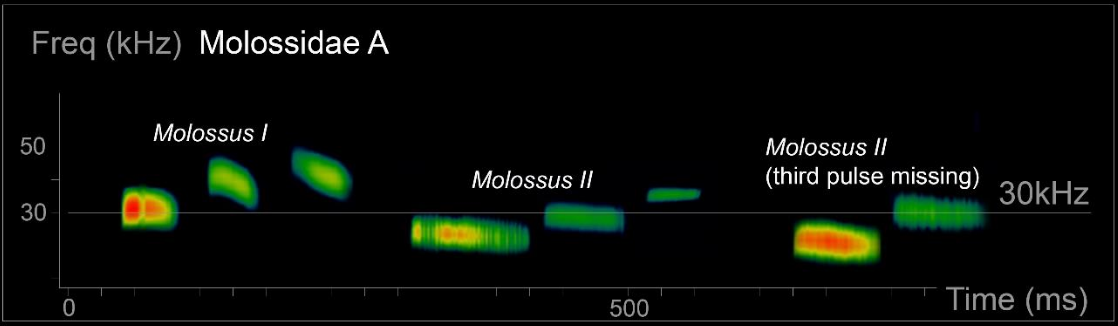
*Molossus sinaloae* / *currentium* / *rufus*

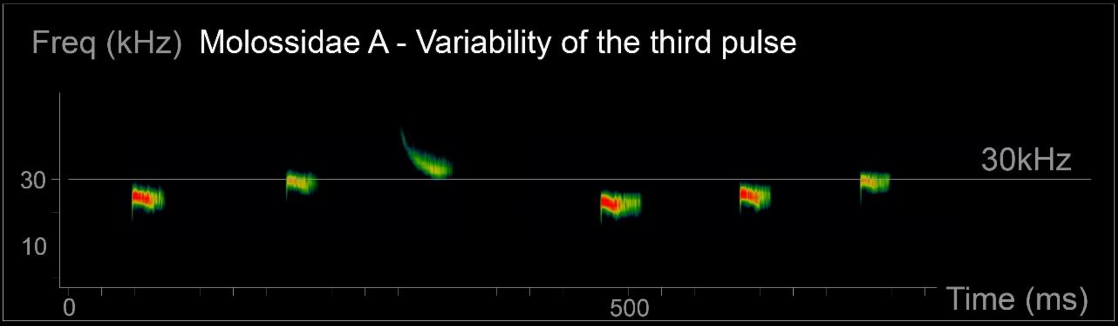
---

* Be careful with the second and third upper pulses, as they sometimes cannot be properly recorded due to their low intensity, which can lead to misidentification. The first FMu part might not be present if the pulse is too faint. *Molossus* I & II can sometimes overlap. In some cases, the higher pulse can be strongly modulated and may be followed by sequences of several similar modulated concave pulses (see figure).


Freq (kHz)
Molossidae A
Molossidae B
Molossidae C
Missing recording
Molossidae D
Time (ms)


Freq (kHz)
Molossidae A
30
20
30kHz
0
600
Time (ms)


Freq (kHz)
Molossidae A
50
30
Molossus I
Molossus II
Molossus II
(third pulse missing)
30kHz
0
500
Time (ms)

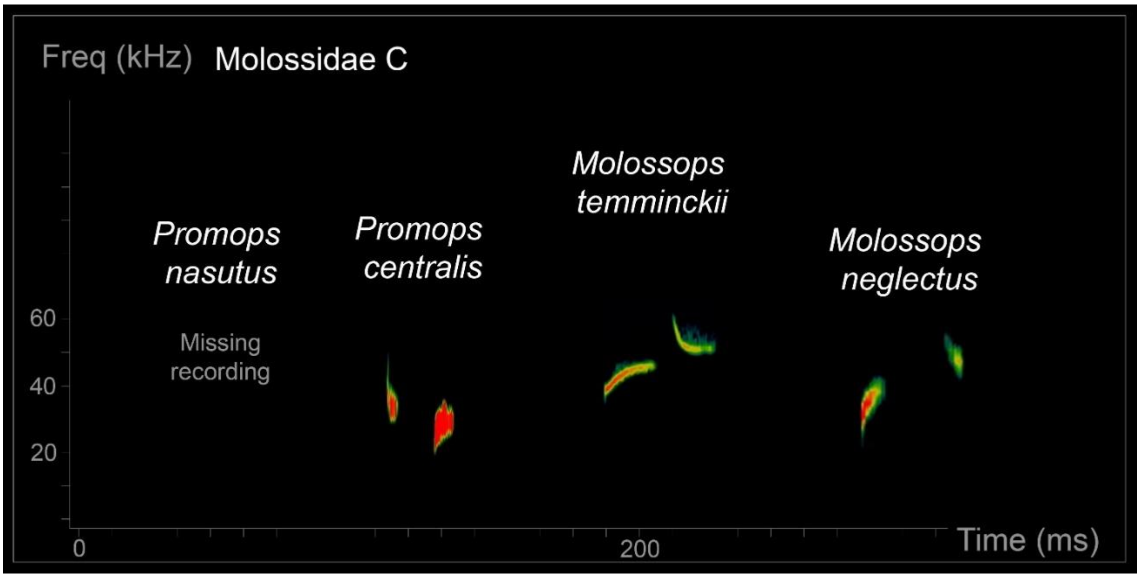

Freq (kHz)
Molossidae A - Variability of the third pulse
30
10
30kHz
0
500
Time (ms)


Freq (kHz)
Molossidae A - Feeding buzz
30
10
30kHz
0
200
Time (ms)

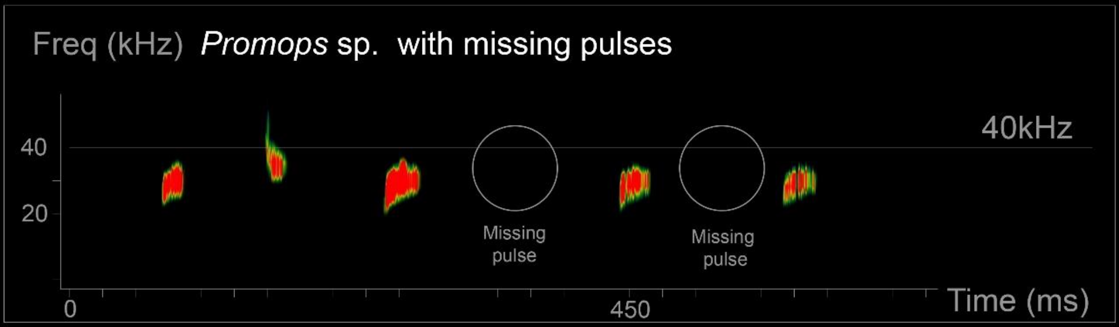
***Molossidae B***

1a. Lower pulse < 40kHz.
  2a. Lower pulse, EF ≈ 34 kHz. *
    Higher pulse, EF ≈ 37 kHz. * *Promops nasutus*
  2a. Lower pulse, EF ≈ 28 kHz. *
    Higher pulse, EF ≈ 30 kHz. * *Promops centralis*
1b. Lower pulse > 40kHz.
  2a. Lower pulse, EF ≈ 54 kHz. *
    Higher pulse, EF ≈ 55 kHz. *
    *Molossops temminckii*

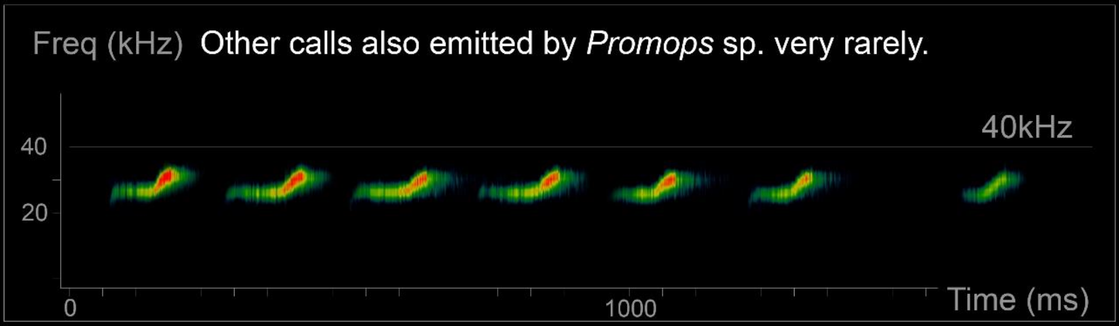
  2a. Lower pulse, EF ≈ 44 kHz. *
    Higher pulse, EF ≈ 46 kHz. *
    *Molossops neglectus*



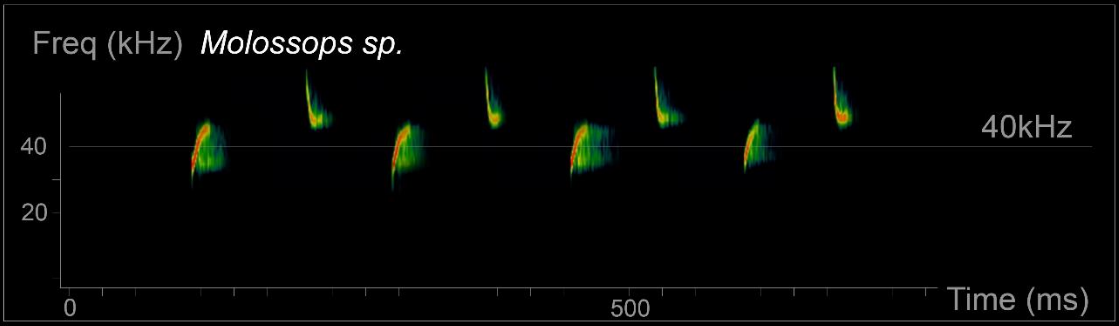
* These groups can sometimes overlap. Therefore, we recommend classification as *Molossus* spp., *Promops* spp or *Molossops* spp. Be careful with the upper pulses, as they sometimes cannot be properly recorded, which can lead to misidentification.



*Promops* sp. with missing pulses



Other calls also emitted by *Promops* sp. very rarely.



*Molossops sp.*

*Molossops* sp. with missing pulses

*Molossops* sp. - Feeding buzz

**Molossidae C**

1a. Only one species with this type of pulse.

*Neoplatymops mattogrossensis*

**Molossidae D**

1a. Only one type of pulse.

*Nyctinomops macrotis*

1b. Two alternating types of two pulse.

2a. Lower pulse, EF ≈ 18 kHz.
Higher pulse, EF≈ 22 kHz. **Molossidae III**

*Eumops auripendulus* / *glaucinus* / *dabbenei* / *hansae* * / *maurus*
*Nyctinomops laticaudatus*, *Tadarida brasiliensis*
*Cynomops planirostris* / *paranus* / *greenhalli* / *abrasus*

## Natalidae

1a. Only one type of pulse.

*Natalus sp.*

## Furipteridae

1a. Only one species with this type of pulse. **

*Furipterus horrens*

## Thyropteridae

1a. Only one genus with this type of pulse. ***

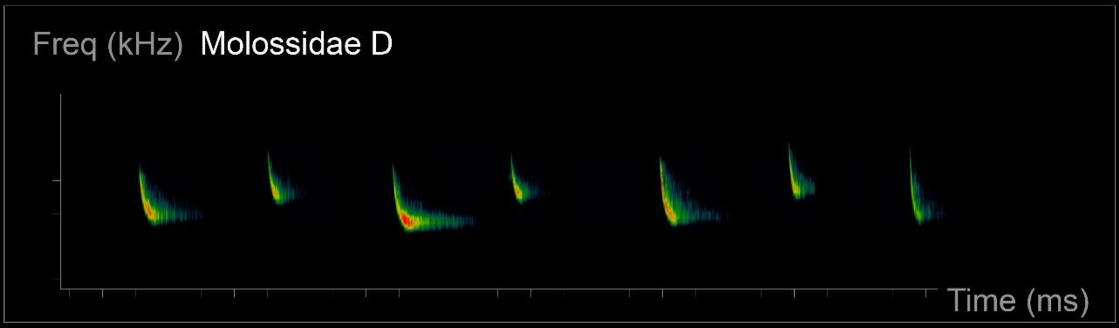
*Thyroptera sp.*

---

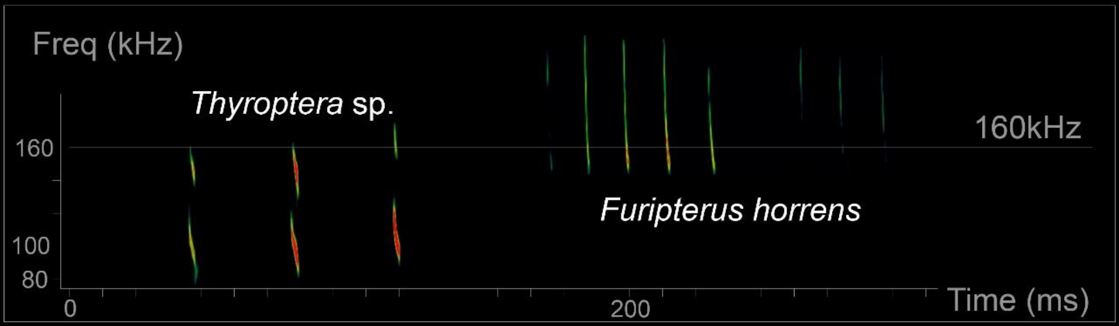
* Sometimes considered as a cryptic species complex with *E. nanus*.

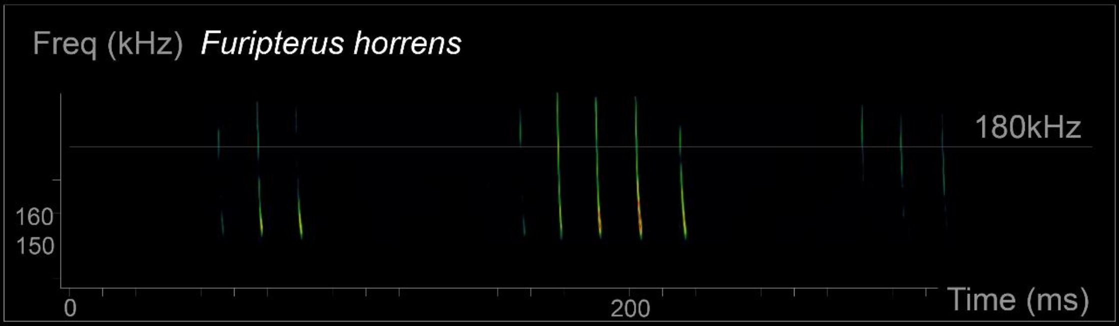
** Sometimes pulses seem to be grouped in sequences of 5-20 pulses during the search calls.

*** Sometimes confused with *Myotis riparius*. Therefore, we recommend classification as *M. riparius/Thyroptera* spp.


Freq (kHz)
Molossidae D
Time (ms)


Freq (kHz)
Molossidae III - Feeding buzz
40
20
20kHz
0
500
Time (ms)


Freq (kHz)
Thyroptera sp.
160kHz
160
Furipterus horrens
100
80
0
200
Time (ms)


Freq (kHz)
Furipterus horrens
180kHz
160
150
0
200
Time (ms)

## *Vespertilionidae*

1a. Pulse mainly FMd; EF 25-45 kHz with irregular and alternating sequences. *

2a. EF ≈ 25-35 kHz.

***Vespertilionidae* I**

*Lasiurus ega* / *castaneus* / *egregius* / *atratus*

2b. EF ≈ 40-45 kHz.

***Vespertilionidae* II**

*Rhogeessa io* / *Lasiurus blossevillii*

1b. Pulse initially FM, but with a considerable QCFd part.
Generally regular low frequencies.

2a. EF ≈ 25-39 kHz.*

***Eptesicus I***

*Eptesicus brasiliensis* / *furinalis* / *chiriquinus*

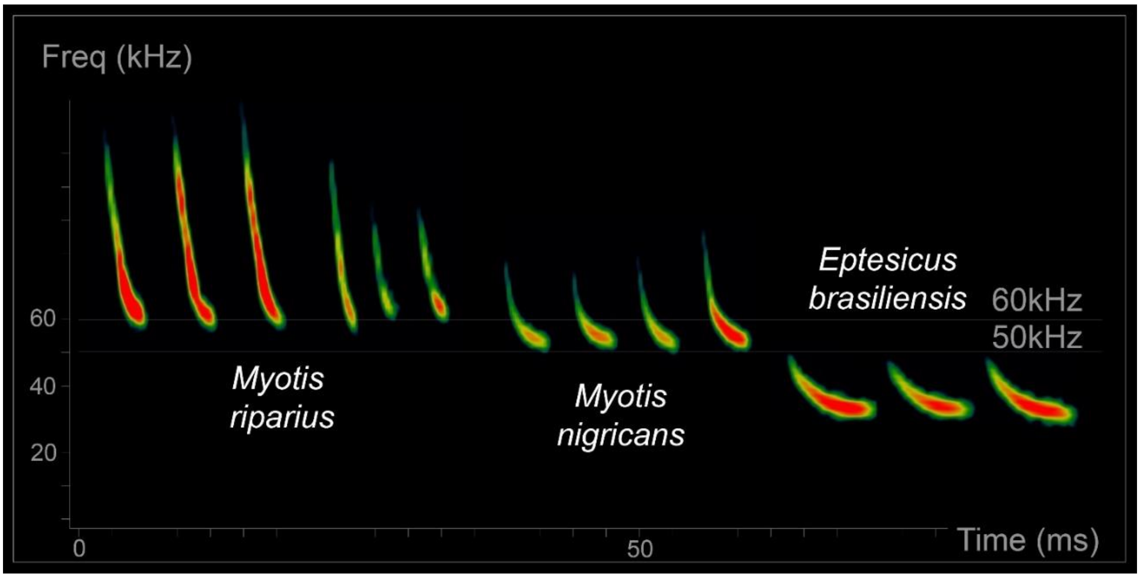
2b. EF > 45 kHz; pulses ending with a QCF tail.

3a. EF > 55 kHz.

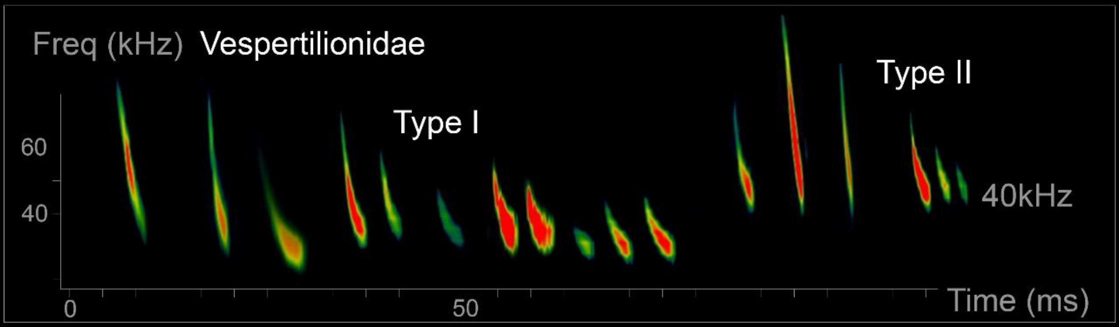
*Myotis riparius*

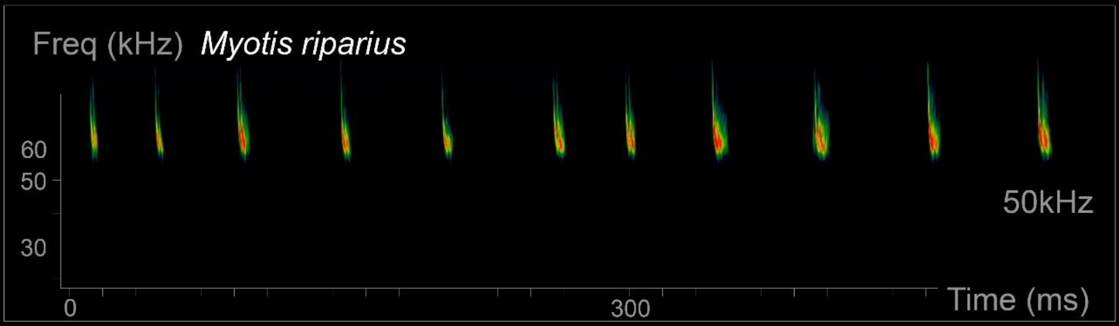
3b. EF ≈ 45-50 kHz.

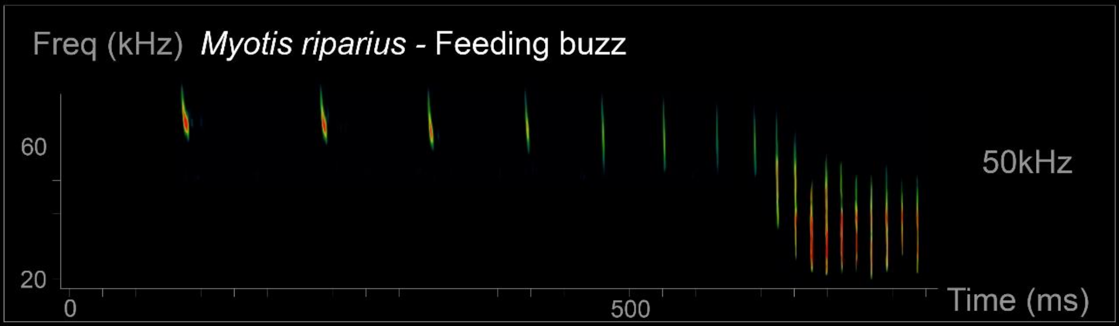
*Myotis nigricans*

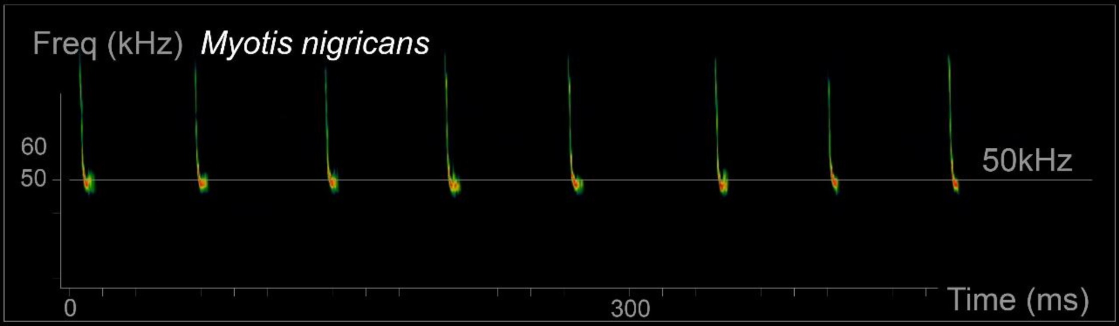


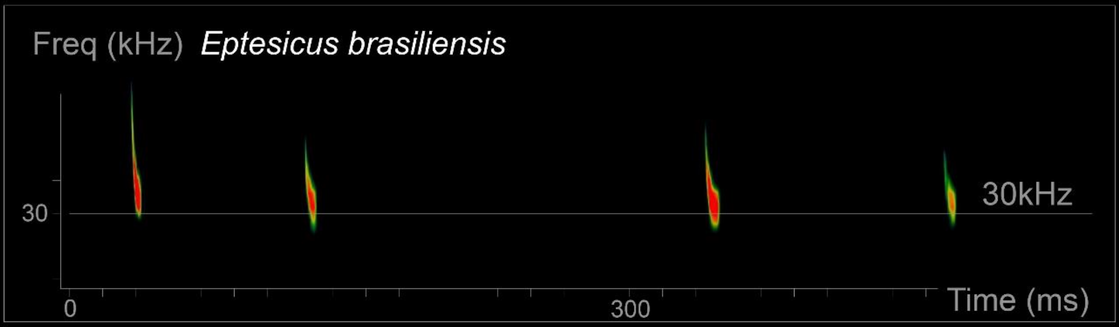
* These species can sometimes overlap which may lead to misidentifications.


Freq (kHz)
Vespertilionidae
Type I
Type II
60
40
40kHz
0
50
Time (ms)


Freq (kHz)
*Myotis riparius*
60
50
30
50kHz
0
300
Time (ms)


Freq (kHz)
*Myotis riparius* - Feeding buzz
60
20
50kHz
0
500
Time (ms)


Freq (kHz)
*Myotis nigricans*
60
50
50kHz
0
300
Time (ms)


Freq (kHz)
*Eptesicus brasiliensis*
30
30kHz
0
300
Time (ms)

# Chapter 3

## Stronger together: Combining automated classifiers with manual post-validation optimizes the workload vs reliability trade-off of species identification in bat acoustic surveys

**López-Baucells, A.**, Torrent, L., Rocha, R., Bobrowiec, P.E.D., Palmeirim, J.M., Meyer, C.F.J., 2018. Published in *Ecological Informatics* DOI: 10.1016/j.ecoinf.2018.11.004

Top: *Saccopteryx leptura;* Bottom: *Rhynchonycteris naso*

# CHAPTER 3

## Stronger together: Combining automated classifiers with manual post-validation optimizes the workload vs reliability trade-off of species identification in bat acoustic surveys

### Abstract

Owing to major technological advances, bioacoustics has become a burgeoning field in ecological research worldwide. Autonomous passive acoustic recorders are becoming widely used to monitor aerial insectivorous bats, and automatic classifiers have emerged to aid researchers in the daunting task of analyzing the resulting massive acoustic datasets. However, the scarcity of comprehensive reference call libraries still hampers their wider application in highly diverse tropical assemblages. Capitalizing on a unique acoustic dataset of more than 650,000 bat call sequences collected over a 3-year period in the Brazilian Amazon, the aims of this study were (a) to assess how pre-identified recordings of free-flying and hand-released bats could be used to train an automatic classification algorithm (random forest), and (b) to optimize acoustic analysis protocols by combining automatic classification with visual post-validation, whereby I evaluated the proportion of sound files to be post-validated for different thresholds of classification accuracy. Classifiers were trained at species or sonotype (group of species with similar calls) level. Random forest models confirmed the reliability of using calls of both free-flying and hand-released bats to train custom-built automatic classifiers. To achieve a general classification accuracy of ~85%, random forest had to be trained with at least 500 pulses per species/sonotype. For seven out of 20 sonotypes, the most abundant in the dataset, I obtained high classification accuracy (>90%). Adopting a desired accuracy probability threshold of 95% for the random forest classifier, I found that the percentage of sound files required for manual post-validation could be reduced by up to 75%, a significant saving in terms of workload. Combining automatic classification with manual ID through fully customizable classifiers implemented in open-source software as demonstrated here shows great potential to help overcome the acknowledged risks and biases associated with the sole reliance on automatic classification.

**Keywords**: Amazon, Bioacoustics, Chiroptera, Echolocation, Machine-learning algorithms.

## Introduction

Bioacoustics is a rapidly expanding field and of increasing importance for informing conservation projects. This is largely due to recent technological advances and the rising number of long-term monitoring programs which are being established for a number of taxa (Dickinson et al. 2010, Kershenbaum et al. 2014), including birds (Gregory et al. 2005), reptiles (Sewell et al. 2012), arthropods (Penone et al. 2013) and bats (Barlow et al. 2015). Interest in bat monitoring has increased over the last decades since bats have been acknowledged to provide important ecosystem services such as pest control (Boyles et al. 2013, Puig-Montserrat et al. 2015) and have been identified as good bioindicators of ecosystem health (Jones et al. 2009, Cunto and Bernard 2012).

Autonomous ultrasound detectors have proven essential for optimizing surveys of aerial insectivorous bats worldwide (Murray et al. 1999, Law et al. 2015). In the Neotropics, in contrast to phyllostomid bats, aerial insectivores are rarely captured in mist-nets (MacSwiney et al. 2008) and therefore, although they represent a high proportion of Neotropical bat diversity (Jung and Kalko 2011), the ecology of many species remains elusive and their echolocation calls poorly described (e.g. López-Baucells et al. 2014, López-Baucells et al. 2018). In fact, despite enormous recent advances in recording technology and equipment, comprehensive regional bat reference call libraries are currently lacking for much of the tropics (Walters et al. 2013, Madhukumar Menon et al. 2018).

Reference call libraries containing echolocation calls from a wide range of locations and habitats are crucial to reliably identify bat species acoustically. Although many species have distinctive echolocation calls, those of others can be very ambiguous due to producing very similar calls with overlapping characteristics (Russo and Voigt 2016). Moreover, weather conditions (e.g. Lawrence and Simmons 1982), geographical location (e.g. López-Baucells et al. 2017), sex (e.g. Puechmaille et al. 2014), body condition (e.g. Puechmaille et al. 2014), age (e.g. Jones and Kokurewicz 1994), reproductive status (e.g. Jones and Ransome 1993) or habitat structure (e.g. Pedro and Simonetti 2014) are all factors that contribute to substantial variation in call structure within species.

Different algorithms such as discriminant function analysis and random forest have already been used to automatically classify bat pulses (i.e. Russo and Jones 2002, MacSwiney et al. 2008, Armitage and Ober 2010, Walters et al. 2012, Zamora-Gutiérrez et al. 2016). However, substantial controversy still exists around the trade-off between the use of automatic classifiers versus manual species identification (Kershenbaum et al. 2014, Russo and Voigt 2016). While the former allows for the rapid analysis of a large number of recordings using an objective and repeatable protocol, manual identification provides more accurate, yet highly subjective and non-reproducible results, apart from being considerably more time-consuming (Kershenbaum et al. 2014). Unfortunately, even though considered a vital analysis step when relying on automated classifiers, posterior visual cross-checking by an expert is all too often neglected (Russo and Voigt 2016). Moreover, no study so far has quantified the potential time savings from using automatic classifiers as a function of the classification accuracy threshold adopted. Automatic classifiers have been criticised because of the inability to distinguish amongst species with similar calls, and because their algorithms are typically trained with calls from hand-released bats (Russo and Voigt 2016). It has been suggested that the use of hand-release calls can compromise the reliability of species identifications since these calls might be strongly affected by handling-related stress of the animal (Szewczak 2000).

Given that automatic classifiers are now widely available, there is a substantial risk that beginners solely rely on automated species identification without proper manual post-validation, which can result in incorrect identifications and thus wrong management decisions and negative conservation outcomes (Russo and Voigt 2016). Automatic classifiers were first applied to bat species identification in temperate areas as a direct consequence of the massive acoustic datasets that are now typically accumulated using passive bat recorders (Russo and Voigt 2016). However, the scarcity of suitable reference call libraries and the controversy around automatic vs. manual classification still hamper their wider application, especially in mega-diverse tropical regions.

The present study is the first to attempt to test the suitability of combining automatic classifiers trained with pre-identified recordings of free-flying bats obtained in the study area (which are much easier to obtain than reference calls from hand-released bats) with posterior manual validation (Fig. 3.1). This approach addresses the aforementioned issues of geographic variability, only classifies calls to the taxonomic level that the researcher can visually confirm with certainty and gives the user full control and flexibility concerning implementation of the algorithm. Capitalizing on a unique acoustic dataset collected over a 3-year period in the Central Amazon, here I use random forest, a machine learning algorithm that has performed well in previous bat acoustic studies (Zamora-Gutiérrez et al. 2016, Bas et al. 2017), to automatically classify aerial insectivorous bats. I evaluated the discriminative ability of the classifier by training it with a) previously identified calls from free-flying bats and those of hand-released bats; and b) datasets of different sizes of reference echolocation calls. To effectively combine the advantages of an automatic classifier with those of manual identification requires establishing a “correct classification probability” threshold below which a recording will need to be visually post-validated. Thus, to evaluate how acoustic studies could be optimized in terms of time commitment for the analyses, I also calculated, for different thresholds, the percentage of sound files from the full dataset that would need to be visually post-validated.

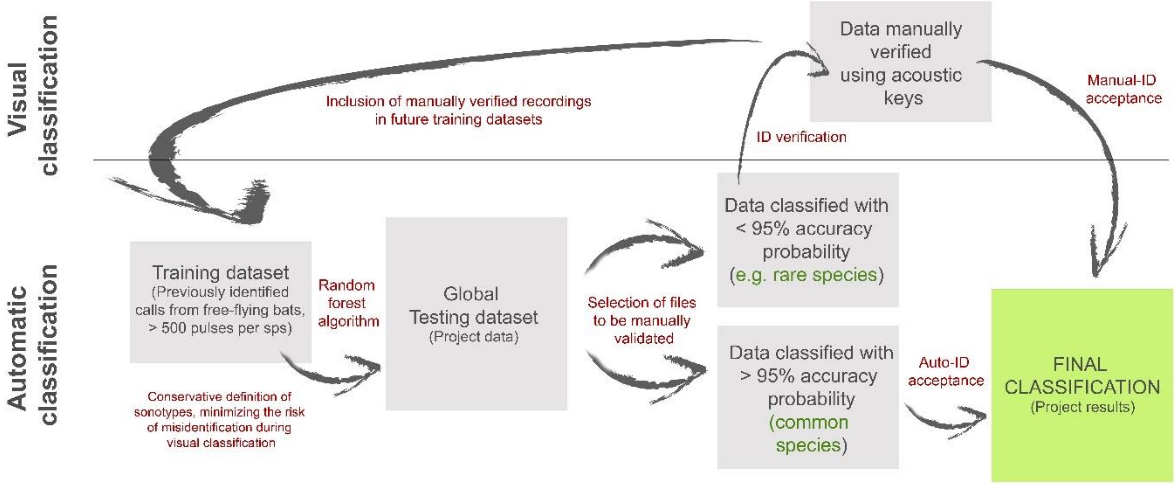


Figure 3.1. Diagram illustrating the classification process from automatic classification to posterior visual validation. Squares represent the datasets and selections of recordings; arrows and red text represent the analytical processes.

## Material and Methods

### 2.1 Study site

The study was conducted at the Biological Dynamics of Forest Fragments Project (BDFFP), a large-scale fragmentation experiment located ~80 km north of Manaus (Brazil) in the Central Amazon (2°20’S, 60°6’W), aimed at assessing the impacts of fragmentation on tropical forest communities (Laurance et al. 2011). Beginning in 1979, the BDFFP established 11 experimental forest fragments, which at the time of isolation were separated from continuous forest by distances of 80-650 m. Nowadays the fragments are surrounded by a matrix of secondary forest at varying successional stages (Laurance et al. 2018). The area is currently composed of a mosaic of unflooded lowland forest (80-160 m a.s.l.), pastures and secondary regrowth forest. Primary forest reaches 30-37 m in mean canopy height, with isolated trees up to 55 m tall (Laurance et al. 2011). Annual rainfall varies between 1900 and 3500 mm per year, with a rainy season between November and June and a dry season from July to

November (Ferreira et al. 2017), while mean annual temperature usually oscillates between 26-30 ºC (de Oliveira and Mori 1999).

## 2.2 Mist-netting and hand-release recordings

Intensive bat sampling was carried out in the context of a larger project assessing fragmentation effects on bats in the BDFFP landscape over a period of four years (2011-2014), using both ground- and canopy-level mist-netting. Sampling covered various types of rainforest habitats including continuous primary forest, forest fragments and secondary regrowth (Farneda et al. 2015, Rocha et al. 2017a, Rocha et al. 2017b). Sporadic sampling was also done over temporary lakes, small ponds and streams, as well as campsites, roads, and pastures (Torrent et al. 2018). Mist-netting was usually conducted from 18:00 to 00:00, except for some lakes where high capture rates sometimes required closing the nets earlier. Captured bats were identified using different keys (Lim and Engstrom 2001, Gardner 2007).

Echolocation call recordings of captured aerial insectivorous bats were made with a Pettersson D1000 bat detector (Pettersson Elektronik, Sweden), using 384 kHz sampling frequency in full spectrum (16-bit resolution) and no triggers or filters. Release calls were obtained after hand release of bats in either clearings or open areas within the forest (N=722 individuals). The detector was placed 5-10 m from the point of release (depending on the species) and once the individual was in flight, the microphone was pointed towards it to record as many search pulses as possible. For analysis, all pulses recorded immediately after release were discarded, as were overloaded calls, those too faint (for which it was impossible to distinct the shape from the background noise), social or stress calls, calls emitted in passive hunting mode and feeding buzzes.

## 2.3 Acoustic monitoring dataset

A total of 50 sites across the BDFFP landscape were acoustically surveyed 2012-2014, including the same sites used for mist-netting as described in Rocha et al (2017a, b). These comprised different-sized forest fragments (N=8), continuous forest (N=9), forest edges (N=11), secondary forest (N=11) and forest clearings (N=11). At each recording point, an automatic SM2Bat detector with an omnidirectional ultrasonic SMX-US microphone (Wildlife Acoustics, Inc., USA) was placed ca. 1.5 m above the ground. Acoustic surveys covered both dry and wet seasons and were conducted twice per season. Detectors were set to automatically record bats from 18:00 to 06:00 in real time with a full spectrum resolution of

16 bit, a high-pass filter set at fs/32 (12 kHz), an adaptive trigger level relative to noise floor of 18 SNR, and for periods of five consecutive nights per site. All recordings were split into five-second long sequences. Within such a five-second sound file, a bat pass was defined as a sequence with a minimum of two recognizable echolocation pulses per species (Millon et al. 2015, Appel et al. 2017, Torrent et al. 2018). This unit was used as a measure of activity levels. A total of 1,088,940 sound files were acquired during the study period in which ~650,000 bat passes were identified.

## 2.4 Echolocation call analysis

Kaleidoscope v.4.0.4 software (Wildlife Acoustics Inc., USA) was used to visualize and manually classify all bat passes from the acoustic monitoring dataset. Call sequences were manually identified to species/sonotype level as in previous studies (Silva and Bernard 2017, Torrent et al. 2018). For the purpose of this study, a sonotype was defined as a category that grouped species with similar calls when it was not possible to clearly assign a call to a particular species (Table 2.S1). Call identification was based on a series of acoustic features and standard measurements - call shape (CS), frequency of maximum energy (FME), start (SF), end (EF), maximum (MaxF) and minimum (MinF) frequency and duration (Dur) - and followed the echolocation key in López-Baucells et al. (2016). Moreover, recordings were also compared with a local reference call library compiled for the same study area over the course of the whole 3-year sampling period. Call sequences or pulses that were too faint for reliable identification (< 10 dB difference in power between background noise and FME of the echolocation pulses) were discarded from the analysis.

In addition to this manual identification, the same recordings were also subjected to an automatic identification process whereby pulse measurements were automatically extracted (~4,178,000 pulses) using SCAN’R (Snapshot Characterization and Analysis Routine) v1.7.4. (Binary Acoustic Technology, USA). Settings were adjusted as specified in Table 2.S2 to minimize the confusion between noise and bat calls. The following measurements were extracted for all pulses: Duration (Dur, ms), Maximum frequency (Fmax, kHz), Minimum frequency (Fmin, kHz), total bandwidth (BW, ms), Frequency at strongest sound pressure level (Fdom, kHz; equivalent to FME or *Frequency of maximum energy*), percentage in duration of Fdom (Ldom, %), High end of characteristic frequency (HiFc, kHz; equivalent of the knee frequency), Low end of characteristic frequency (LowFc, kHz), global slope of the call (Slope, kHz/ms), curvature (Curv) (SCAN'R 2009). After extraction, a Principal

Component Analysis (PCA) was performed, separately for each bat family, in order to visualise how different species/sonotypes clustered based on the similarity of their acoustic parameters.

## 2.5 Supervised machine learning

Supervised classification based on a machine learning algorithm (random forest, RF) was conducted using the R package “caret” (Classification and Regression Training) (Kuhn 2008). Random forest has performed well in several bat studies and is currently the preferred algorithm for the classification of bat echolocation calls (e.g. Zamora-Gutiérrez et al. 2016, Bas et al. 2017). Random forest models are built by comparing and averaging decision tree classifiers that are designed by bootstrapping random samples of the training dataset (Breiman 2001). Amongst its advantages, random forest is not affected by heteroscedasticity, is not strongly affected by outliers or low-informative variables and is relatively easy to use computationally (Olden et al. 2008), which makes it the method of choice for large acoustic datasets. In this case I selected three separate 10-fold cross-validations to tune the training model, with a final value of *mtry* of 2 (chosen for their highest accuracy) (Breiman 2001).

*Data preparation*. All pulse measurements were centred and scaled (Mukherjee and Manna 2006, Kuhn 2008) to make them comparable. The global dataset (~4,178,000 pulses) was split into different training and testing subsets. Training datasets were composed of 50, 100, 500, 1000 and 2000 reference pulses per species/sonotype, which were randomly selected from all recordings (except for *Rhynchonycteris naso* and *Furipterus horrens*, for which I only had data from 12 and 1,000 pulses respectively).

*Data classification*. Using the 1000-pulse training dataset, I evaluated classification accuracy and predictive power of the RF algorithm. Evaluation of performance of the training algorithm on the testing datasets was based on the performance metrics accuracy and kappa. Kappa measures inter-rater agreement for qualitative items (usually considered to be more robust than other measures as it also takes into account the agreement occurring by chance) (Viera and Garrett 2005). The same metrics were then additionally assessed for the different-sized training datasets, ranging from 50 to 2000 pulses/sonotype. Variable (feature) importance scores were also obtained using the R package caret (Kuhn 2008). The contribution of each variable is measured as follows: For each tree, the prediction accuracy is recorded removing each predictor variable. The average of the differences between all accuracies is normalized by the standard error.

Classification success for each species/sonotype was evaluated using 1) a RF model trained with the 2000-pulse dataset based on calls of free-flying bats and 2) a RF model trained with the complete reference call library based on hand-release calls compiled during the whole 3-year study period. The latter unfortunately included less than 2000 pulses for many species (Table 2.S3) due to the inherent difficulty to capture enough individuals from which to obtain release calls. Both training datasets were classified using the same species/sonotype labels in order to make both classifications comparable. Amongst the whole set of metrics commonly used to evaluate classifiers, I selected *sensitivity* and *positive predictive value* (PPV) as the most conservative for evaluating the performance of the acoustic classification task because they highlight the true positives in the classification process (Jennings et al. 2008) (Fig. 3.S1). While sensitivity is the proportion of calls correctly identified as one species/sonotype out of the total number of calls, positive predictive value is the proportion of calls correctly identified as one species/sonotype out of the total number of calls identified as such. Other metrics such as specificity or negative predictive value highlight the certainty of true negatives, which is quite unreliable in multicategory classifications (Fig. 3.S1).

The estimation of the percentage of recordings that would need to be manually checked depending on several classification accuracy thresholds was also based on the 2000-pulse training dataset. Classification accuracy thresholds considered in the analyses ranged from 60 to 95%, in 5% increments.

## Results

### 3.1 Acoustic discrimination at family level

A total of 27 aerial insectivorous bat species from six different families were captured and recorded during the study period, representing 20 different species/sonotypes (Table 3.S1). PCAs based on acoustic features showed that, for mormoopids, automatic parameter extraction often resulted in measurement values coming from different harmonics (Fig. 3.2). *Pteronotus alitonus* and *P. rubiginosus* clearly separated as distinct clusters and, although less evident, *P. personatus* and *P. gymnonotus* were also quite distinctly separated. Similarly, species with modulated calls such as vespertilionid or furipterid bats were split in rather well-defined bands. In contrast, except for *Saccopteryx bilineata* and *S. leptura*, emballonurid and molossid bats showed less defined limits between groups.

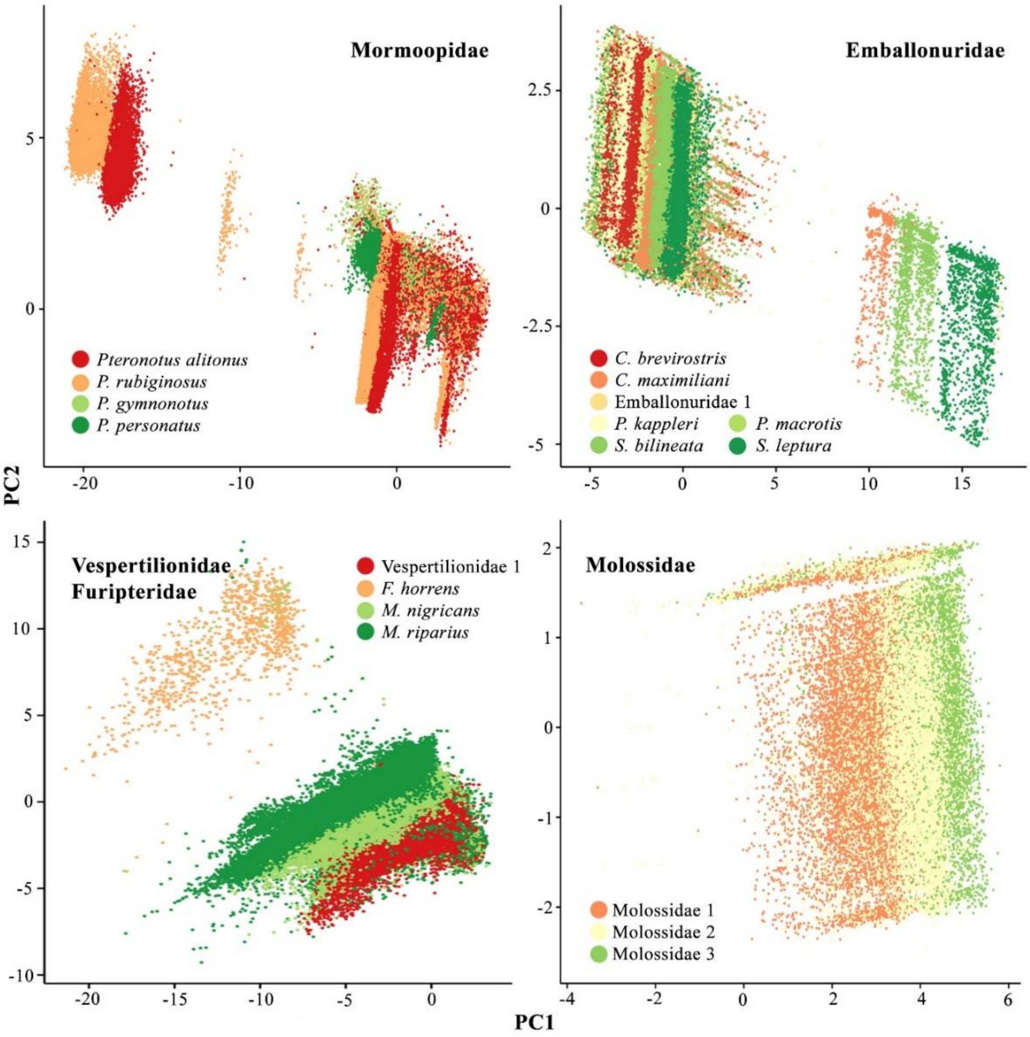


Figure 3.2. Principal component analyses (PCA) based on measurements of a series of acoustic parameters (see Methods) that were automatically extracted with SCAN'R, and manually classified to species/sonotype level following López-Baucells et al. (2016).

## 3.2 Minimum training dataset size and variable importance

I found that, in order to achieve a minimum general accuracy of ~85%, a training dataset of more than 500 pulses per species/sonotype was required (Fig. 3.3A). Classifications undertaken with training datasets based on only 50 pulses showed large variation in accuracy, reaching values below 75%. Classification performance was consistent between accuracy and kappa metrics. “High end of characteristic frequency” (equivalent to the frequency of the knee) was the most important variable in the RF model, followed by “Maximum frequency” and “Dominant frequency” (equivalent to the frequency of maximum energy). However, except for “Length of the dominant frequency”, “Duration”, “Bandwidth” and “Curvature”, all the variables showed quite similar importance values (Fig. 3.3B)

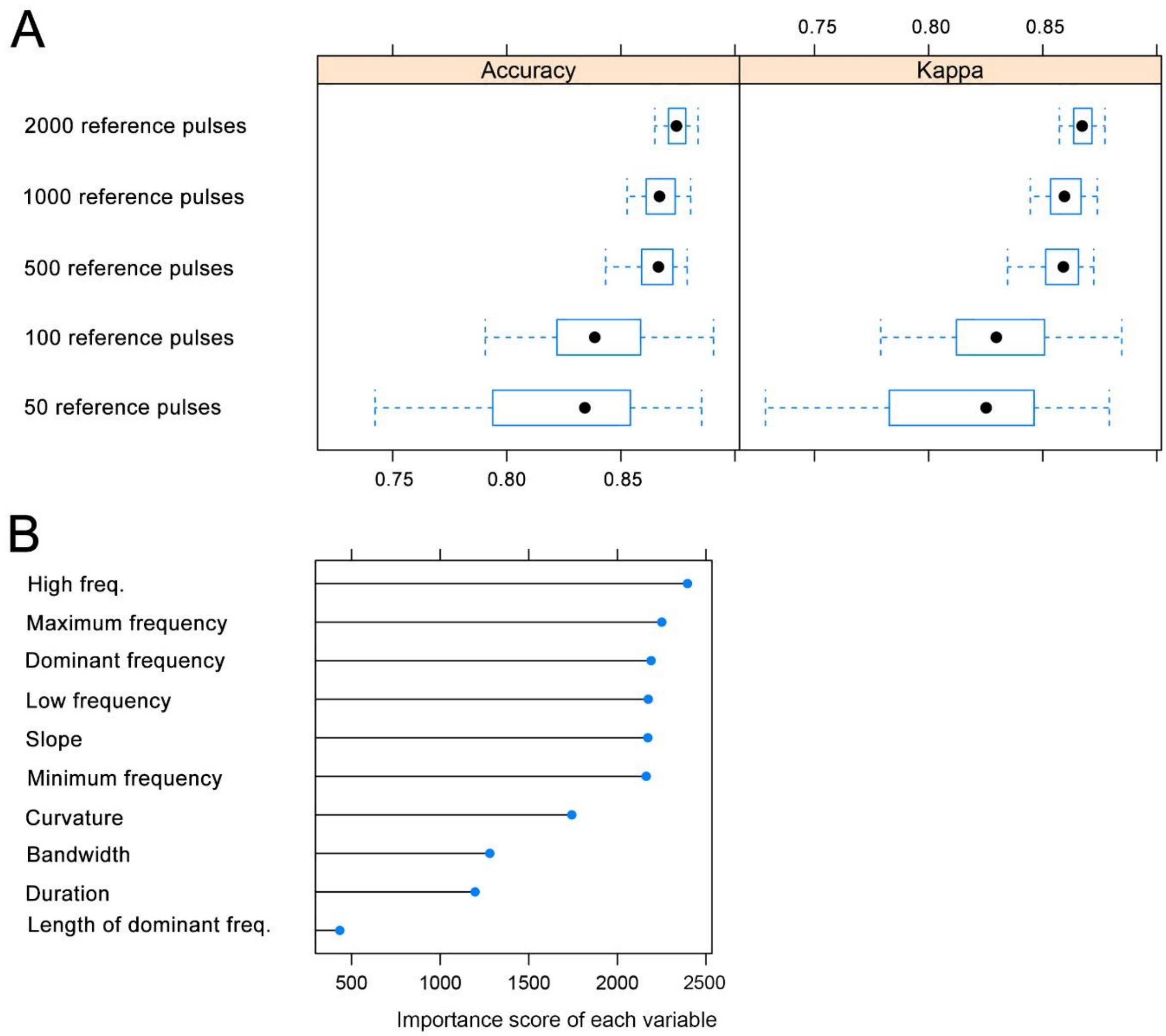


Figure 3.3. A) Classifier performance, evaluated as general accuracy and kappa, for a random forest model built with different-sized training datasets (50 to 2000 pulses/sonotype). The x-axis has been scaled to allow better visualization. Dots are medians, boxes 25% and 75% quartiles and whiskers denote the range. B) Importance of each variable in the random forest model trained with 2000 reference pulses per species/sonotype.

## 3.3 Classifier performance at species/sonotype level

Algorithm performance varied substantially among species/sonotypes (Table 3.S4). Seven had values above 90% for both sensitivity and PPV (*P. alitonus*, *P. rubiginosus*, Vespertilionidae 1, *Myotis nigricans*, *Centronycteris maximiliani*, *Myotis riparius* and *S. bilineata*), indicating not only that most of the recordings were correctly assigned, but also that few other recordings were confused with these species (Table 3.1, Table 3.S4). On the other hand, for other species such as *Furipterus horrens*, Emballonuridae 1, *P. gymnonotus*, Molossidae 3, *Promops* spp. and *P. personatus* there were considerable differences between metrics. For these, I found a low number of false negatives but a large number of false positives (low PPV). Molossidae 1 and 2 were the sonotypes with poorest levels of correct identifications, and *R. naso* (for which I had a very limited number of recordings) was the only species for which the classifier failed. Comparing the RF models trained with calls from free-flying vs. hand-released bats, the former nearly always outperformed the latter (Table 3.1). *Pteronotus alitonus* and *P. rubiginosus* obtained a similar proportion of correct identifications in both HR and FF algorithms, and Molossidae 3 was the only sonotype for which higher sensitivity scores were obtained using calls from hand-released bats, although it also had lower PPV.

Table 3.1. Performance of the random forest classifier for each species/sonotype based on calls from either free-flying (FF) or hand-released (HR) bats. Classification performance is ranked according to sensitivity and positive predictive value (see Methods for an explanation of the rationale underpinning this selection) as > 90% (dark green), 80 - 90% (olive green), and < 80% (light green).

| **Species/sonotypes** | **Acronym** | **Sensitivity** | | **Positive Predictive Value (PPV)** | |
|---|---|---|---|---|---|
| | | FF | HR | FF | HR |
| *Pteronotus alitonus* | PA | 0.96 | 0.92 | 0.99 | 0.80 |
| *Pteronotus rubiginosus* | PR | 0.96 | 0.95 | 0.99 | 0.73 |
| Vespertilionidae 1 | V1 | 0.95 | 0.76 | 0.96 | 0.51 |
| *Myotis nigricans* | MN | 0.93 | 0.32 | 0.96 | 0.70 |
| *Furipterus horrens* | FH | 0.93 | 0.88 | 0.00 | 0.00 |
| *Centronycteris maximiliani* | CM | 0.92 | 0.76 | 0.96 | 0.65 |
| *Myotis riparius* | MR | 0.91 | 0.83 | 0.98 | 0.97 |
| *Saccopteryx bilineata* | SB | 0.91 | 0.42 | 0.93 | 0.96 |
| Emballonuridae 1 | E1 | 0.90 | 0.00 | 0.21 | 0.02 |
| *Cormura brevirostris* | CB | 0.89 | 0.58 | 0.85 | 0.95 |
| *Peropteryx kappleri* | PK | 0.89 | | 0.95 | |
| *Peropteryx macrotis* | PM | 0.88 | 0.27 | 0.60 | 0.02 |
| *Saccopteryx leptura* | SL | 0.88 | 0.76 | 0.95 | 0.89 |
| *Pteronotus gymnonotus* | PG | 0.86 | 0.17 | 0.17 | 0.05 |
| Molossidae 3 | M3 | 0.84 | 0.92 | 0.24 | 0.06 |
| *Promops* sp. | P | 0.82 | | 0.11 | |
| *Pteronotus personatus* | PP | 0.80 | 0.01 | 0.09 | 0.81 |
| Molossidae 1 | M1 | 0.71 | 0.01 | 0.37 | 0.01 |
| Molossidae 2 | M2 | 0.59 | 0.27 | 0.44 | 0.17 |
| *Rhynchonycteris naso* | RN | 0.00 | 0.00 | 0.00 | 0.00 |

## 3.4 Combining automatic classification with manual post-validation

The total number of files to be visually confirmed after automatic classification ranged from ~20%, when the desired accuracy threshold was set to 60%, to ~30%, when it was set to 95% (Fig. 3.4). Following the same pattern found for species/sonotype-specific predictive performance (Table 3.1), for some categories the number of files to be post-validated did not differ substantially for the different accuracy thresholds mentioned previously, while for others, this percentage varied up to 30%. Only in few cases was there marked variation depending on the chosen accuracy threshold (from 40 to 90% for Emballonuridae 1 and *P. personatus*).

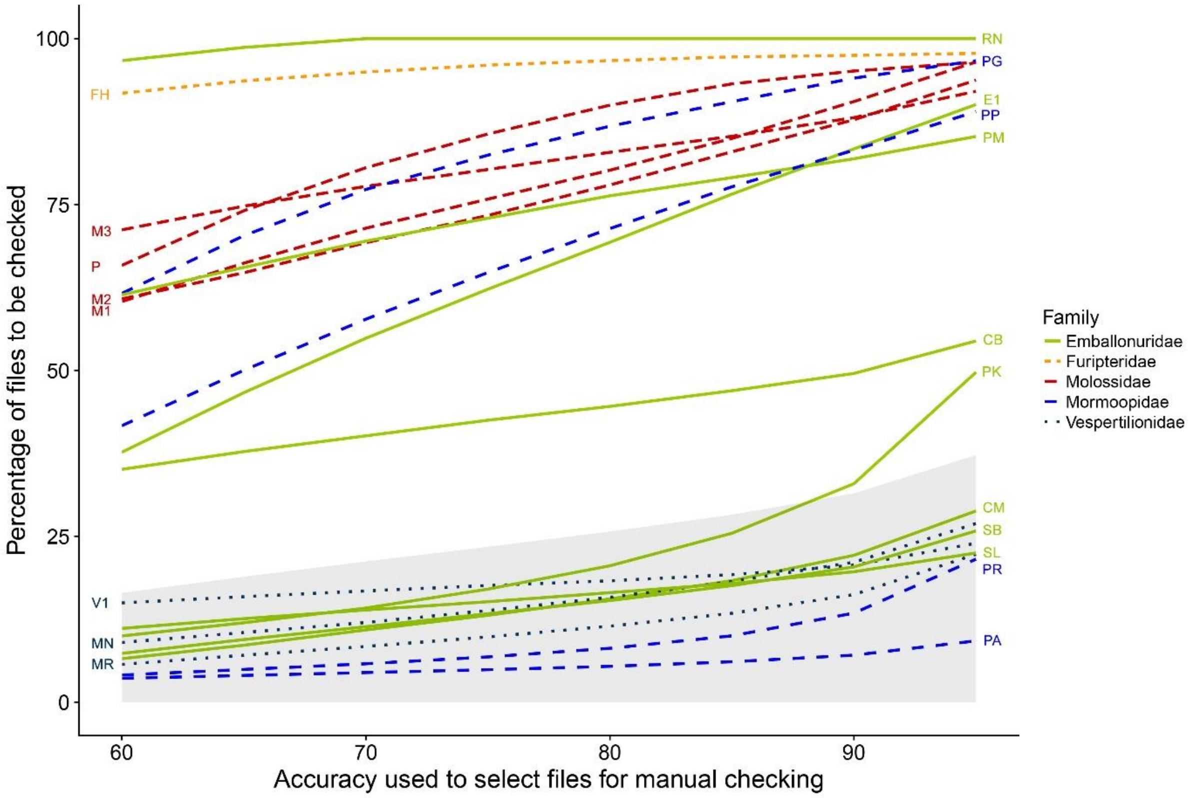


Figure 3.4. Percentage of files requiring visual post-validation as a function of the desired accuracy threshold for identification acceptance. Shaded area: Percentage of the whole dataset. Coloured lines: Percentage for each family; Species acronyms are as given in Table 3.1. Analysis was based on a random forest model, trained with 2000 pulses per species/sonotype (with the exception of Rhynchonycteris naso and Furipterus horrens, for which I had fewer recordings, see Methods).

## Discussion

The results suggest an inexpensive and relatively user-friendly approach (Fig. 3.1) to automatically classify large amounts of bat echolocation data, followed by visual post-validation of a reduced proportion of the original acoustic dataset. This approach overcomes the acknowledged risks and biases associated with the exclusive reliance on current automatic classifiers (Russo and Voigt 2016). With a large dataset of recordings obtained under real field conditions from a 3-year-long study in the Central Amazon, I confirmed the reliability of using locally-recorded echolocation calls from free-flying bats to train a custom-built classifier. This classifier is able to automatically identify the calls of a large subset of the species/sonotypes in the local assemblage with high accuracy (>90%) leaving the rest to be manually classified. The automatic pre-classification reduces the total number of recordings to be visually inspected, therefore optimizing the classification process. This equates to considerable time savings, especially in the case of projects that accumulate massive acoustic data. However, due to the customizable nature of this approach, the advantages of using recordings from free-flying bats hinge on manually pre-identifying a decent amount of calls from free-flying bats using release calls as references, literature and echolocation keys. This obviously entails the risk of including misidentified calls as a source for training the algorithm, a problem I overcame by restricting the classification of the recordings to easily distinguishable species/sonotypes, therefore avoiding misidentifications.

Being non-intrusive, automated recording systems and soundscape studies have recently become very popular and have considerably improved our knowledge about the natural history of elusive bat species, anthropogenic impacts and wildlife conservation in habitats where sampling by traditional methods such as mist-netting would be inefficient or unfeasible (Kubista and Bruckner 2017). However, in developing countries where funding is particularly limited, the widespread use of bioacoustics is still severely hampered by its elevated costs and cost-effective alternatives need to be found quickly. This has inspired a new trend towards developing user-friendly detectors and automatic classifiers which are fully customizable at reduced cost (Whytock et al. 2017, Hill et al. 2018). Reliance on self-built classification algorithms could greatly contribute to studies in regions for which no automatic classifier is available as part of commercially available software packages.

## 4.1 Minimum training dataset size and variable importance

I also identified the minimum number of pulses that should be used in the training dataset in order to achieve general accuracy levels between 75 and 95%. These results show that training the algorithm with 500 pulses per species/sonotype results in average classifier performance > 85%. However, this reference value should be interpreted carefully as it depends on whether the species that are most frequently detected in a region are also those whose echolocation call characteristics are more clearly distinct and thus the species more easily identifiable or not. In my case, the most common species (*P. rubiginosus, Myotis nigricans* and *M. riparius*), all easy to identify, might be positively biasing general accuracy, thus masking lower accuracies for the remaining categories (Biscardi et al. 2004).

The variables that contribute most to separating species may not be the same in all assemblages. For example, Monadjem et al. (2017) found that call duration represented one of the most relevant parameters to distinguish between species, while in this study, I obtained higher importance weights for several other variables. This probably reflects the diversity of pulse shapes and structures found in Neotropical aerial insectivorous bats. By including different variables in the algorithm, one can probably achieve better classification performance in such highly diverse areas (Walters et al. 2013).

## 4.2 Classifier performance at species/sonotype level

Random forest performed very well with the collected dataset, confirming its potential use for analysing bat acoustic datasets. Among the available machine learning algorithms, random forest has already been successfully used in automatic species classification for bats (Armitage and Ober 2010, Zamora-Gutiérrez et al. 2016) and other taxa such as birds (Briggs et al. 2009) and dolphins (Barkley et al. 2011). I obtained similar mean accuracies to those found in previous studies, although results varied among species and families (e.g. MacSwiney et al. 2008, Pio et al. 2010, Britzke et al. 2011, Zamora-Gutiérrez et al. 2016). For Neotropical bats, large variability in predictive power is found for Vespertilionidae and Molossidae, while Emballonuridae and Mormoopidae are usually more accurately identified (Zamora-Gutiérrez et al. 2016). Previous studies have evaluated the performance of automatic algorithms for classifying bat calls at species, genus, family or guild level (Zamora-Gutiérrez et al. 2016, Vassilios et al. 2017). However, it is now widely accepted in the scientific community that automatic classification must be used cautiously (Russo and Jones 2002, Russo and Voigt 2016, Monadjem et al. 2017). In this study, I aimed to optimize the classifiers not at species level but using sonotypes. Although classifying all calls to species level would be ideal, using sonotypes may be sufficient in most cases, obviously depending on a project's specific aims (Redgwell et al. 2009, Armitage and Ober 2010).

For seven out of 20 species/sonotypes I obtained very high values (>90%) for both sensitivity and PPV, proving that random forest algorithm could be used with great confidence to detect and automatically classify them in my recordings. Very few false positives and false negatives were found, indicating that the classifier neither gets them wrong, nor ignores them when they are present (see Table 3.1). These species are also the most predominant in my dataset, which turns this classifier into a great tool due to its potential to greatly reduce the number of files to be manually analysed (Andreassen et al. 2014). One of the main reasons to explain the classification failure of some categories is the limited capacity of SCAN'R to detect and characterize pulses of different lengths (used SCAN'R pulse detection settings were more suitable for long pulses). This will certainly improve soon with new technological advances, or alternatively, could be better implemented through R sound packages. Previous studies have exclusively used accuracy as a means of evaluating algorithm performance and predictive capacity (i.e. Wordley et al. 2014, Zamora-Gutiérrez et al. 2016). However, other more conservative metrics such as positive predictive value and sensitivity are often neglected. I encourage developers of algorithms and researchers to better scrutinize classifier performance by focusing on these more reliable metrics.

## 4.3 Classifier trained with calls from free-flying versus hand-released bats

I compared the performance of the random forest classifier trained with calls from free-flying versus hand-released bats, using only data collected during the 3-year-period of the project. Classifier performance was substantially better using recordings from free-flying bats, probably due to the low number of recordings from hand-released bats for most of the species. In this regard it is important to mention that the effort required to compile complete reference call libraries of good quality using hand-released bats and which cover different environmental situations is titanic (O'Farrell et al. 1999). In fact, this has probably discouraged many researchers from developing their own classifiers so far.

Globally, echolocation call libraries are incomplete, especially in understudied regions such as most of the tropics (Aguilar 2017). Due to species elusiveness, whispering behaviour or rarity, call libraries are usually only built with calls from a few hand-released individuals (Gager et al. 2016, Zamora-Gutiérrez et al. 2016, Monadjem et al. 2017). Although some studies have not found marked differences in automatic classifiers trained with data from distant regions (e.g. Zamora-Gutiérrez et al. 2016), other authors highlight the importance of taking these differences into consideration (Thomas et al. 1987, Barclay et al. 1999, O'Farrell et al. 2000, López-Baucells et al. 2017). Although I urge and support the compilation of comprehensive reference call libraries, this study suggests that training automatic classifiers with manually identified free-flying bats is a very valid option if it is cautiously used in conjunction with conservative classification criteria. As stressed by Jakobsen et al. (2013), it is of vital importance to record calls from naturally behaving bats in the wild and use these recordings to improve classifier performances.

## 4.4 Combining automatic classifiers with manual post-validation

No classifier has proved to provide 100% accuracy so far (Russo and Voigt 2016). Therefore, some authors have recommended to manually validate all sound files (Kubista and Bruckner 2017), which inevitably annihilates or at least greatly reduces the advantages of having automatic algorithms. In other cases, posterior cross-validation is completely neglected, which greatly affects the reliability of the study. According to these findings, even when aiming for an accuracy threshold of 95%, the remaining amount of data to be visually validated could be reduced by up to 75%. This represents a substantial saving in terms of workload.

Different acoustic analysis software with automatic classifiers has been released on the market in the last decades: batIdent (ecoObs, GmbH, Nürnberg, Germany), Kaleidoscope (Wildlife Acoustics, USA), Sonochiro (Sonochiro, France), Sonobat (Sonobat, USA), SCAN’R (Binary Acoustic Technology, USA) and more recently Tadarida (Bas et al. 2017), multiplying the options available to researchers to use technological advances to aid acoustic species identification. The best option for analysing the massive amounts of acoustic data generated by the latest recording devices without compromising the reliability of results, inevitably, lies in finding the right balance between automatic classification and manual cross-validation. This is especially true for threatened or rare species for which false positives will have greater conservation impact (Clement et al. 2014).

## 4.5 Recommendations for effectively combining automatic and manual classification

The approach I have used, while highly versatile, requires that researchers must: A) have good knowledge about the bat fauna of the region (avoiding novice errors that result in misidentifications or passive acceptance of the results from any classifier and acknowledging regional and habitat variation), B) work together with experts on local call libraries and manual identifications, C) be skilled in programming in R or similar software packages, thus being able to adjust machine learning algorithms to particular situations, D) take into consideration both sensitivity and positive predictive values rather than global accuracies, E) define their own sonotypes conservatively (preventing classification to taxonomic levels that are not even visually distinguishable). I also recommend to base selection criteria on the PPV as the most conservative metric of performance (Armitage and Ober 2010) since false negatives are always better than false positives.

## Conclusions

Further research should focus on isolating and analysing individual call sequences instead of pulses and analyse the whole sonogram rather than the pulses one by one (Ren et al. 2009, Damoulas et al. 2010, Kershenbaum et al. 2014). This study shows how open-source statistical tools and software can be used to develop algorithms attaining similar levels of accuracy as commercial classifiers. However, their potential for wider application should be further explored with echolocation datasets from other regions. I also demonstrated that training algorithms with recordings from free-flying bats is possible and advisable if designed to classify recordings at sonotype level. This approach is not conceived to replace the use of calls from hand-released bats, but to aid in data management and classification with massive datasets. Combined with the availability of new low-cost automatic detectors and powerful supervised machine-learning algorithms, this analysis approach opens new opportunities for long-term monitoring programs to be undertaken by researchers in megadiverse regions where echolocation libraries are still scarce. In fact, in these regions, extended acoustic bat monitoring is urgently needed, and fortunately, the technical and analytical tools are now at hand to do so.

## Acknowledgements

I would like to thank Oriol Massana, Diogo Ferreira, Marta Acácio, Fabio Farneda, Madalena Boto, Maria Mas, Gilberto Fernández and Eva Sánchez for fieldwork assistance and José Luis Camargo, Rosely Hipólito, Ary Jorge Ferreira, Luiz Quiroz, Leo and Josimar for logistical support. I thank two anonymous reviewers for helpful comments on an earlier version of the manuscript. Xavier Puig-Montserrat and Carles Flaquer also deserve special mention for their contribution to the discussion and continuous support. This work was supported by the Portuguese Foundation for Science and Technology under grants [PTDC/BIABIC/111184/2009] (CM), [SFRH/BD/80488/2011] (RR), [PD/BD/52597/2014] (ALB) and by the Foundation for Research Support of the State of Amazonas [FAPEAM 062.01173/2015] (PEDB). Additional funding was provided by a Bat Conservation International student research fellowship to ALB and RR.

## References

Aguilar, A. P. A. 2017. Morcegos insetívoros aéreos Neotropicais: identificação acústica e padrões de estruturação de assembleias. PhD Thesis. Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.

Andreassen, T., A. Surlykke, and J. Hallam. 2014. Semi-automatic long-term acoustic surveying: A case study with bats. Ecological Informatics **21**:13-24.

Appel, G., A. López-Baucells, E. Magnusson, and P. E. D. Bobrowiec. 2017. Aerial insectivorous bat activity in relation to moonlight intensity. Mammalian Biology **85**:37-46.

Armitage, D. W., and H. K. Ober. 2010. A comparison of supervised learning techniques in the classification of bat echolocation calls. Ecological Informatics **5**:465-473.

Barclay, R. M. R., J. H. Fullard, and D. S. Jacobs. 1999. Variation in the echolocation calls of the hoary bat (*Lasiurus cinereus*): influence of body size, habitat structure, and geographic location. Canadian Journal of Zoology **77**:530-534.

Barkley, Y., J. N. Oswald, J. V. Carretta, S. Rankin, A. Rudd, and M. O. Lammers. 2011. Comparison of real-time and post-cruise acoustic species identification of dolphin whistles using ROCCA (real-time odontocete call classification algorithm). Department of Commerce, California, United States of America.

Barlow, K. E., P. A. Briggs, K. A. Haysom, A. M. Hutson, N. L. Lechiara, P. A. Racey, A. L. Walsh, and S. D. Langton. 2015. Citizen science reveals trends in bat populations: The National Bat Monitoring Programme in Great Britain. Biological Conservation **182**:14-26.

Bas, Y., D. Bas, and J.-F. Julien. 2017. Tadarida: A toolbox for animal detection on acoustic recordings. Journal of Open Research Software **5**:6.

Biscardi, S., J. Orprecio, M. B. Fenton, A. Tsoar, and J. M. Ratcliffe. 2004. Data, sample sizes and statistics affect the recognition of species of bats by their echolocation calls. Acta Chiropterologica **6**:347-363.

Boyles, J. G., C. L. Sole, P. M. Cryan, and G. F. McCracken. 2013. On estimating the economic value of insectivorous bats: prospects and priorities for biologists. Pages 501-515 *in* S. C. Pedersen and A. R. A., editors. Bat Evolution, Ecology, and Conservation, New York, United States of America.

Breiman, L. 2001. Random forests. Machine Learning **45**:5-32.

Briggs, F., X. Fern, and R. Raich. 2009. Acoustic classification of bird species from syllables: an empirical study. Oregon State University, Oregon, United States of America.

Britzke, E. R., J. E. Duchamp, K. L. Murray, R. K. Swihart, and L. W. Robbins. 2011. Acoustic identification of bats in the eastern United States: A comparison of parametric and nonparametric methods. The Journal of Wildlife Management **75**:660-667.

Clement, M. J., T. J. Rodhouse, P. C. Ormsbee, J. M. Szewczak, and J. D. Nichols. 2014. Accounting for false-positive acoustic detections of bats using occupancy models. Journal of Applied Ecology **51**:1460-1467.

Cunto, G. C., and E. Bernard. 2012. Neotropical bats as indicators of environmental disturbance: What is the emerging message? Acta Chiropterologica **14**:143-151.

Damoulas, T., S. Henry, A. Farnsworth, M. Lanzone, and C. Gomes. 2010. Bayesian classification of flight calls with a novel dynamic time warping kernel. Pages 424-429 *in* Proceedings of the Ninth International Conference on Machine Learning and Applications, Washington, United States of America.

de Oliveira, A. A., and S. A. Mori. 1999. A central Amazonian terra firme forest. I. High tree species richness on poor soils. Biodiversity and Conservation **8**:1219-1244.

Dickinson, J. L., B. Zuckerberg, and D. N. Bonter. 2010. Citizen science as an ecological research tool: challenges and benefits. Annual Review of Ecology and Systematics **41**:149-172.

Farneda, F. Z., R. Rocha, A. López-Baucells, M. Groenenberg, I. Silva, J. M. Palmeirim, P. E. D. Bobrowiec, C. F. J. Meyer, and M. Pocock. 2015. Trait-related responses to habitat fragmentation in Amazonian bats. Journal of Applied Ecology **52**:1381-1391.

Ferreira, D. F., R. Rocha, A. López-Baucells, F. Z. Farneda, J. M. B. Carreiras, J. M. Palmeirim, and C. F. J. Meyer. 2017. Season-modulated responses of Neotropical bats to forest fragmentation. Ecology and Evolution **7**:4059-4071.

Gager, Y., E. Tarland, D. Lieckfeldt, M. Menage, F. Botero-Castro, S. J. Rossiter, R. H. Kraus, A. Ludwig, and D. K. Dechmann. 2016. The value of molecular vs. morphometric and acoustic information for species identification using sympatric molossid bats. PLoS ONE **11**:e0150780.

Gardner, A. L. 2007. Mammals of South America 1: marsupials, xenarthrans, shrews, and bats. University of Chicago Press, Chicago, United States of America.

Gregory, R. D., A. Van Strien, P. Vorisek, A. W. G. Meyling, D. G. Noble, R. P. Foppen, and D. W. Gibbons. 2005. Developing indicators for European birds. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences **360**:269-288.

Hill, A. P., P. Prince, E. P. Covarrubias, C. P. Doncaster, J. L. Snaddon, and A. Rogers. 2018. AudioMoth: Evaluation of a smart open acoustic device for monitoring biodiversity and the environment. Methods in Ecology and Evolution **9**:1199-1211.

Jakobsen, L., S. Brinkløv, and A. Surlykke. 2013. Intensity and directionality of bat echolocation signals. Frontiers in Physiology **4**:89.

Jennings, N., S. Parsons, and M. J. O. Pocock. 2008. Human vs. machine: identification of bat species from their echolocation calls by humans and by artificial neural networks. Canadian Journal of Zoology **86**:371-377.

Jones, G., D. S. Jacobs, T. H. Kunz, M. R. Willig, and P. A. Racey. 2009. Carpe noctem: the importance of bats as bioindicators. Endangered Species Research **8**:93-115.

Jones, G., and T. Kokurewicz. 1994. Sex and age variation in echoloction calls and flight morphology of Daubenton's bats *Myotis daubentonii*. Mammalia **58**:41-50.

Jones, G., and R. D. Ransome. 1993. Echolocation calls of bats are influenced by maternal effects and change over lifetime. Proceedings of the Royal Society - Biological Sciences **252**:125-128.

Jung, K., and E. K. V. Kalko. 2011. Adaptability and vulnerability of high flying Neotropical aerial insectivorous bats to urbanization. Diversity and distributions **17**:262-274.

Kershenbaum, A., D. T. Blumstein, M. A. Roch, C. Akcay, G. Backus, M. A. Bee, K. Bohn, Y. Cao, G. Carter, C. Casar, M. Coen, S. L. DeRuiter, L. Doyle, S. Edelman, I. C. R. Ferrer, T. M. Freeberg, E. C. Garland, M. Gustison, H. E. Harley, C. Huetz, M. Hughes, J. Hyland Bruno, A. Ilany, D. Z. Jin, M. Johnson, C. Ju, J. Karnowski, B. Lohr, M. B. Manser, B. McCowan, E. Mercado, 3rd, P. M. Narins, A. Piel, M. Rice, R. Salmi, K. Sasahara, L. Sayigh, Y. Shiu, C. Taylor, E. E. Vallejo, S. Waller, and V. Zamora-Gutierrez. 2014. Acoustic sequences in non-human animals: a tutorial review and prospectus. Biological Reviews of the Cambridge Philosophical Society **91**:13-52.

Kubista, C. E., and A. Bruckner. 2017. Within-site variability of field recordings from stationary, passively working detectors. Acta Chiropterologica **19**:189-197.

Kuhn, M. 2008. Caret package. Journal of Statistical Software **28**:1-26.

Laurance, W. F., J. L. C. Camargo, P. M. Fearnside, T. E. Lovejoy, G. B. Williamson, R. C. G. Mesquita, C. F. J. Meyer, P. E. D. Bobrowiec, and S. G. W. Laurance. 2018. An Amazonian rainforest and its fragments as a laboratory of global change. Biological Reviews of the Cambridge Philosophical Society **93**:223-247.

Laurance, W. F., J. L. C. Camargo, R. C. C. Luizao, S. G. Laurance, S. L. Pimm, E. M. Bruna, P. C. Stouffer, G. B. Williamson, J. Benitez-Malvido, H. L. Vasconcelos, K. S. Van Houtan, C. E. Zartman, S. A. Boyle, R. K. Didham, A. Andrade, and T. E. Lovejoy. 2011. The fate of Amazonian forest fragments: A 32-year investigation. Biological Conservation **144**:56–67.

Law, B., L. Gonsalves, P. Tap, T. Penman, and M. Chidel. 2015. Optimizing ultrasonic sampling effort for monitoring forest bats. Austral Ecology **40**:886–897.

Lawrence, B. D., and J. A. Simmons. 1982. Measurements of atmospheric attenuation at ultrasonic frequencies and the significance for echolocation by bats. Journal of the Acoustical Society of America **71**:585-590.

Lim, B. K., and M. D. Engstrom. 2001. Bat community structure at Iwokrama Forest, Guyana. Journal of Tropical Ecology **17**:647-665.

López-Baucells, A., R. Rocha, P. E. D. Bobrowiec, E. Bernard, J. Palmeirim, and C. Meyer. 2016. Field Guide to Amazonian Bats. INPA, Manaus, Brazil.

López-Baucells, A., R. Rocha, G. Fernández-Arellano, P. E. D. Bobrowiec, J. M. Palmeirim, and C. F. J. Meyer. 2014. Echolocation of the big red bat *Lasiurus egregius* (Chiroptera: Vespertilionidae) and first record from the Central Brazilian Amazon. Studies on Neotropical Fauna and Environment **49**:18-25.

López-Baucells, A., R. Rocha, V. C. Tavares, L. Mora, P. E. D. Bobrowiec, S. E. Silva, and C. Meyer. 2018. Molecular, morphological and acoustic identification of *Eumops maurus* and *E. hansae* (Chiroptera: Molossidae) with new reports from Central Amazonia. Tropical Zoology **31**:1-20.

López-Baucells, A., L. Torrent, R. Rocha, A. C. Pavan, P. E. D. Bobrowiec, and C. F. J. Meyer. 2017. Geographical variation in the high-duty cycle echolocation of the cryptic common mustached bat *Pteronotus* cf. *rubiginosus* (Mormoopidae). Bioacoustics Journal **27**:341-357.

MacSwiney, G. C., F. M. Clarke, and P. A. Racey. 2008. What you see is not what you get: the role of ultrasonic detectors in increasing inventory completeness in Neotropical bat assemblages. Journal of Applied Ecology **45**:1364-1371.

Madhukumar Menon, A., M. João Ramos Pereira, and L. Moura de Souza Aguiar. 2018. Are automated acoustic identification software reliable for bat surveys in the Neotropical region? PeerJ:(in press).

Millon, L., J.-F. Julien, R. Julliard, and C. Kerbiriou. 2015. Bat activity in intensively farmed landscapes with wind turbines and offset measures. Ecological Engineering **75**:250-257.

Monadjem, A., J. T. Shapiro, F. Mtsetfwa, A. E. Reside, and R. A. McCleery. 2017. Acoustic call library and detection distances for bats of swaziland. Acta Chiropterologica **19**:175-187.

Mukherjee, G., and S. S. Manna. 2006. Weighted scale-free networks in Euclidean space using local selection rule. Physical Review E **74**:036111.

Murray, K. L., E. R. Britzke, B. M. Hadley, and L. W. Robbins. 1999. Surveying bat communities: a comparison between mist nets and the Anabat II bat detector system. Acta Chiropterologica **1**:105-112.

O'Farrell, M. J., C. Corben, and W. L. Gannon. 2000. Geographic variation in the echolocation calls of the hoary bat (*Lasiurus cinereus*). Acta Chiropterologica **2**:185-196.

O'Farrell, M. J., B. W. Miller, and W. L. Gannon. 1999. Qualitative identification of free-flying bats using the Anabat detector. Journal of Mammalogy **80**:11-23.

Olden, J. D., J. J. Lawler, and N. L. Poff. 2008. Machine learning methods without tears: a primer for ecologists. The Quarterly review of biology **83**:171-193.

Pedro, A. R.-S., and J. A. Simonetti. 2014. Variation in search-phase calls of *Lasiurus varius* (Chiroptera: Vespertilionidae) in response to different foraging habitats. Journal of Mammalogy **95**:1004-1010.

Penone, C., I. Le Viol, V. Pellissier, J.-F. Julien, Y. Bas, and C. Kerbiriou. 2013. Use of large-scale acoustic monitoring to assess anthropogenic pressures on orthoptera communities. Conservation Biology **27**:979-987.

Pio, D. V., F. M. Clarke, I. Mackie, and P. A. Racey. 2010. Echolocation calls of the bats of Trinidad, West Indies: is guild membership reflected in echolocation signal design? Acta Chiropterologica **12**:217-229.

Puechmaille, S. J., I. M. Borissov, S. Zsebok, B. Allegrini, M. Hizem, S. Kuenzel, M. Schuchmann, E. C. Teeling, and B. M. Siemers. 2014. Female mate choice can drive the evolution of high frequency echolocation in bats: A case study with *Rhinolophus mehelyi*. PLoS ONE **9**:e103452.

Puig-Montserrat, X., I. Torre, A. López-Baucells, E. Guerrieri, M. M. Monti, R. Rafols-Garcia, X. Ferrer, D. Gisbert, and C. Flaquer. 2015. Pest control service provided by bats in Mediterranean rice paddies: linking agroecosystems structure to ecological functions. Mammalian Biology **80**:237-245.

Redgwell, R. D., J. M. Szewczak, G. Jones, and S. Parsons. 2009. Classification of echolocation calls from 14 species of bat by support vector machines and ensembles of neural networks. Algorithms **2**:907-924.

Ren, Y., M. T. Johnson, P. J. Clemins, M. Darre, S. S. Glaeser, T. S. Osiejuk, and E. Out-Nyarko. 2009. A framework for bioacoustic vocalization analysis using hidden markov models. Algorithms **2**:1410-1428.

Rocha, R., A. López-Baucells, F. Z. Farneda, M. Groenenberg, P. E. D. Bobrowiec, M. Cabeza, J. M. Palmeirim, and C. F. J. Meyer. 2017a. Consequences of a large-scale fragmentation experiment for Neotropical bats: disentangling the relative importance of local and landscape-scale effects. Landscape Ecology **32**:31–45.

Rocha, R., O. Ovaskainen, A. López-Baucells, F. Z. Farneda, D. F. Ferreira, P. E. D. Bobrowiec, M. Cabeza, J. M. Palmeirim, and C. F. J. Meyer. 2017b. Design matters: An evaluation of the impact of small man-made forest clearings on tropical bats using a before-after-control-impact design. Forest Ecology and Management **401**:8-16.

Russo, D., and G. Jones. 2002. Identification of twenty-two bat species (Mammalia: Chiroptera) from Italy by analysis of time-expanded recordings of echolocation calls. Journal of Zoology **258**:91–103.

Russo, D., and C. C. Voigt. 2016. The use of automated identification of bat echolocation calls in acoustic monitoring: A cautionary note for a sound analysis. Ecological Indicators **66**:598-602.

SCAN'R. 2009. SCAN’R Snapshot Characterization and Analysis Software manual. Binary Acoustic Technology, Arizona, United States of America.

Sewell, D., G. Guillera-Arroita, R. A. Griffiths, and T. J. Beebee. 2012. When is a species declining? Optimizing survey effort to detect population changes in reptiles. PLoS ONE **7**:e43387.

Silva, C. R., and E. Bernard. 2017. Bioacoustics as an important complementary tool in bat inventories in the Caatinga drylands of Brazil. Acta Chiropterologica **19**:409-418.

Szewczak, J. 2000. A tethered zip-line arrangement for reliably collecting bat echolocation reference calls. Bat Research News **41**:142.

Thomas, D. W., G. P. Bell, and M. B. Fenton. 1987. Variation in echolocation call frequencies recorded from North American vespertilionid bats: A cautionary note. Journal of Mammalogy **68**:842-847.

Torrent, L., A. López-Baucells, R. Rocha, P. E. D. Bobrowiec, and C. F. J. Meyer. 2018. The importance of lakes for bat conservation in Amazonian rainforests: an assessment using autonomous recorders. Remote Sensing in Ecology and Conservation:(in press).

Vassilios, S., Z.-G. Veronica, J. K. E., and G. Mark. 2017. Bat echolocation call identification for biodiversity monitoring: a probabilistic approach. Journal of the Royal Statistical Society: Series C (Applied Statistics) **67**:165-183.

Viera, A. J., and J. M. Garrett. 2005. Understanding Interobserver Agreement: The Kappa Statistic. Research Series **37**:360.

Vihinen, M. 2012. How to evaluate performance of prediction methods? Measures and their interpretation in variation effect analysis. BMC genomics **13**:S2.

Walters, C. L., A. Collen, T. Lucas, K. Mroz, C. A. Sayer, and K. E. Jones. 2013. Challenges of using bioacoustics to globally monitor bats. Pages 479-499 *in* S. C. Pedersen and R. A. Adams, editors. Bat Evolution, Ecology, and Conservation. Springer, New York, United States of America.

Walters, C. L., R. Freeman, A. Collen, C. Dietz, M. B. Fenton, G. Jones, M. K. Obrist, S. J. Puechmaille, T. Sattler, B. M. Siemers, S. Parsons, and K. E. Jones. 2012. A continental-scale tool for acoustic identification of European bats. Journal of Applied Ecology **49**:1064-1074.

Whytock, R. C., J. Christie, and K. Jones. 2017. Solo: an open source, customizable and inexpensive audio recorder for bioacoustic research. Methods in Ecology and Evolution **8**:308-312.

Wordley, C. F. R., E. K. Foui, D. Mudappa, M. Sankaran, and J. D. Altringham. 2014. Acoustic identification of bats in the Southern Western Ghats, India. Acta Chiropterologica **16**:213-222.

Zamora-Gutiérrez, V., C. López-Gonzalez, M. C. MacSwiney González, B. Fenton, G. Jones, E. K. V. Kalko, S. J. Puechmaille, V. Stathopoulos, K. E. Jones, and R. Freckleton. 2016. Acoustic identification of Mexican bats based on taxonomic and ecological constraints on call design. Methods in Ecology and Evolution **7**:1082-1091.

## Supplementary material

Table 3.S1. Species /sonotypes and their frequency ranges. SF – start frequency; EF – end frequency; FME – frequency of maximum energy; lp – lower pulse; mp – middle pulse; hp – high pulse (López-Baucells et al. 2016).

| Species/sonotype | Species included | Range of FME (kHz) |
|---|---|---|
| **Emballonuridae** | | |
| *Rhynchonycteris naso* | *Rhynchonycteris naso* | 98 – 105 |
| *Saccopteryx leptura* | *Saccopteryx leptura* | lp 46 – 49; hp 43 – 46 |
| *Saccopteryx bilineata* | *Saccopteryx bilineata* | lp 41 – 43; hp 44 – 46 |
| *Centronycteris maximiliani* | *Centronycteris centralis / maximiliani* | 39 – 41 |
| *Peropteryx macrotis* | *Peropteryx macrotis* | 37 – 39 |
| *Peropteryx kappleri* | *Peropteryx kappleri* | 29 – 33 |
| *Cormura brevirostris* | *Cormura brevirostris* | lp 23 – 25; mp 26 – 28; hp 29 – 33 |
| Emballonuridae 1 | *Saccopteryx gymnura / canescens* | 54 –55 |
| **Furipteridae** | | |
| *Furipterus horrens* | *Furipterus horrens* | 117 – 122 |
| **Molossidae** | | |
| Molossidae 1 | *Molossus molossus* | lp 33 – 35; mp 35 – 40; hp 40 – 45 |
| Molossidae 2 | *Molossus sinaloae / currentium / rufus* | lp 25 – 30; mp 30 – 35; hp 35 – 40 |
| Molossidae 3 | *Cynomops planirostris / paranus,*<br>*Cynomops greenhalli /abrasus,*<br>*Eumops auripendulus / glaucinus / dabbenei / hansae / maurus,*<br>*Nyctinomops laticaudatus,*<br>*Tadarida brasiliensis* | lp EF: 17 – 21; hp EF: 21 – 24 |
| *Molossops* 1 | *Molossops neglectus / temminckii* | lp EF: 44 – 54; hp EF: 46 – 55 |
| *Promops* 1 | *Promops centralis / nasutus* | lp EF: 28 – 34; hp EF: 30 – 37 |
| **Mormoopidae** | | |
| *Pteronotus personatus* | *Pteronotus personatus* | SF: 60 – 69 |
| *Pteronotus alitonus* | *Pteronotus alitonus* | 59 – 61 |
| *Pteronotus gymnonotus* | *Pteronotus gymnonotus* | 45 – 60 |
| *Pteronotus rubiginosus* | *Pteronotus rubiginosus* | 54 – 56 |
| **Vespertilionidae** | | |
| Vespertilionidae 1 | *Eptesicus brasiliensis / chiriquinus*<br>*Eptesicus furinalis / Lasiurus* sp. */ Rhogeessa io* | EF: 25 – 45 |
| *Myotis riparius* | *Myotis riparius / Thyroptera tricolor* | EF: 55 – 65 |
| *Myotis nigricans* | *Myotis nigricans* | EF: 45 – 50 |

Table 3.S2. SCAN’R settings used to automatically extract the call measurements from all bat recordings.

| SCAN’R Settings: | |
|---|---|
| Trigger level: | 30 dB |
| Minimum Frequency Cutoff: | 15 kHz |
| Maximum Frequency Cutoff: | 220 kHz |
| Standard view: | 50 ms |
| Peak Power: | 21 dB |
| Minimum duration: | 0.5ms |
| Fit restriction: | Low |
| Pop Filter: | Off |

Table 3.S3. Sample sizes (number of pulses) for the training datasets used in comparing classification performance based on calls from hand-released (HR) vs. free-flying bats (FF).

| Species/sonotype | HR | FF |
|---|---|---|
| *Centronycteris maximiliani* | 2012 | 2000 |
| *Cormura brevirostris* | 16 | 2000 |
| Emballonuridae 1 | 5 | 2000 |
| *Furipterus horrens* | 19 | 1000 |
| Molossidae 1 | 79 | 2000 |
| Molossidae 2 | 316 | 2000 |
| Molossidae 3 | 263 | 2000 |
| *Myotis nigricans* | 309 | 2000 |
| *Myotis riparius* | 737 | 2000 |
| *Peropteryx macrotis* | 361 | 2000 |
| *Pteronotus alitonus* | 624 | 2000 |
| *Pteronotus rubiginosus* | 1736 | 2000 |
| *Pteronotus gymnonotus* | 74 | 2000 |
| *Pteronotus personatus* | 19 | 2000 |
| *Rhynchonycteris naso* | 660 | 12 |
| *Saccopteryx bilineata* | 63 | 2000 |
| *Saccopteryx leptura* | 794 | 2000 |
| Vespertilionidae 1 | 494 | 2000 |

Table 3.S4. Confusion matrix showing the absolute number of real versus predicted identifications based on the random forest model trained with free flying bat calls. Sensitivity (proportion of calls correctly identified as one species/sonotype out of the real number of calls of this category) and PPV (proportion of calls correctly identified as one species/sonotype out of the total number of calls identified as such) values are also given. CB: *Cormura brevirostris*; CM: *Centronycteris maximiliani*; E1: *Emballonuridae* 1; V1: *Vespertilionidae* 1; FH: *Furipterus horrens*; M1: *Molossidae* 1; M2: *Molossidae* 2; M3: *Molossidae* 3; MN: *Myotis nigricans*; MR: *Myotis riparius*; P: *Promops* sp.; PA: *Pteronotus alitonus*; PR: *Pteronotus rubiginosus*; PG: *Pteronotus gymnonotus*; PK: *Peropteryx kappleri*; PM: *Peropteryx macrotis*; PP: *Pteronotus personatus*; RN: *Rhynchonycteris naso*; SB: *Saccopteryx bilineata*; SL: *Saccopteryx leptura*.

| | | REAL IDENTIFICATIONS | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CB | CM | E1 | V1 | FH | M1 | M2 | M3 | MN | MR | P | PA | PR | PG | PK | PM | PP | RN | SB | SL | Total | PPV (%) |
| PREDICTED IDENTIFICATIONS | CB | 35239 | 1191 | 12 | 186 | 1 | 63 | 346 | 17 | 146 | 233 | 148 | 247 | 286 | 6 | 2211 | 43 | 3 | 0 | 616 | 258 | 41252 | 85.4 |
| | CM | 344 | 630409 | 2 | 64 | 0 | 48 | 59 | 5 | 47 | 123 | 33 | 111 | 112 | 0 | 127 | 140 | 2 | 0 | 27303 | 237 | 659166 | 95.6 |
| | E1 | 27 | 243 | 9039 | 34 | 0 | 7 | 32 | 9 | 2343 | 118 | 0 | 9117 | 110 | 14 | 69 | 19 | 6 | 0 | 340 | 22234 | 43761 | 20.7 |
| | V1 | 72 | 479 | 25 | 118020 | 0 | 88 | 331 | 38 | 1356 | 306 | 32 | 204 | 351 | 0 | 565 | 59 | 27 | 0 | 352 | 129 | 122434 | 96.4 |
| | FH | 171 | 1803 | 22 | 219 | 38 | 43 | 220 | 71 | 391 | 1304 | 6 | 1005 | 848 | 27 | 720 | 133 | 32 | 0 | 1879 | 1423 | 10355 | 0 |
| | M1 | 168 | 2354 | 14 | 766 | 0 | 7438 | 3918 | 22 | 829 | 255 | 33 | 129 | 148 | 4 | 2378 | 669 | 0 | 0 | 1018 | 197 | 20340 | 36.6 |
| | M2 | 503 | 1791 | 21 | 1170 | 0 | 979 | 13350 | 289 | 344 | 824 | 67 | 1072 | 793 | 5 | 6551 | 98 | 11 | 0 | 1697 | 784 | 30349 | 44 |
| | M3 | 209 | 2031 | 23 | 493 | 1 | 85 | 1845 | 3436 | 264 | 513 | 35 | 591 | 649 | 2 | 1531 | 104 | 10 | 0 | 1611 | 629 | 14062 | 24.4 |
| | MN | 31 | 524 | 55 | 722 | 1 | 65 | 32 | 6 | 422859 | 12380 | 53 | 888 | 1146 | 332 | 135 | 23 | 22 | 0 | 589 | 1042 | 440905 | 95.9 |
| | MR | 48 | 599 | 2 | 54 | 0 | 0 | 18 | 6 | 6329 | 668632 | 1 | 284 | 6389 | 164 | 39 | 13 | 290 | 16 | 323 | 55 | 683262 | 97.9 |
| | P | 1055 | 8444 | 24 | 429 | 0 | 67 | 256 | 40 | 2417 | 563 | 2223 | 474 | 495 | 2 | 378 | 50 | 2 | 0 | 3690 | 633 | 21242 | 10.5 |
| | PA | 1 | 85 | 175 | 3 | 0 | 0 | 4 | 3 | 1231 | 971 | 0 | 487287 | 2388 | 49 | 26 | 2 | 14 | 0 | 36 | 770 | 493045 | 98.8 |
| | PR | 2 | 151 | 0 | 2 | 0 | 1 | 3 | 2 | 85 | 3737 | 0 | 1175 | 598176 | 340 | 6 | 0 | 283 | 0 | 16 | 13 | 603992 | 99 |
| | PG | 44 | 442 | 29 | 78 | 0 | 12 | 31 | 7 | 10574 | 8301 | 4 | 1834 | 6352 | 6036 | 115 | 16 | 163 | 2 | 337 | 410 | 34787 | 17.4 |
| | PK | 1009 | 445 | 14 | 1305 | 0 | 522 | 1633 | 81 | 182 | 178 | 26 | 155 | 416 | 3 | 132174 | 67 | 19 | 0 | 536 | 190 | 138955 | 95.1 |
| | PM | 172 | 3447 | 24 | 814 | 0 | 913 | 357 | 25 | 138 | 219 | 8 | 151 | 123 | 1 | 570 | 11653 | 71 | 0 | 590 | 225 | 19501 | 59.8 |
| | PP | 22 | 195 | 3 | 34 | 0 | 6 | 19 | 3 | 249 | 35144 | 1 | 111 | 2328 | 29 | 83 | 30 | 3920 | 0 | 179 | 86 | 42442 | 9.2 |
| | RN | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 446 | 0 | 1 | 4 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 459 | 0 |
| | SB | 169 | 29250 | 25 | 56 | 0 | 72 | 97 | 36 | 328 | 124 | 36 | 75 | 104 | 4 | 219 | 50 | 9 | 0 | 463301 | 2906 | 496861 | 93.2 |
| | SL | 111 | 340 | 559 | 41 | 0 | 27 | 32 | 8 | 4550 | 149 | 8 | 604 | 110 | 19 | 110 | 7 | 3 | 0 | 5545 | 229605 | 241828 | 94.9 |
| | Total | 39397 | 684227 | 10068 | 124490 | 41 | 10436 | 22583 | 4104 | 454664 | 734520 | 2714 | 505515 | 621328 | 7037 | 148007 | 13176 | 4887 | 18 | 509960 | 261826 | | |
| | Sensitivity (%). | 89.4 | 92.1 | 89.8 | 94.8 | 92.7 | 71.3 | 59.1 | 83.7 | 93 | 91 | 81.9 | 96.4 | 96.3 | 86.2 | 89.3 | 88.4 | 80.2 | 0 | 90.6 | 87.7 | | |

|  |  | Real identifications |  |  |
|---|---|---|---|---|
|  |  | Sp 1 | Sp 2 |  |
| Predicted identifications | Sp 1 | True positive (TP) | False positive (FP) | Positive predictive value (PPV):<br>TP/(TP+FP) |
|  | Sp 2 | False Negative (FN) | True Negative (TN) | Negative Predictive Value (NPV):<br>TN/(FN+TN) |
|  |  | Sensitivity:<br>TP/(TP+FN) | Specificity:<br>TN/(FP+TN) | Balanced accuracy:<br>(PPV + NPV)/2 |

Figure 3.S1. Diagram summarising the meaning and interpretation of the different metrics commonly used to evaluate automatic classifier performance in bioacoustic studies (Vihinen 2012). Shadowed cells represent correct model decisions. Sensitivity is the proportion of calls correctly identified as one species/sonotype out of the real number of calls of this group). Positive predictive value is the proportion of calls correctly identified as one species/sonotype out of the total number of calls identified as such). Specificity is the proportion of calls correctly identified as another species/sonotype out of the real number of calls that are not this category); Negative predictive value or NPV is the proportion of calls correctly identified as another species/sonotype out of the total number of calls identified as such) and Balanced accuracy (average of the positive predictive value and the negative predictive value).

# Chapter 4

## Optimising bat bioacoustic surveys in human-modified tropical landscapes

**López-Baucells, A.**, Yoh, N., Rocha, R., Bobrowiec, P.E.D.,
Palmeirim, J.M., Meyer, C.F.J.

Top: *Natalus* sp. Bottom: *Saccopteryx leptura*

# CHAPTER 4

## Optimising bat bioacoustic surveys in human-modified tropical landscapes

### Abstract

During the last decades, the use of bioacoustics as a non-invasive and cost-effective sampling method has greatly increased worldwide. For bats, acoustic surveys have long been known to complement traditional mist-netting, however, appropriate protocol guidelines are still lacking for tropical regions. Establishing a minimum sampling effort to detect ecological changes in bat assemblages (e.g. activity, composition, richness) is crucial in view of workload and project cost constraints, and because detecting such changes must be reliable enough to ensure effective conservation actions. Using one of the most comprehensive Neotropical bat acoustic datasets, collected in the Amazon, I assessed the minimum survey effort required to accurately assess (1) the completeness of assemblage inventories and (2) the effects of forest fragmentation on aerial insectivorous bats. I evaluated a combination of 20 different temporal sampling schemes, which differed regarding: (i) number of hours per night, (ii) number of nights per site and (iii) sampling only during the wet or dry season, or both. This was assessed under two different landscape scenarios: (a) in forest fragments embedded in a matrix of secondary forest, and (b) in the same forest fragments, but after they had been re-isolated through clearing of the secondary forest. This study demonstrated that the sampling effort required varied considerably depending on the research aim and the landscape scenario evaluated. In order to achieve 90% inventory completeness, it ranged from ~10 to 80 nights on average during post- and pre- re-isolation, respectively. Recording for more than four hours per night did not result in substantial additional reductions in sampling effort. Regarding the effects of forest fragmentation, except for assemblage composition, bat responses in terms of richness, diversity, and activity were similar across all sampling schemes after the re-isolation. However, before the re-isolation a minimum number of sampling hours per night after dusk (four to six) and number of nights per site (three to five) were crucial to detect significant effects that could otherwise go unnoticed. Based on my results, I propose guidelines that will aid to optimize sampling protocols in bat acoustic surveys in the Neotropics, especially in the context of the funding crisis that science is experiencing.

**Keywords:** Acoustic sampling protocol, Amazon, Chiroptera, Forest fragmentation, Neotropics.

## Introduction

Recent years have seen a rapid increase globally in the use of bioacoustics as a non-invasive and cost-effective method to answer ecological questions, address biodiversity conservation issues or improve habitat management (Froidevaux et al. 2014). This is especially true for bats (e.g.  Kunz et al. 2007, Fischer et al. 2009, Jones et al. 2009, Rodhouse et al. 2011, Frick 2013) due to their widely recognized role as important bioindicators and agents of pest control (Jones et al. 2009, Puig-Montserrat et al. 2015), and their inherent elusiveness, but also for other taxa such as birds (Figueira et al. 2015), amphibians (Alvarez-Berríos et al. 2016) or insects (Jeliazkov et al. 2016). Bioacoustic sampling has great potential to overcome limitations of conventional biodiversity sampling approaches, particularly in the tropics. Megadiverse regions such as the Amazon basin, while hotspots for biodiversity, lag far behind other regions in terms of research and conservation programs (Wilson et al. 2016), and information on more elusive taxonomic groups remains scarce due to limitations inherent to commonly employed sampling approaches.

For bats, acoustic surveys can effectively complement traditional mist-netting (Flaquer et al. 2007, Walters et al. 2013), vastly increasing inventory completeness in bat assemblage studies, especially in the species-rich tropics (MacSwiney et al. 2008, Furey et al. 2009, Meyer et al. 2011, Silva and Bernard 2017). Acoustic surveys can cover large temporal and spatial scales, even in habitats where particular environmental conditions such as vegetation clutter or strong wind make mist-netting inefficient or unfeasible (Murray et al. 1999, MacSwiney et al. 2008, Wordley et al. 2018).

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

Amongst the limitations of acoustic surveys, variability in bat activity levels and imperfect detection are generally difficult to overcome (Duchamp et al. 2006). Bat activity can substantially vary in space and time and is moulded by ecological patterns such as reproductive cycle, proximity of roosts, seasonal migration, swarming activity, habitat clutter or insect abundance (Murray and Kurta 2004, Dzal et al. 2009, Piksa et al. 2011, Adams et al. 2015). A number of studies have already addressed these problems and provided recommendations as to how best to cope with them (Hayes 2000, Duchamp et al. 2006, Yates and Muzika 2006, Gorresen et al. 2008, Fischer et al. 2009). However, whereas sampling design optimization has been an active area of research with regard to more conventional techniques such as mist-netting (e.g. Weller and Lee 2007, Marques et al. 2013, Trevelin et al. 2017), similar comprehensive assessments for bioacoustics are limited (Rodhouse et al. 2011, Froidevaux et al. 2014, Pieretti et al. 2015).

Choosing an effective and statistically robust acoustic sampling protocol is a fundamental issue that researchers must address at the early stages of any bat monitoring or survey project due to time/cost constraints (Rodhouse et al. 2011, Law et al. 2015, Meyer 2015). Despite the ease with which vast acoustic datasets can nowadays be obtained, limited resources force researchers to optimize sampling, taking into consideration the trade-off between temporal vs spatial replicates or the minimum number of seasons sampled, and years or sites required to answer specific ecological questions. Since the accuracy of results depends on how well the real activity patterns of the animals are captured in the data collected (Froidevaux et al. 2014), establishing *a priori* a minimum sampling effort for acoustic surveys to reliably detect ecological changes and disturbance impacts on ecosystems is crucial. Unfortunately, this has rarely been assessed for bats and the few published studies have only focused on optimizing species inventory and rare species detection (Froidevaux et al. 2014). Different acoustic sampling protocols to estimate bat species richness and activity have been evaluated in temperate areas (Skalak et al. 2012, Froidevaux et al. 2014). Some of these focussed on the effects of the position, orientation and number of detectors (Weller and Zabel 2002), while others examined the representativeness of sampling during the dusk peak of bat activity (Froidevaux et al. 2014). Similar assessments are lacking for tropical regions, where much less bioacoustic research has been undertaken, yet the use of passive detectors is rapidly expanding (Silva and Bernard 2017, Arias-Aguilar et al. 2018).

The overall aim of this study was to contribute to the knowledge required to optimize acoustic bat sampling in tropical regions, capitalizing on one of the most comprehensive Neotropical bat acoustic datasets, collected during three years of sampling in the Central Amazon using automatic detectors. The two main goals were to assess the minimum survey effort required to accurately assess 1) the completeness of assemblage inventories and 2) the effects of forest fragmentation on aerial insectivorous bats (in terms of species richness, activity levels, species diversity and assemblage composition). I expected studies merely focused on compiling species lists (i.e. inventories) to generally require less sampling effort than those aimed at assessing responses of bats to anthropogenic habitat loss or modification, mostly because in the latter case tenuous patterns may exist that can easily go unnoticed if sample size was not large enough or the study not appropriately designed. To address the aforementioned objectives, I estimated the minimum survey effort required for a combination of 20 different temporal sampling schemes, considering different time windows or data subsets (i.e., varying (i) the number of hours of sampling per night, (ii) the number of consecutive nights of sampling per site, (iii) sampling only during the wet or dry season, or both). Moreover, the performance of all sampling schemes was assessed under two different landscape scenarios: (1) in forest fragments embedded in a matrix of old secondary forest (low patch-matrix contrast), and (2) in the same forest fragments following their recent re-isolation through clearing of the secondary forest around them (high patch-matrix contrast). I hypothesized that optimised inventories (with 90% of the species present detected) would include only recordings during the first nocturnal activity peak (18:00 – 22:00) for a reasonable number of nights (~30 nights) (Froidevaux et al. 2014). Furthermore, due to the marked differences in bat activity between seasons (Skalak et al. 2012, Ferreira et al. 2017) I expected sampling during both dry and wet season to be crucial, probably more important than repeating the acoustic sampling in the same sites during the same season. Since detecting bat responses to forest fragmentation might be more challenging than simply inventorying the bat fauna of a particular location, I expected that the same sampling schemes as those identified for inventories would be best, but that greater sampling effort in terms of number of nights would be required to obtain robust results. Finally, I predicted sampling effort required to be strongly dependent on matrix contrast. For fragments embedded in secondary forest I expected the required sampling effort to be greater due to the greater homogeneity of the landscape, compared to sampling within fragments surrounded by cleared areas.

## Material and Methods

### Study area and site selection

The study was conducted at the Biological Dynamics of Forest Fragments Project (BDFFP), located ~80 km north of Manaus (2º30'S, 60ºW) (Laurance et al. 2018). The forest in the area was experimentally fragmented during the 1980s, which resulted in a mosaic landscape containing about 1000 km$^2$ of continuous primary terra firme forest and a series of different-sized forest fragments. Canopy height in continuous forest is about 30-37 m, and annual precipitation ranges from 1900 to 3500 mm, with a dry season between July and October and a wet season from November to June (Ferreira et al. 2017). Forest fragments were initially surrounded by cattle pasture, which due to abandonment regenerated into tall secondary forest (Farneda et al. 2018, Rocha et al. 2018). However, fragment isolation was experimentally preserved by periodically clearing a 100m-wide strip around each of the fragments (cf. Rocha et al. 2017). The most recent fragment re-isolation occurred between November 2013 and March 2014, and the acoustic data used herein were collected immediately before (from June 2011 to September 2013) and after this (from April to October 2014). Prior to fragment re-isolation, I sampled bats in the interior of continuous primary forest (N=9 sites) and forest fragments (N=8) as well as in the adjacent secondary forest matrix (N=8). Following fragment re-isolation, the same sites were sampled, with detectors during both study periods being placed in the exact same location. Due to logistical constraints, after the re-isolation, only six of the control sites were sampled.

### Acoustic sampling

At each sampling site, one SM2Bat detector with an omnidirectional ultrasonic SMX-US microphone (Wildlife Acoustics, Inc., USA) was placed ca. 1.5 m above the ground. Detectors recorded from sunset to sunrise, at 384kHz sampling rate in full spectrum with 16-bit resolution. The high pass filter was set at 12kHz (fs/32), with a trigger level of 18SNR. At each site, I recorded bats during five (2012-2013) and three (2014) consecutive nights, always twice during the dry and wet seasons. I standardized the sampling unit using bat passes, which were defined as 5 sec long sequences with a minimum of two distinguishable pulses of a certain species (Millon et al. 2015, Appel et al. 2017, Torrent et al. 2018).

## Echolocation call analysis

All bat passes were manually analysed using Kaleidoscope v.4.0.4 software (Wildlife Acoustics Inc., USA). Classification was done manually to species/sonotype level following the key in López-Baucells et al. (2016). A sonotype was defined as a category that grouped species with similar calls when it was not possible to clearly assign a call to a particular species (Table 4.S1). Species/sonotype identification was based on several acoustic parameters such as shape of the calls, frequency of maximum energy (FME), start (StFreq), end (EFreq), maximum (MaxFreq) and minimum (MinFreq) frequency, and call duration (D). Additionally, some calls which were difficult to identify were compared to those from a local reference call library compiled by the authors during the study period (López-Baucells, unpublished). All calls recorded immediately after release were discarded, as were overloaded calls, those too faint (for which it was impossible to distinguish the shape from the background noise), social or stress calls, calls emitted in passive hunting mode and feeding buzzes. This led to a total of 1,088,940 analysed recordings, and ~650,000 identified bat passes, with a total of 21 species/sonotypes (Table 4.S1): 598,502 files to species level (93.6%), and 40,532 to sonotype level (6.3%).

## Statistical analysis

### PROTOCOL OPTIMIZATION FOR SPECIES RICHNESS EVALUATION:

In order to derive minimum sampling effort to determine species richness (inventory completeness), I compared the results of species accumulation curves (SAC), considering sampling nights as the unit of sampling effort (Moreno and Halffter 2000, Froidevaux et al. 2014, Law et al. 2015) and splitting the full dataset to reflect a set of different temporal sampling schemes, varying: (i) the number of hours of sampling per night, (ii) the number of consecutive full nights of sampling per site (in that case I subset the dataset by sequentially increasing the number of recorded nights per site; up to five during the 2012-13 period and up to three for 2014), (iii) sampling only during the wet or dry season, or both (also using full nights). Different time windows were chosen from 18:00 to 6:00 by gradually increasing the number of recording hours (e.g. 18:00-18:59, 18:00-19:59, 18:00-20:59…, 18:00-06:00) (Banks-Leite et al. 2012). SACs were computed (with randomization of the samples; 100 permutations) for the datasets collected prior to and after fragment re-isolation using the *specaccum* function from the R package “vegan” (Oksanen et al. 2015). Because achieving 100% inventory completeness usually requires a prohibitively large sampling effort (Moreno and Halffter 2000), I compared the number of sampling nights per site required to reach 90% of the estimated total number of species in the survey area, calculated with the jackknife estimator (jack1) from the *specaccum* function in the R package *vegan* (e.g. Skalak et al. 2012, Froidevaux et al. 2014). The Jackknife method uses subsets by successively deleting x individuals from the main dataset and it is widely used in ecological studies (Heltshe and Forrester 1983, Chao and Chiu 2014).

### PROTOCOL OPTIMIZATION TO ASSESS THE EFFECTS OF HABITAT MODIFICATION

I modelled the effect of habitat type on richness, bat activity (used as a surrogate of abundance, as suggested by Lintott et al. 2014), diversity and assemblage composition for each of the temporal sampling schemes previously described. Because sampling effort per sampling unit (night) was equal, in all analyses (species accumulation curves and statistical models) richness was considered as the number of species/sonotypes present at a sampling site. Bat activity was defined as the number of bat passes per night. Species diversity was assessed with the Simpson index (calculated using the R package *vegan*), which combines species richness and evenness (Borcard et al. 2011). Assemblage comparisons were performed using the Bray-Curtis index as an abundance-based measure of dissimilarity (Borcard et al. 2011). In order to use this index to characterize each sampling site, I first calculated a baseline assemblage composition by pooling the data from all continuous forest interiors. Then I quantified similarity in species composition between each site and this reference assemblage using the function *vegdist* (Bray-Curtis distance metric) from the R package “vegan” (Jost et al. 2011). To model the effect of habitat type on the aforementioned response variables, I performed Monte Carlo Markov Chain generalized linear mixed models using the R package “MCMCglmm”, specifying a Poisson (for bat activity) or Gaussian (for richness, evenness and assemblage composition) error distribution. When the response variable showed signs of overdispersion or zero-inflation, the priors were set to be weakly informative in order to deal with over-dispersion (inverse gamma parameters: nu=0.002 and V=1) (Kryvokhyzha et al. 2016, Hadfield 2017). Acoustic data, in particular bat activity data, are usually overdispersed and do not adhere to normality assumptions, resulting in greater variability than expected in the response variable (variance > mean, Richards 2008) and therefore traditional statistical methods are not recommended (Adams et al. 2015). Habitat type was specified as a categorical fixed effect (categories: continuous forest, forest fragments and secondary forest/clearings), and sampling site as a random effect (Adams et al. 2015). Models were run with 50,000 iterations, and the first 10,000 were discarded from the results. All statistical analyses were carried out using R software, version 3.2.4. (R Core Team 2017), and all plots built with the “ggplot2” package (Wickham 2009).

## Results

### PROTOCOL OPTIMIZATION FOR SPECIES RICHNESS EVALUATION

The SAC computed with the whole dataset estimated through the jack1 estimator a total of 21 different species/sonotypes both before and after the re-isolation periods. When assessing different temporal sampling schemes, the SACs varied considerably prior to and after fragment re-isolation (Fig. 4.1A). The rate of decrease in the estimated sampling effort required with increasing number of hours of recording varied between both scenarios, being much more gradual before re-isolation compared to the very marked decline after re-isolation (Fig. 4.1B). When recordings from only the first three hours after sunset were considered, >150 nights before the re-isolation and >50 nights after the re-isolation were needed to achieve 90% inventory completeness (Fig. 4.1B), while with datasets with more than four hours required sampling effort remained almost constant (~80 and 10 nights respectively) (Fig. 4.1B). In terms of the number of consecutive nights per site, under both landscape scenarios, sampling only for one night was sufficient to achieve 90% inventory completeness. In fact, recording more consecutive nights per site did not significantly improve the rate of new species detections, but increased the workload, thus making these schemes generally less efficient (Table 4.1). In the seasonal analysis, before fragment re-isolation almost no differences were found between dry and wet season datasets, while after the re-isolation, the estimated sampling effort for the wet season was almost double that required during the dry season (Table 4.1). When using the data from both seasons together, the minimum estimated sampling effort was reduced by one third or by half respectively.

Table 4.1. Estimated sampling effort (number of nights) to reach 90% inventory completeness under different subsampling schemes: (i) the number of consecutive nights of sampling per site and (ii) sampling only during the wet or dry season, or both). *Because I recorded only three consecutive nights after fragment re-isolation, schemes with four and five nights of sampling were not evaluated during this period.

| Scheme | **Estimated sampling effort to reach 90% inventory completeness** | |
|---|---|---|
| | Before Re-isolation | After Re-isolation* |
| 1 night / site | 59 | 9 |
| 2 night / site | 74 | 10 |
| 3 night / site | 80 | 10 |
| 4 night / site | 81 | |
| 5 night / site | 87 | |
| Dry season | 127 | 8 |
| Wet season | 128 | 14 |
| Both seasons | 80 | 10 |

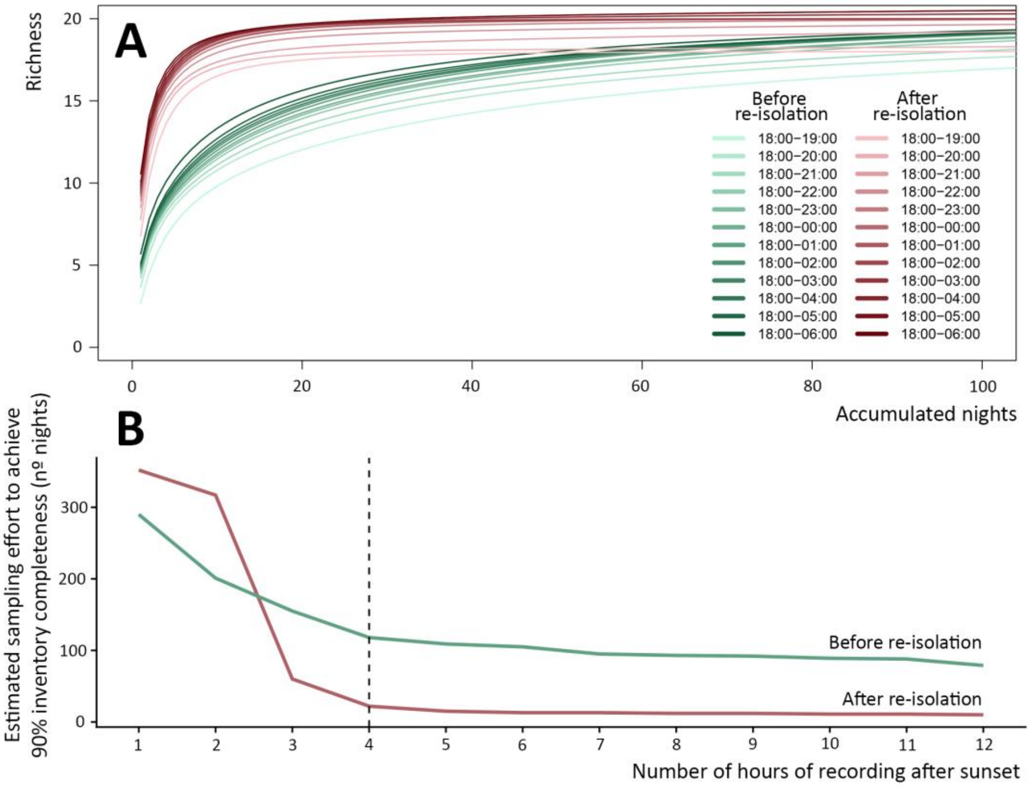


Figure 4.1. A) Species accumulation curves under different temporal sampling schemes using a varying number of hours of recording per night; B) Estimated sampling effort measured as total number of nights required to detect 90% of the species present in the study area. GREEN lines: data collected during the 1st **sampling period** in continuous forest, forest fragments and secondary forest; RED lines: data collected during the 2nd **sampling period** in continuous forest, forest fragments and clearings. Vertical line in B at x=4 h of recording.

Although for some response variables the results of the different models were quite consistent (e.g. bat activity in forest fragments or richness in the clearings, after the re-isolation), effect sizes for other response variables varied substantially depending on the temporal sampling scheme (e.g. for Simpson diversity both before and after the re-isolation, in the forest fragments, secondary forest and clearings, Fig. 4.2). This variability sometimes led to contradictory results with regard to significance of the effect.

Before fragment re-isolation, in the secondary forest a minimum of four hours was necessary to detect a significant negative effect (i.e. reduction relative to CF) on richness and species diversity (Simpson), while five hours of recording were needed for bat activity. In contrast, assemblage composition of the secondary forest was significantly distinct from CF only when it was evaluated with the full night dataset. After re-isolation, sampling only the first four and three hours per night, respectively, were insufficient to detect significant responses with regard to species composition (Bray-Curtis) in forest fragments and clearings. However, except for species composition, during this period the effect of the clearings on all response variables examined were very robust and always showed significant differences between clearings and CF, independently of the sampling scheme.

Using different numbers of consecutive nights per site resulted in consistent results for some response variables (e.g. all results after the re-isolation), whereas there were large inconsistencies for others (e.g. bat activity or assemblage composition in forest fragments before the re-isolation) (Fig. 4.2). Before the re-isolation, in the secondary forest up to five consecutive nights were needed to detect a significant negative effect on richness and species diversity. In forest fragments one night was insufficient to detect positive effects on bat activity, but in the secondary forest, with more than two nights, the significant effects detected with smaller datasets disappeared. Likewise, with five nights the significant effect found with smaller datasets disappeared for assemblage composition.

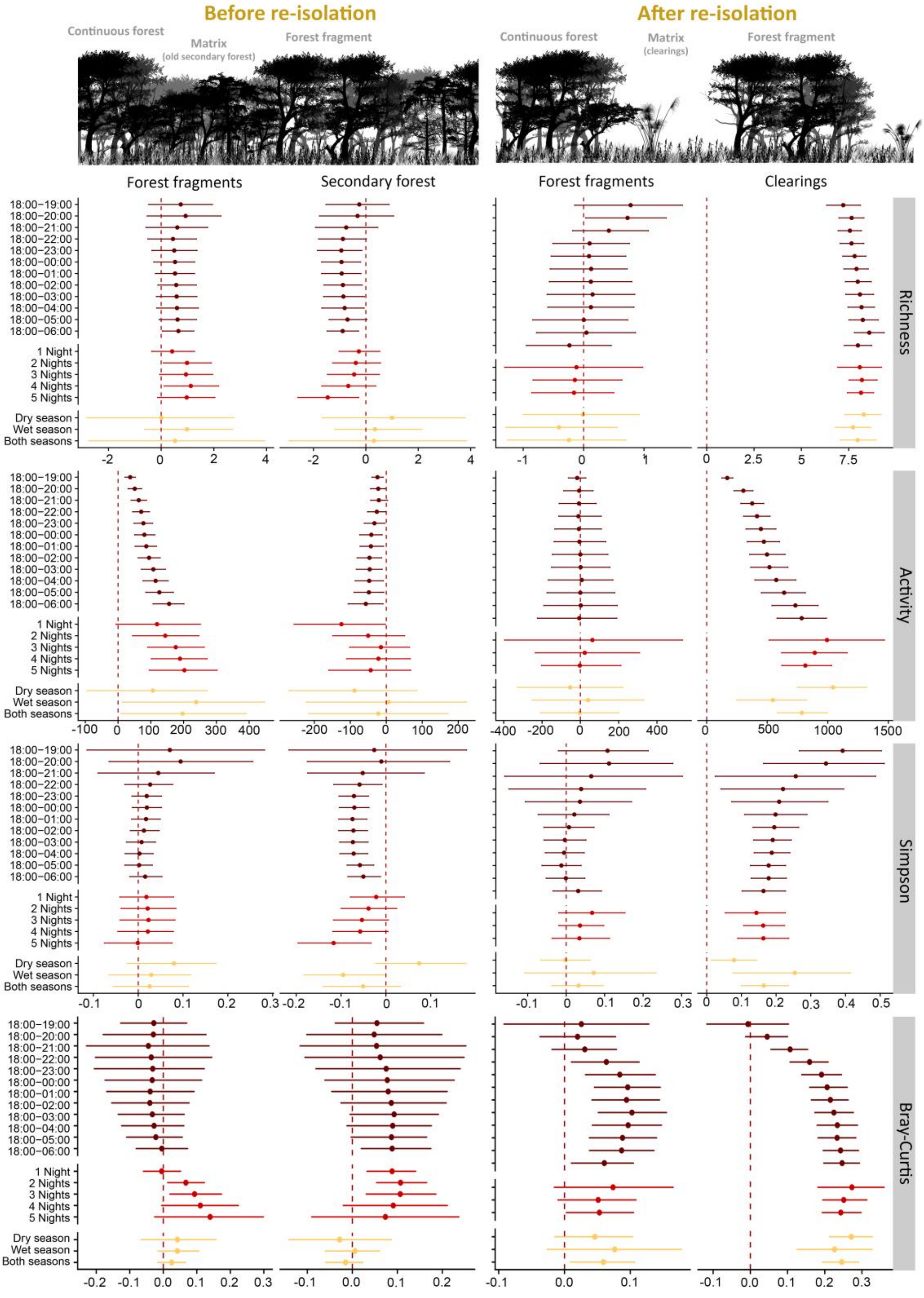


Figure 4.2. Effects of habitat modification on richness, activity, diversity and assemblage composition of aerial insectivorous bats during the two sampling periods (2012-2013 and 2014), evaluated using generalized linear mixed models. Effect estimates are considered as the fixed effect posterior distribution, characterized by its mean (dot) and credible intervals (95% CI, lines). The effects of habitat modification are calculated as the magnitude of change between the sampled habitat types and continuous forest interiors.

Results based on datasets collected during different seasons were quite consistent (Fig. 4.2). In all models performed after the re-isolation data, the direction of the effect was always the same independently of the sampling scheme (except for assemblage composition in the forest fragments).

I found pronounced differences between subsets (e.g. bat activity in the forest fragments for which using only data from the dry season resulted in a non-significant effect, as opposed to a significant positive response when using data from both seasons or the wet season only). Species diversity was lower in secondary than in continuous forest during the wet season, however, this effect was non-significant if only the dry season data or both seasons were considered (Fig. 4.2).

## Discussion

This study demonstrated that in my study area, the sampling effort required to achieve species inventories at 90% completeness for aerial insectivorous bats varies substantially with habitat type, being about eight times higher in forest fragments embedded in a matrix of old secondary forest than in the same forest fragments re-isolated by clearing the surrounding secondary forest. I showed that recording longer than four hours after sunset per night does not significantly reduce the number of nights required to inventory 90% of the species present in the study area. In fact, the rate of decrease in the estimated sampling effort required varied considerably before and after re-isolation, being much more gradual in the former compared to the very marked decline in the latter. The results indicate that, for inventories, the sampling season and the number of consecutive nights per site are less important than the nightly time window during which sampling is conducted. When modelling the effect of forest fragmentation on insectivorous bats, after the re-isolation, the significance and direction of the effect was consistent across all sampling schemes (except for assemblage composition). However, before fragment re-isolation, choosing the most appropriate temporal sampling scheme was crucial to detect significant effects that otherwise might go unnoticed.

## PROTOCOL OPTIMIZATION FOR SPECIES RICHNESS EVALUATION

In order to detect at least 90% of the species present, the analyses indicated a minimum sampling requirement of ~80 nights for the first landscape scenario (forest fragments embedded in a matrix of old secondary forest), but only ~10 nights for the second (forest fragments that were re-isolated by clearing the surrounding secondary vegetation). Similar results were found by Froidevaux et al. (2014) in temperate habitats where diversity in forest gaps was much more easily assessed than in forest interiors. This is explained by the fact that open or edge space foragers, which usually are rare in the cluttered forest interiors, seemingly increase in activity when the forest is cleared as they become more easily detected (Estrada-Villegas et al. 2010). Alternatively, insect abundance might have substantially increased in the cleared areas after fragment re-isolation, resulting in a localized burst in bat activity (Salvarina et al. 2018). However, it is worth to notice that small increases in species richness can still be expected after 100 nights of surveys. As reported in previous studies (Skalak et al. 2012, Froidevaux et al. 2014), the maximum number of species is rarely reached with any reduced subset with a reasonable number of sampling nights (<50).

Aerial hawking bats tend to be more active during the first hours of the night due to the peak in aerial insect availability, during what is commonly known as the highest nocturnal activity peak (Bernard 2002, Appel et al. 2017). Sometimes researchers assume that sampling during this period is sufficiently representative to describe the diversity of aerial insectivorous bats (Stahlschmidt and Bruehl 2012). Froidevaux et al. (2014) evaluated the differences between three sampling schemes in Europe: full night, four hours after sunset, and two hours after sunset plus two hours before sunrise and found the best outcome was achieved when recording the entire night. Trevelin et al. (2017), who assessed the effectiveness of capturing phyllostomids with mist-nets during the first six hours in the Neotropics, also found that restricting the sampling scheme frequently resulted in incomplete inventories. This study is the first to address similar hypotheses for Neotropical aerial insectivorous bats, evaluating how completeness changed in response to cumulative increases in effort (i.e. number of sampling hours) instead of discrete temporal sampling schemes such as used by Froidevaux et al. (2014). The fact that in all comparisons I found a marked difference between the results using the first four hours after sunset and those with more than four hours per night suggests that this time-window is the minimum necessary sampling effort to ensure an adequate representativeness (90%) of these bat assemblages.

If sampling effort needs to be optimised for inventorying bats, reducing the nightly time window was more effective than reducing the number of consecutive nights per site. Law et al. (2015) recommended to repeat the number of visits in certain sampling points rather than distributing the equivalent sampling effort in new sampling sites. The spatial dimension of their study is an important aspect that I was not able to address due to the fact that the BDFFP landscape offers limited scope for spatial replication, and also because my study was primarily designed to investigate the effect of forest fragmentation on insectivorous bats. Law et al. (2015) found that to adequately capture spatial heterogeneity it is always preferable to use up to three detectors for two nights rather than using one single detector for six nights, i.e. prioritizing spatial over temporal replication. It would be essential to test this again in the Neotropics and conduct a similar study by using several detectors within the same sampling sites, and also by substantially increasing the number of sampling sites across the study area, as Law et al. (2015) did in Australia and Froidevaux et al. (2014) in Europe.

In terms of seasonal sampling window, especially before the re-isolation, the combination of data from both dry and wet season allowed me to record significantly more species using less sampling effort than using data from only one season. I recorded fewer species in the dry season (although at higher rates of detection) than in the wet season, probably due to reduced insect availability (Taylor et al. 2013). This implies that to accurately describe bat assemblages in the tropics, studies would be required to sample during both seasons.

## PROTOCOL OPTIMIZATION FOR BAT STUDIES IN MODIFIED HABITATS

Choosing the right sampling scheme to accurately describe ecological patterns and to detect the consequences of habitat modification on wildlife is more important in a conservation context, although inherently more difficult than choosing the right protocol for inventories. I showed that with inappropriate sampling designs, differences not only became more difficult to detect but can also point into the opposite direction, potentially undermining conservation efforts. Describing ecological phenomena goes beyond the limits of missing some species within an inventory (Law et al. 2015). And full and accurate knowledge of these processes is needed to ensure appropriate conservation actions.

Before fragment re-isolation, different nightly sampling schemes provided contradictory results, underlining the importance of not restricting the time of recording within the night if weak ecological patterns are to be detected. Variability in model effect sizes was sometimes so large that significant results were only found with full night of sampling. Similarly, when I considered datasets with five entire nights, some significant results obtained with smaller data subsets turned to be non-significant. The large variability on the results can potentially mask significant results which turns the interpretation of some specific results quite confusing. In some cases, in contrast to what I found with the SACs, increasing the number of consecutive nights per site up to five substantially increased the accuracy of my results. This was especially true for secondary forest (e.g. richness, bat activity, species diversity and assemblage composition), which suggests that the number of consecutive nights might be more important for detecting ecological patterns than for inventories. In agreement with Law et al. (2015), I found that night to night variation in activity was high, however, I argue that within-night variation might even exceed any bias linked to the former. The fact that, before the re-isolation, I did not find clear differences between the results of the models that considered both seasons together or separately, suggests that those ecological patterns might be consistent all year long.

After the re-isolation, for almost all response metrics, accurate results were already obtained with the smallest subsets evaluated (e.g. one hour after sunset, one season or one night per site). The only exception was for assemblage composition in forest fragments and clearings, in which case four and three hours, respectively, were necessary to detect significant effects. This supports the fact that methodological requirements (i.e. minimum sampling effort) to answer specific ecological questions strongly depend on the variation in the studied variables, landscape heterogeneity or the presence of open areas (Bros and Cowell 1987, Chao et al. 2009, Johnson et al. 2014), a problem that has usually been addressed through *a priori* power analysis (Toft and Shea 1983, Steidl et al. 1997, Johnson et al. 2014). Besides the environmental heterogeneity, specific characteristics of local bat assemblages will also influence the required sampling effort. More detailed information about the species phenology or foraging strategies in the Neotropics would fill current knowledge gaps that currently hamper the reliability of monitoring programmes.

## Recommendations

Optimizing sampling protocols in monitoring programmes, surveys or any other scientific study implies that sampling effort, methodology, equipment costs and workload associated with field work need to be carefully balanced (Guillera-Arroita and Lahoz-Monfort 2012) as time dedicated to field work and data analyses is directly linked to project costs (Gardner et al. 2008, Froidevaux et al. 2014). Sampling design will mainly depend on the scientific questions that the study aims to address. Based on these findings, I propose guidelines that can aid to streamline bat acoustic surveys in the Neotropics, by reducing project workload and costs, while maintaining high accuracy and representativeness. I believe these following recommendations are valuable to guide future research, especially in the context of the funding crisis that science is experiencing:

1) For species inventories:
    a. I recommend reducing, in decreasing order of priority, the number of consecutive nights per site, the sampling hours per night; as well as distribute the sampling effort equally between both seasons.

    b. The minimum time-window to record a minimum of 90% of bat species present in the study area should include the first four hours after sunset. However, if rare or elusive species also are to be targeted, I recommend recording the complete night (Moreno and Halffter 2000).

    c. The total time required to achieve 90% complete inventories highly depends on the matrix contrast and the heterogeneity of the landscape. Although in low-contrast landscapes a total of ~80 entire nights spread in both seasons can be enough, in high-contrast landscapes, only ~10 entire nights would be sufficient.

2) For studies on forest fragmentation:
    a. I recommend reducing, in decreasing order of priority, the number of seasons sampled, the number of consecutive nights per site, and finally the sampling hours per night.

b. To detect the consequences of habitat modification in low-contrast landscapes, recording the first four to six hours after sunset and recording during three to five nights in the same sites is essential. However, in high-contrast landscapes, the consequences of forest fragmentation are generally detected even with the shortest sampling scheme (e.g. one hour after sunset or even one night per site).

c. Assessing the effect of spatial heterogeneity on the required sampling effort by using several detectors within the sampling sites, and also by sequentially increasing the number of sampling sites across the study area is crucial. This research question, however, is yet to be answered.

## Acknowledgements

I would like to thank Oriol Massana, Diogo Ferreira, Marta Acácio and Fabio Farneda, Madalena Boto, Maria Mas, Gilberto Fernández, Eva Sánchez for fieldwork assistance and José Luis Camargo, Rosely Hipólito, Ary Jorge Ferreira, Luiz Quiroz, Leo and Josimar for logistical support. Xavier Puig-Montserrat and Carles Flaquer also deserve special mention for their contribution on the discussion and continuous support. This work was supported by the Portuguese Foundation for Science and Technology under grants [PTDC/BIABIC/111184/2009] (CM), [SFRH/BD/80488/2011] (RR), [PD/BD/52597/2014] (ALB) and by the CNPq by the fellowship [160049/2013-0] (PEDB). Additional funding was provided by a Bat Conservation International student research fellowship to ALB and RR.

## References

Adams, A. M., L. McGuire, L. Hooton, and M. B. Fenton. 2015. How high is high? Using percentile thresholds to identify peak bat activity. Canadian Journal of Zoology **93**:307-3013.

Alvarez-Berríos, N., M. Campos-Cerqueira, A. Hernández-Serna, C. Amanda Delgado, F. Román-Dañobeytia, and T. M. Aide. 2016. Impacts of small-scale gold mining on birds and anurans near the Tambopata Natural Reserve, Peru, assessed using passive acoustic monitoring. Tropical Conservation Science **9**:832-851.

Appel, G., A. López-Baucells, E. Magnusson, and P. E. D. Bobrowiec. 2017. Aerial insectivorous bat activity in relation to moonlight intensity. Mammalian Biology **85**:37-46.

Arias-Aguilar, A., F. Hintze, L. M. S. Aguiar, V. Rufray, E. Bernard, and M. J. R. Pereira. 2018. Who’s calling? Acoustic identification of Brazilian bats. Mammal Research **63**:231-253.

Banks-Leite, C., R. M. Ewers, R. G. Pimentel, and J. P. Metzger. 2012. Decisions on temporal sampling protocol influence the detection of ecological patterns. Biotropica **44**:378-385.

Bernard, E. 2002. Diet, activity and reproduction of bat species (Mammalia, Chiroptera) in Central Amazonia, Brazil. Revista Brasileira De Zoologia **19**:173-188.

Borcard, D., F. Gillet, and P. Legendre. 2011. Numerical ecology with R. Springer, New York, United States of America.

Bros, W., and B. Cowell. 1987. A technique for optimizing sample size (replication). Journal of Experimental Marine Biology and Ecology **114**:63-71.

Chao, A., R. K. Colwell, C.-W. Lin, and N. J. Gotelli. 2009. Sufficient sampling for asymptotic minimum species richness estimators. Ecology **90**:1125-1133.

Chao, A., and C. H. Chiu. 2014. Species richness: estimation and comparison. Wiley StatsRef: Statistics Reference Online:1-26.

Duchamp, J. E., M. Yates, R. M. Muzika, and R. K. Swihart. 2006. Estimating probabilities of detection for bat echolocation calls: An application of the double-observer method. Wildlife Society Bulletin **34**:408-412.

Dzal, Y., L. A. Hooton, E. L. Clare, and M. B. Fenton. 2009. Bat activity and genetic diversity at Long Point, Ontario, an important bird stopover site. Acta Chiropterologica **11**:307-315.

Estrada-Villegas, S., C. F. Meyer, and E. K. Kalko. 2010. Effects of tropical forest fragmentation on aerial insectivorous bats in a land-bridge island system. Biological Conservation **143**:597-608.

Farneda, F. Z., R. Rocha, A. López-Baucells, E. M. Sampaio, J. M. Palmeirim, P. E. D. Bobrowiec, C. E. V. Grelle, and C. F. J. Meyer. 2018. Functional recovery of Amazonian bat assemblages following secondary forest succession. Biological Conservation **218**:192-199.

Ferreira, D. F., R. Rocha, A. López-Baucells, F. Z. Farneda, J. M. B. Carreiras, J. M. Palmeirim, and C. F. J. Meyer. 2017. Season-modulated responses of Neotropical bats to forest fragmentation. Ecology and Evolution **7**:4059-4071.

Figueira, L., J. L. Tella, U. M. Camargo, and G. Ferraz. 2015. Autonomous sound monitoring shows higher use of Amazon old growth than secondary forest by parrots. Biological Conservation **184**:27-35.

Fischer, J., J. Stott, B. S. Law, M. D. Adams, and R. I. Forrester. 2009. Designing effective habitat studies: quantifying multiple sources of variability in bat activity. Acta Chiropterologica **11**:127-137.

Flaquer, C., I. Torre, and A. Arrizabalaga. 2007. Comparison of sampling methods for inventory of bat communities. Journal of Mammalogy **88**:526–533.

Frick, W. F. 2013. Acoustic monitoring of bats, considerations of options for long-term monitoring. Therya **4**:69-78.

Froidevaux, J. S. P., F. Zellweger, K. Bollmann, and M. K. Obrist. 2014. Optimizing passive acoustic sampling of bats in forests. Ecology and Evolution **4**:4690-4700.

Furey, N. M., I. J. Mackie, and P. A. Racey. 2009. The role of ultrasonic bat detectors in improving inventory and monitoring surveys in Vietnamese karst bat assemblages. Current Zoology **55**:327-341.

Gardner, T. A., J. Barlow, I. S. Araujo, T. C. Ávila-Pires, A. B. Bonaldo, J. E. Costa, M. C. Esposito, L. V. Ferreira, J. Hawes, and M. I. Hernandez. 2008. The cost-effectiveness of biodiversity surveys in tropical forests. Ecology Letters **11**:139-150.

Gorresen, P. M., A. C. Miles, C. M. Todd, F. J. Bonaccorso, and T. J. Weller. 2008. Assessing bat detectability and occupancy with multiple automated echolocation detectors. Journal of Mammalogy **89**:11-17.

Guillera-Arroita, G., and J. J. Lahoz-Monfort. 2012. Designing studies to detect differences in species occupancy: power analysis under imperfect detection. Methods in Ecology and Evolution **3**:860-869.

Hadfield, T. 2017. MCMCglmm Course Notes. Report, United Kingdom.

Hayes, J. P. 2000. Assumptions and practical considerations in the design and interpretation of echolocation-monitoring studies. Acta Chiropterologica **2**:225-236.

Heltshe, J. F., and N. E. Forrester. 1983. Estimating species richness using the jackknife procedure. Biometrics **39**:1-11.

Hill, A. P., P. Prince, E. P. Covarrubias, C. P. Doncaster, J. L. Snaddon, and A. Rogers. 2018. AudioMoth: Evaluation of a smart open acoustic device for monitoring biodiversity and the environment. Methods in Ecology and Evolution **9**:1199-1211.

Jeliazkov, A., Y. Bas, C. Kerbiriou, J.-F. Julien, C. Penone, and I. Le Viol. 2016. Large-scale semi-automated acoustic monitoring allows to detect temporal decline of bush-crickets. Global Ecology and Conservation **6**:208-218.

Johnson, P. C. D., S. J. E. Barry, H. M. Ferguson, P. Müller, and H. Schielzeth. 2014. Power analysis for generalized linear mixed models in ecology and evolution. Methods in Ecology and Evolution **6**:133-142.

Jones, G., D. S. Jacobs, T. Kunz, M. R. Willig, and P. A. Racey. 2009. Carpe noctem: the importance of bats as bioindicators. Endangered Species Research **8**:93-115.

Jost, L., A. Chao, and R. L. Chazdon. 2011. Compositional similarity and β (beta) diversity. Pages 66-84 *in* A. E. Magurran and B. J. McGill, editors. Biological diversity: frontiers in measurement and assessment. Oxford University Press, New York, United States of America.

Kryvokhyzha, D., K. Holm, J. Chen, A. Cornille, S. Glemin, S. I. Wright, U. Lagercrantz, and M. Lascoux. 2016. The influence of population structure on gene expression and flowering time variation in the ubiquitous weed *Capsella bursa-pastoris* (Brassicaceae). Molecular Ecology **25**:1106-1121.

Kunz, T. H., E. B. Arnett, W. P. Erickson, A. R. Hoar, G. D. Johnson, R. P. Larkin, M. D. Strickland, R. W. Thresher, and M. D. Tuttle. 2007. Ecological impacts of wind energy development on bats: questions, research needs, and hypotheses. Frontiers in Ecology and the Environment **5**:315-324.

Kunz, T. H., and S. Parsons. 2009. Ecological and behavioral methods for the study of bats. 2nd edition. Johns Hopkins University Press, Baltimore, United States of America.

Laurance, W. F., J. L. C. Camargo, P. M. Fearnside, T. E. Lovejoy, G. B. Williamson, R. C. G. Mesquita, C. F. J. Meyer, P. E. D. Bobrowiec, and S. G. W. Laurance. 2018. An Amazonian rainforest and its fragments as a laboratory of global change. Biological Reviews of the Cambridge Philosophical Society **93**:223-247.

Law, B., L. Gonsalves, P. Tap, T. Penman, and M. Chidel. 2015. Optimizing ultrasonic sampling effort for monitoring forest bats. Austral Ecology **40**:886–897.

Lintott, P. R., E. Fuentes-Montemayor, D. Goulson, and K. J. Park. 2014. Testing the effectiveness of surveying techniques in determining bat community composition within woodland. Wildlife Research **40**:675-684.

López-Baucells, A., R. Rocha, P. E. D. Bobrowiec, E. Bernard, J. Palmeirim, and C. Meyer. 2016. Field Guide to Amazonian Bats. INPA, Manaus, Brazil.

MacSwiney, G. C., F. M. Clarke, and P. A. Racey. 2008. What you see is not what you get: the role of ultrasonic detectors in increasing inventory completeness in Neotropical bat assemblages. Journal of Applied Ecology **45**:1364-1371.

Marques, J. T., M. J. Ramos Pereira, T. A. Marques, C. D. Santos, J. Santana, P. Beja, and J. M. Palmeirim. 2013. Optimizing sampling design to deal with mist-net avoidance in Amazonian birds and bats. PLoS ONE **8**:e74505.

Meyer, C. F. J. 2015. Methodological challenges in monitoring bat population- and assemblage-level changes for anthropogenic impact assessment. Mammalian Biology **80**:159-169.

Meyer, C. F. J., L. M. S. Aguiar, L. F. Aguirre, J. Baumgarten, F. M. Clarke, J. F. Cosson, S. E. Villegas, J. Fahr, D. Faria, N. Furey, M. Henry, R. Hodgkison, R.

K. B. Jenkins, K. G. Jung, T. Kingston, T. H. Kunz, M. C. MacSwiney, I. Moya, B. D. Patterson, J. M. Pons, P. A. Racey, K. Rex, E. M. Sampaio, S. Solari, K. E. Stoner, C. C. Voigt, D. von Staden, C. D. Weise, and E. K. V. Kalko. 2011. Accounting for detectability improves estimates of species richness in tropical bat surveys. Journal of Applied Ecology **48**:777-787.

Millon, L., J.-F. Julien, R. Julliard, and C. Kerbiriou. 2015. Bat activity in intensively farmed landscapes with wind turbines and offset measures. Ecological Engineering **75**:250-257.

Moreno, C. E., and G. Halffter. 2000. Assessing the completeness of bat biodiversity inventories using species accumulation curves. Journal of Applied Ecology **37**:149-158.

Murray, K. L., E. R. Britzke, B. M. Hadley, and L. W. Robbins. 1999. Surveying bat communities: a comparison between mist nets and the Anabat II bat detector system. Acta Chiropterologica **1**:105-112.

Murray, S. W., and A. Kurta. 2004. Nocturnal activity of the endangered Indiana bat (*Myotis sodalis*). Journal of Zoology **262**:197-206.

Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, and H. Wagner. 2015. vegan: community ecology package. R package version 2.3-3.

Pieretti, N., M. Duarte, R. Sous-Lima, M. Rodrigues, R. Young, and A. Farina. 2015. Determining temporal sampling schemes for passive acoustic studies in different tropical ecosystems. Tropical Conservation Science **8**:215-234.

Piksa, K., W. Bogdanowicz, and A. Tereba. 2011. Swarming of bats at different elevations in the Carpathian Mountains. Acta Chiropterologica **13**:113-122.

Puig-Montserrat, X., I. Torre, A. López-Baucells, E. Guerrieri, M. M. Monti, R. Rafols-Garcia, X. Ferrer, D. Gisbert, and C. Flaquer. 2015. Pest control service provided by bats in Mediterranean rice paddies: linking agroecosystems structure to ecological functions. Mammalian Biology **80**:237-245.

Richards, S. A. 2008. Dealing with overdispersed count data in applied ecology. Journal of Applied Ecology **45**:218-227.

Rocha, R., A. López-Baucells, F. Z. Farneda, M. Groenenberg, P. E. D. Bobrowiec, M. Cabeza, J. M. Palmeirim, and C. F. J. Meyer. 2017. Consequences of a large-scale fragmentation experiment for Neotropical bats: disentangling the relative importance of local and landscape-scale effects. Landscape Ecology **32**:31–45.

Rocha, R., O. Ovaskainen, A. López-Baucells, F. Z. Farneda, E. M. Sampaio, P. E. D. Bobrowiec, M. Cabeza, J. M. Palmeirim, and C. F. J. Meyer. 2018. Secondary forest regeneration benefits old-growth specialist bats in a fragmented tropical landscape. Scientific Reports **8**:3819.

Rodhouse, T. J., K. T. Vierling, and K. M. Irvine. 2011. A practical sampling design for acoustic surveys of bats. The Journal of Wildlife Management **75**:1094-1102.

Salvarina, I., D. Gravier, and K. O. Rothhaupt. 2018. Seasonal bat activity related to insect emergence at three temperate lakes. Ecology and Evolution **8**:3738–3750.

Silva, C. R., and E. Bernard. 2017. Bioacoustics as an important complementary tool in bat inventories in the Caatinga drylands of Brazil. Acta Chiropterologica **19**:409-418.

Skalak, S. L., R. E. Sherwin, and R. M. Brigham. 2012. Sampling period, size and duration influence measures of bat species richness from acoustic surveys. Methods in Ecology and Evolution **3**:490-502.

Stahlschmidt, P., and C. A. Bruehl. 2012. Bats as bioindicators - the need of a standardized method for acoustic bat activity surveys. Methods in Ecology and Evolution **3**:503-508.

Steidl, R. J., J. P. Hayes, and E. Schauber. 1997. Statistical power analysis in wildlife research. The Journal of Wildlife Management **61**:270-279.

Taylor, P. J., A. Monadjem, and J. N. Steyn. 2013. Seasonal patterns of habitat use by insectivorous bats in a subtropical African agro-ecosystem dominated by macadamia orchards. African Journal of Ecology **51**:552-561.

Toft, C. A., and P. J. Shea. 1983. Detecting community-wide patterns: Estimating power strengthens statistical inference. The American Naturalist **122**:618-625.

Torrent, L., A. López-Baucells, R. Rocha, P. E. D. Bobrowiec, and C. F. J. Meyer. 2018. The importance of lakes for bat conservation in Amazonian rainforests: an assessment using autonomous recorders. Remote Sensing in Ecology and Conservation:(in press).

Towsey, M., J. Wimmer, I. Williamson, and P. Roe. 2014. The use of acoustic indices to determine avian species richness in audio-recordings of the environment. Ecological Informatics **21**:110-119.

Trevelin, L. C., R. L. Novaes, P. F. Colas-Rosas, T. C. Benathar, and C. A. Peres. 2017. Enhancing sampling design in mist-net bat surveys by accounting for sample size optimization. PLoS ONE **12**:e0174067.

Walters, C. L., A. Collen, T. Lucas, K. Mroz, C. A. Sayer, and K. E. Jones. 2013. Challenges of using bioacoustics to globally monitor bats. Pages 479-499 *in* S. C. Pedersen and R. A. Adams, editors. Bat Evolution, Ecology, and Conservation. Springer, New York, United States of America.

Weller, T. J., and D. C. Lee. 2007. Mist net effort required to inventory a forest bat species assemblage. Journal of Wildlife Management **71**:251-257.

Weller, T. J., and C. J. Zabel. 2002. Variation in bat detections due to detector orientation in a forest. Wildlife Society Bulletin **30**:922-930.

Wickham, H. 2009. ggplot2: elegant graphics for data analysis. R package version 2.1.0.

Wilson, K. A., N. A. Auerbach, K. Sam, A. G. Magini, A. S. L. Moss, S. D. Langhans, S. Budiharta, D. Terzano, and E. Meijaard. 2016. Conservation research is not happening where it is most needed. PLoS Biol **14**:e1002413.

Wordley, C. F. R., M. Sankaran, D. Mudappa, and J. D. Altringham. 2018. Heard but not seen: Comparing bat assemblages and study methods in a mosaic landscape in the Western Ghats of India. Ecology and Evolution **8**:3883-3894.

Yates, M. D., and R. M. Muzika. 2006. Effect of forest structure and fragmentation on site occupancy of bat species in Missouri Ozark forests. Journal of Wildlife Management **70**:1238-1248.

Zamora-Gutiérrez, V., C. López-Gonzalez, M. C. MacSwiney González, B. Fenton, G. Jones, E. K. V. Kalko, S. J. Puechmaille, V. Stathopoulos, K. E. Jones, and R. Freckleton. 2016. Acoustic identification of Mexican bats based on taxonomic and ecological constraints on call design. Methods in Ecology and Evolution **7**:1082-1091.

## Supplementary material

Table 4.S1. Sonotype classification used in this study, based on López-Baucells et al. (2016).

| **Sonotype/Species** | **Species included in the sonotype** |
|---|---|
| Emballonuridae | |
| *Rhynchonycteris naso* | *Rhynchonycteris naso* |
| *Saccopteryx leptura* | *Saccopteryx leptura* |
| *Saccopteryx bilineata* | *Saccopteryx bilineata* |
| *Centronycteris maximiliani* | *Centronycteris centralis / maximiliani* |
| *Peropteryx macrotis* | *Peropteryx macrotis* |
| *Peropteryx kappleri* | *Peropteryx kappleri* |
| *Cormura brevirostris* | *Cormura brevirostris* |
| *Emballonuridae* 1 | *Saccopteryx gymnura / canescens* |
| Furipteridae | |
| *Furipterus horrens* | *Furipterus horrens* |
| Molossidae | |
| *Molossidae I* | *Molossus molossus* |
| *Molossidae II* | *Molossus rufus / sinaloae / currentium* |
| *Molossidae III* | *Cynomops planirostris / paranus / greenhalli /abrasus,*<br>*Eumops auripendulus / glaucinus / dabbenei / hansae / maurus,*<br>*Nyctinomops laticaudatus,*<br>*Tadarida brasiliensis* |
| *Molossops* | *Molossops neglectus,/ temminckii* |
| *Promops* | *Promops centralis / nasutus* |
| Mormoopidae | |
| *Pteronotus personatus* | *Pteronotus personatus* |
| *Pteronotus alitonus* | *Pteronotus alitonus* |
| *Pteronotus gymnonotus* | *Pteronotus gymnonotus* |
| *Pteronotus rubiginosus* | *Pteronotus rubiginosus* |
| Vespertilionidae | |
| *Myotis riparius* | *Myotis riparius / Thyroptera tricolor* |
| *Myotis nigricans* | *Myotis nigricans* |
| *Vespertilionidae 1* | *Eptesicus brasiliensis / chiriquinus*<br>*Eptesicus furinalis*<br>*Lasiurus sp.*<br>*Rhogeessa io* |

# Chapter 5

## Recovery of taxonomic, functional and phylogenetic diversity of insectivorous bats in old secondary Neotropical rainforest

**López-Baucells, A.**, Rocha, R., Bobrowiec, P.E.D., Palmeirim, J.M., Meyer, C.F.J.

Top: *Pteronotus rubiginosus;* Bottom: Brazilian rainforest in Central Amazon

# CHAPTER 5

## Recovery of taxonomic, functional and phylogenetic diversity of insectivorous bats in old secondary Neotropical rainforest

### Abstract

Regenerating secondary forests are an expanding type of land cover in many parts of the tropics and increasingly being recognised for their important role in conserving biodiversity in human-modified tropical landscapes, although the time required for ecosystem recovery depends on numerous landscape characteristics. Due to increased law enforcement, deforestation rates in the Amazon dropped by about 25,000 km$^2$/year between the 1980s and 2013, which was accompanied by a rapid increase in secondary regrowth. I surveyed aerial insectivorous bats in the Central Amazon over a period of two years using passive acoustic detectors in a mosaic of continuous forest and forest fragments of different sizes surrounded by a matrix of ~30-year-old secondary forest to investigate their responses to forest fragmentation and vegetation regrowth. I modelled bat responses in terms of bat activity, as well as taxonomic, functional, and phylogenetic α and β diversity. Activity was highly variable and independent of both fragment size and habitat, but diversity metrics revealed clear negative fragmentation effects upon bat assemblages. Bat assemblages in secondary forest almost always had lower taxonomic, functional and phylogenetic diversity than in continuous forest, whereby differences became gradually more pronounced with decreasing fragment size. Almost all fragment edges showed a significant peak in the diversity metrics compared to the forest interiors, probably because edges harbour more heterogeneous microhabitats. Changes in β diversity was mainly related to species and trait loss, which is likely to result in an impoverishment of the ecosystem services provided by them. Although bat ensemble richness in forest fragments was not markedly different from that in continuous forest interiors, the time lapse of ~30 years was clearly not enough for a full diversity recovery in the secondary forest. Due to high variability in species responses, legal protection must be adapted to the most vulnerable ones in order to improve their conservation.

**Keywords**: Amazon, Bioacoustics, Brazil, Chiroptera, Echolocation, Fragmentation, Logging.

## Introduction

Humans are decimating wildlife on Earth at unprecedented rates, with a recent massive loss of species during what we now recognise as the “Anthropocene” (Dirzo et al. 2014). Habitat loss and fragmentation are some of the most severe threats to living species and habitats (Foley et al. 2011, Haddad et al. 2015), turning natural landscapes into mosaics composed of patches of human-modified habitats (Numata and Cochrane 2012). Island Biogeographic Theory (MacArthur and Wilson 1967), long invoked to explore the effects of habitat fragmentation on natural communities, has now been superseded by Countryside Biogeography (Daily 1997, Mendenhall et al. 2013) as the theoretical framework underpinning recent fragmentation studies, which adequately recognizes the importance of the matrix in determining species responses in human-modified dynamic landscapes (Mendenhall et al. 2014, Wolfe et al. 2015).

Across the tropics, secondary forests are increasingly being recognised for their important role in biodiversity conservation (Chazdon et al. 2009, Lennox et al. 2018). Secondary forests are a kind of matrix habitat that has been shown to facilitate movement of many species between remnants of primary habitat, some of them recolonizing degraded habitats (e.g. Stouffer and Bierregaard 1995). However, although species richness can recover as a result of forest regeneration, the capacity of secondary forests to buffer the consequences of forest fragmentation and habitat loss is still widely debated (Barlow et al. 2007, Chazdon 2014). The time required for a regrowth forest to return to a pre-disturbance state depends on the number, sizes, and shapes of remaining habitat patches, structural complexity of the vegetation, landscape configuration, matrix structure, edge effects or the presence of natural corridors (Bowen et al. 2007).

In the Amazon, deforestation rates have dropped from 30,000 km$^2$/year in the 1980s - mostly caused by road and agricultural expansion (Rosa et al. 2017) - to 5843 km$^2$/year in 2013, due to increased law enforcement and the improvement of the protected area network (Davidson et al. 2012, Nepstad et al. 2014). As a result of the abandonment of formerly deforested land, between 1978 and 2002 the area under secondary regrowth has quintuplicated from 29,000 to 161,000 km$^2$ (Neeff et al. 2006). However, new threats such as oil palm plantations (Butler and Laurance 2009), expanding soy agriculture (Rosa et al. 2017) or the rapid development of dams (Lees et al. 2016) are currently increasing the risk of reverting these somewhat positive trends.

Brazil represents one of the major global hotspots for bats (Nogueira et al. 2014), with almost 150 species occurring in the Amazon alone (Bernard et al. 2011, López-Baucells et al. 2016). However, although the consequences of forest fragmentation on phyllostomid bat assemblages in the Neotropics have been a topic of intensive research (Klingbeil and Willig 2009, Klingbeil and Willig 2010, Avila-Cabadilla et al. 2012, Avila-Cabadilla et al. 2014, García-García et al. 2014, Farneda et al. 2015, Arroyo-Rodríguez et al. 2016, Muylaert et al. 2016, Ferreira et al. 2017, Rocha et al. 2017a, Rocha et al. 2018), aerial insectivorous bats, i.e. their non-phyllostomid counterparts, have largely been neglected and their responses to habitat fragmentation remain poorly understood (but see Estrada-Villegas et al. 2010, who evaluated the effects of habitat isolation on species taxonomic diversity). Importantly, unlike for phyllostomid bats (Cisneros et al. 2014b, Garcia-Morales et al. 2016, Farneda et al. 2018b), there are no studies assessing the consequences of habitat fragmentation for aerial insectivorous bats in terms of functional and phylogenetic diversity. Amongst the traditional research questions posed by fragmentation studies, the role of fragment size, edge effects, and compositional vs configurational aspects of the landscape have rarely been addressed for this ensemble. Due to the lack of information about their natural history, we are still far from being able to identify the most sensitive species to devise effective conservation plans.

In this chapter, I surveyed aerial insectivorous bats with automatic acoustic detectors in a mosaic of continuous primary forest, forest fragments of different sizes and a matrix of old secondary forest in Central Amazon. The main aim of the study was to quantify between-habitat differences in terms of overall activity, (used as a surrogate of abundance; Lintott et al. 2014), as well as regarding the three complementary biodiversity facets taxonomic diversity (TD), functional diversity (FD), and phylogenetic diversity (PD). Specifically, I compared assemblage responses between fragment and continuous forest interiors, secondary forests and continuous forest interiors, and between fragments interiors and edges.

Since primary and secondary forest become progressively more similar in structure and composition with time of regeneration, and due to the concomitant reduction in fragment-matrix contrast, I expected species composition to be roughly similar in all habitats, but still not completely equal to that in continuous primary forest interiors. In terms of α diversity, I expected assemblages in the interior of continuous primary forest to be taxonomically, functionally and phylogenetically most diverse, followed by forest fragment interiors and secondary forest. Regarding fragment sizes, I predicted that larger fragments (100 ha) would host bat assemblages that are more similar to continuous forest than smaller fragments (1 and 10 ha) in terms of TD, FD and PD. I also predicted higher bat activity, TD, FD and PD at the fragment edges due to the presence of species that take advantage of both habitats along the ecotone. In terms of β diversity I expected higher values for secondary forests than for forest fragments, as their assemblages might be more distinct from continuous forests. I predicted that the old secondary forest would represent new available habitat for high duty cycle echolocating bats, since those species are more adapted to hunt in cluttered habitats (Schnitzler and Kalko 2001) and secondary forests tend to be more cluttered than primary forest. Conversely, I expected edge-space foragers to be strongly affected by vegetation clutter, hence being more abundant in primary forest than in secondary regrowth. Because open-space foragers fly above the forest canopy and may travel long distances every night, I did not predict substantial differences between habitat types or between interiors and edges.

## Material and Methods

### Study area

The study was conducted at the Biological Dynamics of Forest Fragments Project (BDFFP) in the Central Brazilian Amazon, ~80km north of Manaus (2º30'S, 60ºW, Fig. 5.1). Th BDFFP is the largest and longest-running experimental study of forest fragmentation in the world (Laurance et al. 2017). Since fragment isolation in the early 1980s, research projects have been carried out on a diverse range of taxa including plants (Fáveri et al. 2008, Sousa et al. 2017), arthropods (Morato 2001, Querino et al. 2011), birds (Stouffer et al. 2009, Powell et al. 2013, Powell et al. 2015, Moura et al. 2016, Powell et al. 2016), primates (Boyle et al. 2013), and bats (Farneda et al. 2015, Rocha et al. 2017a, Rocha et al. 2018). The BDFFP extends over an area of more than 1000 km$^2$ and is characterized by a mosaic of continuous *terra firme* rainforest, and forest fragments surrounded by secondary regrowth. The area was intensively logged and burned during the 1980s, resulting in forest fragments of different sizes (1, 10 and 100 ha) that are isolated from the continuous forest by distances of 80-650 m. Since their initial isolation, fragments were re-isolated on several occasions by clearing a 100 m wide strip of vegetation around them, with the last re-isolation event before the present study occurring between 1999 and 2001 (Laurance et al. 2011). Due to these activities, the landscape matrix is composed of secondary forest in different successional stages (Carreiras et al. 2014). Annual rainfall ranges from 1900-3500mm, with temperatures oscillating around 30ºC (Laurance et al. 2011). The dry season is from July to October and the wet season from November to June (Laurance et al. 2011).

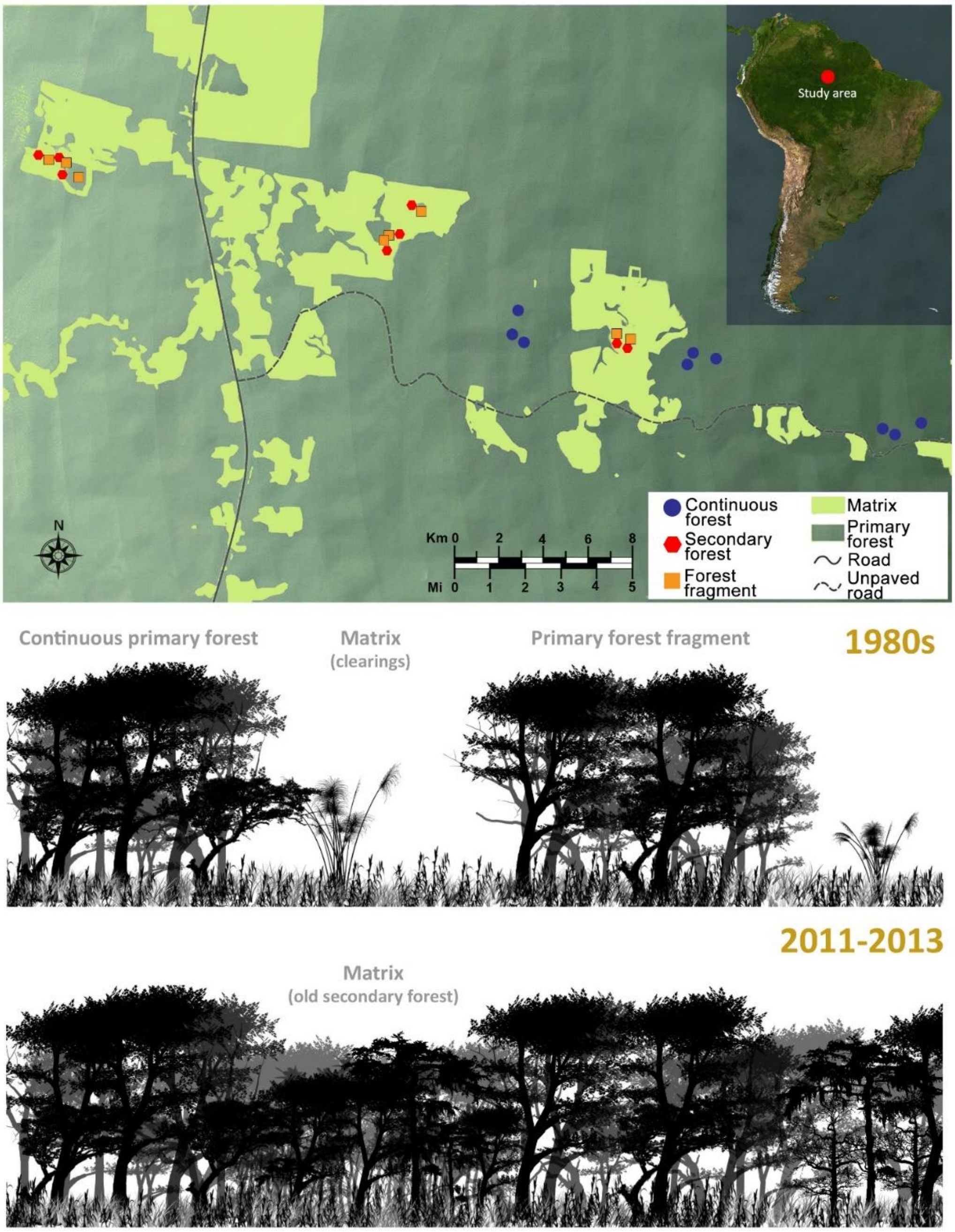


Figure 5.1 Map indicating the location of the Biological Dynamics of Forest Fragments Project (BDFFP) in the Central Amazon, Brazil (inset) and the distribution of the sampling sites in continuous forest, forest fragments, and secondary forest across the study area. Drawings illustrate the landscape structure after initial fragment isolation in the 1980s and during the sampling period of this study (2011-2013).

## Acoustic surveys

I sampled aerial insectivorous bats between 2011 and 2013 using autonomous passive detectors deployed in the interiors and at the edges of eight forest fragments of 1 ha (3), 10 ha (3) and 100 ha (2), and in the interiors of nine control sites in continuous primary forest. In addition, I sampled eight secondary forest sites, which were located ~100m from the nearest fragment edges into the matrix. Recordings were made with automatic ultrasound detectors SM2Bat+ with omnidirectional microphones SMX-US (Wildlife Acoustics Inc., Massachusetts, USA). At each of the 33 recording points, the detector was placed at ~1.5 m height. Detectors were programmed to record from 18:00 to 06:00 in real time with a full spectrum resolution of 16-bit, a high pass filter set at fs/32 (12kHz), trigger level 18SNR, and for periods of one night per site during the first year and five consecutive nights thereafter. Surveys were conducted twice in the wet season and twice in the dry season per year.

## Sound analysis

All recordings were manually analysed using the software Kaleidoscope 4.0.4 (Wildlife Acoustics Inc., Massachusetts, USA). Species were identified from the recordings following the key in López-Baucells et al. (2016) and by comparing them with a reference call library compiled during the study. Whenever possible, calls were assigned to species level, otherwise they were classified into groups of species with similar calls, i.e. sonotypes. A total of 21 different species/sonotypes were used – including 14 identified to species level and seven to mixed-species groups (Supplementary material Table 5.S1). Nomenclature and taxonomic classification follow Burgin et al. (2018).

Due to the intrinsic limitations of acoustic data, which do not provide information on true species abundance, the sample unit was a bat pass. I defined a bat pass as any sequence of a maximum of 5” duration with a minimum of two recognizable echolocation pulses per species, with a clearly distinct shape from the background noise (Millon et al. 2015, Appel et al. 2017, Torrent et al. 2018). Bat activity was quantified as the number of bat passes per night per sonotype (Wickramasinghe et al. 2003), corrected for differences in sound detectability between habitat types. These corrections were made given the different levels of attenuation of sound intensity (dB) in the focal habitats. To estimate attenuation, I used a constant frequency speaker (SM2-Cal, Wildlife Acoustics Inc., Massachusetts, USA), placed along five 30 m long, linear transects randomly distributed in primary forest, secondary regrowth and open areas. I then recorded its signal with an SM2Bat+ detector at 1 m intervals along each transect. With the gathered data I generated linear regressions between sound intensity and distance from the speaker for each habitat. Finally, I used the habitat-specific regression equations to calculate the distance at which I considered that the signal (at 40 kHz) got theoretically dissipated (-50dB). I used this correction to calculate a relative proportion of acoustic volume sampled. Bat call frequency and the intensity at which the animals vocalize determine the maximum detection distance, and thus, this correction might not be highly accurate for all the species, especially those emitting at high frequencies. However, it will probably be appropriate for most of the species, which have echolocation calls at around 40 kHz. Due to the different detectability of each species, I always compared results within species, but avoided to do any comparison between them (see Fig. 5.S1 for more information about this choice and the rationale behind it).

## Calculation of response metrics

Taxonomic α diversity (TD), functional α diversity (FD) and phylogenetic α diversity (PD) were calculated using the R package 'BAT' (Cardoso et al. 2015). TD is calculated as a mean species diversity from a sites x species matrix with activity data. Calculations of PD and FD were both based on the average total branch lengths of the respective phylogenetic and functional trees that link the species/sonotypes in each sampling location.

Phylogenetic data was extracted from the most recent species-level supertree for bats (Shi and Rabosky 2015), and PD was calculated following Faith (1992). Since it was not present in the supertree, *Promops nasutus* was replaced by its closest congener, *P. centralis*, an approach that does not cause serious changes in the distance matrices (Cisneros et al. 2014a).

Functional data were based on Fraixedas (2017) who published a set of functional traits to study the vulnerability of aerial insectivorous bats to forest fragmentation in the same study area. Calculation of FD was based on Petchey and Gaston (2002) and Petchey and Gaston (2006). Due to the high relevance of echolocation and flight manoeuvrability shaping habitat and niche selection and foraging behaviour in insectivorous bats, I used functional traits closely linked to these abilities. The rationale used for the selection of traits follows Fraixedas (2017). I included both quantitative (body mass, frequency of maximum energy, wing aspect ratio and relative wing loading) and qualitative (vertical stratification and call shape) traits. Vertical stratification was divided into *understorey*, *canopy* and *above canopy* categories, and call shape classified as *constant*, *modulated* and *quasi-constant* frequency (Table 5.S1, Supplementary material). Using the function *daisy* from the 'Cluster' R package (Maechler et al. 2012), I calculated functional pairwise dissimilarity matrices based on Gower distances, which allow the inclusion of both categorical and numerical variables (Botta-Dukát 2005). Using these matrices, I performed a hierarchical cluster analysis to create a new tree based on functional traits (analogous to the phylogenetic one) which was subsequently used to calculate FD.

Additionally, β diversity, measured through the Jaccard index, was also assessed at taxonomic, functional and phylogenetic levels between continuous primary forest and forest fragment interiors as well as between continuous primary forest and secondary forest. Total beta diversity ($\beta_{total}$) was partitioned into its richness difference (i.e. due to species loss/gain, βrich) and replacement (i.e. turnover, $\beta_{repl}$) components (Cardoso et al. 2014).

## Modelling the effect of habitat type and fragment size on bat activity, TD, FD and PD

In order to gain general insight into how bat species were distributed amongst the different habitats, all sonotypes were sorted according to their frequency of occurrence in each habitat type: continuous primary forest, forest fragments and secondary forest (including fragment edges). Subsequently, levels of total bat activity and diversity (TD, FD and PD) between habitats were statistically compared with Monte Carlo Markov Chain generalized linear mixed models, using the "MCMCglmm" R package (Hadfield 2017). I included a categorical habitat type variable, with 10 levels (continuous forest interior, 100 ha interior, 100 ha edge, 100 ha matrix, 10 ha interior, 10 ha edge, 10 ha matrix, 1 ha interior, 1 ha edge, 1 ha matrix), as fixed effect in all models, and sampling site as a random effect. When I detected overdispersion or zero-inflated distributions in the dependent variables, modified priors that can deal with over-dispersion (defined with the following values: nu=0.002 and V=1) were used (Kryvokhyzha et al. 2016, Hadfield 2017). When bat activity is quantified using bat passes, data tend to be overdispersed and therefore rarely follow normality (Torrent et al. 2018). Models were run with 50,000 iterations with the first 10,000 discarded as burn-in. All models were checked to have converged based on each parameter time series representations in the trace plots (Hadfield 2017). In all analyses I considered "sonotypes" as the sampling unit. Additionally, in order to visualize species-specific differences, I plotted each bat species' activity average per habitat type (Fig. 5.S2). Models were not run for each species. All statistical analyses were carried out using R software 3.2.4. (R Core Team, 2017).

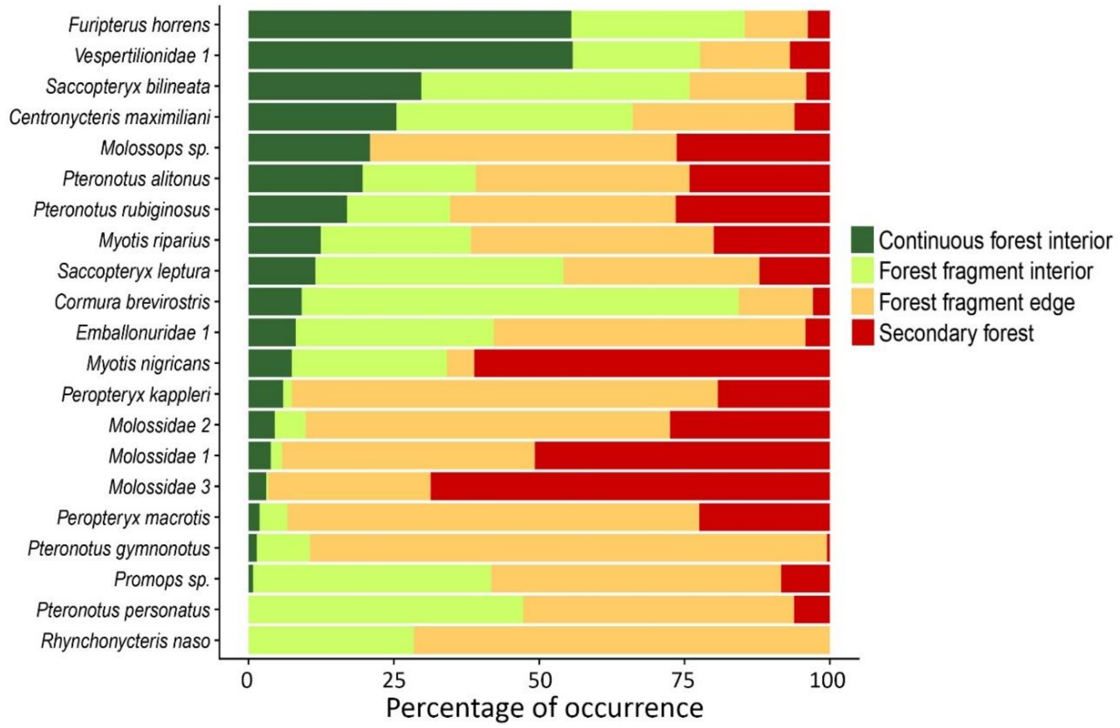


Figure 5.2 Sonotypes detected over the 2-year study period, sorted by their frequency of occurrence in continuous forest interiors, forest fragment interiors, forest fragment edges, and secondary forest. Percentage of occurrence was calculated using detectability-corrected bat activities in each site per habitat type (see Supplementary material for more information about the detectability correction). Differences in sampling effort between habitat categories were accounted for in the percentage calculations.

## Results

I obtained a total of ~1,088,000 recordings in which I identified ~650,000 bat passes belonging to 21 different sonotypes from six different families (Table 5.S1). Although almost all sonotypes were recorded in the four habitat types, for many species there were more recordings in secondary forest than in primary forest (Fig. 5.2). In fact, only six out of 21 sonotypes were more frequently recorded in primary forest (continuous or fragments) than in secondary forest (*Cormura brevirostris*, *Vespertilionidae 1*, *Furipterus horrens*, *Saccopteryx bilineata*, *Centronycteris maximiliani* and *S. leptura*), and only two species (*F. horrens* and *Vespertilionidae 1*) had occurrence frequencies greater than 50% in continuous forest. Most of the recordings were in fact either in forest fragment interiors or in forest fragment edges, although the patterns were highly species- specific. For example, both *Peropteryx kappleri and P. macrotis* had more recordings in forest edges while its sister species *C. brevirostris* was more represented in forest fragment interiors. No sonotype was exclusively recorded in a single habitat and almost all of them were recorded at least once in all four habitats (Fig. 5.2).

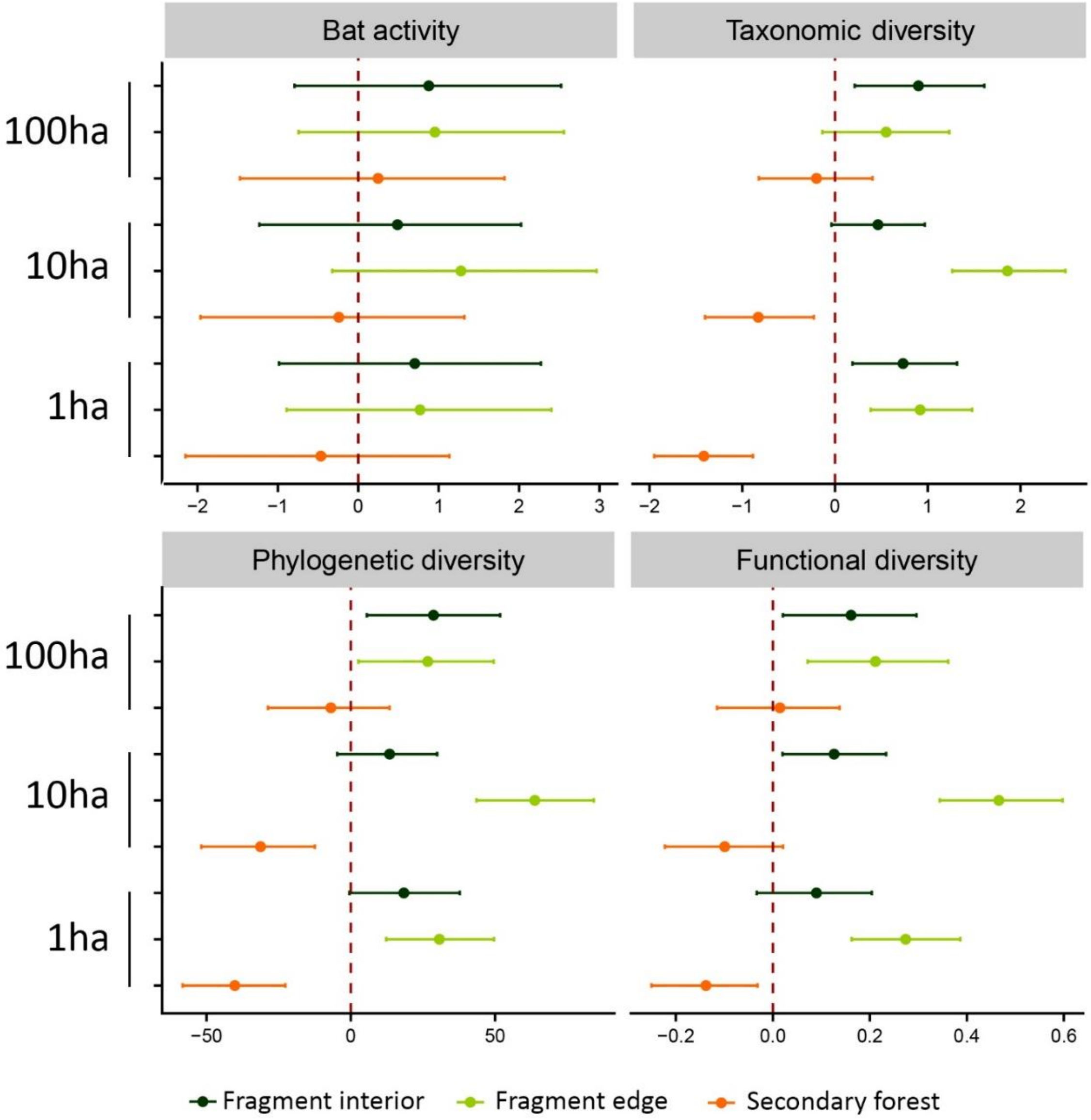


Figure 5.3. Comparison of total bat activity and three biodiversity metrics across different habitat types found at the Biological Dynamics of Forest Fragments Project (continuous forest interior, forest fragment interiors, forest fragment edges and adjoining secondary forest). The predicted differences between each habitat and continuous forest interior, modelled using MCMC GLMMs, are plotted with their corresponding 95% credible interval. Results are split for the three different forest fragment sizes.

The modelling results revealed that total bat activity was highly variable and independent of both fragment size and habitat (Fig. 5.3), but at the species level varied greatly amongst habitat types (Fig. 5.2 and S3). For several species commonly classified as open- or edge-space flyers (e.g. *Cormura brevirostris. Centronycteris maximiliani and Saccopteryx leptura*) activity in secondary forest, especially at sites adjacent to the smallest fragments, was consistently lower than in continuous forest. *Molossus* spp and *Myotis nigricans* were frequently recorded in secondary forest (Fig. 5.2). Both *Pteronotus rubiginosus and P. alitonus* showed high activity levels in edge habitats as well as in some secondary forest sites. Activity at the fragment edges was considerably higher compared to the other sampled habitats for species such as *Centronycteris maximiliani*, *Saccopteryx leptura* and *Peropteryx kappleri* (Fig. 5.S2), and markedly lower in continuous forest for most of the species (e.g. *S. leptura* and *P. rubiginosus*).

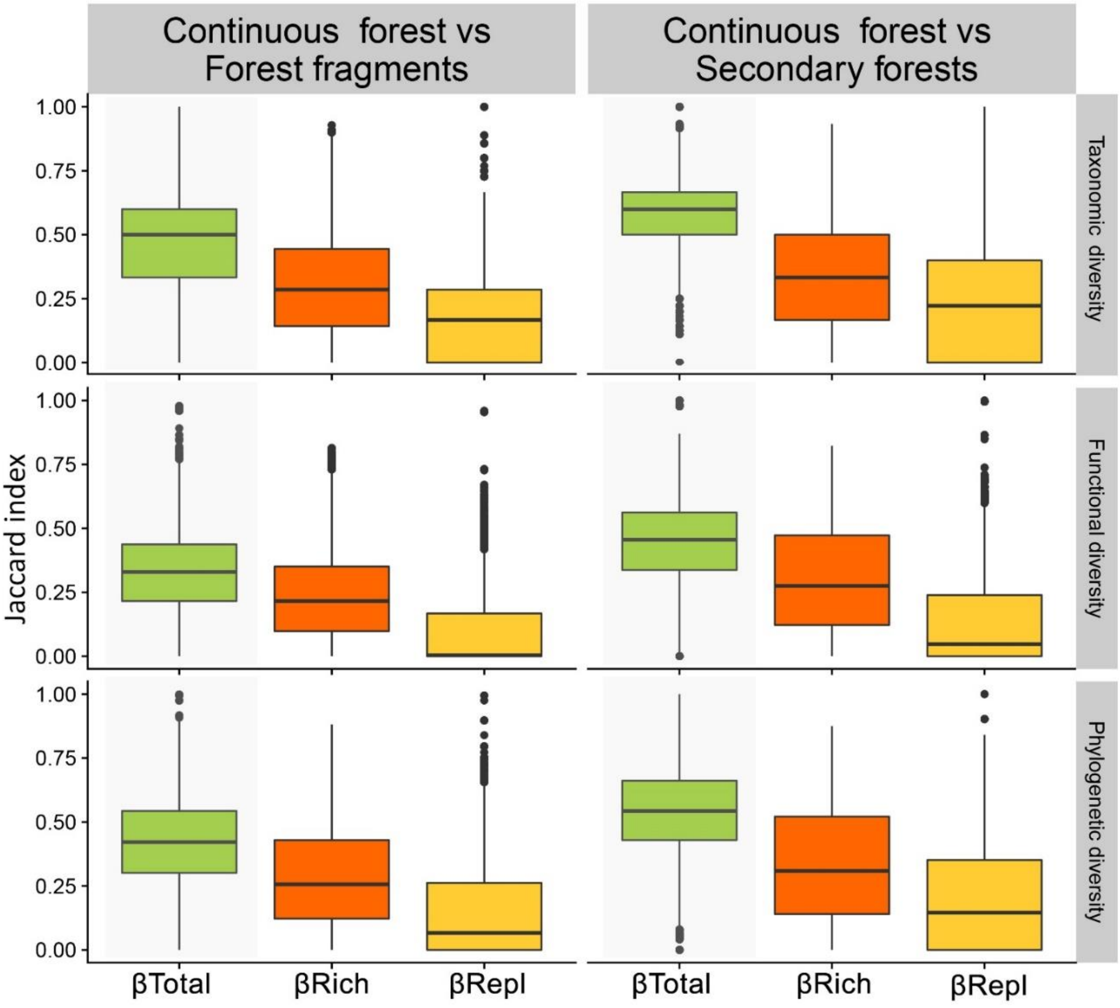


Figure 5.4 Comparison of the components of taxonomic, functional, and phylogenetic β diversity, between continuous forest, forest fragments and secondary forest. Values represent means (horizontal lines) ± 95% confidence levels (colour bars) and maximum and minimum (vertical lines). Points represent outliers. $\beta_{Total}$: total beta diversity; $\beta_{Repl}$, beta diversity due to replacement of species; $\beta_{Rich}$: beta diversity due to loss or gain of species.

Secondary forest harboured significantly lower TD, FD and PD than continuous forest interiors (Fig.5.3), with the exception of secondary forest adjacent to the 100 ha fragments for all three response variables, and secondary forest adjacent to the 10 ha fragment for FD. In these cases, I obtained slightly but non-significantly lower FD values (Fig.5.3). In fact, the negative effects found for secondary forest became gradually more pronounced with a reduction in fragment size, from 100 to 1 ha. Compared to continuous forest interiors, (i) 100 ha fragment interiors had higher TD, PD and FD, (ii) 10 ha fragment interiors had slightly higher FD, (iii) and 1 ha fragment interiors had higher TD. Edges of the smaller fragments had slightly but significantly higher TD, FD and PD than continuous forest (Fig. 5.3).

The differences in β total TD, FD and PD were always higher between continuous and secondary forest than between continuous forest and forest fragments (Fig. 5.4). β diversity partitioning showed that in all cases differences were due to the loss of species rather than to replacement by distinct species (Fig. 5.4).

## Discussion

In this study, I assessed TD, FD and PD of aerial insectivorous bat assemblages in forest fragments and secondary forests after ~30 years of natural regrowth and compared them with those in continuous forest. I show that, as predicted, although some recovery could be detected in the secondary forest (94% for TD, 84% for FD and 87% for PD), this time lapse was not enough for a full species recovery. However, and contrary to my hypotheses, continuous forest interiors were not significantly more diverse than fragment interiors. In terms of fragment size, I confirmed that the larger fragments (10 & 100 ha) and the secondary forest around them tended to harbour higher TD, FD and PD than the smaller (1 ha) fragments. The analyses also indicated that fragment edges are generally more diverse in terms of TD, FD and PD than the interiors of continuous forest and fragments. Conversely, bat activity was unaffected by habitat or fragment size. Regarding species-specific responses, and according to my predictions, high duty-cycle echolocators were more associated with the secondary forests (together with other species with peak frequencies above 100kHz). However, edge-foraging species such as emballonurid bats were more commonly detected in the fragment edges and forest fragment interiors (highly affected by near edges).

## Aerial insectivorous bat diversity in secondary forest

Despite the rapid forest regrowth typical in the tropics, aerial insectivorous bat assemblages in the secondary forest matrix were still far from being fully recovered regarding TD, FD and PD to levels observed in continuous forest interiors. These findings support similar studies carried out in the same area for phyllostomid bats (Rocha et al. 2017a, Farneda et al. 2018a, Farneda et al. 2018b) and others reporting slow species recovery worldwide (Dunn 2004, Derroire et al. 2016). For instance, about 88% of dung beetles and bird diversity was recovered only after 40 years of regeneration (Lennox et al. 2018); 50-80 years were required to recover tree species richness as reported by Martin et al. (2013), 40-50 years to recover ant assemblages (Belshaw and Bolton 1993), 100 for birds (Raman et al. 1998), or 30 for the full recovery of bird assemblages at the BDFFP (Powell et al. 2013, Powell et al. 2015).

Regenerating forests can provide new habitat for forest-dwelling species and contribute to the recovery of ecosystems in fragmented primary forests (Derroire et al. 2016). However, this process is strongly influenced by long-term successional dynamics, which result in successive changes or stages in their communities over time (González del Pliego et al. 2016). Although some species start foraging in early-stage regrowth, most forest-dwelling specialists remain restricted to continuous patches of old-growth forest and only start using secondary forests after long periods of time. This has been reported for ant assemblages in a mosaic of different secondary forests (Bihn et al. 2008). Slow recovery processes have also been reported in terms of microhabitat diversity between 14 and 35 year-old secondary forests (González del Pliego et al. 2016). Assessing species recovery in secondary forests after a major disturbance allows us to understand the resilience of these habitats through successional processes and predict their conservation status at each specific recovery stage (Derroire et al. 2016).

The loss of TD and FD in the secondary forests sampled in the BDFFP is likely to result in an impoverishment of the ecosystem services provided by aerial insectivorous bats such as insect population control (Kunz et al. 2011, Puig-Montserrat et al. 2015). In terms of PD, the fact that it is also lower in the secondary forests reflects a depletion of evolutionary richness due to the loss of certain species (Struebig et al. 2011, Rivera-Ortíz et al. 2014, Valente et al. 2017). The close relationship between phylogenetic diversity decrease and the lower complexity of secondary forests compared to continuous forest suggests that habitat fragmentation reduces total evolutionary history by eliminating distantly-related species in less complex habitat. However, other studies provide, to some extent, different results. Edwards et al. (2017) found that bird assemblages in Colombia were fully recovered in terms of phylogenetic diversity in 30-year-old secondary forests, highlighting the importance of secondary forests, not only regarding carbon uptake or as species reservoirs (Bongers et al. 2015), but also for maintaining the evolutionary history within a community.

## Aerial insectivorous bat diversity in forest fragments

I did not find any local extinctions in the BDFFP forest fragments. In fact, fragment assemblages were either not significantly different from continuous forest or slightly more diverse, especially the 100 ha fragments. However, similar studies conducted at the BDFFP on phyllostomid bat assemblages found significantly lower TD, FD and PD in the fragments than in continuous forest (Anita et al. submitted, Rocha et al. 2017a, Farneda et al. 2018a), suggesting that the effects of fragmentation are strongly species- or guild-specific. The fact that TD, FD and PD in secondary forests next to the largest fragments (10 and 100 ha) were higher than in those adjoining smaller fragments (1 ha) indicates that in the study area, the largest fragments act as important reservoirs of aerial insectivorous bat diversity for the regenerating areas.

Local extinctions due to fragmentation have mostly been reported for forest fragments isolated by a “hard” matrix like water in recently-constructed dams (e.g. Estrada-Villegas et al. 2010, Benchimol and Peres 2015a, Benchimol and Peres 2015b, Aurélio-Silva et al. 2016, Jones et al. 2016). However, in continental forests, the extinction or the return of sensitive animal species in secondary forests are processes usually targeted by conservationists. The extinction of one common insectivorous bird species in secondary forests younger than 27-31 years was reported in Brazil (Powell et al. 2016). Similarly, 47 bird species went extinct around the Brazilian city of Belem after more than 200 years of forest loss and degradation (Moura et al. 2014). The major differences between these scenarios lie in the nature of the matrix. While water matrices often create impermeable barriers, secondary forests positively contribute to the recovery of fragmented natural communities by facilitating animal movements (Chazdon et al. 2009). In addition to matrix type, time since fragment isolation is another critical factor influencing ecosystem dynamics, as it takes a very long time for the full effects of isolation to manifest (Ewers and Didham 2006a, Rainho and Palmeirim 2017).

## Aerial insectivorous bats diversity at fragment edges

For phyllostomids, Rocha et al. (2018) found that old-growth specialists greatly benefited from the secondary forest regrowth as they were captured significantly more often in fragment interiors (where they roost and forage) after several years of vegetation recovery in the surrounding secondary forest. Regarding phyllostomid bats, forest interiors almost always had higher TD and FD compared to the fragment edges and secondary forest. However, for aerial insectivorous bats, TD, FD and PD peaked at the primary-secondary forest interface, probably because these habitats simultaneously harbour edge-, open- and interior- specialists, and because edges can also function as corridors. Edges being used as commuting corridors has already been reported for bats (Jantzen and Fenton 2013, Kalcounis-Rueppell et al. 2013) as well as for birds (Zurita et al. 2012, Zurita and Bellocq 2012, Fonderflick et al. 2013, Powell et al. 2015). Although some research has addressed the effects of fragmentation on FD of phyllostomid bats (Cisneros et al. 2014b, Farneda et al. 2015, Garcia-Morales et al. 2016, Farneda et al. 2018a), none of them has specifically looked at the edges of the forest fragments.

In line with my results, in a land-bridge island system, Estrada-Villegas et al. (2010) found that small islands (equivalent to small terrestrial fragments embedded in a high-contrast matrix) had higher richness than mainland sites (equivalent to continuous forest). Edge effects can penetrate large distances into the forest, influencing animal assemblages in several ways, up until 150 m for primates (Lenz et al. 2014), 1.5 km for birds (Zurita et al. 2012), 1 km for invertebrates (Ewers and Didham 2006b); or 1.5 km (Delaval and Charles-Dominique 2006), 80 m (Jantzen and Fenton 2013) and 1 km (Yoh 2018) for bats. In general, these distances are remarkably larger than my smaller sampled fragments size. However, at guild-level, forest bat species tended to show clear negative responses to fragmentation, some of them being severely affected by island size and isolation (Estrada-Villegas et al. 2010). Bat activity in this land-bridge island system was highly related to species-specific requirements and their particular functional traits.

## Species-specific responses to fragmentation

Areas that were cleared in the 1980s at the BDFFP soon became densely covered by young tree stems and lianas (Berenguer et al. 2018), turning them into highly cluttered habitats, a characteristic that was still discernible during my sampling period (see Rocha et al. 2017a). The nature of secondary forests inevitably hinders foraging by some bat species. This has also been described by Blakey et al. (2016) for Australian forests, who established a clutter threshold of 1100 stems/ha, above which bat activity was markedly lower. Flight performance and manoeuvrability are some of the most limiting factors for bats to select their foraging habitats (Norberg and Rayner 1987, Kalko and Handley 2001). Some edge-foraging species such as *Centronycteris maximiliani, Cormura brevirostris, Saccopteryx bilineata* or vespertilionid bats selected primary forest interiors and edges to forage (Fig. 5.S2), probably because the presence of old dead and fallen trees might have created small forest gaps, where they are assumed to forage (Jakobsen et al. 2012, Asner et al. 2013, Rodríguez-Posada 2016).

The observed differences in β FD between continuous forest and forest fragments as well as between continuous forest and secondary forest might be directly linked to each family´s flight performance and/or type of echolocation, which are adapted to specific habitats (Norberg and Rayner 1987, Fraixedas 2017). Lower functional beta diversity for secondary forests than for fragments compared to continuous forest was mostly due to the loss of certain species rather than species turnover (Fig. 5.4). While species adapted to secondary regrowth are likely to also be eventually detected in primary forest, the same does not happen the other way around. High duty-cycle echolocators such as *Pteronotus rubiginosus* and *P. alitonus* (*Pteronotus* sp. 3 in López-Baucells et al. 2017) are well-adapted to forage in cluttered forests where they minimize competitive pressure for food resources as they occupy a relatively free acoustic niche (Siemers and Schnitzler 2004). Therefore, although they were also found in primary forest interiors, I can assume they are generally favoured by forest recovery, since vegetation regrowth tends to be dense and cluttered.

In this study, almost all molossid bats seemed to be mostly associated with the secondary forest (Fig. 5.S2), which could probably be due to changes in their flight behaviour related to each of the sampled habitats and thus, due to differences in detectability. These species tend to forage in open areas at high altitude over the forest canopy, or over disturbed habitats such as pastures or urban spaces (Adams 2012, Bader et al. 2015, Jung and Threlfall 2016). Due to the lower height of secondary regrowth, they probably fly lower and therefore, their calls become more easily detectable in secondary forest compared to continuous forest. This bias likely also applies to other species due to variation in assemblage composition across vertical forest strata (Pereira et al. 2010, Marques et al. 2015). Insect abundance at the highest forest layers could also vary substantially between primary and secondary forests, and consequently influence patterns of bat foraging activity (Brandon-Mong et al. 2018).

This study complements similar work conducted previously in the same area (Rocha 2017, Rocha et al. 2017a, Rocha et al. 2017b, Farneda et al. 2018a, Rocha et al. 2018), in which the role of landscape composition was studied for phyllostomid bats. The combination of bat works carried out until today in the BDFFP represents a unique project for which information about the consequences of forest fragmentation are assessed at multiple taxonomic levels simultaneously (including phyllostomid and non-phyllostomid bats), with data at species-, assemblage- level as well as for a variety of diversity metrics.

## Overall fragmentation effects on aerial insectivorous bats and landscape management

The mosaic of forests of different ages in the study landscape might help to boost aerial insectivorous bat diversity, favouring most of the species and disfavouring just a few. Which ones are favoured mainly depends on how their intrinsic functional traits and phylogenetic history increase their adaptation capacity (Klingbeil and Willig 2009, Fraixedas 2017, Farneda et al. 2018b). This has already been described by Connell (1978) as the Intermediate Disturbance Hypothesis, which suggests that richness peaks at intermediate intensities or frequencies of disturbance. Nevertheless, despite these apparent positive effects, it is essential to gain further insights into which species' populations are most sensitive to fragmentation and design management plans according to their respective conservation status (Vieira et al. 2014).

Deforestation in the Amazon is likely to increase again due to the expansion of new agricultural opportunities such as soya or palm oil (Hansen et al. 2013, FAO 2016, Rosa et al. 2017). Favouring diverse habitat mosaics is increasingly recognized as an appropriate landscape management tool to promote conservation and improve sustainability practices (Chazdon 2014). However, since species responses are very specific (Rodríguez-San Pedro and Simonetti 2015, Moura et al. 2016), legal protection should especially target the most sensitive species (Vieira et al. 2014). This study adds evidence to the fact that assemblage recovery in terms of TD, FD and PD can only be achieved over the very long term (>30 years at least). Old secondary forests might only represent intermediate stages in the middle of a very long process of regeneration. Therefore, the irreplaceable value of continuous primary forest and large fragments should never be forgotten (Barlow et al. 2007, Gibson et al. 2011, Laurance et al. 2017).

## Acknowledgements

I would like to thank Oriol Massana, Diogo Ferreira, Marta Acácio and Fabio Farneda, Madalena Boto, Maria Mas, Gilberto Fernández, Eva Sánchez for fieldwork assistance and José Luis Camargo, Rosely Hipólito, Ary Jorge Ferreira, Luiz Quiroz, Leo and Josimar for logistical support. Xavier Puig-Montserrat and Carles Flaquer also deserve special mention for their contribution on the discussion and continuous support. This work was supported by the Portuguese Foundation for Science and Technology under grants [PTDC/BIABIC/111184/2009] (CM), [SFRH/BD/80488/2011] (RR), [PD/BD/52597/2014] (ALB) and by the CNPq by the fellowship [160049/2013-0] (PEDB). Additional funding was provided by a Bat Conservation International student research fellowship to ALB and RR.

# References

Adams, M. D. 2012. Vertical stratification of insectivorous bats (Microchiroptera) in haversted forests: assessing the role of structural clutter in shaping patterns of flight activity. PhD Thesis. University of Wollongong, Wollongong, Australia.

Appel, G., A. López-Baucells, E. Magnusson, and P. E. D. Bobrowiec. 2017. Aerial insectivorous bat activity in relation to moonlight intensity. Mammalian Biology **85**:37-46.

Arroyo-Rodríguez, V., C. Rojasa, R. A. Saldaña-Vázqueza, and K. E. Stonerb. 2016. Landscape composition is more important than landscape configuration for phyllostomid bat assemblages in a fragmented biodiversity hotspot. Biological Conservation **198**:84-92.

Asner, G. P., J. R. Kellner, T. Kennedy-Bowdoin, D. E. Knapp, C. Anderson, and R. E. Martin. 2013. Forest Canopy Gap Distributions in the Southern Peruvian Amazon. PLoS ONE **8**:e60875.

Aurélio-Silva, M., M. Anciães, L. M. P. Henriques, M. Benchimol, and C. A. Peres. 2016. Patterns of local extinction in an Amazonian archipelagic avifauna following 25years of insularization. Biological Conservation **199**:101-109.

Avila-Cabadilla, L. D., G. A. Sanchez-Azofeifa, K. E. Stoner, M. Y. Alvarez-Añorve, M. Quesada, and C. A. Portillo-Quintero. 2012. Local and landscape factors determining occurrence of phyllostomid bats in tropical secondary forests. PLoS ONE **7**:e35228.

Avila-Cabadilla, L. D., K. E. Stoner, J. M. Nassar, M. M. Espírito-Santo, M. Y. Alvarez-Añorve, C. I. Aranguren, M. Henry, J. A. González-Carcacía, L. A. Dolabela Falcão, and G. A. Sanchez-Azofeifa. 2014. Phyllostomid bat occurrence in successional stages of Neotropical dry forests. PLoS ONE **9**:e84572.

Bader, E., K. Jung, E. K. V. Kalko, R. A. Page, R. Rodriguez, and T. Sattler. 2015. Mobility explains the response of aerial insectivorous bats to anthropogenic habitat change in the Neotropics. Biological Conservation **186**:97-106.

Barlow, J., L. A. Mestre, T. A. Gardner, and C. A. Peres. 2007. The value of primary, secondary and plantation forests for Amazonian birds. Biological Conservation **136**:212-231.

Belshaw, R., and B. Bolton. 1993. The effect of forest disturbance on the leaf litter ant fauna in Ghana. Biodiversity & Conservation **2**:656-666.

Benchimol, M., and C. A. Peres. 2015a. Predicting local extinctions of Amazonian vertebrates in forest islands created by a mega dam. Biological Conservation **187**:61-72.

Benchimol, M., and C. A. Peres. 2015b. Widespread forest vertebrate extinctions induced by a mega-hydroelectric dam in lowland Amazonia. PLoS ONE **10**:e0129818.

Berenguer, E., T. A. Gardner, J. Ferreira, L. E. O. C. Aragão, R. Mac Nally, J. R. Thomson, I. C. G. Vieira, and J. Barlow. 2018. Seeing the woods through the saplings: Using wood density to assess the recovery of human-modified Amazonian forests. Journal of Ecology **106**:2190-2203.

Bernard, E., V. C. Tavares, and E. Sampaio. 2011. Updated compilation of bat species (Chiroptera) for the Brazilian Amazonia. Biota Neotropica **11**:35-46.

Bihn, J. H., M. Verhaagh, M. Brändle, and R. Brandl. 2008. Do secondary forests act as refuges for old growth forest animals? Recovery of ant diversity in the Atlantic forest of Brazil. Biological Conservation **141**:733-743.

Blakey, R. V., B. S. Law, R. T. Kingsford, J. Stoklosa, P. Tap, and K. Williamson. 2016. Bat communities respond positively to large-scale thinning of forest regrowth. Journal of Applied Ecology **53**:1694-1703.

Bongers, F., R. Chazdon, L. Poorter, and M. Peña-Claros. 2015. The potential of secondary forests. Science **348**:642-643.

Botta-Dukát, Z. 2005. Rao's quadratic entropy as a measure of functional diversity based on multiple traits. Journal of Vegetation Science **16**:533-540.

Bowen, M. E., C. A. McAlpine, A. P. N. House, and G. C. Smith. 2007. Regrowth forests on abandoned agricultural land: A review of their habitat values for recovering forest fauna. Biological Conservation **140**:273-296.

Boyle, S. A., B. B. Lenz, K. A. Gilbert, W. R. Sprionello, M. S. Gómez, E. Z. Setz, A. M. dos Reis, O. F. da Silva, A. Keuroghlian, and F. Pinto. 2013. Primates of the biological dynamics of forest fragments project: A history. Pages 57-74 *in* L. K. Marsh and C. A. Chapman, editors. Primates in Fragments. Springer, New York, United States of America.

Brandon-Mong, G. J., J. E. Littlefair, K. W. Sing, Y. P. Lee, H. M. Gan, E. L. Clare, and J. J. Wilson. 2018. Temporal changes in arthropod activity in tropical anthropogenic forests. Bulletin of Entomological Research:1-8.

Burgin, C. J., J. P. Colella, P. L. Kahn, and N. S. Upham. 2018. How many species of mammals are there? Journal of Mammalogy **99**:1-14.

Butler, R. A., and W. F. Laurance. 2009. Is oil palm the next emerging threat to the Amazon? Tropical Conservation Science **2**:1-10.

Cardoso, P., F. Rigal, J. C. Carvalho, M. Fortelius, P. A. Borges, J. Podani, and D. Schmera. 2014. Partitioning taxon, phylogenetic and functional beta diversity into replacement and richness difference components. Journal of Biogeography **41**:749-761.

Cardoso, P., F. Rigal, J. C. Carvalho, and S. Kembel. 2015. BAT – Biodiversity Assessment Tools, an R package for the measurement and estimation of alpha and beta taxon, phylogenetic and functional diversity. Methods in Ecology and Evolution **6**:232-236.

Carreiras, J. M. B., J. Jones, R. M. Lucas, and C. Gabriel. 2014. Land use and land cover change dynamics across the Brazilian Amazon: insights from extensive time-series analysis of remote sensing data. PLoS ONE **9**:e104144.

Cisneros, L. M., K. R. Burgio, L. M. Dreiss, B. T. Klingbeil, B. D. Patterson, S. J. Presley, and M. R. Willig. 2014a. Multiple dimensions of bat biodiversity along an extensive tropical elevational gradient. Journal of Animal Ecology **83**:1124-1136.

Cisneros, L. M., M. E. Fagan, and M. R. Willig. 2014b. Effects of human-modified landscapes on taxonomic, functional and phylogenetic dimensions of bat biodiversity. Diversity and distributions **21**:523-533.

Connell, J. H. 1978. Diversity in tropical rain forests and coral reefs. Science **199**:1302-1310.

Chazdon, R. L. 2014. Second growth: The promise of tropical forest regeneration in an age of deforestation. University of Chicago Press, Connecticut, United States of America.

Chazdon, R. L., C. A. Peres, D. Dent, D. Sheil, A. E. Lugo, D. Lamb, N. E. Stork, and S. E. Miller. 2009. The potential for species conservation in tropical secondary forests. Conservation Biology **23**:1406-1417.

Daily, G. C. 1997. Countryside biogeography and the provision of ecosystem services. Pages 104-113 *in* Raven, editor. Nature and human society: The quest for a sustainable world. National Academy Press, Washington, United States of America.

Davidson, E. A., A. C. de Araujo, P. Artaxo, J. K. Balch, I. F. Brown, M. M. C. Bustamante, M. T. Coe, R. S. DeFries, M. Keller, M. Longo, J. W. Munger, W. Schroeder, B. S. Soares-Filho, C. M. Souza, and S. C. Wofsy. 2012. The Amazon basin in transition. Nature **481**:321-328.

Delaval, M., and P. Charles-Dominique. 2006. Edge effects on frugivorous and nectarivorous bat communities in a neotropical primary forest in French Guiana. Revue d'écologie **61**:343-352.

Derroire, G., P. Balvanera, C. Castellanos-Castro, G. Decocq, D. K. Kennard, E. Lebrija-Trejos, J. A. Leiva, P.-C. Odén, J. S. Powers, V. Rico-Gray, M. Tigabu, and J. R. Healey. 2016. Resilience of tropical dry forests - a meta-analysis of changes in species diversity and composition during secondary succession. Oikos **125**:1386-1397.

Dirzo, R., H. S. Young, M. Galetti, G. Ceballos, N. J. B. Isaac, and B. Collen. 2014. Defaunation in the Anthropocene. Science **345**:401-406.

Dunn, R. R. 2004. Recovery of faunal communities during tropical forest regeneration. Conservation Biology **18**:302-309.

Edwards, D. P., M. R. Massam, T. Haugaasen, and J. J. Gilroy. 2017. Tropical secondary forest regeneration conserves high levels of avian phylogenetic diversity. Biological Conservation **209**:432-439.

Estrada-Villegas, S., C. F. Meyer, and E. K. Kalko. 2010. Effects of tropical forest fragmentation on aerial insectivorous bats in a land-bridge island system. Biological Conservation **143**:597-608.

Ewers, R. M., and R. K. Didham. 2006a. Confounding factors in the detection of species responses to habitat fragmentation. Biological Reviews **81**:117-142.

Ewers, R. M., and R. K. Didham. 2006b. Continuous response functions for quantifying the strength of edge effects. Journal of Applied Ecology **43**:527-536.

Faith, D. P. 1992. Conservation evaluation and phylogenetic diversity. Biological Conservation **61**:1-10.

FAO. 2016. State of the World's forests. Food and Agriculture Organization of the United Nations, Rome, Italy.

Farneda, F. Z., R. Rocha, A. López-Baucells, M. Groenenberg, I. Silva, J. M. Palmeirim, P. E. D. Bobrowiec, C. F. J. Meyer, and M. Pocock. 2015. Trait-related responses to habitat fragmentation in Amazonian bats. Journal of Applied Ecology **52**:1381-1391.

Farneda, F. Z., R. Rocha, A. López-Baucells, E. M. Sampaio, J. M. Palmeirim, P. E. D. Bobrowiec, C. E. V. Grelle, and C. F. J. Meyer. 2018a. Functional recovery of Amazonian bat assemblages following secondary forest succession. Biological Conservation **218**:192-199.

Farneda, F. Z., R. Rocha, A. López-Baucells, E. M. Sampaio, J. M. Palmeirim, P. E. D. Bobrowiec, C. E. V. Grelle, and C. F. J. Meyer. 2018b. The road to functional recovery: temporal effects of matrix regeneration on Amazonian bats. Tropical Conservation Science **11**:1-4.

Fáveri, S. B., H. L. Vasconcelos, and R. Dirzo. 2008. Effects of Amazonian forest fragmentation on the interaction between plants, insect herbivores, and their natural enemies. Journal of Tropical Ecology **24**:57-64.

Ferreira, D. F., R. Rocha, A. López-Baucells, F. Z. Farneda, J. M. B. Carreiras, J. M. Palmeirim, and C. F. J. Meyer. 2017. Season-modulated responses of Neotropical bats to forest fragmentation. Ecology and Evolution **7**:4059-4071.

Foley, J. A., N. Ramankutty, K. A. Brauman, E. S. Cassidy, J. S. Gerber, M. Johnston, N. D. Mueller, C. O'Connell, D. K. Ray, P. C. West, C. Balzer, E. M. Bennett, S. R. Carpenter, J. Hill, C. Monfreda, S. Polasky, J. Rockstrom, J. Sheehan, S. Siebert, D. Tilman, and D. P. M. Zaks. 2011. Solutions for a cultivated planet. Nature **478**:337-342.

Fonderflick, J., A. Besnard, and J.-L. Martin. 2013. Species traits and the response of open-habitat species to forest edge in landscape mosaics. Oikos **122**:42-51.

Fraixedas, S. 2017. Which functional traits make Neotropical aerial insectivorous bats more vulnerable to forest fragmentation? MSc Thesis. Autonomous University of Barcelona, Barcelona, Spain.

García-García, J. L., A. Santos-Moreno, and C. Kraker-Castañeda. 2014. Ecological traits of phyllostomid bats associated with sensitivity to tropical forest fragmentation in Los Chimalapas, Mexico. Tropical Conservation Science **7**:457-474.

Garcia-Morales, R., C. E. Moreno, E. I. Badano, I. Zuria, J. Galindo-Gonzalez, A. E. Rojas-Martinez, and E. S. Avila-Gomez. 2016. Deforestation impacts on bat functional diversity in tropical landscapes. PLoS ONE **11**:e0166765.

Gibson, L., T. M. Lee, L. P. Koh, B. W. Brook, T. A. Gardner, J. Barlow, C. A. Peres, C. J. A. Bradshaw, W. F. Laurance, T. E. Lovejoy, and N. S. Sodhi. 2011.

Primary forests are irreplaceable for sustaining tropical biodiversity. Nature **478**:378.

González del Pliego, P., B. R. Scheffers, E. W. Basham, P. Woodcock, C. Wheeler, J. J. Gilroy, C. A. Medina Uribe, T. Haugaasen, R. P. Freckleton, and D. P. Edwards. 2016. Thermally buffered microhabitats recovery in tropical secondary forests following land abandonment. Biological Conservation **201**:385-395.

Haddad, N. M., L. A. Brudvig, J. Clobert, K. F. Davies, A. Gonzalez, R. D. Holt, T. E. Lovejoy, J. O. Sexton, M. P. Austin, and C. D. Collins. 2015. Habitat fragmentation and its lasting impact on Earth's ecosystems. Science advances **1**:e1500052.

Hadfield, T. 2017. MCMCglmm Course Notes. Report, United Kingdom.

Hansen, M. C., P. V. Potapov, R. Moore, M. Hancher, S. A. Turubanova, A. Tyukavina, D. Thau, S. V. Stehman, S. J. Goetz, T. R. Loveland, A. Kommareddy, A. Egorov, L. Chini, C. O. Justice, and J. R. G. Townshend. 2013. High-resolution global maps of 21st-century forest cover change. Science **342**:850-853.

Jakobsen, L., E. K. V. Kalko, and A. Surlykke. 2012. Echolocation beam shape in emballonurid bats, *Saccopteryx bilineata* and *Cormura brevirostris*. Behavioral Ecology and Sociobiology **66**:1493-1502.

Jantzen, M. K., and M. B. Fenton. 2013. The depth of edge influence among insectivorous bats at forest–field interfaces. Canadian Journal of Zoology **91**:287-292.

Jones, I. L., N. Bunnefeld, A. S. Jump, C. A. Peres, and D. H. Dent. 2016. Extinction debt on reservoir land-bridge islands. Biological Conservation **199**:75-83.

Jung, K., and C. G. Threlfall. 2016. Urbanisation and its effects on bats - A global meta-analysis. Pages 13-33 *in* C. Voigt, editor. Bats in the Anthropocene: Conservation of Bats in a Changing World Springer, New York, United States of America.

Kalcounis-Rueppell, M. C., K. M. Briones, J. A. Homyack, R. Petric, M. M. Marshall, and D. A. Miller. 2013. Hard forest edges act as conduits, not filters, for bats. Wildlife Society Bulletin **37**:571-576.

Kalko, E. K. V., and C. O. Handley. 2001. Neotropical bats in the canopy: diversity, community structure, and implications for conservation. Plant Ecology **153**:319-333.

Klingbeil, B. T., and M. R. Willig. 2009. Guild-specific responses of bats to landscape composition and configuration in fragmented Amazonian rainforest. Journal of Applied Ecology **46**:203-213.

Klingbeil, B. T., and M. R. Willig. 2010. Seasonal differences in population-, ensemble- and community- level responses of bats to landscape structure in Amazonia. Oikos **119**:1654-1664.

Kryvokhyzha, D., K. Holm, J. Chen, A. Cornille, S. Glemin, S. I. Wright, U. Lagercrantz, and M. Lascoux. 2016. The influence of population structure on gene expression and flowering time variation in the ubiquitous weed *Capsella bursa-pastoris* (Brassicaceae). Molecular Ecology **25**:1106-1121.

Kunz, T. H., E. B. de Torrez, D. Bauer, T. Lobova, and T. H. Fleming. 2011. Ecosystem services provided by bats. Annals of the New York Academy of Sciences **1223**:1-38.

Laurance, W. F., J. L. C. Camargo, P. M. Fearnside, T. E. Lovejoy, G. B. Williamson, R. C. G. Mesquita, C. F. J. Meyer, P. E. D. Bobrowiec, and S. G. W. Laurance. 2017. An Amazonian rainforest and its fragments as a laboratory of global change. Biological Reviews of the Cambridge Philosophical Society **93**:223-247.

Laurance, W. F., J. L. C. Camargo, R. C. C. Luizao, S. G. Laurance, S. L. Pimm, E. M. Bruna, P. C. Stouffer, G. B. Williamson, J. Benitez-Malvido, H. L. Vasconcelos, K. S. Van Houtan, C. E. Zartman, S. A. Boyle, R. K. Didham, A. Andrade, and T. E. Lovejoy. 2011. The fate of Amazonian forest fragments: A 32-year investigation. Biological Conservation **144**:56–67.

Lees, A. C., C. A. Peres, P. M. Fearnside, M. Schneider, and J. A. Zuanon. 2016. Hydropower and the future of Amazonian biodiversity. Biodiversity & Conservation **25**:451-466.

Lennox, G. D., T. A. Gardner, J. R. Thomson, J. Ferreira, E. Berenguer, A. C. Lees, R. Mac Nally, L. Aragao, S. F. B. Ferraz, J. Louzada, N. G. Moura, V. H. F. Oliveira, R. Pardini, R. R. C. Solar, F. Z. Vaz-de Mello, I. C. G. Vieira, and J. Barlow. 2018. Second rate or a second chance? Assessing biomass and biodiversity recovery in regenerating Amazonian forests. Global Change Biology:(in press).

Lenz, B. B., K. M. Jack, and W. R. Spironello. 2014. Edge effects in the primate community of the biological dynamics of forest fragments project, Amazonas, Brazil. American Journal of Physical Anthropology **155**:436-446.

Lintott, P. R., E. Fuentes-Montemayor, D. Goulson, and K. J. Park. 2014. Testing the effectiveness of surveying techniques in determining bat community composition within woodland. Wildlife Research **40**:675-684.

López-Baucells, A., R. Rocha, P. E. D. Bobrowiec, E. Bernard, J. Palmeirim, and C. Meyer. 2016. Field Guide to Amazonian Bats. INPA, Manaus, Brazil.

López-Baucells, A., L. Torrent, R. Rocha, A. C. Pavan, P. E. D. Bobrowiec, and C. F. J. Meyer. 2017. Geographical variation in the high-duty cycle echolocation of the cryptic common mustached bat *Pteronotus* cf. *rubiginosus* (Mormoopidae). Bioacoustics Journal **27**:341-357.

MacArthur, R. H., and E. O. Wilson. 1967. Theory of Island Biogeography. Princeton University Press, Princeton, United States of America.

Maechler, M., P. Rousseeuw, A. Struyf, M. Hubert, and K. Hornik. 2012. Cluster: cluster analysis basics and extensions. R package version 2.0.7-1.

Marques, J. T., M. J. Ramos Pereira, and J. M. Palmeirim. 2015. Patterns in the use of rainforest vertical space by Neotropical aerial insectivorous bats: all the action is up in the canopy. Ecography **39**:476-486.

Martin, P. A., A. C. Newton, and J. M. Bullock. 2013. Carbon pools recover more quickly than plant biodiversity in tropical secondary forests. Proceedings of the Royal Society B-Biological Sciences **280**:20132236.

Mendenhall, C. D., C. V. Kappel, and P. R. Ehrlich. 2013. Countryside Biogeography. Pages 347-360 *in* S. A. Levin, editor. Encyclopedia of Biodiversity. Academic Press, Waltham, United States of America.

Mendenhall, C. D., D. S. Karp, C. F. J. Meyer, E. A. Hadly, and G. C. Daily. 2014. Predicting biodiversity change and averting collapse in agricultural landscapes. Nature **509**:213-217.

Millon, L., J.-F. Julien, R. Julliard, and C. Kerbiriou. 2015. Bat activity in intensively farmed landscapes with wind turbines and offset measures. Ecological Engineering **75**:250-257.

Morato, E. F. 2001. Efeitos da fragmentação florestal sobre vespas e abelhas solitárias na Amazônia Central. II. estratificação vertical. Revista Brasileira De Zoologia **18**:737-747.

Moura, N. G., A. C. Lees, A. Aleixo, J. Barlow, E. Berenguer, J. Ferreira, R. Mac Nally, J. R. Thomson, and T. A. Gardner. 2016. Idiosyncratic responses of Amazonian birds to primary forest disturbance. Oecologia **180**:903-916.

Moura, N. G., A. C. Lees, A. Aleixo, J. O. S. Barlow, S. M. Dantas, J. Ferreira, M. D. E. F. C. Lima, and T. A. Gardner. 2014. Two hundred years of local avian extinctions in Eastern Amazonia. Conservation Biology **28**:1271-1281.

Muylaert, R. L., R. D. Stevens, and M. C. Ribeiro. 2016. Threshold effect of habitat loss on bat richness in cerrado-forest landscapes. Ecological Applications **26**:1854-1867.

Neeff, T., R. M. Lucas, J. R. d. Santos, E. S. Brondizio, and C. C. Freitas. 2006. Area and age of secondary forests in Brazilian Amazonia 1978-2002: An Empirical Estimate. Ecosystems **9**:609-623.

Nepstad, D., D. McGrath, C. Stickler, A. Alencar, A. Azevedo, B. Swette, T. Bezerra, M. DiGiano, J. Shimada, R. Seroa da Motta, E. Armijo, L. Castello, P. Brando, M. C. Hansen, M. McGrath-Horn, O. Carvalho, and L. Hess. 2014. Slowing Amazon deforestation through public policy and interventions in beef and soy supply chains. Science **344**:1118-1123.

Nogueira, M. R., I. P. de Lima, R. Moratelli, V. C. Tavares, R. Gregorin, and A. L. Peracchi. 2014. Checklist of Brazilian bats, with comments on original records. Check List **10**:808-821.

Norberg, U. M., and J. M. V. Rayner. 1987. Ecological morphology and flight in bats (Mammalia, Chiroptera) - wing adaptations, flight performance, foraging strategy and echolocation. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences **316**:337-419.

Numata, I., and M. A. Cochrane. 2012. Forest fragmentation and its potential implications in the Brazilian Amazon between 2001 and 2010. Open Journal of Forestry **2**:265-271.

Pereira, M. J. R., J. T. Marques, and J. M. Palmeirim. 2010. Vertical stratification of bat assemblages in flooded and unflooded Amazonian forests. Current Zoology **56**:469-478.

Petchey, O. L., and K. J. Gaston. 2002. Functional diversity (FD), species richness and community composition. Ecology Letters **5**:402-411.

Petchey, O. L., and K. J. Gaston. 2006. Functional diversity: back to basics and looking forward. Ecology Letters **9**:741-758.

Powell, L. L., P. C. Stouffer, and E. I. Johnson. 2013. Recovery of understory bird movement across the interface of primary and secondary amazon rainforest. The Auk **130**:459-468.

Powell, L. L., J. D. Wolfe, E. I. Johnson, and P. C. Stouffer. 2016. Forest recovery in post-pasture Amazonia: Testing a conceptual model of space use by insectivorous understory birds. Biological Conservation **194**:22-30.

Powell, L. L., G. Zurita, J. D. Wolfe, E. I. Johnson, and P. C. Stouffer. 2015. Changes in habitat use at rain forest edges through succession: a case study of understory birds in the Brazilian Amazon. Biotropica **47**:723-732.

Puig-Montserrat, X., I. Torre, A. López-Baucells, E. Guerrieri, M. M. Monti, R. Rafols-Garcia, X. Ferrer, D. Gisbert, and C. Flaquer. 2015. Pest control service provided by bats in Mediterranean rice paddies: linking agroecosystems structure to ecological functions. Mammalian Biology **80**:237-245.

Querino, R., S. Couceiro, L. Queiroz, and A. Penteado-Dias. 2011. The spatial distribution of Hymenoptera parasitoids in a forest reserve in Central Amazonia, Manaus, AM, Brazil. Brazilian Journal of Biology **71**:865-871.

Rainho, A., and J. M. Palmeirim. 2017. Understanding the long term consequences of fragmentation: lessons from the bats of Bijagós (Guinea-Bissau, West Africa). Hystrix **28**:173–179.

Raman, T. R. S., G. S. Rawat, and A. J. T. Johnsingh. 1998. Recovery of tropical rainforest avifauna in relation to vegetation succession following shifting cultivation in Mizoram, north-east India. Journal of Applied Ecology **35**:214-231.

Rivera-Ortíz, F. A., R. Aguilar, M. D. C. Arizmendi, M. Quesada, and K. Oyama. 2014. Habitat fragmentation and genetic variability of tetrapod populations. Animal Conservation **18**:249-258.

Rocha, R. 2017. Tropical forest fragmentation: effects on the spatio-temporal dynamics of its bat communities. PhD Thesis. University of Lisbon, Lisbon, Portugal.

Rocha, R., A. López-Baucells, F. Z. Farneda, M. Groenenberg, P. E. D. Bobrowiec, M. Cabeza, J. M. Palmeirim, and C. F. J. Meyer. 2017a. Consequences of a large-scale fragmentation experiment for Neotropical bats: disentangling the relative importance of local and landscape-scale effects. Landscape Ecology **32**:31-45.

Rocha, R., O. Ovaskainen, A. López-Baucells, F. Z. Farneda, D. F. Ferreira, P. E. D. Bobrowiec, M. Cabeza, J. M. Palmeirim, and C. F. J. Meyer. 2017b. Design matters: An evaluation of the impact of small man-made forest clearings on tropical bats using a before-after-control-impact design. Forest Ecology and Management **401**:8-16.

Rocha, R., O. Ovaskainen, A. López-Baucells, F. Z. Farneda, E. M. Sampaio, P. E. D. Bobrowiec, M. Cabeza, J. M. Palmeirim, and C. F. J. Meyer. 2018. Secondary forest regeneration benefits old-growth specialist bats in a fragmented tropical landscape. Scientific Reports **8**:3819.

Rodríguez-Posada, M. E. 2016. Confirmación de la presencia de *Centronycteris maximiliani* (Chiroptera: Emballonuridae) en Colombia, con comentarios sobre la distribución de *Centronycteris*. Mastozoologia Neotropical **23**:179-184.

Rodríguez-San Pedro, A., and J. A. Simonetti. 2015. The relative influence of forest loss and fragmentation on insectivorous bats: does the type of matrix matter? Landscape Ecology **30**:1561-1572.

Rosa, I. M. D., C. Gabriel, and J. M. B. Carreiras. 2017. Spatial and temporal dimensions of landscape fragmentation across the Brazilian Amazon. Regional Environmental Change **17**:1687-1699.

Schnitzler, H. U., and E. K. V. Kalko. 2001. Echolocation by insect-eating bats: We define four distinct functional groups of bats and find differences in signal structure that correlate with the typical echolocation tasks faced by each group Bioscience **51**:557-569.

Shi, J. J., and D. L. Rabosky. 2015. Speciation dynamics during the global radiation of extant bats. Evolution **69**:1528-1545.

Siemers, B. M., and H.-U. Schnitzler. 2004. Echolocation signals reflect niche differentiation in five sympatric congeneric bat species. Nature **429**:657-661.

Sousa, T. R., F. R. C. Costa, T. V. Bentos, N. Leal Filho, R. C. G. Mesquita, and I. O. Ribeiro. 2017. The effect of forest fragmentation on the soil seed bank of Central Amazonia. Forest Ecology and Management **393**:105-112.

Stouffer, P. C., and R. O. Bierregaard. 1995. Use of Amazonian forest fragments by understory insectivorous birds. Ecology **76**:2429-2445.

Stouffer, P. C., C. Strong, and L. N. Naka. 2009. Twenty years of understorey bird extinctions from Amazonian rain forest fragments: consistent trends and landscape-mediated dynamics. Diversity and distributions **15**:88-97.

Struebig, M. J., T. Kingston, E. J. Petit, S. C. Le Comber, A. Zubaid, A. Mohd-Adnan, and S. J. Rossiter. 2011. Parallel declines in species and genetic diversity in tropical forest fragments. Ecology Letters **14**:582-590.

Torrent, L., A. López-Baucells, R. Rocha, P. E. D. Bobrowiec, and C. F. J. Meyer. 2018. The importance of lakes for bat conservation in Amazonian rainforests: an assessment using autonomous recorders. Remote Sensing in Ecology and Conservation:(in press).

Valente, L., R. S. Etienne, and L. M. Dávalos. 2017. Recent extinctions disturb path to equilibrium diversity in Caribbean bats. Nature Ecology & Evolution **1**:0026.

Vieira, I., T. Gardner, J. Ferreira, A. Lees, and J. Barlow. 2014. Challenges of governing second-growth forests: a case study from the Brazilian Amazonian State of Para. Forests **5**:1737-1752.

Wickramasinghe, L. P., S. Harris, G. Jones, and N. Vaughan. 2003. Bat activity and species richness on organic and conventional farms: impact of agricultural intensification. Journal of Applied Ecology **40**:984-993.

Wolfe, J. D., P. C. Stouffer, K. Mokross, L. L. Powell, and M. M. Anciães. 2015. Island vs. countryside biogeography: an examination of how Amazonian birds respond to forest clearing and fragmentation. Ecosphere **6**:1-14.

Yoh, N. 2018. The impact of edge-effects on aerial insectivorous bat assemblages in the Amazon. MSc thesis. University of Salford, Salford, United Kingdom.

Zurita, G., G. Pe’er, M. I. Bellocq, and M. M. Hansbauer. 2012. Edge effects and their influence on habitat suitability calculations: a continuous approach applied to birds of the Atlantic forest. Journal of Applied Ecology **49**:503-512.

Zurita, G. A., and M. I. Bellocq. 2012. Bird assemblages in anthropogenic habitats: identifying a suitability gradient for native species in the Atlantic Forest. Biotropica **44**:412-419.

## Supplementary material

Table 5.S1. Sonotype classification used in this study, based on López-Baucells et al. (2016).

| **Sonotype/Species** | **Species included in the sonotype** |
|---|---|
| Emballonuridae | |
| *Rhynchonycteris naso* | *Rhynchonycteris naso* |
| *Saccopteryx leptura* | *Saccopteryx leptura* |
| *Saccopteryx bilineata* | *Saccopteryx bilineata* |
| *Centronycteris maximiliani* | *Centronycteris centralis / maximiliani* |
| *Peropteryx macrotis* | *Peropteryx macrotis* |
| *Peropteryx kappleri* | *Peropteryx kappleri* |
| *Cormura brevirostris* | *Cormura brevirostris* |
| *Emballonuridae* 1 | *Saccopteryx gymnura / canescens* |
| Furipteridae | |
| *Furipterus horrens* | *Furipterus horrens* |
| Molossidae | |
| *Molossidae I* | *Molossus molossus* |
| *Molossidae II* | *Molossus rufus / sinaloae / currentium* |
| *Molossidae III* | *Cynomops planirostris / paranus / greenhalli /abrasus,*<br>*Eumops auripendulus / glaucinus / dabbenei / hansae / maurus,*<br>*Nyctinomops laticaudatus,*<br>*Tadarida brasiliensis* |
| *Molossops* | *Molossops neglectus,/ temminckii* |
| *Promops* | *Promops centralis / nasutus* |
| Mormoopidae | |
| *Pteronotus personatus* | *Pteronotus personatus* |
| *Pteronotus alitonus* | *Pteronotus alitonus* |
| *Pteronotus gymnonotus* | *Pteronotus gymnonotus* |
| *Pteronotus rubiginosus* | *Pteronotus rubiginosus* |
| Vespertilionidae | |
| *Myotis riparius* | *Myotis riparius / Thyroptera tricolor* |
| *Myotis nigricans* | *Myotis nigricans* |
| *Vespertilionidae 1* | *Eptesicus brasiliensis / chiriquinus*<br>*Eptesicus furinalis*<br>*Lasiurus sp.*<br>*Rhogeessa io* |

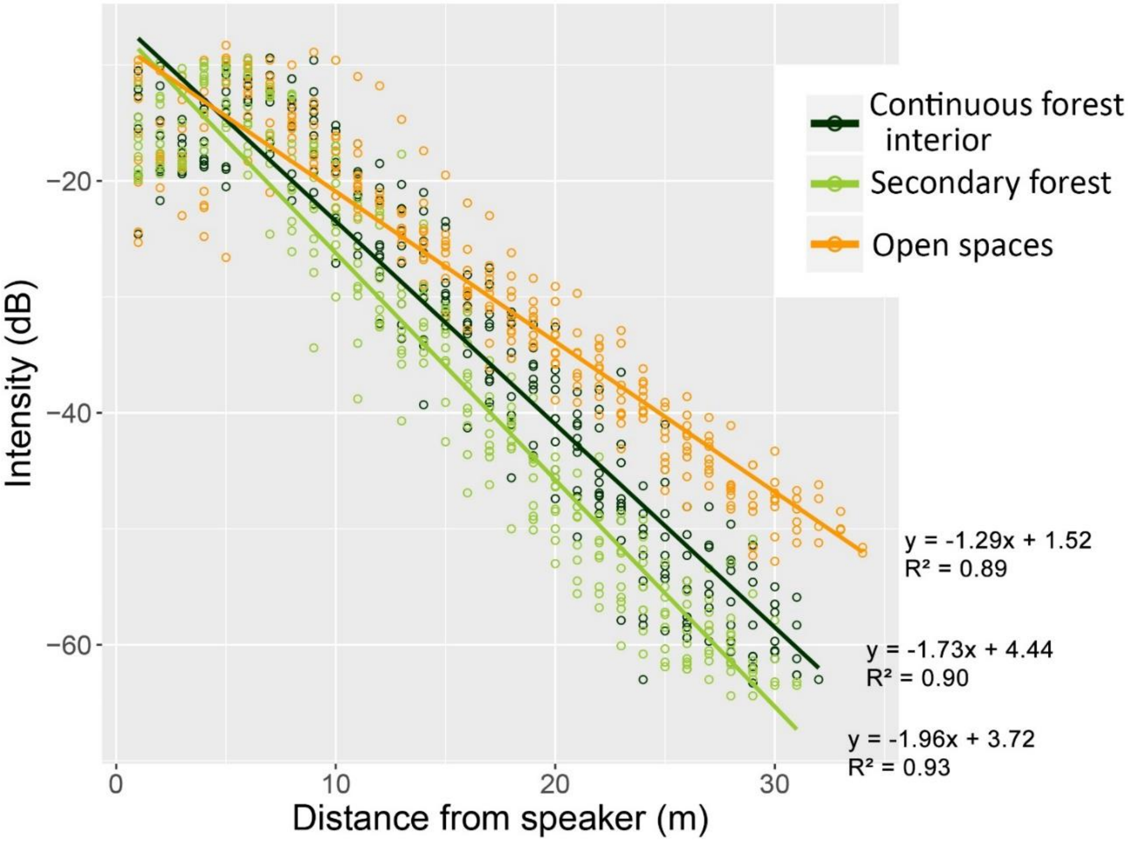


Figure 5.S1. Acoustic detectability correction per habitat type. The differences in acoustic detectability between continuous forest interior and secondary forest were compensated for by calculating the real sampled volume in each habitat. For this, a constant frequency speaker (SM2-Cal, Wildlife Acoustics Inc., Massachusetts, USA), was placed along five 30 m long, linear transects randomly distributed in continuous forest interior, secondary forest, and additionally in open spaces as controls. I then recorded its signal with an SM2Bat+ detector at 1 m intervals along each transect. In order to quantify sound dissipation, linear regressions between the distance from the speaker (m) and sound intensity (dB) were calculated for all habitats. Through the regression equation, I calculated the distance at which I considered that the signal got theoretically dissipated (-50dB) in each habitat. From this, I calculated what I define as the ‘acoustic detectability range’ (ADR), the spherical volume in space in which a single signal at 40kHz is detected if emitted at 94dB sound pressure level (+/- 3dB). The difference in ADR between habitats was assessed using the formula of the regular sphere volume ($V = \frac{4}{3}\pi r^3$) where r is the maximum detection distance. Depending on call frequency and the inherent call intensity with which each bat species/individual vocalizes the maximum detection distance might change. For that reason, I use this correction to calculate a relative proportion of acoustic volume sampled within species but not between them. I also decided to use a constant signal of 40 kHz, which is among the most common bat echolocation frequencies in the study area (López-Baucells et al. 2016).

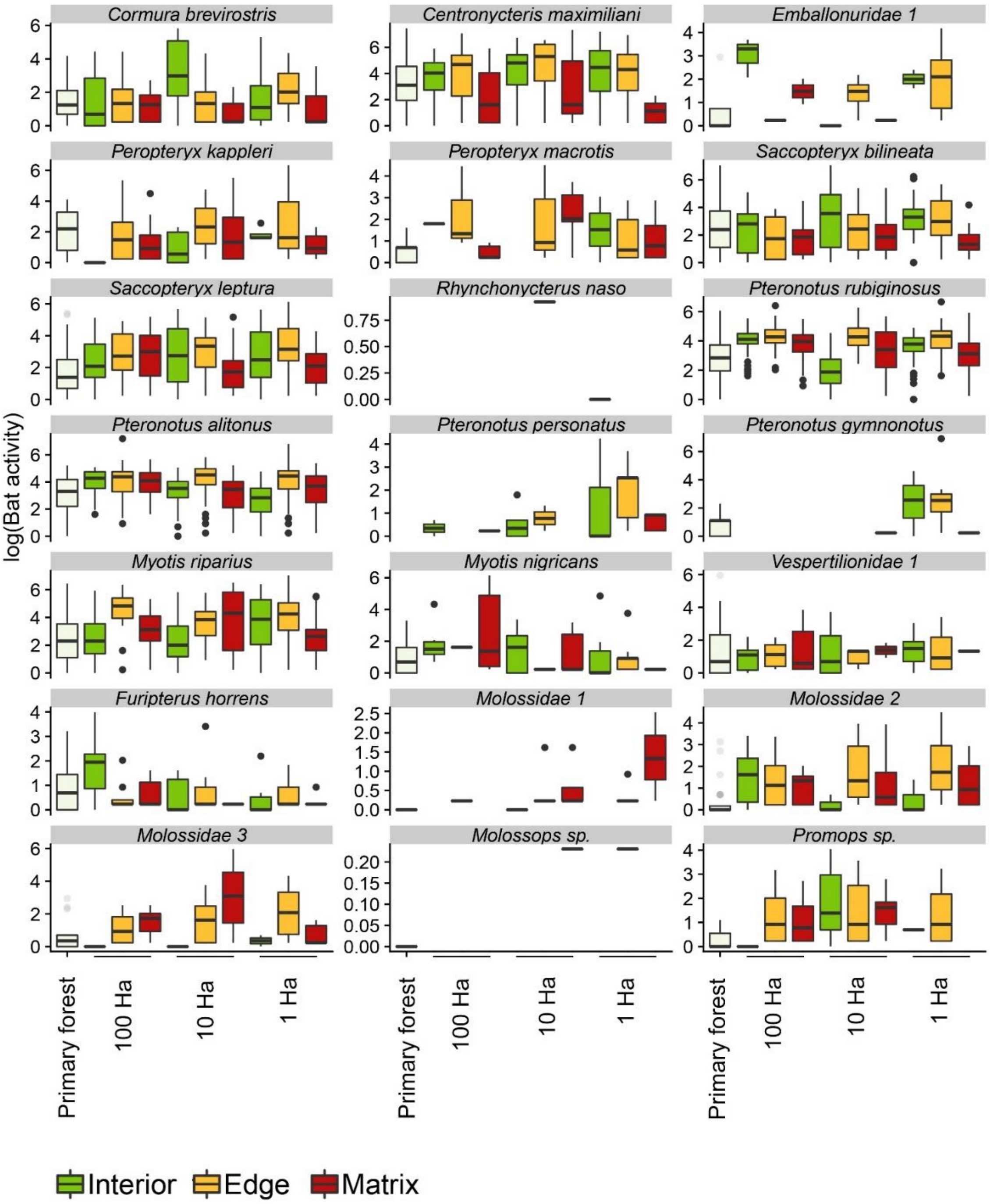


Figure 5.S2 Bat activity per night for each bat species in all habitat categories recorded between 2011 and 2013. Log-transformation was applied to the y-axis since the data were not normally distributed. Box-plot centres are medians, boxes 25% and 75% quartiles and whiskers denote the range. Dots at the end of the boxplot represent outliers.

# Chapter 6

---

## Echolocation and wing morphology: key trait correlates of vulnerability of aerial insectivorous bats to tropical forest

Fraixedas, S., **López-Baucells, A.**$^{+}$, Rocha, R., Farneda, F.Z., Bobrowiec, P.E.D.,
Palmeirim, J.M., Meyer, C.F.J., 2018

Top: *Thyroptera tricolor;* Bottom: *Pteronotus rubiginosus*

# CHAPTER 6

## Echolocation and wing morphology: key trait correlates of vulnerability of aerial insectivorous bats to tropical forest fragmentation

## Abstract

Fragmentation and habitat loss rank high amongst the most pressing threats to biodiversity. Understanding how variation in functional traits is associated with species vulnerability in fragmented landscapes is central to the design of effective conservation strategies. Here, I used a whole-ecosystem ecological experiment in the Central Amazon to investigate which functional traits of aerial insectivorous bats best predict their sensitivity to forest fragmentation. During 2013 and 2014, bats were surveyed using passive bat recorders in six continuous forest sites, eight forest fragments, eight fragment edges, and eight forest clearings. The interaction between functional traits, environmental characteristics, and species distribution was investigated using a combination of RLQ and fourth-corner analyses. These results showed that echolocation call structure, vertical stratification and wing aspect ratio were the strongest predictors of sensitivity to forest fragmentation. Frequency of maximum energy, body mass and relative wing loading did not show any correlation with the environmental traits. Bat species with constant-frequency calls were associated with high vegetation density, being more susceptible to forest fragmentation than species with frequency-modulated calls. The trait "vertical stratification" was also correlated with vegetation structure, indicating that understory species were more sensitive to forest loss than canopy species. Finally, species with high aspect ratio wings were linked to forest edges and clearings. These findings suggest that species functional traits determine the vulnerability of aerial insectivorous bats towards fragmentation and, similarly, environmental condition determine if a species is likely to become extinct due to fragmentation in a particular context. Preserving structurally complex forests might be crucial to ensure the long-term persistence of the most sensitive and vulnerable species of this bat ensemble in fragmented landscapes across the Neotropics.

**Keywords:** Amazon, Passive bat recorders, Bioacoustics, Chiroptera, Conservation, Deforestation, Echolocation, Species functional traits, Wing morphology.

## Introduction

The exponential increase of the human population and growing per capita consumption of resources are causing widespread habitat loss and degradation, which are threatening the survival of numerous species worldwide (Dirzo et al. 2014, Laurance et al. 2014). In fact, human-caused environmental changes are among the most significant and immediate threats to biodiversity (Ewers and Didham 2006, Dobrovolski et al. 2011, Haddad et al. 2015), and its impacts on biota are predicted to be more pronounced in species-rich tropical areas (Malhi et al. 2014). Across the tropics, human-induced habitat modification is creating increasingly fragmented landscapes composed of smaller and isolated primary habitat patches (Haddad et al. 2015, Taubert et al. 2018), usually embedded in a matrix with a mosaic of different habitats that affect both species diversity and composition (Ewers and Didham 2006, Kupfer et al. 2006, Kennedy et al. 2011, Loureiro and Gregorin 2015). Matrix quality and permeability are key determinants of biodiversity persistence in degraded landscapes (Ewers and Didham 2006, Kennedy et al. 2011, Mendenhall et al. 2014, Rocha et al. 2017b). Matrix permeability refers to the capacity of any landscape to promote movement of animal populations and to sustain key ecological services and processes (Ray et al. 2002). In general, low matrix permeability negatively affects local biodiversity as it reduces connectivity and increases the effects of isolation of remnant patches (Ricketts 2001, Estrada-Villegas et al. 2010).

Understanding how habitat alterations affect biodiversity and ecosystem functioning is crucial for the creation of effective conservation strategies (Cadotte et al. 2011). Since most ecological processes are determined by species functional traits (Cardinale et al. 2012), trait-based approaches have lately become popular to predict the anthropogenic effects on ecosystem functioning in fragmented tropical landscapes. Functional traits are measurable characteristics of organisms or species (morphological, physiological, phenological, or behavioural) that determine their performance, fitness, and ecological functions in a particular habitat (Violle et al. 2007). Because some species are more susceptible to habitat loss than others (Davies et al. 2000, Henle et al. 2004), identifying which traits make species more sensitive to habitat fragmentation is essential to minimize local extinctions (Barbaro and Van Halder 2009, Hanspach et al. 2012).

The tropics harbour the greatest diversity of bats (Mickleburgh et al. 2002, Altringham 2011), which are responsible for key ecological functions. They act as pollinators and seed dispersers of a large number of plant species, promoting the restoration of forest in disturbed areas. Moreover, they are both important prey and biological control agents of animal populations (Kunz et al. 2011). The recent increase in published studies on tropical bats has considerably advanced our knowledge about the effects of habitat loss and fragmentation on this taxon at different ecological levels. However, most studies have analysed biodiversity changes from a taxonomic point of view, rarely focusing on a functional trait-based dimension (e.g. Cisneros et al. 2015, Farneda et al. 2015, Meyer et al. 2016, Frank et al. 2017, Wordley et al. 2017, Farneda et al. 2018a).

In the Neotropics, aerial insectivorous bats represent 30-50% of local bat assemblages (Kalko 1998, MacSwiney et al. 2008, Estrada-Villegas et al. 2010). Although aerial insectivorous bats are usually classified into one ensemble, they exhibit high variability in foraging and behavioural strategies. However, they have rarely been considered in fragmentation studies as most of them were based on captures with mist-nets, a sampling method poorly suited for this group (Estrada-Villegas et al. 2010, Silva and Bernard 2017). Although acoustic methods are the most suitable to sample aerial insectivorous bats, the costs and time commitment involved in acoustic surveys is still considerable. Moreover, the echolocation calls of many species and the variation among them have not yet been adequately documented, and reference call libraries for tropical regions are scarce (MacSwiney et al. 2008, López-Baucells et al. 2016). As a result of these limitations, aerial insectivorous bats continue to be underrepresented in inventories and ecological studies (Cunto and Bernard 2012), and there is a lack of data about their vulnerability to habitat fragmentation (but see Estrada-Villegas et al. 2010, Bader et al. 2015a, Bader et al. 2015b). Moreover, other ecological characteristics like hunting strategies remain poorly studied (Marques et al. 2013). Despite the aforementioned limitations, the recent improvement of acoustic recording devices and new data analysis approaches have stimulated the increased inclusion of aerial insectivorous bats in surveys and monitoring programmes (Browning et al. 2017).

Here, I used an ecosystem-wide ecological experiment in the Brazilian Amazon to investigate which functional traits are linked to fragmentation sensitivity in aerial insectivorous bats. I employed the framework introduced by Dray et al. (2014) that integrates RLQ and fourth-corner methods to examine trait-environment links and their association with patterns of species distribution. My main research questions were: (i) Are species with high frequency calls better adapted to forested habitats and thus, species with low frequency calls less sensitive to fragmentation? (ii) Are the echolocation call types (modulated/quasi-modulated/constant frequency calls) related to bat species distribution in a fragmented landscape and therefore to their vulnerability towards fragmentation? (iii) Are morphological characteristics such as body mass, wing loading and aspect ratio filtering bat assemblages in a fragmented landscape? and (iv) Does vertical stratification foraging habits influence the adaptability of bats to deforestation?

## Materials and Methods

### Study area

The study was conducted at the Biological Dynamics of Forest Fragments Project (BDFFP), located in the Central Amazon, approximately 80 km north of Manaus (2º30'S, 60ºW), Brazil (Fig. 6.1). Commencing in 1979, the BDFFP is the longest-running experimental study of habitat fragmentation (Laurance et al. 2017). The study area covers 1,000 km$^2$ of moist tropical forest characterized by a mosaic of primary *terra firme* rainforest and forest fragments (1, 10, and 100 ha) embedded in a matrix of late-stage secondary regrowth (~30 years old). In the early 1980s, the region underwent an intense process of forest clearing, resulting in a set of fragments of primary forest isolated from continuous forest by distances of 80-650 m.

Since their creation, these experimental fragments were re-isolated 4 to 5 times by clearing a 100m-wide strip around each fragment. The most recent re-isolation occurred in 2013-2014, resulting in a landscape comprised of both forest-dominated habitats and small clearings around the fragments (Rocha et al. 2017c). Yearly rainfall fluctuates between 1,900 and 3,500 mm, with a dry season from June to October and a wet season from November to June (Ferreira et al. 2017). Forest canopy height varies from 30 to 37 m, with some emergent trees reaching 55 m (Laurance et al. 2011).

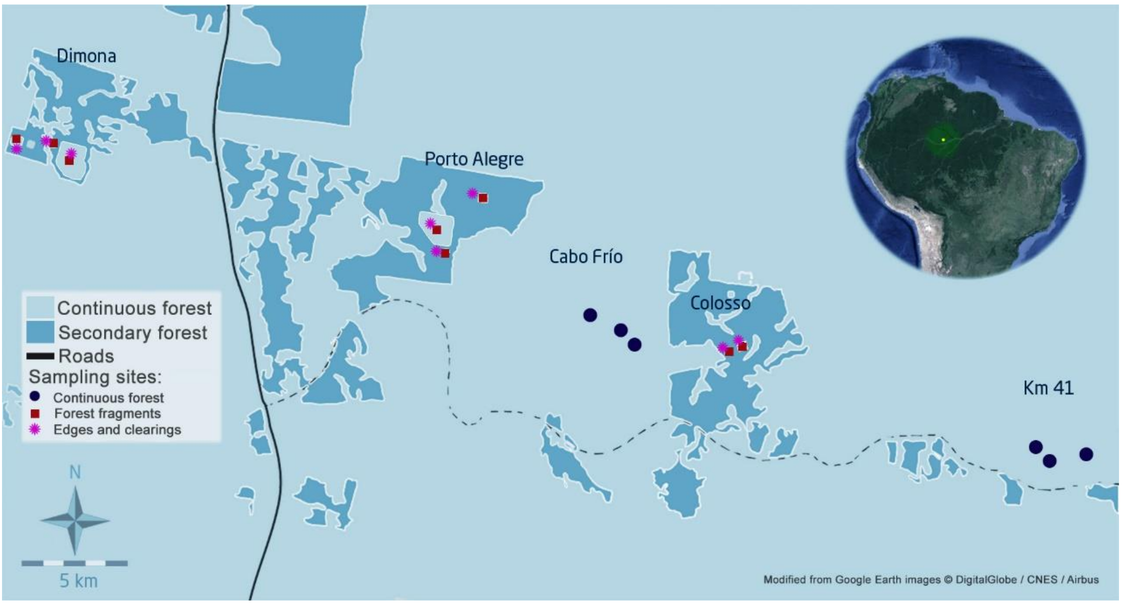


Figure 6.1. Map of the study area in the central Amazon. Image (c) DigitalGlobe / CNES / Airbus. All sampling sites are represented (Km 41 & Cabo Frio for the continuous forest and Dimona, Colosso & Porto Alegre for forest fragments); continuous forest is represented in light blue and secondary vegetation in dark blue. The image in the upper right corner shows the location of the study area.

## Sound recordings

Acoustic surveys were carried out in eight forest fragments during the first year after the re-isolation that took place in 2013-2014 (Rocha et al. 2017b), and six control sites in continuous primary forest (Fig. 6.1). Data from all the fragments were pooled together independently of their size within the habitat category "fragment", due to the limited number of replicates. Automatic bat recorders Song Meter 2 (SM2Bat+) equipped with omnidirectional ultrasonic SMX-US microphones (Wildlife Acoustics, Inc. Massachusetts, USA) were used to acoustically sample bats. Recorders were placed at 1.5 m height, in the interiors (N=8) and edges (N=8) of all forest fragments, as well as in the adjacent clearings (N=8) (for more information regarding the experimental re-isolation of the forest fragments see Rocha et al. 2017c). With the six recorders placed in the interior of continuous forest I had a total of 30 sampling sites. Detectors were installed for three complete nights per recording site (from 18:00 to 6:00 h), and all sites were sampled twice during both the dry and wet season in 2014.

## Acoustic analysis

Kaleidoscope Pro Software v4.0.4 (Wildlife Acoustics, In. Massachusetts, USA) was used to manually classify all recordings based on López-Baucells et al. (2016) and a regional reference call library compiled by A. López-Baucells (unpublished). In the case of 14 species it was possible to identify the calls to species level. However, some species had to be grouped into sonotypes (comprising several species with similar calls that could not be reliably distinguished, N=5; see Appendix). Owing to inherent constraints of acoustic data (i.e. the fact that it is not possible to count individuals), I used “bat activity” as a proxy for bat abundance. The sample unit was a *bat pass* (Wickramasinghe et al. 2003), which I considered as any sequence/recording with a minimum of two distinguishable echolocation pulses per species during a maximum time of five seconds (Millon et al. 2015). I only considered those pulses whose intensity exceeded 10dB from the background noise.

## Environmental variables

Vegetation structure was measured at each sampling site within three plots of 100 m$^2$ (5 x 20 m) located next to the detector locations. Four environmental variables were used in the analyses to quantify species sensitivity to fragmentation based on their functional traits: *Number of stems* (number of vertical branches and trees higher than breast height), *Tree height* (height of all trees ≥ 10 cm diameter at breast height), *Shortest distance to water* (distance in km between each site and the nearest water body calculated using Google Earth), and *Habitat type* (qualitative variable including four categories: continuous forest interior, fragment interior and fragment edge, clearing).

## Species traits

The relative scarcity of knowledge on aerial insectivorous bats, together with the difficulty in gathering information about most of the species, led me to exclude some of traits from the analyses such as diet or roosting strategy/guilds (Borkin and Parsons 2011, Bohnenstengel 2012, Farneda et al. 2015, Coronado et al. 2018) focusing on only five well-known functional traits as potential predictors of their vulnerability to habitat fragmentation:

1. *Echolocation: frequency of maximum energy*

Frequency of maximum energy (FME), also known as peak frequency, is the most intense frequency in the call (López-Baucells et al. 2016). It determines how quickly the intensity of a call will diminish and therefore, will influence the kind of habitat in which the bat will be able to fly and forage. Data on FME for all species were obtained from López-Baucells et al. (2016). Whenever species with similar peak frequencies had to be grouped under the same sonotype, the average FME of all species was calculated and used in subsequent analyses.

2. *Echolocation: call structure*

This predictor was based on an existing guild classification of bats that links habitat type and foraging mode with their echolocation calls (Schnitzler and Kalko 2001, Schnitzler et al. 2003). Following this, I established the following categories: A) FM signals: usually emitted by edge and cluttered space foragers; B) QCF signals: mostly emitted by edge and open space foragers; and C) CF signals: emitted by cluttered space foragers. Species were assigned to each category following López-Baucells et al. (2016).

3. *Body mass*

Body mass plays a key role in the physiology, ecology and diversification of species and also influences life-history traits of individuals (Safi et al. 2013). Species with large body size tend to have smaller populations and larger home ranges and are therefore more prone to extinction (Purvis et al. 2000). Body mass was measured in captured bats (data collected at the BDFFP between 2012-2014, Rocha et al. 2017a, Rocha et al. 2017b, Rocha et al. 2017c, Rocha et al. 2018) and averaged for each species (excluding juveniles and reproductive females). Missing data for *Molossops* spp., *Peropteryx kappleri* and *P. macrotis*, *Promops* spp., and *Pteronotus personatus* were obtained from the literature (Eisenberg and Redford 1989).

4. *Vertical stratification (VertS)*

Bat activity and richness differ among tropical forest strata, whereby species are generally divided into canopy, sub-canopy or understory specialists. However, this classification is mostly based in studies with data collected for phyllostomids bats (Carvalho et al. 2013, Marques 2016). I assigned species to different strata based on the results obtained by Mas (2014), who studied vertical stratification at the BDFFP using acoustic data: A) All bats for which M. Mas found more contacts or recordings in the understory than in other strata; B) Bats flying significantly more often between 20-30 m but still under the canopy (sub-canopy) ; and C) All molossid bats that according to Freeman (1981) are assumed to fly above the canopy.

5. *Wing morphology*

Manoeuvrability and flight speed in bats are limited by wing morphology (Marinello and Bernard 2014), which usually determines access to suitable foraging habitat (Arita and Fenton 1997). I calculated aspect ratio (AR) and relative wing loading (RWL) of each species following Norberg and Rayner (1987). Measurements were obtained from extended-wing photographs of bats captured at the BDFFP (Rocha 2017, Torrent et al. 2018) and analysed with *ImageJ* (National Institute of Health, USA). Missing data were obtained from the literature.

## Statistical analysis

Several analytical methods have historically been used to assess the associations between species traits and environmental gradients (Peres-Neto et al. 2017). Amongst them, RLQ and fourth-corner methods provide an excellent approach to assess trait–environment relationships at the species level and have been widely used in ecological and conservation research (Kleyer et al. 2012). Dray et al. (2014) recently developed an analytical framework that combines both methods and so far is considered the most appropriate approach to study the relation between environmental variables and functional traits.

Applied to my dataset, this approach tests the relation of the data contained in three different matrices: table L (species activity levels per sampling site), table Q (functional traits of species) and table R (environmental variables per sampling site). A correspondence analysis (CA) is applied to table L (species abundance table), providing a common organisation of the samples and the species (Dray et al. 2014). Both the table of environmental variables (R) and traits (Q) contained a mix of qualitative and quantitative variables and were therefore analysed using Hill-Smith principal component analysis (PCA; Hill and Smith 1976). The RLQ method maximizes the covariance between the traits and the environmental variables through the species relative abundance (Dray et al. 2014). While RLQ consists of a multivariate analysis of the associations between the three tables, the fourth-corner method tests the significance of bivariate associations.

I used two permutation models in the RLQ-fourth corner analysis. Model 2 tests the null hypothesis that there is no relationship between the distribution of species and environmental conditions (i.e. no association between R and L matrices). In contrast, Model 4 assumes no association between L and Q and the null hypothesis here is that species composition is independent of species traits (Dray et al. 2014). To adjust p-values for multiple comparisons, I used the false discovery method (Benjamini and Hochberg 1995, Dray et al. 2014), determining significance based on 50,000 permutations. All analyses were carried out using R v3.4.1 (R Core Team 2017), specifically package ade4 (Dray and Dufour 2007).

## Results

The RLQ analysis showed that the first axis of the ordination (considering the three tables R, L and Q) explained 93.8% of the variance and the second axis 5.3%. The first and second axes of the Hill-Smith PCA of the functional traits table (Q table) account for 44.7% and 26.7% respectively. Regarding the R table (environmental variables), the first axis of the PCA explained 48.3% of the variance, and the second axis 23.1%. Finally, in the L table (species activity) the first axis of the correspondence analysis (CA) explained 43.1% of the variance and the second 15.3% (Table 6.2).

In the PCA of the R table (Fig. 6.2), sites grouped according to the gradient made up by the different habitat categories, with all forest interior sites (continuous forest and forest fragments) well separated from clearings and fragment edges along the first axis. In the PCA of the Q ordination table (Fig. 6.3), no clear species groupings could be distinguished. However, all species were mostly spatially organised based on two of their functional traits, call structure (CF or FM calls) and frequency of maximum energy (FME). The eigenvalues of the first axis of the RLQ analysis from species traits and environmental variables (Fig. 6.4) showed that the variables that explained most variability in species occurrence among sites were aspect ratio, call structure (CF), vertical stratification, and two categories of the habitat type variable, forest fragment and continuous forest.

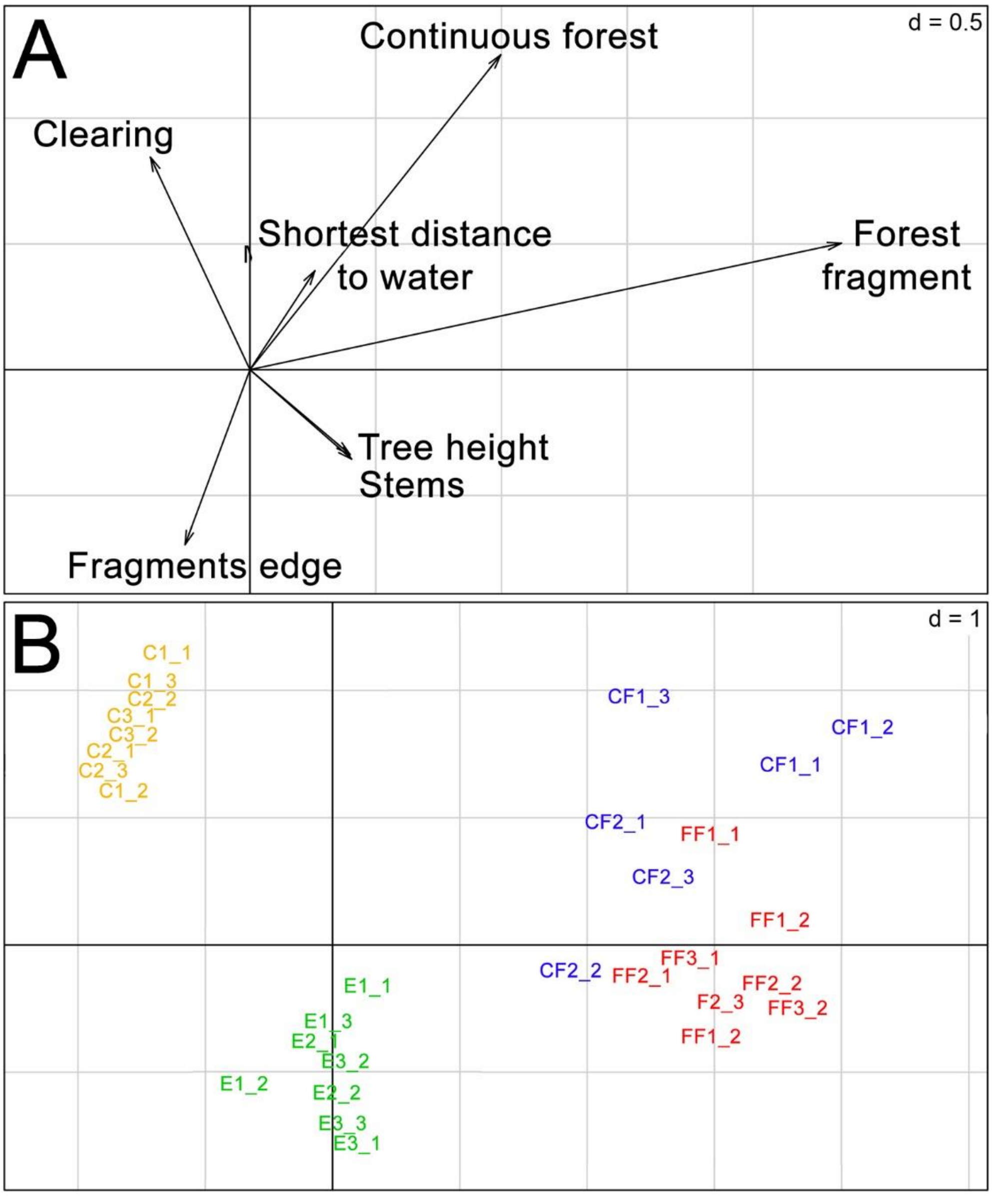


Figure 6.2. Biplots from the Hill-Smith PCA of the environmental gradients (R table). A) Environmental variables. B) Sites. Abbreviations of the sites refer to: C= Clearing; FF=forest fragment, CF= continuous forest, E= edge.

Fourth corner analysis revealed a highly significant relationship between species distribution and environmental variables (model 2, $p<0.0001$), and a significant association between species composition and functional traits (model 4, $p=0.04$). Based on 50,000 iterations, the total inertia of the RLQ analysis indicates a significant general relation ($p=0.04$) between environmental variables and functional traits in the combined models 2 and 4.

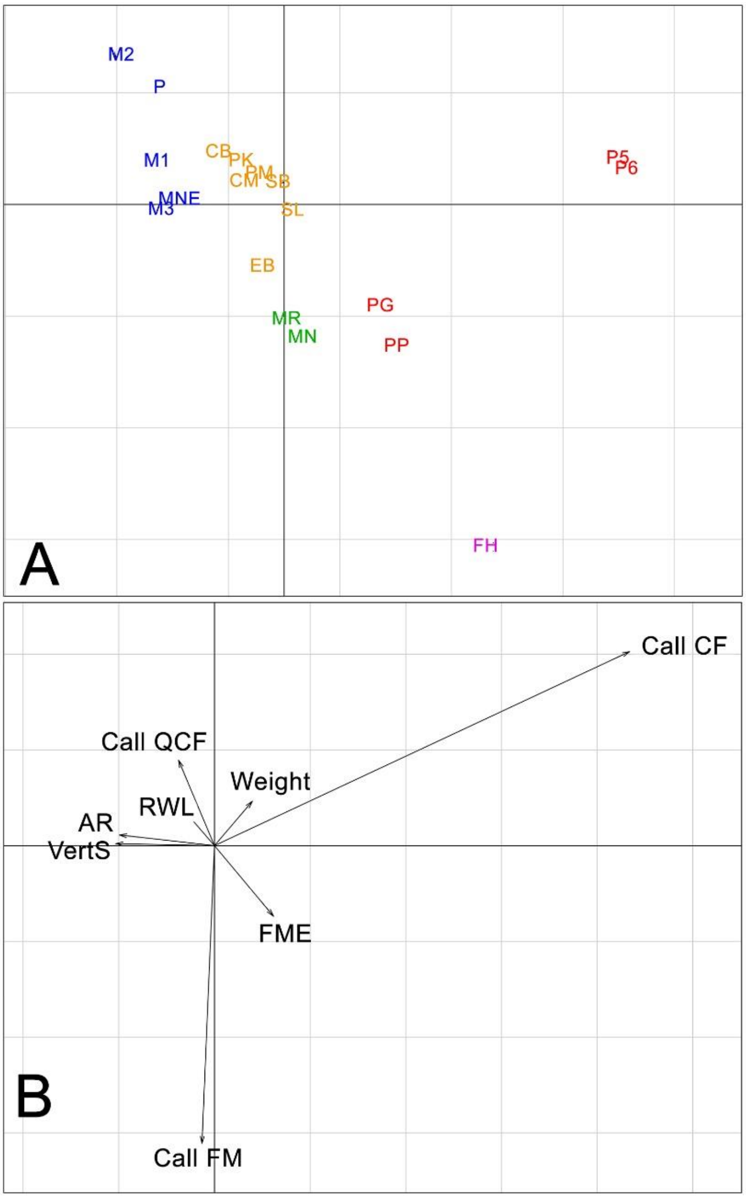


Figure 6.3. Biplots from the Hill-Smith PCA of the functional traits (Q table). A) Species. Red: Mormoopidae; Pink: Furipteridae; Green: Vespertilionidae; Orange: Emballonuridae; Blue: Molossidae (see Table 7.S1 for species abbreviations). B) Traits (see Materials and Methods for trait abbreviations).

Finally, the combination of RLQ and fourth-corner analysis yielded four significant ($p<0.05$) trait-environment relationships (Fig. 6.5): CF call structure showed a positive correlation with the first axis (AxR1) for the environmental gradient; vertical stratification and aspect ratio revealed a negative correlation with the AxR1 axis for the environmental gradient; and the assemblages recorded in the forest fragments were significantly associated with the AxQ1 axis that represented the pool of bat functional traits.

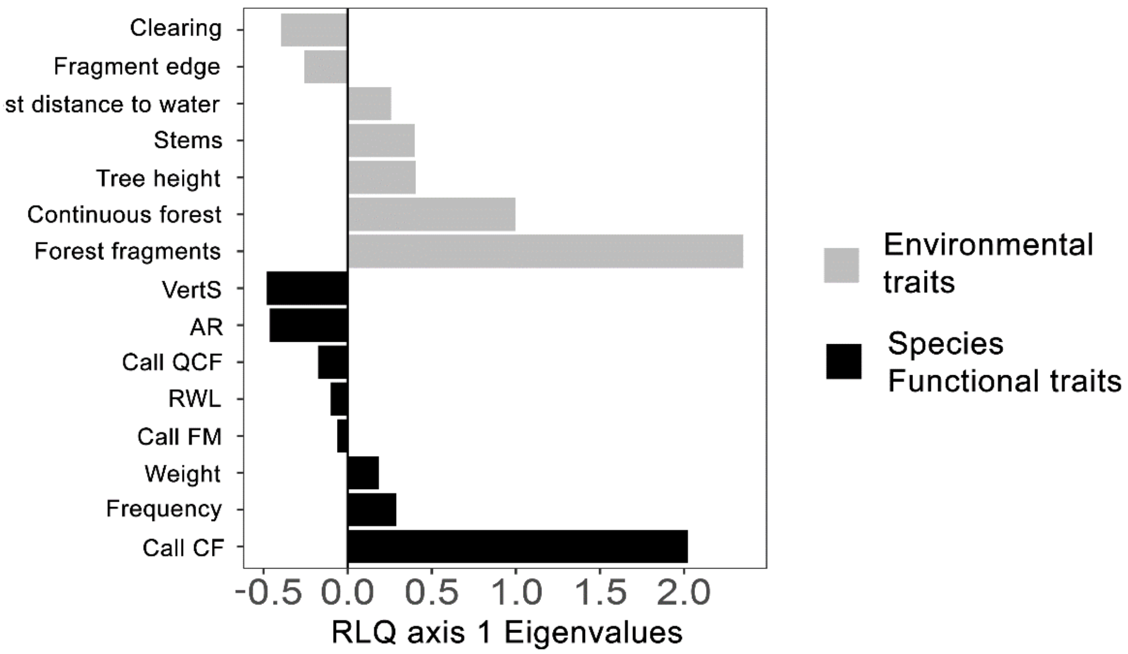
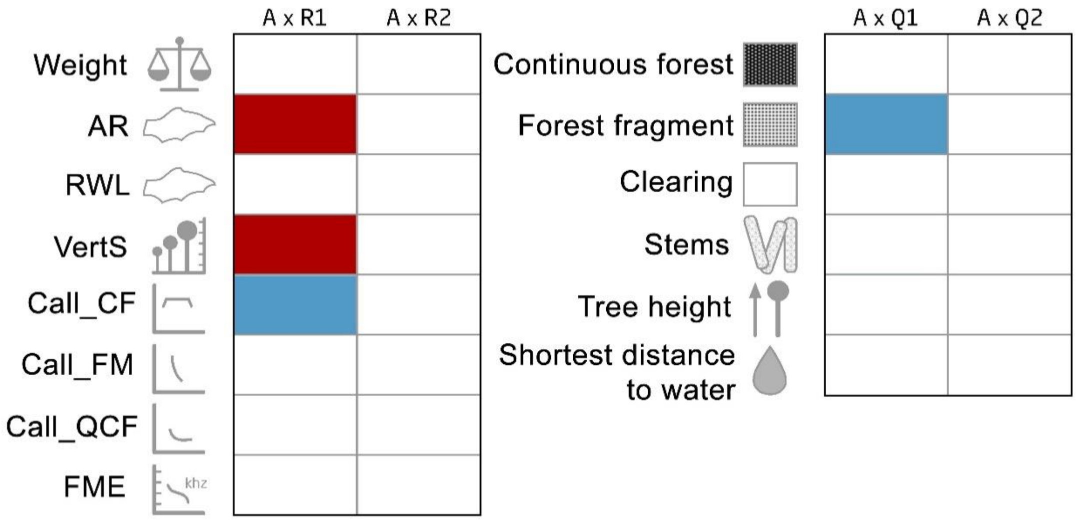


Figure 6.4. Eigenvalues of species traits and environmental variables along the first axis of the RLQ analysis. See Materials and Methods for trait abbreviations.



| | A x R1 | A x R2 |
|---|---|---|
| Weight | | |
| AR | 🟥 | |
| RWL | | |
| VertS | 🟥 | |
| Call_CF | 🟦 | |
| Call_FM | | |
| Call_QCF | | |
| FME | | |

| | A x Q1 | A x Q2 |
|---|---|---|
| Continuous forest | | |
| Forest fragment | 🟦 | |
| Clearing | | |
| Stems | | |
| Tree height | | |
| Shortest distance to water | | |

Figure 6.5. Results of the RLQ and fourth-corner analysis. A) Significant relations between the species traits and the first two RLQ ordination axes for environmental gradients. B) Significant relations between environmental characteristics and the first two RLQ ordination axes for species traits. Significant (p<0.05) positive associations are represented by blue cells and negative by red cells.

## Discussion

The diversity of species functional traits in a given assemblage is a key predictor of an ecosystem's functioning, resistance and resilience (Petchey and Gaston 2006), and therefore, functional traits are also likely to influence the vulnerability of species to the ongoing process of habitat destruction and fragmentation. However, trait-mediated environmental filters are still seldom considered in studies based on human-modified tropical landscapes. This is especially true in the case of poorly known animal groups, such as aerial insectivorous bats. I used autonomous bat detectors and a modern statistical approach to identify how traits of Neotropical aerial insectivorous bats correlate with environmental features and species distributions in a fragmented landscape. The results revealed that several traits were significantly linked to environmental variables, potentially affecting the vulnerability of the species to habitat fragmentation. I found strong associations between echolocation call type and wing aspect ratio with features of aerial insectivorous bats foraging habitat that are likely to be affected by forest fragmentation.

The responses of phyllostomid bats to habitat modification have been extensively studied across the Neotropics (Meyer et al. 2016, Frank et al. 2017). In the BDFFP recent long-term studies have provided important insights into the dynamics of bat responses to landscape fragmentation associated with matrix regeneration (Farneda et al. 2018a, Farneda et al. 2018b, Rocha et al. 2018). Farneda et al. (2015) found that, in the case of Amazonian phyllostomid bats, body mass and trophic level are good predictors of fragmentation sensitivity. However, this issue is poorly studied in aerial insectivorous bats, and the present study is one of the few investigating the link between these species' traits and vulnerability to fragmentation.

Although bat echolocation calls are specifically adapted to different habitat types (Schnitzler et al. 2003), only a few studies have analysed the correlation between echolocation traits and habitat characteristics. For example, Wordley et al. (2017) linked echolocation and morphological traits to habitat use in the Paleotropics and found that functional traits filtered bat assemblages in tea plantations.

A recent study tested the relation between echolocation traits and forest structure as quantified through LiDAR technology (Blakey et al. 2017). In accordance with my results, the authors did not find a significant correlation between forest structure variables and frequency of maximum energy. Frank et al. (2017) found that this echolocation parameter was not predictive of a bat's habitat usage. Although Wordley et al. (2017) found that bats with highest FME were mostly found in forest, they suggested that this variable might be less useful than others to predict bat vulnerability towards habitat loss and fragmentation. The consistency between these results and those in the available literature (Blakey et al. 2016, Frank et al. 2017), suggest that this trait is not a key predictor of bat vulnerability to fragmentation.

Froidevaux et al. (2016) studied the relation between bat guilds (each one of them with different echolocation types) and forest structure through the combination of LiDAR and acoustic sampling. They found that guild-specific activity was strongly influenced by the 3D habitat structure, highlighting the importance of echolocation in habitat selection. This result is in line with those obtained by Jung et al. (2012) who found that bat assemblages were affected by canopy structure, height, surface roughness and edge fraction in Germany.

Bats belonging to the same guild might not use calls with similar frequencies, but unquestionably tend to share similar echolocation call shapes, which are highly related to habitat type and foraging mode (Schnitzler and Kalko 2001, Denzinger and Schnitzler 2013). Following a widely used classification of bats into guilds (Schnitzler and Kalko 2001), I used the term "clutter foragers" for those species that emit CF calls (e.g. *Pteronotus alitonus* and *Pteronotus* cf. *rubiginosus*). They emit pulses of long duration that allow them to obtain accurate information about the environment, and detect small, mobile prey in dense vegetation (Barclay 1999, Barclay et al. 1999, López-Baucells et al. 2017, Pavan and Marroig 2017). These species might be more susceptible to the landscape-wide increase in clearings and the reduction of vegetation density associated with forest fragmentation. These results show that CF species are more abundant in forested areas than in clearings or fragment edges, regardless of whether the area is a continuous forest or a forest fragment. This suggests that vegetation cover is a major factor shaping habitat use and indicates that these bats are capable to persist even in relatively small forest patches. Wordley et al. (2017) obtained similar results in India, with bats that use CF calls being much more common in protected forests than in altered habitats. A better knowledge of the extent to which these species can adapt to fragmentation, or which types of remnant habitats offer suitable environmental conditions for long-term persistence is essential to improve their conservation. Larger distances between remnant patches in landscapes that are heavily fragmented would likely constitute a substantial barrier for these CF species, reducing their survival capacity (Ethier and Fahrig 2011).

My results confirm that species with high aspect ratio wings are linked to clearings and forest edges, as suggested by Marinello and Bernard (2014) and other authors before them. In contrast, species with low aspect ratios (short and broad wings), low body mass, slow and highly manoeuvrable flight (Aldridge and Rautenbach 1987, Norberg and Rayner 1987), are well adapted to structurally complex environments such as forest interiors (Marinello and Bernard 2014). These species usually fly short distances and have small home ranges (Fenton 1997, Meyer et al. 2005). Therefore, the fact that fragmented forests are often surrounded by open habitats may represent a relevant threat to them, as they are reluctant to make long displacements and cross open areas. Farneda et al. (2015) also found a significant correlation between wing morphology in phyllostomid bats and the environmental characteristics of the sampled habitats, indicating that wing morphology is a common key predictor of fragmentation sensitivity across Neotropical bat ensembles.

I found a strong link between the strata in which each bat species tends to forage (vertical stratification) and the vegetation structure of the forest. Primary tropical forests are characterised by strong vertical complexity that creates a pattern of differential use of space by organisms (Bernard 2001, Marques et al. 2015). Bats move through space three-dimensionally and consequently, forest structure and vegetation clutter may affect both horizontal and vertical patterns (Hayes and Gruver 2000, Jung et al. 2012, Mas 2014). In fact, most of the foraging activity of bats occurs in the canopy, the upper forest layer (Kalko and Handley 2001, Vetter et al. 2011, Marques et al. 2015, Marques 2016). Deforestation is not likely to have a large impact on bats that usually forage in open spaces because they mostly fly above the forest canopy (Marques et al. 2015). However, for bats that typically forage in cluttered habitats, a reduction of the canopy may result in a contraction of suitable foraging habitat.

Studies addressing the link between bat functional traits and their sensitivity to habitat fragmentation are still few (Meyer et al. 2008, Farneda et al. 2015, Garcia-Morales et al. 2016, Blakey et al. 2017) compared to the extensive number of studies on fragmentation effects on tropical bats that focused on the taxonomic diversity dimension. Patterns of habitat use in aerial insectivorous bats are strongly influenced by two main functional traits, wing morphology and echolocation call structure (Almeida et al. 2014, Marques et al. 2015). Bats with high wing loading and aspect ratio (long and pointed wings) tend to emit quasi-constant frequency calls, suitable to detect prey at long distances in open habitats. In contrast, bats with low wing loading and aspect ratio (short and rounded wings) emit either modulated or constant frequency calls, characterised by a shorter range of detection, more suitable for cluttered habitats (Aldridge and Rautenbach 1987, Emrich et al. 2014). This intricate relation among wing morphology, echolocation call design, habitat type and feeding ecology hampers the clear separation of the influence of each functional trait on fragmentation vulnerability. Studies on tropical bats that have tried to unravel the relation between functional traits and environmental gradients so far mostly targeted phyllostomid species, therefore neglecting the acoustic component (Meyer et al. 2008, Farneda et al. 2015, Garcia-Morales et al. 2016). More research is needed to reveal to which extent and under which circumstances a particular trait is more important than another.

Farneda et al. (2015) show that diet type is an important predictor of vulnerability to fragmentation in phyllostomids, and this may also be true for aerial insectivorous bats. In fact, insectivorous bats have distinct diets that include a broad diversity of insect groups (Kalka and Kalko 2006, Lawer and Darkoh 2016) and there is evidence that some of these groups are substantially impacted by forest fragmentation (Didham et al. 1996, Golden and Crist 1999, Rösch et al. 2013, Benítez-Malvido et al. 2016). Diet type may thus influence their vulnerability to fragmentation, but I could not include this trait in the study due to the lack of knowledge about the diet of the species involved. Roosting habits is also a potentially important trait that should be included in future studies. In fact, bat species that roost in crevices, under bark or in hollow tree trunks, may depend on the presence of old trees and therefore be highly vulnerable to habitat loss and fragmentation. In this context, it is important to improve our understanding of the natural history of aerial insectivorous bats, to allow studies that include a more complete set of predictors that potentially influence the vulnerability of species to fragmentation.

## Conclusions

The new analytical framework developed by Dray et al. (2014) is an excellent tool for researchers studying the vulnerability of species towards environmental stressors such as habitat loss and fragmentation. I found that a combination of species functional traits and environmental characteristics were relevant predictors of the sensitivity of aerial insectivorous bats towards fragmentation, a finding that may apply across Neotropical landscapes. In this context, the type of echolocation calls used by the species proved to be particularly important. Species with QCF echolocation calls, adapted to open habitats or forest edges, are likely to be less affected by forest fragmentation. In contrast, species with CF echolocation calls, typically understory foragers with low wing loading and aspect ratio, are potentially more affected. The latter are thus potentially particularly threatened by deforestation, although the fact that they manage to persist even in small fragments (i.e. 1ha fragments) is encouraging. Promoting natural forest regeneration and human-assisted restoration seem to be promising management strategies in the Neotropics for maintaining high taxonomic and functional bat diversity at the landscape-scale (Farneda et al. 2018a, Farneda et al. 2018b, Rocha et al. 2018). My results indicate that preserving the structural complexity of tropical forests is important to facilitate the persistence of the most fragmentation-sensitive species and to avoid general functional trait loss and associated loss in ecosystem services provided by bats in human-modified tropical landscapes.

## Acknowledgements

I would like to thank the volunteers and field assistants that participated in data collection as well as the BDFFP for logistical support. I am also thankful to Isabel Núñez for her contribution to the figure design and to Sara Fraixedas and Álvaro Fernández-Llamazares for their help with the proofreading. Research was conducted under permit (26877-2) issued by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio). This work was supported by the Portuguese Foundation for Science and Technology under grants [PTDC/BIABIC/111184/2009] (CM), [SFRH/BD/80488/2011] (RR), [PD/BD/52597/2014] (ALB) and by the Foundation for Research Support of the State of Amazonas [FAPEAM 062.01173/2015] (PEDB). Additional funding was provided by a Bat Conservation International student research fellowship to ALB and RR.

## References

Aldridge, H., and I. L. Rautenbach. 1987. Morphology, echolocation and resource partitioning in insectivorous bats. Journal of Animal Ecology **56**:763–778.

Almeida, M. H., A. D. Ditchfield, and R. S. Tokumaru. 2014. Habitat characteristics and insectivorous bat activity. Chiroptera Neotropical **20**:1264-1270.

Altringham, J. 2011. Bats. From Evolution to Conservation. Second edition edition. Oxford University Press, New York, United States of America.

Arita, H. T., and M. B. Fenton. 1997. Flight and echolocation In the ecology and evolution of bats. Trends in Ecology & Evolution **12**:53-58.

Bader, E., M. Acácio, and A. Monadjem. 2015a. The importance of water bodies for insectivorous bats in a Malagasy dry deciduous forest: A case example from Kirindy (Cnferef). Malagasy Nature **9**:88-96.

Bader, E., K. Jung, E. K. V. Kalko, R. A. Page, R. Rodriguez, and T. Sattler. 2015b. Mobility explains the response of aerial insectivorous bats to anthropogenic habitat change in the Neotropics. Biological Conservation **186**:97-106.

Barbaro, L., and I. Van Halder. 2009. Linking bird, carabid beetle and butterfly life-history traits to habitat fragmentation in mosaic landscapes. Ecography **32**:321-333.

Barclay, R. M. R. 1999. Bats are not birds - A cautionary note on using echolocation calls to identify bats: A comment. Journal of Mammalogy **80**:290-296.

Barclay, R. M. R., J. H. Fullard, and D. S. Jacobs. 1999. Variation in the echolocation calls of the hoary bat (*Lasiurus cinereus*): influence of body size, habitat structure, and geographic location. Canadian Journal of Zoology **77**:530-534.

Benítez-Malvido, J., W. Dáttilo, A. P. Martínez-Falcón, C. Durán-Barrón, J. Valenzuela, S. López, and R. Lombera. 2016. The multiple impacts of tropical forest fragmentation on arthropod biodiversity and on their patterns of interactions with host plants. PLoS ONE **11**:e0146461.

Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society. Series B Methodological **57**:289-300.

Bernard, E. 2001. Vertical stratification of bat communities in primary forests of Central Amazon, Brazil. Journal of Tropical Ecology **17**:115-126.

Blakey, R. V., B. S. Law, R. T. Kingsford, and J. Stoklosa. 2017. Terrestrial laser scanning reveals below-canopy bat trait relationships with forest structure. Remote Sensing of Environment **198**:40-51.

Blakey, R. V., B. S. Law, R. T. Kingsford, J. Stoklosa, P. Tap, and K. Williamson. 2016. Bat communities respond positively to large-scale thinning of forest regrowth. Journal of Applied Ecology **53**:1694-1703.

Bohnenstengel, T. 2012. Roost selection by the forest-dwelling bat *Myotis bechsteinii* (Mammalia: Chiroptera): implications for its conservation in managed woodlands. Bulletin de la Société Neuchâteloise des Sciences Naturelles **132**:47-62.

Borkin, K. M., and S. Parsons. 2011. Sex-specific roost selection by bats in clearfell harvested plantation forest: improved knowledge advises management. Acta Chiropterologica **13**:373-383.

Browning, E., R. Gibb, P. Glover-Kapfer, and K. Jones. 2017. Passive acoustic monitoring in ecology and conservation. WWF, Woking, United Kingdom.

Cadotte, M. W., K. Carscadden, and N. Mirotchnick. 2011. Beyond species: functional diversity and the maintenance of ecological processes and services. Journal of Applied Ecology **48**:1079-1087.

Cardinale, B. J., J. E. Duffy, A. Gonzalez, D. U. Hooper, C. Perrings, P. Venail, A. Narwani, G. M. Mace, D. Tilman, and D. A. Wardle. 2012. Corrigendum: Biodiversity loss and its impact on humanity. Nature **489**:326-326.

Carvalho, F., M. E. Fabián, and J. O. Menegheti. 2013. Vertical structure of an assemblage of bats (Mammalia: Chiroptera) in a fragment of Atlantic Forest in Southern Brazil. Zoologia **30**:491-498.

Cisneros, L. M., M. E. Fagan, and M. R. Willig. 2015. Effects of human-modified landscapes on taxonomic, functional and phylogenetic dimensions of bat biodiversity. Diversity and distributions **21**:523-533.

Coronado, A., C. Flaquer, X. Puig-Montserrat, E. Barthe, M. Mas, A. Arrizabalaga, and A. López-Baucells. 2018. The role of secondary trees in Mediterranean mature forests for the conservation of the forest-dwelling bat *Myotis alcathoe*. Are current logging guidelines appropriate? Hystrix, the Italian Journal of Mammalogy **28**:240–246.

Cunto, G. C., and E. Bernard. 2012. Neotropical bats as indicators of environmental disturbance: What is the emerging message? Acta Chiropterologica **14**:143-151.

Davies, K. F., C. R. Margules, and J. F. Lawrence. 2000. Which traits of species predict population declines in experimental forest fragments? Ecology **81**:1450-1461.

Denzinger, A., and H.-U. Schnitzler. 2013. Bat guilds, a concept to classify the highly diverse foraging and echolocation behaviors of microchiropteran bats. Frontiers in Physiology **4**:164.

Didham, R. K., J. Ghazoul, N. E. Stork, and A. J. Davis. 1996. Insects in fragmented forests: a functional approach. Trends in Ecology & Evolution **11**:255-260.

Dirzo, R., H. S. Young, M. Galetti, G. Ceballos, N. J. B. Isaac, and B. Collen. 2014. Defaunation in the Anthropocene. Science **345**:401-406.

Dobrovolski, R., J. A. F. Diniz-Filho, R. D. Loyola, and P. De Marco Júnior. 2011. Agricultural expansion and the fate of global conservation priorities. Biodiversity & Conservation **20**:2445-2459.

Dray, S., P. Choler, S. Dolédec, P. R. Peres-Neto, W. Thuiller, S. Pavoine, and C. J. F. ter Braak. 2014. Combining the fourth-corner and the RLQ methods for assessing trait responses to environmental variation. Ecology **95**:14-21.

Dray, S., and A.-B. Dufour. 2007. The ade4 package: implementing the duality diagram for ecologists. Journal of Statistical Software **22**:1-20.

Eisenberg, J. F., and K. H. Redford. 1989. Mammals of the Neotropics. Volume 3 Ecuador, Peru, Bolivia, Brazil, Chicago, United States of America.

Emrich, M. A., E. L. Clare, W. O. Symondson, S. E. Koenig, and M. B. Fenton. 2014. Resource partitioning by insectivorous bats in Jamaica. Molecular Ecology **23**:3648-3656.

Estrada-Villegas, S., C. F. Meyer, and E. K. Kalko. 2010. Effects of tropical forest fragmentation on aerial insectivorous bats in a land-bridge island system. Biological Conservation **143**:597-608.

Ethier, K., and L. Fahrig. 2011. Positive effects of forest fragmentation, independent of forest amount, on bat abundance in eastern Ontario, Canada. Landscape Ecology **26**:865-876.

Ewers, R. M., and R. K. Didham. 2006. Confounding factors in the detection of species responses to habitat fragmentation. Biological Reviews **81**:117-142.

Farneda, F. Z., R. Rocha, A. López-Baucells, M. Groenenberg, I. Silva, J. M. Palmeirim, P. E. D. Bobrowiec, C. F. J. Meyer, and M. Pocock. 2015. Trait-related responses to habitat fragmentation in Amazonian bats. Journal of Applied Ecology **52**:1381-1391.

Farneda, F. Z., R. Rocha, A. López-Baucells, E. M. Sampaio, J. M. Palmeirim, P. E. D. Bobrowiec, C. E. V. Grelle, and C. F. J. Meyer. 2018a. Functional recovery of Amazonian bat assemblages following secondary forest succession. Biological Conservation **218**:192-199.

Farneda, F. Z., R. Rocha, A. López-Baucells, E. M. Sampaio, J. M. Palmeirim, P. E. D. Bobrowiec, C. E. V. Grelle, and C. F. J. Meyer. 2018b. The road to functional recovery: temporal effects of matrix regeneration on Amazonian bats. Tropical Conservation Science **11**:1-4.

Fenton, M. B. 1997. Science and the conservation of bats. Journal of Mammalogy **78**:1-14.

Ferreira, D. F., R. Rocha, A. López-Baucells, F. Z. Farneda, J. M. B. Carreiras, J. M. Palmeirim, and C. F. J. Meyer. 2017. Season-modulated responses of Neotropical bats to forest fragmentation. Ecology and Evolution **7**:4059-4071.

Frank, H. K., L. O. Frishkoff, C. D. Mendenhall, G. C. Daily, and E. A. Hadly. 2017. Phylogeny, traits, and biodiversity of a Neotropical bat assemblage: Close relatives show similar responses to local deforestation. The American Naturalist **190**:200-212.

Freeman, P. W. 1981. A multivariate study of the family Molossidae (Mammalia, Chiroptera): morphology, ecology, evolution. Field Museum of Natural History Lincoln, United States of America.

Froidevaux, J. S. P., F. Zellweger, K. Bollmann, G. Jones, and M. K. Obrist. 2016. From field surveys to LiDAR: Shining a light on how bats respond to forest structure. Remote Sensing of Environment **175**:242-250.

Garcia-Morales, R., C. E. Moreno, E. I. Badano, I. Zuria, J. Galindo-Gonzalez, A. E. Rojas-Martinez, and E. S. Avila-Gomez. 2016. Deforestation Impacts on Bat Functional Diversity in Tropical Landscapes. PLoS ONE **11**:e0166765.

Golden, D. M., and T. O. Crist. 1999. Experimental effects of habitat fragmentation on old-field canopy insects: community, guild and species responses. Oecologia **118**:371-380.

Haddad, N. M., L. A. Brudvig, J. Clobert, K. F. Davies, A. Gonzalez, R. D. Holt, T. E. Lovejoy, J. O. Sexton, M. P. Austin, and C. D. Collins. 2015. Habitat fragmentation and its lasting impact on Earth’s ecosystems. Science advances **1**:e1500052.

Hanspach, J., J. Fischer, K. Ikin, J. Stott, and B. S. Law. 2012. Using trait-based filtering as a predictive framework for conservation: a case study of bats on farms in southeastern Australia. Journal of Applied Ecology **49**:842-850.

Hayes, J. P., and J. C. Gruver. 2000. Vertical stratification of bat activity in an old-growth forest in western Washington. Northwest Science **74**:102-108.

Henle, K., K. F. Davies, M. Kleyer, C. Margules, and J. Settele. 2004. Predictors of species sensitivity to fragmentation. Biodiversity & Conservation **13**:207-251.

Hill, M. O., and A. J. E. Smith. 1976. Principal component analysis of taxonomic data with multi-state discrete characters. Taxon **25**:249-255.

Jung, K., S. Kaiser, S. Böhm, J. Nieschulze, and E. K. V. Kalko. 2012. Moving in three dimensions: effects of structural complexity on occurrence and activity of insectivorous bats in managed forest stands. Journal of Applied Ecology **49**:523-531.

Kalka, M., and E. K. V. Kalko. 2006. Gleaning bats as underestimated predators of herbivorous insects: diet of *Micronycteris microtis* (Phyllostomidae) in Panama. Journal of Tropical Ecology **22**:1-10.

Kalko, E. 1998. Organisation and diversity of tropical bat communities through space and time. Zoology **101**:281-297.

Kalko, E. K. V., and C. O. Handley. 2001. Neotropical bats in the canopy: diversity, community structure, and implications for conservation. Plant Ecology **153**:319-333.

Kennedy, C. M., E. H. C. Grant, M. C. Neel, W. F. Fagan, and P. P. Marra. 2011. Landscape matrix mediates occupancy dynamics of Neotropical avian insectivores. Ecological Applications **21**:1837-1850.

Kleyer, M., S. Dray, F. Bello, J. Lepš, R. J. Pakeman, B. Strauss, W. Thuiller, and S. Lavorel. 2012. Assessing species and community functional responses to environmental gradients: which multivariate methods? Journal of Vegetation Science **23**:805-821.

Kunz, T. H., E. B. de Torrez, D. Bauer, T. Lobova, and T. H. Fleming. 2011. Ecosystem services provided by bats. Annals of the New York Academy of Sciences **1223**:1-38.

Kupfer, J. A., G. P. Malanson, and S. B. Franklin. 2006. Not seeing the ocean for the islands: the mediating influence of matrix-based processes on forest fragmentation effects. Global Ecology and Biogeography **15**:8-20.

Laurance, W. F., J. L. C. Camargo, P. M. Fearnside, T. E. Lovejoy, G. B. Williamson, R. C. G. Mesquita, C. F. J. Meyer, P. E. D. Bobrowiec, and S. G. W. Laurance. 2017. An Amazonian rainforest and its fragments as a laboratory of global change. Biological Reviews of the Cambridge Philosophical Society **93**:223-247.

Laurance, W. F., J. L. C. Camargo, R. C. C. Luizao, S. G. Laurance, S. L. Pimm, E. M. Bruna, P. C. Stouffer, G. B. Williamson, J. Benitez-Malvido, H. L. Vasconcelos, K. S. Van Houtan, C. E. Zartman, S. A. Boyle, R. K. Didham, A. Andrade, and T. E. Lovejoy. 2011. The fate of Amazonian forest fragments: A 32-year investigation. Biological Conservation **144**:56–67.

Laurance, W. F., J. Sayer, and K. G. Cassman. 2014. Agricultural expansion and its impacts on tropical nature. Trends in Ecology & Evolution **29**:107-116.

Lawer, E. A., and E. L. Darkoh. 2016. Effects of agroecosystems on insect and insectivorous bat activity: a preliminary finding based on light trap and mist net captures. Turkish Journal of Zoology **40**:423-432.

López-Baucells, A., R. Rocha, P. E. D. Bobrowiec, E. Bernard, J. Palmeirim, and C. Meyer. 2016. Field Guide to Amazonian Bats. INPA, Manaus, Brazil.

López-Baucells, A., L. Torrent, R. Rocha, A. C. Pavan, P. E. D. Bobrowiec, and C. F. J. Meyer. 2017. Geographical variation in the high-duty cycle echolocation of the cryptic common mustached bat *Pteronotus* cf. *rubiginosus* (Mormoopidae). Bioacoustics Journal **27**:341-357.

Loureiro, L. O., and R. Gregorin. 2015. Structure of a bat assemblage from a fragmented landscape in the state of Minas Gerais, southeastern Brazil. Mastozoologia Neotropical **22**:35-42.

MacSwiney, G. C., F. M. Clarke, and P. A. Racey. 2008. What you see is not what you get: the role of ultrasonic detectors in increasing inventory completeness in Neotropical bat assemblages. Journal of Applied Ecology **45**:1364-1371.

Malhi, Y., T. A. Gardner, G. R. Goldsmith, M. R. Silman, and P. Zelazowski. 2014. Tropical Forests in the Anthropocene. Annual Review of Environment and Resources **39**:125-159.

Marinello, M. M., and E. Bernard. 2014. Wing morphology of Neotropical bats: a quantitative and qualitative analysis with implications for habitat use. Canadian Journal of Zoology **92**:141-147.

Marques, J. T. 2016. Structuring Amazonian bat assemblages: importance of horizontal and vertical dimensions of habitat. University of Lisbon, Lisbon, Portugal.

Marques, J. T., M. J. Ramos Pereira, T. A. Marques, C. D. Santos, J. Santana, P. Beja, and J. M. Palmeirim. 2013. Optimizing sampling design to deal with mist-net avoidance in Amazonian birds and bats. PLoS ONE **8**:e74505.

Marques, J. T., M. J. Ramos Pereira, and J. M. Palmeirim. 2015. Patterns in the use of rainforest vertical space by Neotropical aerial insectivorous bats: all the action is up in the canopy. Ecography **39**:476-486.

Mas, M. 2014. Vertical stratification on insectivorous bats ensembles in Central Amazon. MSc Thesis. Universitat de Barcelona, Barcelona, Spain.

Mendenhall, C. D., D. S. Karp, C. F. J. Meyer, E. A. Hadly, and G. C. Daily. 2014. Predicting biodiversity change and averting collapse in agricultural landscapes. Nature **509**:213-217.

Meyer, C. F. J., J. Fruend, W. P. Lizano, and E. K. V. Kalko. 2008. Ecological correlates of vulnerability to fragmentation in Neotropical bats. Journal of Applied Ecology **45**:381-391.

Meyer, C. F. J., M. J. Struebig, and M. R. Willig. 2016. Responses of tropical bats to habitat fragmentation, logging, and deforestation. Pages 63-103 *in* C. C. Voigt and T. Kingston, editors. Bats in the Anthropocene: Conservation of bats in a changing world. Springer, New York, United States of America.

Meyer, C. F. J., M. Weinbeer, and E. K. V. Kalko. 2005. Home-range size and spacing patterns of *Macrophyllum macrophyllum* (Phyllostomidae) foraging over water. Journal of Mammalogy **86**:587-598.

Mickleburgh, S. P., A. M. Hutson, and P. A. Racey. 2002. A review of the global conservation status of bats. Oryx **36**:18-34.

Millon, L., J.-F. Julien, R. Julliard, and C. Kerbiriou. 2015. Bat activity in intensively farmed landscapes with wind turbines and offset measures. Ecological Engineering **75**:250-257.

Norberg, U. M., and J. M. V. Rayner. 1987. Ecological morphology and flight in bats (*Mammalia, Chiroptera*) - wing adaptations, flight performance, foraging strategy and echolocation. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences **316**:337-419.

Pavan, A. C., and G. Marroig. 2017. Timing and patterns of diversification in the Neotropical bat genus *Pteronotus* (Mormoopidae). Molecular Phylogenetics and Evolution **108**:61-69.

Peres-Neto, P. R., S. Dray, and C. J. ter Braak. 2017. Linking trait variation to the environment: critical issues with community-weighted mean correlation resolved by the fourth-corner approach. Ecography **40**:806-816.

Petchey, O. L., and K. J. Gaston. 2006. Functional diversity: back to basics and looking forward. Ecology Letters **9**:741-758.

Purvis, A., J. L. Gittleman, G. Cowlishaw, and G. M. Mace. 2000. Predicting extinction risk in declining species. Proceedings of the Royal Society of London. Series B: Biological Sciences **267**:1947-1952.

R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ray, N., A. Lehmann, and P. Joly. 2002. Modeling spatial distribution of amphibian populations: a GIS approach based on habitat matrix permeability. Biodiversity & Conservation **11**:2143-2165.

Ricketts, T. H. 2001. The matrix matters: effective isolation in fragmented landscapes. The American Naturalist **158**:87-99.

Rocha, R. 2017. Tropical forest fragmentation: effects on the spatio-temporal dynamics of its bat communities. PhD Thesis. University of Lisbon, Lisbon, Portugal.

Rocha, R., D. F. Ferreira, A. López-Baucells, F. Z. Farneda, J. Carreiras, J. M. Palmeirim, and C. F. Meyer. 2017a. Does sex matter? Gender-specific responses to forest fragmentation in Neotropical bats. Biotropica **49**:881-890.

Rocha, R., A. López-Baucells, F. Z. Farneda, M. Groenenberg, P. E. D. Bobrowiec, M. Cabeza, J. M. Palmeirim, and C. F. J. Meyer. 2017b. Consequences of a large-scale fragmentation experiment for Neotropical bats: disentangling the relative importance of local and landscape-scale effects. Landscape Ecology **32**:31–45.

Rocha, R., O. Ovaskainen, A. López-Baucells, F. Z. Farneda, D. F. Ferreira, P. E. D. Bobrowiec, M. Cabeza, J. M. Palmeirim, and C. F. J. Meyer. 2017c. Design matters: An evaluation of the impact of small man-made forest clearings on tropical bats using a before-after-control-impact design. Forest Ecology and Management **401**:8-16.

Rocha, R., O. Ovaskainen, A. López-Baucells, F. Z. Farneda, E. M. Sampaio, P. E. D. Bobrowiec, M. Cabeza, J. M. Palmeirim, and C. F. J. Meyer. 2018. Secondary forest regeneration benefits old-growth specialist bats in a fragmented tropical landscape. Scientific Reports **8**:3819.

Rösch, V., T. Tscharntke, C. Scherber, and P. Batary. 2013. Landscape composition, connectivity and fragment size drive effects of grassland fragmentation on insect communities. Journal of Applied Ecology **50**:387-394.

Safi, K., S. Meiri, and K. E. Jones. 2013. Evolution of body size in bats. Pages 95-151 *in* F. A. Smith and S. K. Lyons, editors. Animal body size: Linking pattern and process across space, time, and taxonomic group. University of Chicago Press Chicago, United States of America.

Schnitzler, H. U., and E. K. V. Kalko. 2001. Echolocation by insect-eating bats: We define four distinct functional groups of bats and find differences in signal structure that correlate with the typical echolocation tasks faced by each group Bioscience **51**:557-569.

Schnitzler, H. U., C. F. Moss, and A. Denzinger. 2003. From spatial orientation to food acquisition in echolocating bats. Trends in Ecology & Evolution **18**:386-394.

Silva, C. R., and E. Bernard. 2017. Bioacoustics as an important complementary tool in bat inventories in the Caatinga drylands of Brazil. Acta Chiropterologica **19**:409-418.

Taubert, F., R. Fischer, J. Groeneveld, S. Lehmann, M. S. Muller, E. Rodig, T. Wiegand, and A. Huth. 2018. Global patterns of tropical forest fragmentation. Nature **554**:519-522.

Torrent, L., A. López-Baucells, R. Rocha, P. E. D. Bobrowiec, and C. F. J. Meyer. 2018. The importance of lakes for bat conservation in Amazonian rainforests: an assessment using autonomous recorders. Remote Sensing in Ecology and Conservation:(in press).

Vetter, D., M. M. Hansbauer, Z. Végvári, and I. Storch. 2011. Predictors of forest fragmentation sensitivity in Neotropical vertebrates: a quantitative review. Ecography **34**:1-8.

Violle, C., M. L. Navas, D. Vile, E. Kazakou, C. Fortunel, I. Hummel, and E. Garnier. 2007. Let the concept of trait be functional! Oikos **116**:882-892.

Wickramasinghe, L. P., S. Harris, G. Jones, and N. Vaughan. 2003. Bat activity and species richness on organic and conventional farms: impact of agricultural intensification. Journal of Applied Ecology **40**:984-993.

Wordley, C. F. R., M. Sankaran, D. Mudappa, and J. D. Altringham. 2017. Bats in the Ghats: Agricultural intensification reduces functional diversity and increases trait filtering in a biodiversity hotspot in India. Biological Conservation **210**:48-55.

# Chapter 7

## The importance of lakes for bat conservation in Amazonian rainforests: an assessment using autonomous recorders

Torrent, L.*, **López-Baucells, A.***, Rocha, R., Bobrowiec, P. E. D., Meyer, C. F. J.

Published in *Remote Sensing in Ecology and Conservation* DOI: 10.1002/rse2.83

Top: *Cynomops planirostris;* Bottom: *Molossus rufus*

# CHAPTER 7

## The importance of lakes for bat conservation in Amazonian rainforests: an assessment using autonomous recorders

## Abstract

Recent studies predict a future decrease in precipitation across the tropics, particularly the Amazon, likely causing significant droughts that have negative consequences for Amazonian freshwater biomes, especially lakes. Furthermore, immediate consequences of global warming for terrestrial fauna associated with tropical lakes are poorly understood as the vast majority of studies come from temperate regions. Here, I assess the seasonal importance of lakes for the conservation of aerial insectivorous bats in the Central Amazon using passive bat recorders. I compared richness, general bat activity and foraging activity between lakes and adjacent forest. Of a total of 21 species/sonotypes recorded in both habitats, all were detected over lakes, and 18 were significantly more active over lakes than in forest. Only two species had significantly higher activity levels in the forest than at the lakes. Species richness and general bat activity over the lakes were higher in the dry than in the rainy season. Foraging activity was also greater over the lakes than within the forest in both seasons. Moreover, both variables were positively correlated with lake size, although the effect on activity was species-specific. Climate change-driven shrinking of lakes may have detrimental consequences for aerial insectivorous bats, especially for the most water-dependent species. Compared to permanent water bodies of other regions, the value of tropical lakes for functionally important taxa, such as bats, has been understudied. Higher bat activity levels over lakes than in forest in both seasons and comprising the whole ensemble of aerial insectivorous bats of the study region, indicate that lakes embedded in Amazonian terra firme forests deserve special attention for future bat conservation.

**Keywords**: Acoustic sampling, Aerial insectivorous bats, Amazon, Climate change, Passive acoustic, Monitoring, Tropical lakes.

## Introduction

The Amazon Basin, comprising 6.9 million km2 of watershed (Macedo and Castello 2015), harbors both the Earth's largest river drainage system and largest contiguous area of rainforest. Throughout the Amazon Basin, lakes usually occur in areas that are seasonally flooded and are formed of two main elements: a body of open water that varies seasonally in extent and an area of inundated vegetation (Melack and Coe 2013). Upland forests (terra firme) cover an area of about 50% of the hole Amazonian wetland in the basin (Junk et al. 2010) and although they do not get flooded as other regions, streams and lakes are also present. Amazonian lakes face a series of threats due to (1) lack of agreement on their classification and management; (2) dam construction; (3) land-cover change; and (4) anthropogenic climate change (Melack and Coe 2013, Jiménez-Cisneros et al. 2014, Castello and Macedo 2016, Salvarina 2016).

Predicted temperature increases due to climate change are usually considered to be less important in the tropics than in boreal (Sala et al. 2000, Solomon 2007), arid (Root et al. 2003, Parmesan 2007) or temperate regions (Sala et al. 2000, Root et al. 2003). Tropical ecosystems are interannually influenced by El Niño events (Trenberth and Hoar 1996) which cause extreme climate variability in the form of floods and droughts (Williams et al. 2005, Marengo et al. 2013). Therefore, due to these marked, recurring fluctuations, it is generally accepted that within a safe threshold, tropical ecosystems will be less sensitive to future climatic changes than those at higher latitudes (Collins et al. 2013, IPCC 2014). However, for the Neotropics, some climate models predict a decrease in precipitation and harsher droughts during the dry season (Beaumont et al. 2011, Marengo and Espinoza 2016), especially in the eastern and southern Amazon Basin (e.g. Shiogama et al. 2011, Staal et al. 2016, Aragão et al. 2018). This decrease in precipitation might have severe consequences for species that rely on lakes to fulfil their life cycles, especially during the dry season.

The vast majority of studies related to lakes and their associated fauna come from temperate habitats in Europe (Zacharias et al. 2007), Australia (Warwick and Brock 2003), Western USA (Kneitel and Lessin 2010) and South and North Africa (Rhazi et al. 2006, Rhazi et al. 2009). For Amazonia, studies on lakes have focused on those in floodplain forests and mainly targeted phytoplankton and fish (e.g. De Melo and Huszar 2000, Chellappa et al. 2005, Pazin et al. 2006, Moresco et al. 2017). Several studies have been conducted on a variety of taxa (e.g. birds and mammals) in flooded

Amazonian forests (e.g. Haugaasen and Peres 2007, Haugaasen and Peres 2008, Beja et al. 2010), indicating for instance that the availability of water attracts more fauna than fruit presence (Paredes et al. 2017). For bats, Pereira et al. (2009) documented a large number of species associated with seasonally flooded forests, emphasizing the importance of this habitat for insectivorous bats. Almeida et al. (2014) demonstrated that open grassland habitat with scattered fruit trees with a water reservoir from a river dam had greater presence of insectivorous bats than open habitats without water in southeastern Brazil. Little is known about the abundance and distribution of lakes in unflooded terra firme forest (Sioli 2012) and no study yet has assessed the ecological importance of such lakes for aerial insectivorous bats.

Freshwater bodies are essential for many bat species as they provide appropriate habitat for drinking and foraging (Seibold et al. 2013, Korine et al. 2016, Russo et al. 2017) as well as important habitats during their reproductive cycles (McLean and Speakman 1999, Adams and Thibault 2006, Adams and Hayes 2008, Cisneros et al. 2015). Hence, a reduction in the number of lakes as a result of climate change might have potentially severe consequences for those bat species that depend on them.

The overarching aim of this study was to use bioacoustics to identify the ecological role of lakes for aerial insectivorous bats in Central Amazonian terra firme rainforest and to establish whether their importance varies seasonally. My specific objectives were to: (1) compare species richness, general bat activity and foraging activity between habitat types (forest and lakes) and between seasons (dry and rainy); (2) determine the influence of lake size on species richness and general bat activity; and (3) quantify differences in species-specific activity levels between habitats and seasons. I predicted higher levels of richness, general bat activity and foraging activity at lakes than in forest because, based on existing bibliography from other regions, bats commute to the former to drink and forage. Moreover, I expected richness and general bat activity to be higher at lakes during the dry months due to the lower water availability within the forest. Regarding my second objective, I predicted greater activity and species richness over larger lakes than small ones because, aside from providing more food resources, the former are structurally less complex habitats (open areas without overhanging foliage) and thus are also accessible to less manoeuvrable species. Thirdly, I hypothesized that although all species would be present at the lakes at least to drink, some species would forage more often in the forest (i.e. mormoopid bats), while others (molossid, emballonurid and vespertilionid bats), given their morphological adaptations for foraging in open habitats, would be more frequently recorded at the lakes.

## Material and Methods

### Study area

The study was conducted at the Biological Dynamics of Forest Fragments Project (BDFFP), 80 km north of Manaus, in the Brazilian Amazon (Fig. 7.S1). The area encompasses approximately 1000 km2 of primary and secondary unflooded upland (terra firme) rainforest surrounding forest fragments that were isolated between 1980 and 1984 (Marengo et al. 2013, Laurance et al. 2017, Rocha et al. 2017b). Average annual temperature is 25.8°C and there is a well-defined dry season from June to October when precipitation drops below 100 mm/month and a rainy season from November to May when precipitation can exceed 300 mm/month (Ferreira et al. 2017, Kunert et al. 2017). The topography of the surveyed terrain and its surroundings is relatively flat, divided by many small streams. Large rivers are almost absent; however, there are permanent lakes whose water level fluctuates seasonally (Laurance et al. 2017). The primary forest canopy is between 30 and 37 m tall, with emergent trees reaching up to 55 m. For a more detailed description of the study area, see Laurance et al. (2017).

### Acoustic surveys

Bats were sampled at seven sites in primary forest (continuous forest and 100 ha forest fragments: Forest 1 to Forest 7), located in the reserves Cabo Frio, Dimona, Porto Alegre and Km 41, and at eight adjacent lakes, one in Dimona (Lake 1), two in Porto Alegre (Lake 2 and 3), one close to the main BDFFP road (Lake 4), one in Colosso (Lake 5), and three in Km 41 (Lakes 6, 7 and 8) (Figs. S1 and S2 and Table 7.1). Forest sites were selected based on their proximity to the known lakes in the study area. At each forest site, a passive bat recorder (Song Meter SM2Bat+) with an omnidirectional ultrasonic SMX-US microphone (Wildlife Acoustics, In. Maynard, Massachusetts, USA) was attached to the branches or the trunk of a tree at 1–2 m height, pointing in the direction with least vegetation clutter. At the lakes, the recorders were set at their shores, at the same height, attached to the branches or trunks of trees, with the microphone facing the centre of the water body. During each sampling session, the detectors were programmed to continuously record from 18:00 h to 06:00 h, for a period of three consecutive nights. Recordings were made in real time, with a full spectrum (fs) resolution of 16-bit, a high-pass filter set at fs/32 (12 kHz) and a trigger level of 18 signal to noise ratio. Each site was sampled twice per season and acoustic surveys were conducted during the dry season (August to October) of 2013 and the rainy season (March to May) of 2014. The total number of nights surveyed was 72 for the lakes and 79 for the forest sites as I had to discard some of the data when the detectors only recorded during half of the night due to technical problems or intense rain.

Table 7.1. General information about the lakes surveyed, including the oscillation of the water level between the rainy and dry seasons, at the Biological Dynamics of Forest Fragments Project, Central Amazon, Brazil.

| **Site** | **Reserve** | **Size: rainy season ($m^2$)** | **Size: dry season ($m^2$)** | **Geographical coordinates** |
|---|---|---|---|---|
| *Lake 1* | Dimona | ~200 | ~200 | -2.3398381°, -60.1016720° |
| *Lake 2* | Porto Alegre | ~2500 | ~400 | -2.3574670°, -59.9593178° |
| *Lake 3* | Porto Alegre | ~2300 | ~1300 | -2.3851464°, -59.9841295° |
| *Lake 4* | Km 21 | ~9600 | ~9600 | -2.4051716°, -59.9704628° |
| *Lake 5* | Colosso | ~2100 | ~440 | -2.4109754°, -59.8690231° |
| *Lake 6* | Km 41 | ~2900 | ~860 | -2.4305276°, -59.7741627° |
| *Lake 7* | Km 41 | ~60 | ~60 | -2.4492385°, -59.7701420° |
| *Lake 8* | Km 41 | ~9700 | ~2400 | -2.4476869°, -59.7505882° |
| *Forest 1* | Cabo Frio | - | - | -2.380979°, -59.928022° |
| *Forest 2* | Cabo Frio | - | - | -2.399757°, -59.900202° |
| *Forest 3* | Cabo Frio | - | - | -2.416985°, -59.926773° |
| *Forest 4* | Km 41 | - | - | -2.416634°, -59.780269° |
| *Forest 5* | Km 41 | - | - | -2.427005°, -59.749305° |
| *Forest 6* | Porto Alegre | - | - | -2.363252°, -59.975384° |
| *Forest 7* | Dimona | - | - | -2.343255°, -60.095072° |

## Bioacoustic analysis

I defined sample unit as a ‘bat pass’ – a sequence with a minimum of two recognizable echolocation pulses per species emitted by a flying bat within a 5-sec sound file (Azam et al. 2015, Millon et al. 2015) – which was used as a surrogate of activity levels. Species-specific activity was quantified as the number of bat passes per night recorded for each species and total activity as the sum of all bat passes per night. I also quantified foraging activity as the number of feeding buzzes detected per species (Kalko and Schnitzler 1989).

I used Kaleidoscope Pro Software (version 4.0.4.) (Wildlife Acoustics, Inc. Maynard, Massachusetts, USA) to analyze and classify the recordings. Call sequences were manually identified to species level or left as mixed species groups, that is, sonotypes, when it was not possible to clearly assign a call to a particular species (Table 7.S1). Classification was undertaken following (López-Baucells et al. 2016), and by comparing my recordings with a local reference call library compiled in the same study area during 2011–2014. Species were manually identified based on a series of acoustic features and standard measurements: call shape, frequency of maximum energy (FME), start, end, maximum and minimum frequency, and call duration. If call sequences or pulses were too faint (<10 dB difference from the background noise) to obtain the information needed for reliable species identification they were discarded. The analysis thus included a total of 21 sonotypes from the families Emballonuridae, Furipteridae, Molossidae, Mormoopidae and Vespertilionidae of which 14 were classified to species level, whereas seven sonotypes grouped several species (Table 7.S1).

## Statistical analysis

To assess the effect of habitat type (lakes vs. forest) and season (dry vs. rainy) on richness, total activity and foraging activity, I used Generalized Linear Mixed-effects Models (GLMMs) with a Poisson distribution, fitted in a Bayesian framework using Markov Chain Monte Carlo analysis (MCMC). Response variables in the GLMMs were richness (total number of sonotypes registered in a single night), bat activity (number of bat passes registered in a single night in total and per species) and foraging activity (number of feeding buzzes registered in a single night in total). Habitat type and season were used as fixed factors and reserve (Dimona, Porto Alegre, Km 21, Km 41, Colosso and Cabo Frio) was included as a random factor. I also included the interaction between both fixed-effect variables. Modelling was performed with the ‘MCMCglmm’ R package (Hadfield 2010), with default priors for the fixed effects (zero mean, high variance). Interaction effects were plotted with the ‘effects’ package (Fox 2003).

Lake sizes were estimated measuring their water surface areas with Google Earth. As the size of the lakes did not vary continuously (Table 7.1), I grouped them into three categories: ‘Small’ (<200 m2); ‘Medium’ (2000–3000 m2); and ‘Large’ (>9000 m2). GLMMs were also used to analyze the effect of lake size (fixed effect) on richness and total bat activity. For this particular analysis, species from the family Molossidae were excluded as they are known to not forage in cluttered environments and over small lakes (Mora et al. 2004, Kalka et al. 2008, Kalko et al. 2008). All plots were built using the package ‘ggplot2’ in R (Wickham 2009) and all statistical analyses were performed with R version 3.3.2 for Windows (R Core-Team 2015).

## Results

From a total of 290 899 sound files, I identified 353 099 bat passes and 69 454 feeding buzzes (Table 7.S1). Although all 21 sonotypes were recorded at the lakes, only 15 were detected in the forest (Fig. 7.1). Specifically, only *Pteronotus* sp1, *Pteronotus rubiginosus* and *Furipterus horrens* were more often recorded in forest than at lakes (Fig. 7.1).

### Effect of habitat type and season on aerial insectivorous bats

*Richness, total activity and foraging activity*

Species richness, total activity and the number of feeding buzzes were significantly higher at lakes than in forest sites ($P < 0.05$) (Fig. 7.2a and Table 7.S2). In fact, habitat had a stronger effect than season for all response variables (Fig. 7.2b). Total bat activity and foraging activity were generally three and four times, respectively, higher over lakes than in forest sites, while richness was remarkably greater over lakes than in forest (Fig. 7.2). Season and its interaction with habitat only had a significant effect on richness. While at the lakes, richness increased during the dry season compared with the rainy season, in primary forest richness was higher in the rainy than in the dry season (Fig. 7.3).

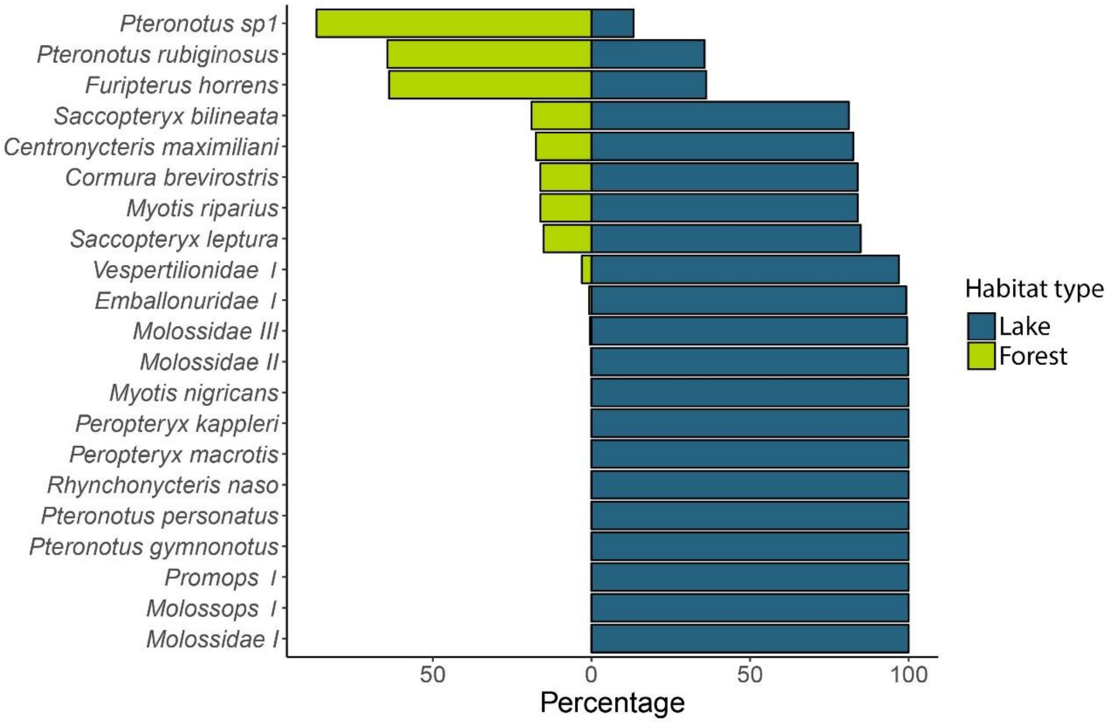


Figure 7.1. Percentage of bat species occurrence at eight lakes and in seven primary forest sites at the Biological Dynamics of Forest Fragments Project, Central Amazon.

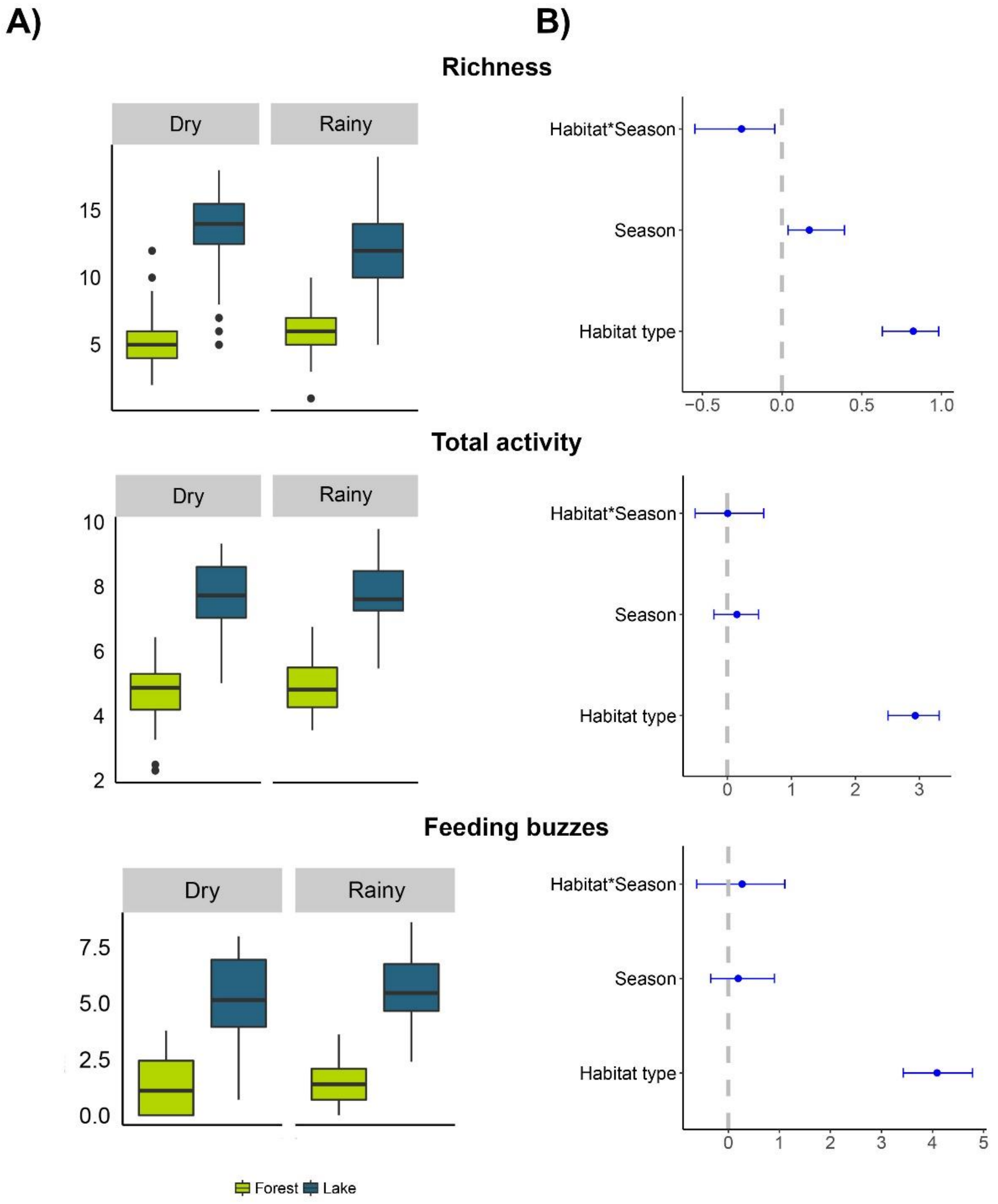


Figure 7.2. (A) Richness, total bat activity and feeding buzzes per night in primary forest and at lakes for each season at the Biological Dynamics of Forest Fragments Project, Central Amazon. (B) Parameter estimates of the fixed effects habitat type and season as well as their interaction.

### *Species-specific responses to habitat type and season*

Only *Furipterus horrens* and *Pteronotus* sp1 had significantly higher activity levels in forest, whereas all other sonotypes, with the exception of *P. rubiginosus*, were detected significantly more often at lakes than in forest (Fig. 7.4 and Table 7.S3). Thirty-five percent of sonotypes showed significant differences in activity levels depending on the season between lakes and forest. While the activity of *Centronycteris maximiliani*, *Peropteryx kappleri* and *Vespertilionidae* I in the forest showed a significant peak in the rainy season, it remained almost constant along the year at the lakes (Figs. S4 and S7).

The activity of *Myotis nigricans*, *Molossops* I and *Promops* I in the rainy season significantly increased at the lakes, and simultaneously decreased in forests (Figs. S5 and S7). *Pteronotus rubiginosus* and *Molossidae* III showed lower activity in the rainy season in both habitats, but more markedly so at the lakes (Figs. S5, S6 and Table 7.S3).

*Effect of lake size*

Both richness and total activity scaled significantly and positively with lake size (Fig. 7.5 and Table 7.S4). The variance associated with the reserve was minimal, and thus, its effect on the differences between lake size negligible (Table 7.S2 and Table 7.S5). *Myotis nigricans* and *Furipterus horrens* were detected significantly more often over large lakes than medium and small ones (Table 7.S6). Others (*Rhynchonycteris naso*, *Saccopteryx bilineata, Emballonuridae* I, *Peropteryx kappleri*, *Peropteryx macrotis*) had greatest activity at both medium-sized and large lakes. In contrast, *Centronycteris maximiliani*, *Pteronotus rubiginosus*, *Pteronotus* sp1 and *Myotis riparius* were detected significantly more at the small than the large lakes (Fig. 7.S3 and Table 7.S6). Activity levels for *Cormura brevirostris*, *Pteronotus gymnonotus* and *Pteronotus personatus* did not vary among lake size categories (Fig. 7.S3 and Table 7.S6).

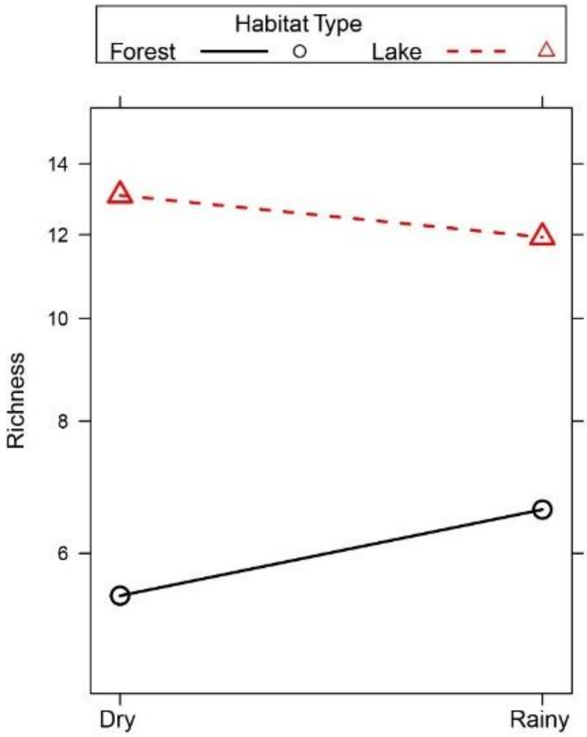


Figure 7.3. Interaction plot illustrating the inter-dependence of habitat type (forest and lake) and season (dry and rainy) in determining richness of aerial insectivorous bats at the Biological Dynamics of Forest Fragments Project, Central Amazon, Brazil.

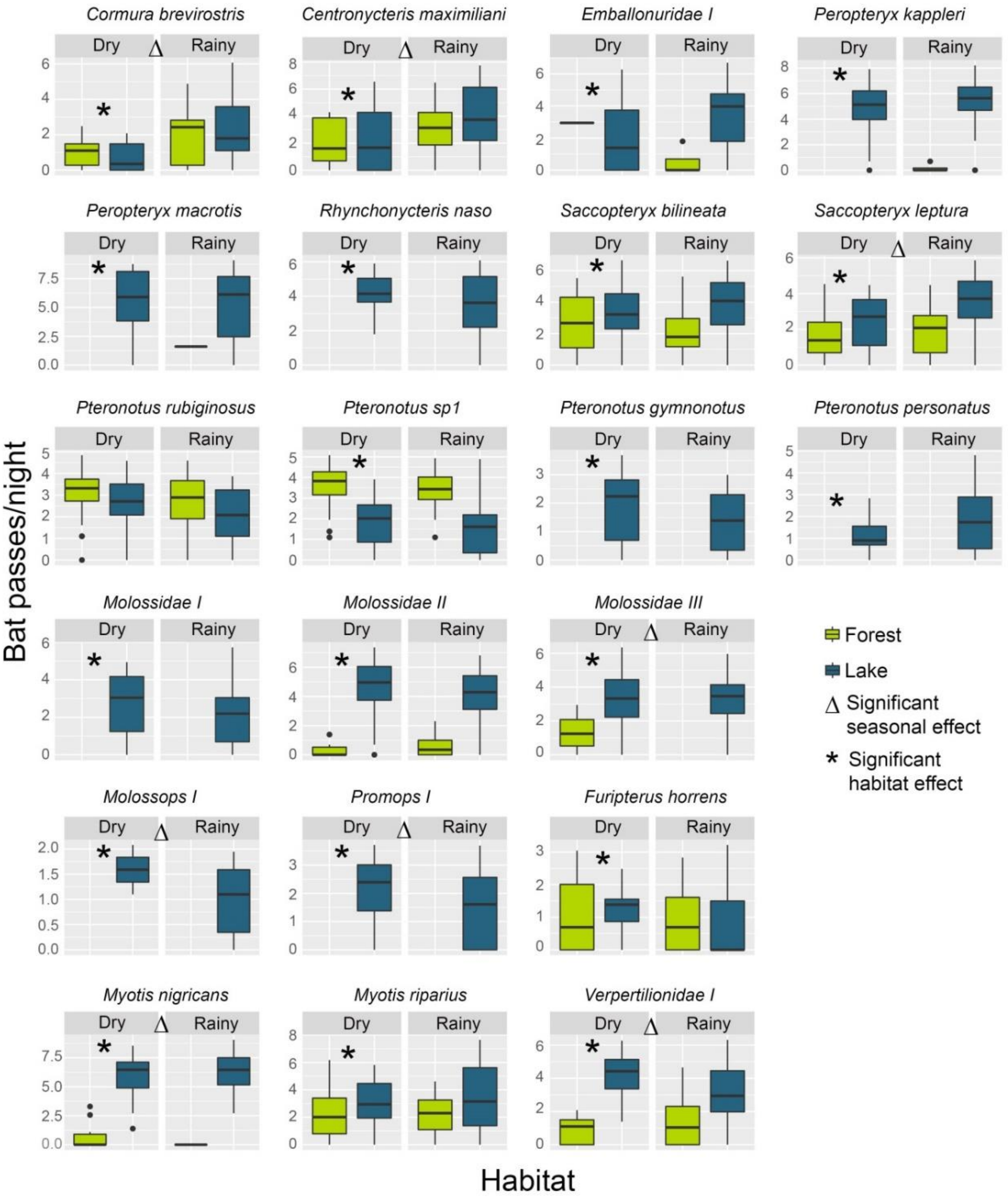


Figure 7.4. Comparison of the number of bat passes per species per night between primary forest and lakes for each season at the Biological Dynamics of Forest Fragments Project, Central Amazon, Brazil. Significant differences ($p<0.05$) are indicated with an asterisk (habitat effect) or triangle (seasonal effect).

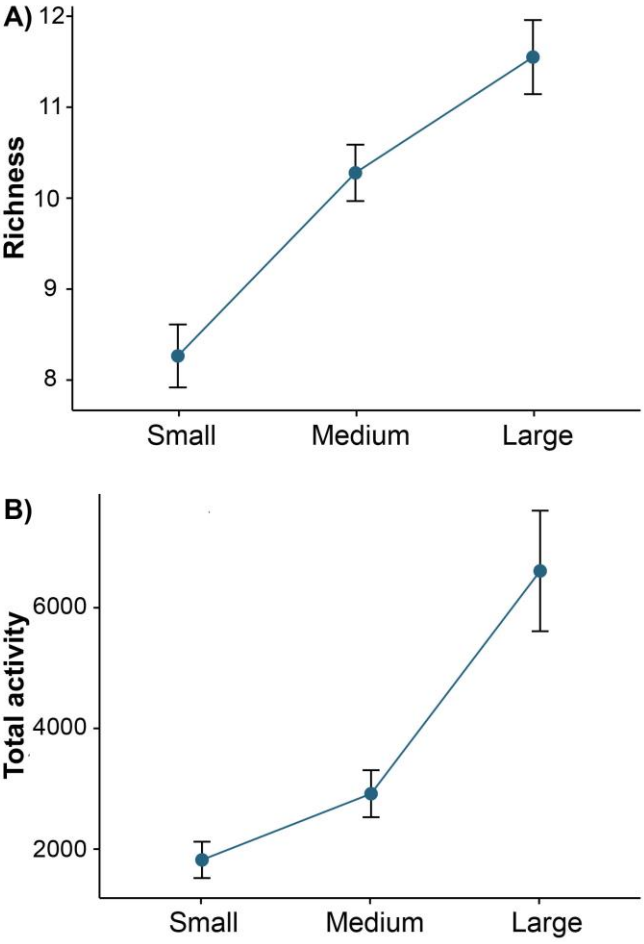


Figure 7.5. Richness and total activity (mean ± 95% CI) of aerial insectivorous bats (excluding molossids) per site and night for “small” (up to 200 m2), “medium” (2000–3000 m2) and “large” (> 9000 m2) lakes at the Biological Dynamics of Forest Fragments Project, Central Amazon, Brazil.

## Discussion

As predicted, richness, general bat activity and foraging activity were significantly higher over lakes than in forest, especially during the dry season. This highlights the fundamental importance of lakes as key foraging habitat for aerial insectivorous bats and consequently for their conservation in Amazonian terra firme forests. As expected, all 21 species/sonotypes identified in the study area were detected over the lakes (either while foraging, drinking or commuting), whereas none was exclusively found in the forest. In fact, only three species were detected significantly more in forest than at the lakes, supporting the hypothesis that most of the species might at least to some extent be dependent on lakes and that their long-term persistence in terra firme forest thus might be strongly linked to the future of these aquatic habitats. Additionally, the results confirmed that sonotype richness and general bat activity were positively correlated with lake size.

### *Effect of habitat, season and lake size on richness and total activity*

The lakes sampled had higher richness compared to the adjacent forests, including both open-space foragers and strict forest-dwelling species that were recorded while foraging. Pereira et al. (2009) undertook a similar study in Amazonian flooded forest and found that aerial insectivorous bats were more abundant in seasonally inundated than in terra firme forest. During both seasons, the total number of feeding buzzes recorded in this study was four times higher over the lakes than within the forest (Fig. 7.2). This adds to previous evidence that most Neotropical aerial insectivorous bats actively forage over water bodies (MacSwiney et al. 2009, de Moraes Costa et al. 2012, Staal et al. 2016). In general, riparian habitats and water bodies in the tropics are characterized by higher insect abundances than dense forests (Fukui et al. 2006, Chan et al. 2008, Hagen and Sabo 2011), especially during the rainy season (Dudgeon 1997, Chan et al. 2008). However, as lakes do not provide as much vegetation cover as forests during moonlit nights, they also expose bats to increased risk of predation (Appel et al. 2017). Therefore, higher richness in forest during the rainy season than during the driest months suggests that some species (probably strict forest-dwelling bats) might not need to commute to the lakes if enough resources are available within the forest. In contrast, richness at the lakes was higher during the dry than the rainy season, probably because some species were forced to commute to the lakes for drinking due to the lack of smaller water sources within the forest. Activity levels of some Neotropical bat species vary seasonally in response to higher energetic requirements associated with pregnancy and lactation in females (Cisneros et al. 2015, Rocha et al. 2017a). Greater abundance of insects over lakes might offer better foraging opportunities to female bats during their reproductive season. However, lack of phenological data for the species included in this study and the impossibility to disentangle males and females based on acoustic data precludes a sex-specific analysis in relation to reproductive patterns.

The lakes sampled during this study were less encroached by overhanging vegetation than other aquatic habitat associated with rainforests, such as streams or small ponds. Less overhanging vegetation allows less manoeuvrable species, such as molossid bats, to use these lakes for drinking and foraging, giving them enough space to manoeuvre, despite their wing morphology (Greif and Siemers 2010). Species-specific bat activity levels are greatly influenced by habitat complexity (Sleep and Brigham 2003, Farneda et al. 2015). When foraging, bats receive information about both potential prey and their immediate surroundings (Schnitzler and Kalko 2001). As a consequence, flight costs to navigate and to detect and catch prey are higher within structurally complex forest habitats than over lakes and other open areas. Moreover, open, deep (1–1.5 m), smooth and calm surfaces of water have been suggested to be ideal for all types of bats to drink compared to flowing rivers and streams (Almenar et al. 2006, Linton et al. 2011, Seibold et al. 2013). As some of the lakes surveyed were also partially connected to streams, bats could have also used these linear corridors as commuting routes (Palmeirim and Etherdige 1985, Meyer et al. 2005). Although detectability in open spaces should be higher than in cluttered environments, I tested ultrasound attenuation between forests and lakes and found the difference at distances up to 10 m to be so negligible, that I assumed they could be directly compared (Fig. 7.S8).

### *Species-specific responses to habitat type, season and lake size*

#### Emballonuridae

*Rhynchonycteris naso* was the emballonurid bat most strongly associated with lakes, as it was never recorded in the forest. As described by Ceballos (2014), this species tends to forage and roost over water bodies. The other emballonurids identified were also more commonly detected over lakes, in agreement with existing literature which considers *Saccopteryx leptura* an open-space forager (Jung et al. 2007) and *S. bilineata*, *Centronycteris maximiliani*, *Peropteryx kappleri* and *P. macrotis* edge specialists (Jung et al. 2007, Kalka et al. 2008, Barboza-Marquez et al. 2014). However, in contrast to previous studies, *Cormura brevirostris*, a forest interior forager (Estrada-Villegas et al. 2010), was also detected significantly more over lakes, probably because higher densities of aquatic insects during the rainy season accumulated above water bodies (Chan et al. 2008).

#### Molossidae

These results agree with previous studies in which molossid were commonly found over water bodies (de Moraes Costa et al. 2012), especially above larger lakes that allow them to easily manoeuvre near the water surface (Adams and Simmons 2002, de Moraes Costa et al. 2012). Although *Promops*, *Molossops* and other rare molossid species are widely distributed across Central and South America, little is known about these genera (González-Terrazas et al. 2016, López-Baucells et al. 2018) and this study provides new information about their presence and feeding behaviour at Amazonian lakes.

### Mormoopidae

The species complex *Pteronotus* cf. *parnellii* includes two sympatric sister species in my study area (López-Baucells et al. 2017). This data provides the first comparative quantification of species-specific activity and occurrence of these species (*Pteronotus rubiginosus* and *P.* sp1) and suggest that both are associated with highly cluttered habitats. *Pteronotus personatus* and *P. gymnonotus* were rarely recorded and only over lakes, which agrees with previous literature where they are defined as edge specialists (Kalka et al. 2008).

### Furipteridae

*Furipterus horrens* is one of the least known insectivorous species in the Neotropics due to its elusiveness and the difficulty to detect it acoustically or with capture techniques (Falcão et al. 2015). The species was only captured once in the study area during extensive mist netting surveys that were conducted parallel to this study (Rocha et al. 2017b). Using bioacoustics, I passively and more efficiently monitored presence and activity of *F. horrens,* whereby my data suggest greater activity within the forest than over lakes.

### Vespertilionidae

As expected, *Myotis nigricans* and *M. riparius* were both significantly more active over lakes (de Moraes Costa et al. 2012) than in forest, particularly during the rainy season when insect availability is greater (Chan et al. 2008). Despite both species foraging over the water, these results indicate differential preferences regarding lake size. Although *M. nigricans* was frequently detected over large lakes, *M. riparius* was more often recorded over small lakes in closed environments. These are cryptic species whose identification in the field is far from easy due to similarities in morphology and echolocation. Their capacity to forage in lower forest strata, over water and both in open spaces and edge-and gap situations (Siemers et al. 2001, Sampaio et al. 2003) as well as their diet (Siemers et al. 2001, Laval and Rodríguez 2002) should be further investigated to unveil what factors allow such similar species to coexist.

## Conservation implications

Studies indicate that climate change can affect the emergence of aquatic insects and also cause physiological changes in tropical insects (Greig et al. 2012, Jonsson et al. 2015). Hence, longer drought periods associated with climate change might imperil tropical fauna that exploit insects associated with freshwater ecosystems. As insectivorous bats extensively use lakes in the tropics to forage and drink, it is essential to study their response to seasonal fluctuations in water levels. In fact, not only aerial insectivorous bats but also other guilds, such as gleaning animalivorous, frugivorous and nectarivorous bats (Giannini and Kalko 2004, Meyer and Kalko 2008) might be seasonally relying on Amazonian lakes. During the rainy season, lakes may be good foraging areas for animalivorous bats for two main reasons: (1) their prey, such as terrestrial arthropods and frogs, migrate to escape the water (Adis and Junk 2002) and (2) lakes are more accessible habitats than dense forest as they have less obstacles which facilitates manoeuvrability and prey detection (Schnitzler and Kalko 2001, Bobrowiec et al. 2014). Lakes can accumulate nutrient-rich sediments during rainy periods, enhancing the productivity of flowers and fruits of pioneer trees (Haugaasen and Peres 2007, Pereira et al. 2009), which could attract frugivorous and nectarivorous bats. The available evidence therefore seems to suggest that potentially negative consequences of anthropogenic climate change upon Neotropical lakes will not only affect aerial insectivorous bats but also various other Neotropical bat guilds.

A better understanding of bat phenology would allow us to more reliably predict the consequences of climate change for this species-rich group. These findings indicate that the climate change-driven shrinking or disappearance of many lakes across the Amazon Basin will have negative repercussions for aerial insectivorous bats, probably leading to local population declines, if not extinctions, of the most water-dependent species. Compared to permanent lakes in other regions of the world, the role of Amazonian lakes for bat conservation so far has clearly been underappreciated. Higher levels of bat activity over lakes than in forest sites in both seasons and comprising the whole ensemble of aerial insectivorous bats of the study region, indicate that lakes, although seasonally variable in terms of water surface and sparsely distributed in unflooded Amazonian rainforest, deserve special attention for conservation. In the face of anthropogenic climate change, protection of floodplains and riparian forest, as well as buffer zones along rivers and lakes should be encouraged across the Amazon Basin to sustain populations of not only aerial insectivorous bats, but the whole diversity of taxa associated with these aquatic ecosystems.

## Acknowledgments

I would like to thank Oriol Massana, Diogo Ferreira, Marta Acácio and Fabio Farneda for fieldwork assistance and the Instituto Nacional de Pesquisas da Amazonia (INPA) and the Biological Dynamics of Forest Fragments Project (BDFFP), especially Jose Luis Camargo, Rosely Hipólito and Ary Jorge Ferreira for logistical support. This work was supported by the Portuguese Foundation for Science and Technology under research grant PTDC/BIABIC/111184/2009 (C.F.J.M.) and scholarships SFRH/BD/80488/2011 (R.R.) and PD/BD/52597/2014 (A.L.B.). Additional funding was provided by Bat Conservation International to A.L.B. and R.R. Research was conducted under permit (26877-2) issued by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio). This is publication 737 in the Technical Series of the BDFFP.

# References

Adams, R. A., and M. A. Hayes. 2008. Water availability and successful lactation by bats as related to climate change in arid regions of western North America. Journal of Animal Ecology **77**:1115-1121.

Adams, R. A., and J. A. Simmons. 2002. Directionality of drinking passes by bats at water holes: is there cooperation? Acta Chiropterologica **4**:195-199.

Adams, R. A., and K. M. Thibault. 2006. Temporal resource partitioning by bats at water holes. Journal of Zoology **270**:466-472.

Adis, J., and W. J. Junk. 2002. Terrestrial invertebrates inhabiting lowland river floodplains of Central Amazonia and Central Europe: a review. Freshwater Biology **47**:711-731.

Almeida, M. H., A. D. Ditchfield, and R. S. Tokumaru. 2014. Habitat characteristics and insectivorous bat activity. Chiroptera Neotropical **20**:1264-1270.

Almenar, D., J. Aihartza, U. Goiti, E. Salsamendi, and I. Garin. 2006. Habitat selection and spatial use by the trawling bat *Myotis capaccinii* (Bonaparte, 1837). Acta Chiropterologica **8**:157-167.

Appel, G., A. López-Baucells, E. Magnusson, and P. E. D. Bobrowiec. 2017. Aerial insectivorous bat activity in relation to moonlight intensity. Mammalian Biology **85**:37-46.

Aragão, L. E., L. O. Anderson, M. G. Fonseca, T. M. Rosan, L. B. Vedovato, F. H. Wagner, C. V. Silva, C. H. S. Junior, E. Arai, and A. P. Aguiar. 2018. 21st Century drought-related fires counteract the decline of Amazon deforestation carbon emissions. Nature Communications **9**:536.

Azam, C., C. Kerbiriou, A. Vernet, J. F. Julien, Y. Bas, L. Plichard, J. Maratrat, and I. Le Viol. 2015. Is part-night lighting an effective measure to limit the impacts of artificial lighting on bats? Global Change Biology **21**:4333-4341.

Barboza-Marquez, K., L. F. Aguirre, J. C. Pérez Zubieta, and E. K. Kalko. 2014. Habitat use by aerial insectivorous bats of external areas of barro colorado nature monument, panama. Chiroptera Neotropical **19**:44-56.

Beaumont, L. J., A. Pitman, S. Perkins, N. E. Zimmermann, N. G. Yoccoz, and W. Thuiller. 2011. Impacts of climate change on the world's most exceptional ecoregions. Proceedings of the National Academy of Sciences:201007217.

Beja, P., C. D. Santos, J. Santana, M. J. Pereira, J. T. Marques, H. L. Queiroz, and J. M. Palmeirim. 2010. Seasonal patterns of spatial variation in understory bird assemblages across a mosaic of flooded and unflooded Amazonian forests. Biodiversity and Conservation **19**:129.

Bobrowiec, P. E. D., L. d. S. Rosa, J. Gazarini, and T. Haugaasen. 2014. Phyllostomid bat assemblage structure in Amazonian flooded and unflooded forests. Biotropica **46**:312-321.

Castello, L., and M. N. Macedo. 2016. Large-scale degradation of Amazonian freshwater ecosystems. Global Change Biology **22**:990-1007.

Ceballos, G. 2014. Mammals of Mexico. Johns Hopkins University Press, Baltimore, United States of America.

Cisneros, L. M., M. E. Fagan, and M. R. Willig. 2015. Effects of humans-modified landscapes on taxonomic, functional and phylogenetic dimensions of bat biodiversity. Diversity and distributions **21**:523-533.

Collins, M., R. Knutti, J. Arblaster, J.-L. Dufresne, T. Fichefet, P. Friedlingstein, X. Gao, W. Gutowski, T. Johns, and G. Krinner. 2013. Long-term climate change: projections, commitments and irreversibility.*in* IPCC, editor. Climate Change 2013: The Physical Science Basis. IPCC Working Group I Contribution to AR5. Cambridge University Press, Cambridge, United Kingdom.

Chan, E. K., Y. T. Yu, Y. Zhang, and D. Dudgeon. 2008. Distribution patterns of birds and insect prey in a tropical riparian forest. Biotropica **40**:623-629.

Chellappa, S., J. Sa-Oliveira, and N. Chellappa. 2005. Fish fauna of a temporary lake in an Amazonian Conservation Area. Acta Limnologica Brasiliensia **17**:283-289.

De Melo, S., and V. L. M. Huszar. 2000. Phytoplankton in an Amazonian flood-plain lake (Lago Batata, Brasil): diet variation and species strategies. Journal of Plankton Research **22**:63-76.

de Moraes Costa, L., J. L. Luz, and C. E. L. Esbérard. 2012. Riqueza de morcegos insetívoros em lagoas no Estado do Rio de Janeiro, Brasil. Papéis Avulsos de Zoologia **52**:7-19.

Dudgeon, D. 1997. Life histories, secondary production and microdistribution of hydropsychid caddisflies (Trichoptera) in a tropical forest stream. Journal of Zoology **243**:191-210.

Estrada-Villegas, S., C. F. Meyer, and E. K. Kalko. 2010. Effects of tropical forest fragmentation on aerial insectivorous bats in a land-bridge island system. Biological Conservation **143**:597-608.

Falcão, F., J. A. Ugarte-Núñez, D. Faria, and C. B. Caselli. 2015. Unravelling the calls of discrete hunters: acoustic structure of echolocation calls of furipterid bats (Chiroptera, Furipteridae). Bioacoustics Journal **24**:175-183.

Farneda, F. Z., R. Rocha, A. López-Baucells, M. Groenenberg, I. Silva, J. M. Palmeirim, P. E. D. Bobrowiec, C. F. J. Meyer, and M. Pocock. 2015. Trait-related responses to habitat fragmentation in Amazonian bats. Journal of Applied Ecology **52**:1381-1391.

Ferreira, D. F., R. Rocha, A. López-Baucells, F. Z. Farneda, J. M. B. Carreiras, J. M. Palmeirim, and C. F. J. Meyer. 2017. Season-modulated responses of Neotropical bats to forest fragmentation. Ecology and Evolution **7**:4059-4071.

Fox, J. 2003. Effect displays in R for generalised linear models. Journal of Statistical Software **8**:1-27.

Fukui, D., M. Murakami, S. Nakano, and T. Aoi. 2006. Effect of emergent aquatic insects on bat foraging in a riparian forest. Journal of Animal Ecology **75**:1252-1258.

Giannini, N. P., and E. K. V. Kalko. 2004. Trophic structure in a large assemblage of phyllostomid bats in Panama. Oikos **105**:209-220.

González-Terrazas, T. P., L. R. Víquez, A. Ibarra-Macías, A. T. Ruíz, L. Torres-Knoop, K. Jung, M. Tschapka, and R. A. Medellín. 2016. New records and range extension of *Promops centralis* (Chiroptera: Molossidae). Revista Mexicana De Biodiversidad **87**:1407-1411.

Greif, S., and B. M. Siemers. 2010. Innate recognition of water bodies in echolocating bats. Nature Communications **1**:107.

Greig, H. S., P. Kratina, P. L. Thompson, W. J. Palen, J. S. Richardson, and J. B. Shurin. 2012. Warming, eutrophication, and predator loss amplify subsidies between aquatic and terrestrial ecosystems. Global Change Biology **18**:504-514.

Hagen, E. M., and J. L. Sabo. 2011. A landscape perspective on bat foraging ecology along rivers: does channel confinement and insect availability influence the response of bats to aquatic resources in riverine landscapes? Oecologia **166**:751-760.

Haugaasen, T., and C. A. Peres. 2007. Vertebrate responses to fruit production in Amazonian flooded and unflooded forests. Biodiversity & Conservation **16**:4165.

Haugaasen, T., and C. A. Peres. 2008. Population abundance and biomass of large-bodied birds in Amazonian flooded and unflooded forests. Bird Conservation International **18**:87-101.

IPCC. 2014. Climate change 2014: synthesis report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the intergovernmental panel on Climate Change.*in* R. Pachauri and L. Meyer, editors. Climate change 2014: synthesis report. IPCC, Geneva, Switzerland.

Jiménez-Cisneros, B., T. Oki, N. Arnell, G. Benito, J. Cogley, P. Döll, T. Jiang, and S. Mwakalila. 2014. Freshwater resources in climate change 2014: Impacts, adaptation, and vulnerability. Part a: global and sectoral aspects. Page 1132 *in* C. Field, V. Barros, D. Dokken, K. Mach, M. Mastrandrea, T. Bilir, M. Chatterjee, K. Ebi, Y. Estrada, R. Genova, B. Girma, E. Kissel, A. Levy, S. MacCracken, P. Mastrandrea, and L. White, editors. Contribution of working group II to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, United Kingdom.

Jonsson, M., P. Hedström, K. Stenroth, E. R. Hotchkiss, F. R. Vasconcelos, J. Karlsson, and P. Byström. 2015. Climate change modifies the size structure of assemblages of emerging aquatic insects. Freshwater Biology **60**:78-88.

Jung, K., E. K. Kalko, and O. Von Helversen. 2007. Echolocation calls in Central American emballonurid bats: signal design and call frequency alternation. Journal of Zoology **272**:125-137.

Junk, W. J., M. T. Piedade, F. Wittmann, J. Schöngart, and P. Parolin. 2010. Amazonian floodplain forests: ecophysiology, biodiversity and sustainable management. Springer, New York, United States of America.

Kalka, M. B., A. R. Smith, and E. K. V. Kalko. 2008. Bats limit arthropods and herbivory in a tropical forest. Science **320**:71-71.

Kalko, E. K., S. Estrada Villegas, M. Schmidt, M. Wegmann, and C. F. Meyer. 2008. Flying high - assessing the use of the aerosphere by bats. Integrative and Comparative Biology **48**:60-73.

Kalko, E. K., and H.-U. Schnitzler. 1989. The echolocation and hunting behavior of Daubenton's bat, *Myotis daubentoni*. Behavioral Ecology and Sociobiology **24**:225-238.

Kneitel, J. M., and C. L. Lessin. 2010. Ecosystem-phase interactions: aquatic eutrophication decreases terrestrial plant diversity in California vernal pools. Oecologia **163**:461-469.

Korine, C., R. Adams, D. Russo, M. Fisher-Phelps, and D. Jacobs. 2016. Bats and water: anthropogenic alterations threaten global bat populations. Pages 215-241 *in* C. C. Voigt and T. Kingston, editors. Bats in the Anthropocene: Conservation of bats in a changing world. Springer, New York, United States of America.

Kunert, N., L. M. T. Aparecido, S. Wolff, N. Higuchi, J. d. Santos, A. C. d. Araujo, and S. Trumbore. 2017. A revised hydrological model for the Central Amazon: The importance of emergent canopy trees in the forest water budget. Agricultural and Forest Meteorology **239**:47-57.

Laurance, W. F., J. L. C. Camargo, P. M. Fearnside, T. E. Lovejoy, G. B. Williamson, R. C. G. Mesquita, C. F. J. Meyer, P. E. D. Bobrowiec, and S. G. W. Laurance. 2017. An Amazonian rainforest and its fragments as a laboratory of global change. Biological Reviews of the Cambridge Philosophical Society **93**:223-247.

Laval, R. K., and B. Rodríguez. 2002. Murciélagos de Costa Rica. Instituto Nacional de Biodiversidad, San José, Costa Rica.

Linton, D., K. Haysom, R. Angel, M. Dodds, and S. Laurence. 2011. Creating ponds for bats No. 1107708. Charity, United Kingdom.

López-Baucells, A., R. Rocha, P. E. D. Bobrowiec, E. Bernard, J. Palmeirim, and C. Meyer. 2016. Field Guide to Amazonian Bats. INPA, Manaus, Brazil.

López-Baucells, A., R. Rocha, V. C. Tavares, L. Mora, P. E. D. Bobrowiec, S. E. Silva, and C. Meyer. 2018. Molecular, morphological and acoustic identification of *Eumops maurus* and *E. hansae* (Chiroptera: Molossidae) with new reports from Central Amazonia. Tropical Zoology **31**:1-20.

López-Baucells, A., L. Torrent, R. Rocha, A. C. Pavan, P. E. D. Bobrowiec, and C. F. J. Meyer. 2017. Geographical variation in the high-duty cycle echolocation of the cryptic common mustached bat *Pteronotus* cf. *rubiginosus* (Mormoopidae). Bioacoustics Journal **27**:341-357.

Macedo, M., and L. Castello. 2015. State of the Amazon: Freshwater connectivity and ecosystem health. WWF Living Amazon Initiative, Brasilia, Brazil.

MacSwiney, M. C., B. B. Cime, F. M. Clarke, and P. A. Racey. 2009. Insectivorous bat activity at cenotes in the Yucatan Peninsula, Mexico. Acta Chiropterologica **11**:139-147.

Marengo, J. A., L. M. Alves, W. R. Soares, D. A. Rodriguez, H. Camargo, M. P. Riveros, and A. D. Pabló. 2013. Two contrasting severe seasonal extremes in tropical South America in 2012: flood in Amazonia and drought in northeast Brazil. Journal of climate **26**:9137-9154.

Marengo, J. A., and J. Espinoza. 2016. Extreme seasonal droughts and floods in Amazonia: causes, trends and impacts. International Journal of Climatology **36**:1033-1050.

McLean, J., and J. Speakman. 1999. Energy budgets of lactating and non-reproductive brown long-eared bats (*Plecotus auritus*) suggest females use compensation in lactation. Functional Ecology **13**:360-372.

Melack, J. M., and M. T. Coe. 2013. Climate change and the floodplain lakes of the Amazon basin. Pages 295-310 *in* C. R. Goldman, M. Kumagai, and R. D. Robarts, editors. Climatic change and global warming of inland waters: Impacts and Mitigation for Ecosystems and Societies. Wiley, Oxford, United Kingdom.

Meyer, C. F. J., and E. K. V. Kalko. 2008. Assemblage-level responses of phyllostomid bats to tropical forest fragmentation: land-bridge islands as a model system. Journal of Biogeography **35**:1711-1726.

Meyer, C. F. J., M. Weinbeer, and E. K. V. Kalko. 2005. Home-range size and spacing patterns of *Macrophyllum macrophyllum* (Phyllostomidae) foraging over water. Journal of Mammalogy **86**:587-598.

Millon, L., J.-F. Julien, R. Julliard, and C. Kerbiriou. 2015. Bat activity in intensively farmed landscapes with wind turbines and offset measures. Ecological Engineering **75**:250-257.

Mora, E., S. Macías, M. Vater, F. Coro, and M. Kössl. 2004. Specializations for aerial hawking in the echolocation system of *Molossus molossus* (Molossidae, Chiroptera). Journal of Comparative Physiology A **190**:561-574.

Moresco, G. A., J. C. Bortolini, J. D. Dias, A. Pineda, S. Jati, and L. C. Rodrigues. 2017. Drivers of phytoplankton richness and diversity components in Neotropical floodplain lakes, from small to large spatial scales. Hydrobiologia **799**:203-215.

Palmeirim, J., and K. Etherdige. 1985. The influence of man-made trails on foraging by tropical frugivorous bats. Biotropica **17**:82-83.

Paredes, O. S., D. Norris, T. G. Oliveira, and F. Michalski. 2017. Water availability not fruitfall modulates the dry season distribution of frugivorous terrestrial vertebrates in a lowland Amazon forest. PLoS ONE **12**:e0174049.

Parmesan, C. 2007. Influences of species, latitudes and methodologies on estimates of phenological response to global warming. Global Change Biology **13**:1860-1872.

Pazin, V. F., W. E. Magnusson, J. Zuanon, and F. P. Mendonca. 2006. Fish assemblages in temporary ponds adjacent to ‘terra-firme’ streams in Central Amazonia. Freshwater Biology **51**:1025-1037.

Pereira, M. J. R., J. T. Marques, J. Santana, C. D. Santos, J. Valsecchi, H. L. De Queiroz, P. Beja, and J. M. Palmeirim. 2009. Structuring of Amazonian bat assemblages: the roles of flooding patterns and floodwater nutrient load. Journal of Animal Ecology **78**:1163-1171.

Rhazi, L., P. Grillas, M. Rhazi, and J.-C. Aznar. 2009. Ten-year dynamics of vegetation in a Mediterranean temporary pool in western Morocco. Hydrobiologia **634**:185-194.

Rhazi, L., M. Rhazi, P. Grillas, and D. El Khyari. 2006. Richness and structure of plant communities in temporary pools from western Morocco: influence of human activities. Hydrobiologia **570**:197-203.

Rocha, R., D. F. Ferreira, A. López-Baucells, F. Z. Farneda, J. Carreiras, J. M. Palmeirim, and C. F. Meyer. 2017a. Does sex matter? Gender-specific responses to forest fragmentation in Neotropical bats. Biotropica **49**:881-890.

Rocha, R., A. López-Baucells, F. Z. Farneda, M. Groenenberg, P. E. D. Bobrowiec, M. Cabeza, J. M. Palmeirim, and C. F. J. Meyer. 2017b. Consequences of a large-scale fragmentation experiment for Neotropical bats: disentangling the relative importance of local and landscape-scale effects. Landscape Ecology **32**:31-45.

Root, T. L., J. T. Price, K. R. Hall, S. H. Schneider, C. Rosenzweig, and J. A. Pounds. 2003. Fingerprints of global warming on wild animals and plants. Nature **421**:57-60.

Russo, D., L. Cistrone, N. Libralato, C. Korine, G. Jones, and L. Ancillotto. 2017. Adverse effects of artificial illumination on bat drinking activity. Animal Conservation **20**:492-501.

Sala, O. E., F. S. Chapin, J. J. Armesto, E. Berlow, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, L. F. Huenneke, R. B. Jackson, and A. Kinzig. 2000. Global biodiversity scenarios for the year 2100. Science **287**:1770-1774.

Salvarina, I. 2016. Bats and aquatic habitats: a review of habitat use and anthropogenic impacts. Mammal Review **46**:131-143.

Sampaio, E. M., E. K. V. Kalko, E. Bernard, B. Rodríguez-Herrera, and C. O. Handley. 2003. A biodiversity assessment of bats (Chiroptera) in a tropical lowland rainforest of Central Amazonia, including methodological and conservation considerations. Studies on Neotropical Fauna and Environment **38**:17-31.

Schnitzler, H. U., and E. K. V. Kalko. 2001. Echolocation by insect-eating bats: We define four distinct functional groups of bats and find differences in signal structure that correlate with the typical echolocation tasks faced by each group Bioscience **51**:557-569.

Seibold, S., J. Buchner, C. Bässler, and J. Müller. 2013. Ponds in acidic mountains are more important for bats in providing drinking water than insect prey. Journal of Zoology **290**:302-308.

Shiogama, H., S. Emori, N. Hanasaki, M. Abe, Y. Masutomi, K. Takahashi, and T. Nozawa. 2011. Observational constraints indicate risk of drying in the Amazon basin. Nature Communications **2**:253-258.

Siemers, B., E. Kalko, and H. U. Schnitzler. 2001. Echolocation behavior and signal plasticity in the Neotropical bat *Myotis nigricans* (Schinz, 1821)

(Vespertilionidae): a convergent case with European species of *Pipistrellus*? Behavioral Ecology and Sociobiology **50**:317-328.

Sioli, H. 2012. The Amazon: limnology and landscape ecology of a mighty tropical river and its basin. Springer, New York, United States of America.

Sleep, D. J., and R. M. Brigham. 2003. An experimental test of clutter tolerance in bats. Journal of Mammalogy **84**:216-224.

Solomon, S. 2007. Intergovernmental Panel on Climate Change (IPCC), climate change 2007: the physical science basis. Working group I contribution. Cambridge University Press, Cambridge, United Kingdom.

Staal, A., S. C. Dekker, C. Xu, and E. H. van Nes. 2016. Bistability, spatial interaction, and the distribution of tropical forests and savannas. Ecosystems **19**:1080-1091.

Trenberth, K. E., and T. J. Hoar. 1996. The 1990-1995 El Niño-southern oscillation event: longest on record. Geophysical Research Letters **23**:57-60.

Warwick, N. W., and M. A. Brock. 2003. Plant reproduction in temporary wetlands: the effects of seasonal timing, depth, and duration of flooding. Aquatic Botany **77**:153-167.

Wickham, H. 2009. ggplot2: elegant graphics for data analysis. R package version 2.1.0.

Williams, E., A. Dall'Antonia, V. Dall'Antonia, J. M. d. Almeida, F. Suarez, B. Liebmann, and A. C. M. Malhado. 2005. The drought of the century in the Amazon Basin: An analysis of the regional variation of rainfall in South America in 1926. Acta Amazonica **35**:231-238.

Zacharias, I., E. Dimitriou, A. Dekker, and E. Dorsman. 2007. Overview of temporary ponds in the Mediterranean region: threats, management and conservation issues. Journal of Environmental Biology **28**:1-9.

# Supplementary material

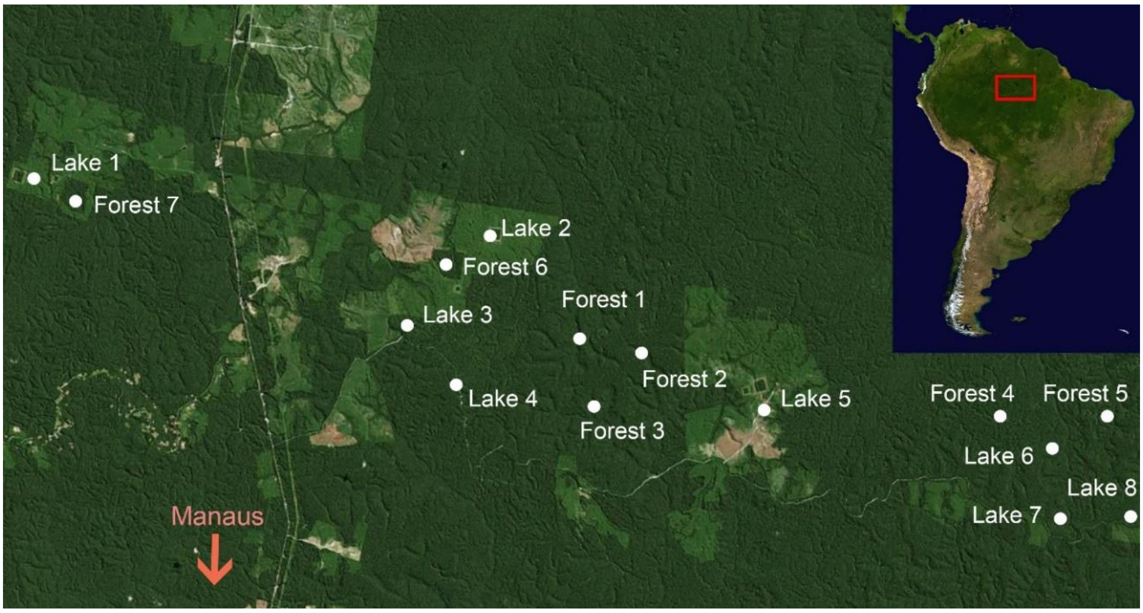


Figure 7.S1. Location of the Biological Dynamics of Forest Fragments Project and the lakes (*Lake 1* to *8*) and primary forest sites (*Forest 1* to *7*) sampled in the Central Amazon, Brazil. Dark green areas: primary rainforest; light green: secondary regrowth forest and pastures. Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community.

Figure 7.S2. Photos of each lake surveyed for bat activity in this study at the Biological Dynamics of Forest Fragments Project, Central Amazon, Brazil. Numbers correspond to each lake in Fig.7. S1.

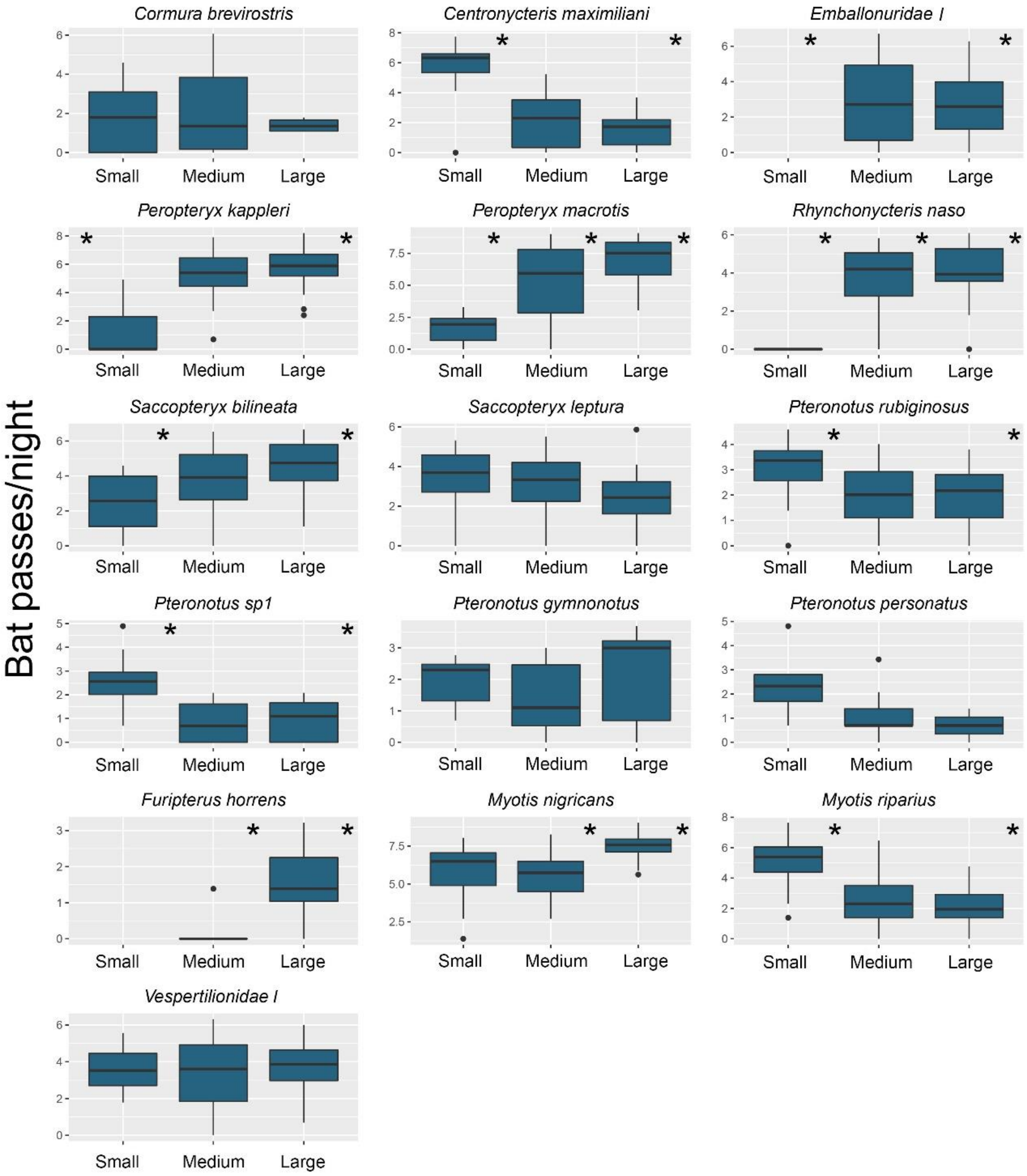


Figure 7.S3. Species/sonotype activity per site per night between lakes of different size for all bat families except Molossidae, at the Biological Dynamics of Forest Fragments Project, Central Amazon, Brazil. The asterisk indicates significant differences ($p<0.05$) between size categories based on GLMMs. An asterisk at the right of the “large” box indicates significant differences between the categories “large” and “small”.

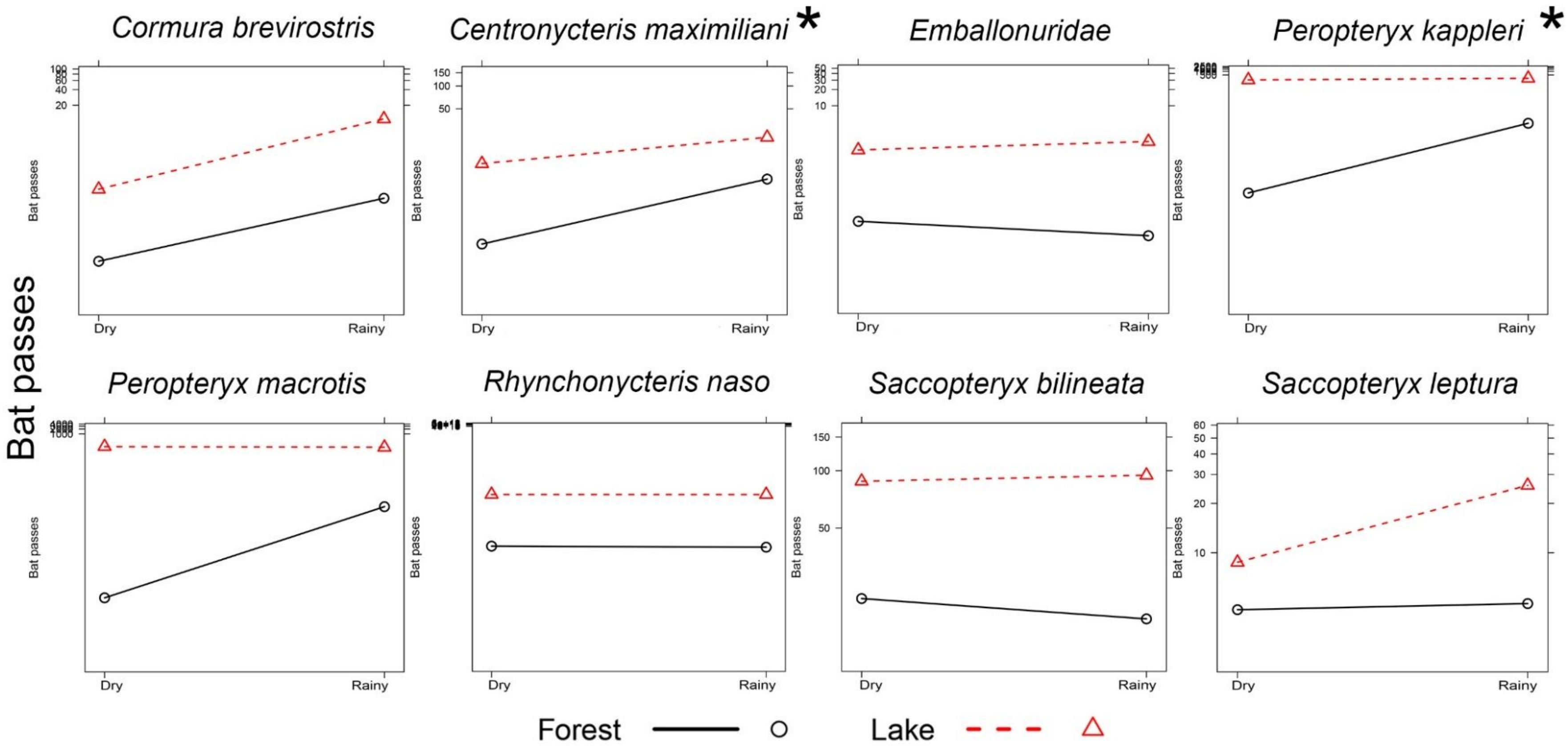


Figure 7.S4. Interaction effect between habitat type and season as determinants of species-specific activity of emballonurid bats. The asterisk indicates significance (p<0.05).

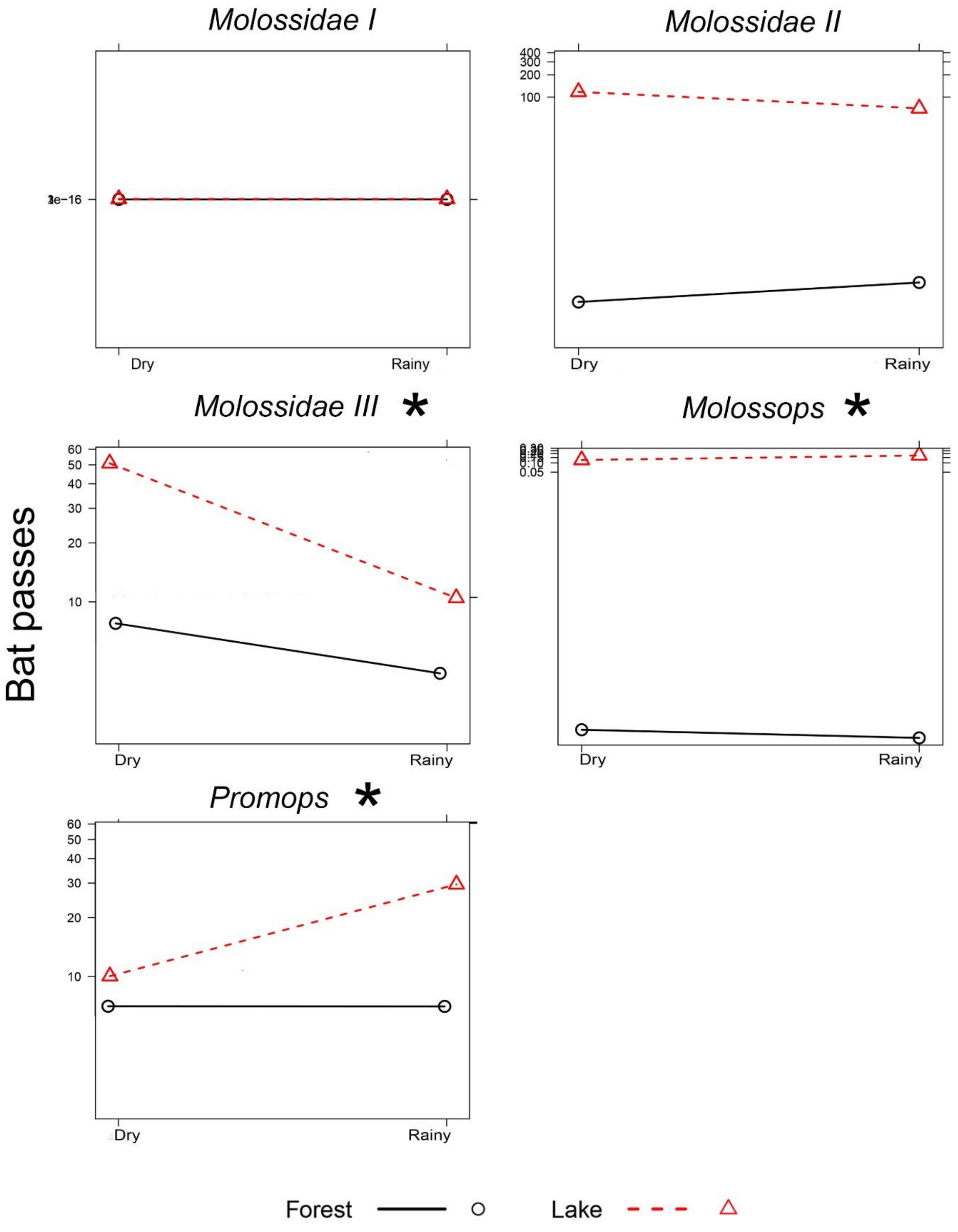


Figure 7.S5. Interaction effect between habitat type and season as determinants of species-specific activity of molossid bats. The asterisk indicates significance (p<0.05).

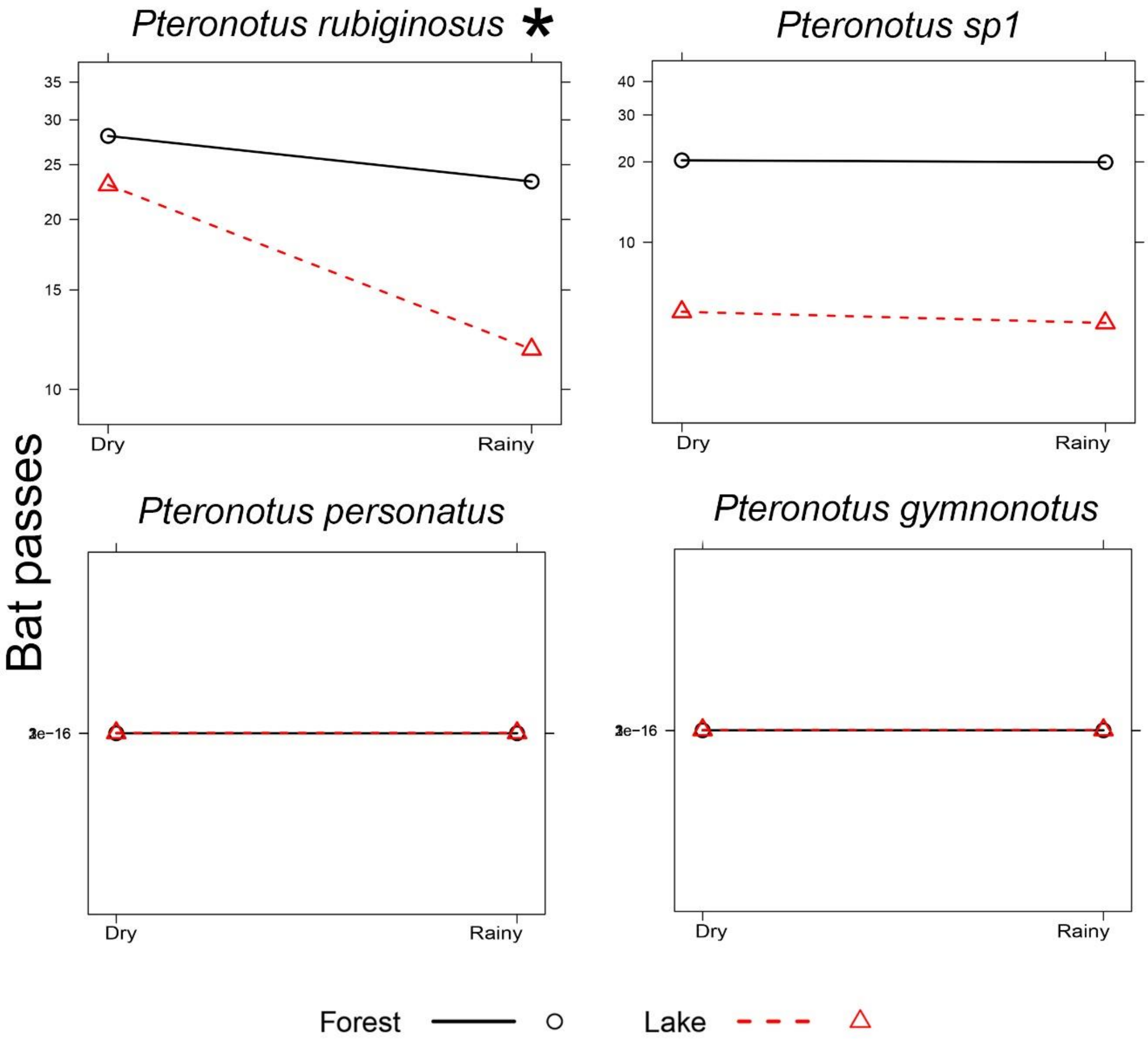


Figure 7.S6. Interaction effect between habitat type and season as determinants of species-specific activity of mormoopid bats. The asterisk indicates significance ($p<0.05$).

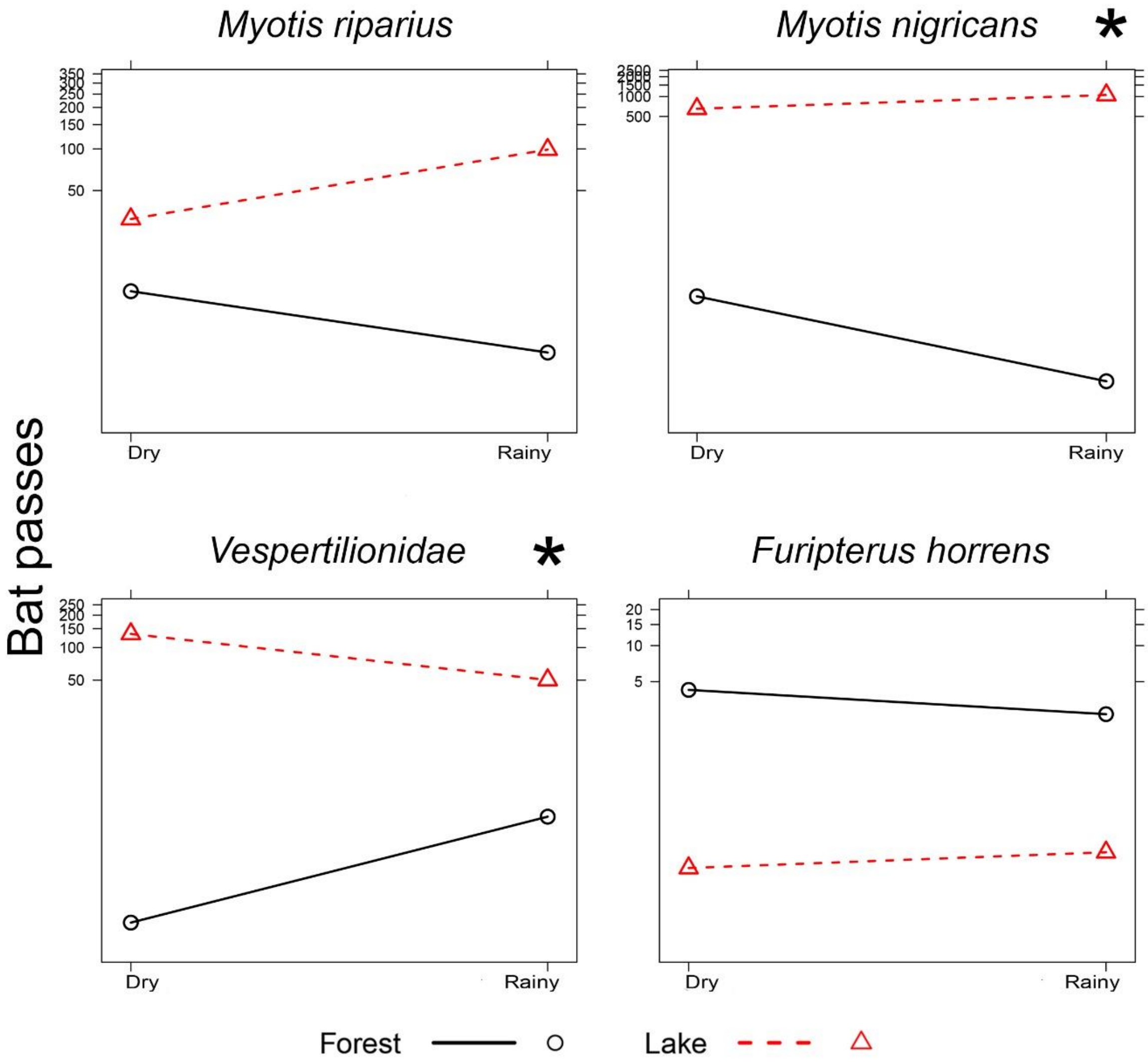


Figure 7.S7. Interaction effect between habitat type and season as determinants of species-specific activity of vespertilionid and furipterid bats. The asterisk indicates significance (p<0.05).

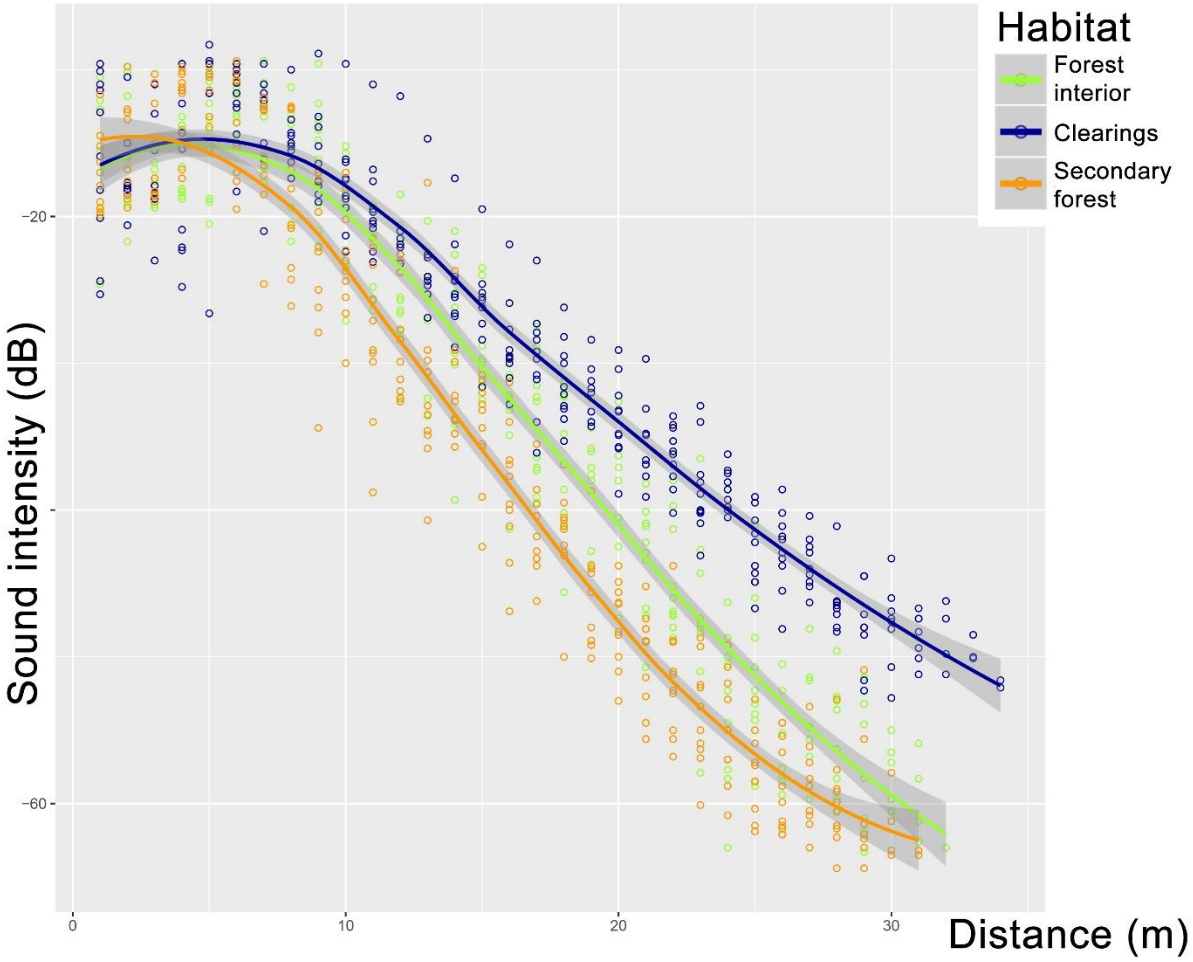


Figure 7.S8. Ultrasound attenuation along 30-meters transects at the different habitats (forest interior, clearings and secondary forest) in the Biological Dynamics of Forest Fragments Project, Central Amazon, Brazil. Attenuation was calculated using a constant ultrasound emitter (SM2 ultrasonic calibrator; Wildlife Acoustics, Inc., Maynard, USA) placed in a constant position, and an SM2 recorder set at 2 meters intervals across the landscape. Sound intensity was calculated using Kaleidoscope Pro Software (version 4.0.4.) (Wildlife Acoustics, Inc. Maynard, Massachusetts, USA), measured in decibels.

Table 7.S1. Bat echolocation call sonotypes detected at the Biological Dynamics of Forest Fragments Project, Central Amazon, and their frequency ranges. SF – start frequency; EF – end frequency; FME – frequency of maximum energy; lp – lower pulse; mp – middle pulse; hp – high pulse (López-Baucells et al., 2016).

| Sonotype | Species included | Range of FME (kHz) | Total number bat passes | Total number feeding buzzes |
|---|---|---|---|---|
| **Emballonuridae** | | | | |
| *Rhynchonycteris naso* | *Rhynchonycteris naso* | 98 – 105 | 4,292 | 415 |
| *Saccopteryx leptura* | *Saccopteryx leptura* | lp: 46 – 49; hp: 43 – 46 | 4,312 | 522 |
| *Saccopteryx bilineata* | *Saccopteryx bilineata* | lp: 41 – 43; hp: 44 – 46 | 12,480 | 1,392 |
| *Centronycteris maximiliani* | *Centronycteris centralis / maximiliani* | 39 – 41 | 14,396 | 1,202 |
| *Peropteryx macrotis* | *Peropteryx macrotis* | 37 – 39 | 114,959 | 4,2451 |
| *Peropteryx kappleri* | *Peropteryx kappleri* | 29 – 33 | 35,501 | 3,222 |
| *Cormura brevirostris* | *Cormura brevirostris* | lp: 23 – 25; mp: 26 – 28; hp: 29 – 33 | 1,459 | 58 |
| *Emballonuridae I* | *Saccopteryx gymnura / canescens* | 54 –55 | 3,408 | 1,040 |
| **Furipteridae** | | | | |
| *Furipterus horrens* | *Furipterus horrens* | 117 – 122 | 267 | 0 |
| **Molossidae** | | | | |
| *Molossidae I* | *Molossus molossus* | lp 33 – 35; mp 35 – 40; hp 40 – 45 | 2,083 | 40 |
| *Molossidae II* | *Molossus sinaloae* / *currentium* / *rufus* | lp 25 – 30; mp 30 – 35; hp 35 – 40 | 20,410 | 694 |
| *Molossidae III* | *Cynomops planirostris* / *paranus*,<br>*Cynomops greenhalli* /*abrasus*,<br>*Eumops auripendulus* / *glaucinus* / *dabbenei* / *hansae* / *maurus*,<br>*Nyctinomops laticaudatus*,<br>*Tadarida brasiliensis* | lp EF: 17 – 21; hp EF: 21 – 24 | 4,804 | 49 |
| *Molossops I* | *Molossops neglectus*,<br>*Molossops temminckii* | lp EF: 44 – 54; hp EF: 46 – 55 | 35 | 2 |
| *Promops I* | *Promops centralis*,<br>*Promops nasutus* | lp EF: 28 – 34; hp EF: 30 – 37 | 576 | 17 |
| **Mormoopidae** | | | | |
| *Pteronotus personatus* | *Pteronotus personatus* | SF: 60 – 69 | 245 | 19 |
| *Pteronotus* sp1 | *Pteronotus* sp1 | 59 – 61 | 5,590 | 126 |
| *Pteronotus gymnonotus* | *Pteronotus gymnonotus* | 45 – 60 | 363 | 20 |
| *Pteronotus rubiginosus* | *Pteronotus rubiginosus* | 54 – 56 | 4,757 | 92 |
| **Vespertilionidae** | | | | |
| *Vespertilionidae I* | *Eptesicus brasiliensis / chiriquinus*<br>*Eptesicus furinalis / Lasiurus sp. / Rhogeessa io* | EF: 25 – 45 | 7,694 | 209 |
| *Myotis riparius* | *Myotis riparius / Thyroptera tricolor* | EF: 55 – 65 | 15,138 | 1,542 |
| *Myotis nigricans* | *Myotis nigricans* | EF: 45 – 50 | 102,190 | 16,310 |
| **TOTAL** | | | 353,099 | 69,454 |

Table 7.S2. Summary of the MCMC-GLMMs testing for the effect of habitat type (forest vs. lake) and season (dry vs. rainy) on bat sonotype richness and total bat activity. Significant p-values (<0.05) are marked in bold. "CI" stands for confidence intervals, "Eff. samp" for effective sample size and "pMCMC" for particle MCMC.

| **Model: Y ~ Season * Habitat + (1\| Reserve)** | | | | | |
|---|---|---|---|---|---|
| **Richness** | | | | | |
| Coefficients: | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Season | 0.15 | 0.06 | 0.26 | 8.96 | **p<0.05** |
| Habitat type | 0.85 | 0.72 | 0.98 | 5.06 | **p<0.05** |
| Habitat*Season | -0.19 | -0.35 | -0.07 | 4.69 | **p<0.05** |
| Random factor: Reserve | 0.0005 | 3.541e-06 | 0.002 | 14.49 | |
| **Total activity** | | | | | |
| Coefficients: | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Season | 0.14 | -0.20 | 0.48 | 1000 | 0.42 |
| Habitat type | 2.96 | 2.55 | 3.32 | 1000 | **p<0.05** |
| Habitat*Season | -0.02 | -0.49 | 0.56 | 1000 | 0.94 |
| Random factor: Reserve | 0.02 | 5.48e-17 | 0.15 | 58.88 | |
| **Foraging activity (feeding buzzes)** | | | | | |
| Coefficients: | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Season | 0.14 | -0.44 | 0.77 | 420.3 | 0.62 |
| Habitat type | 4.04 | 3.22 | 4.64 | 838.9 | **p<0.05** |
| Habitat*Season | 0.33 | -0.45 | 1.22 | 485.1 | 0.47 |
| Random factor: Reserve | 1.41 | 1.03 | 1.88 | 796.3 | |

Table 7.S3. Summary of the results of MCMC-GLMMs modelling species-specific acoustic bat activity in relation to habitat type (forest vs. lake), season (dry vs. rainy) and the interaction of both. Significant p-values (<0.05) are marked in bold. “CI” stands for confidence intervals, “Eff. samp” for effective sample size and “pMCMC” for particle MCMC.

| **Model: Y ~ Season * Habitat + (1\| Reserve)** | | | | | |
|---|---|---|---|---|---|
| ***Cormura brevirostris*** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Season | 2.59 | 0.60 | 4.62 | 188.81 | **p<0.05** |
| Habitat type | 4.40 | 1.31 | 6.85 | 76.25 | **p<0.05** |
| Habitat*Season | 0.11 | -2.59 | 2.74 | 251.49 | 0.92 |
| ***Centronycteris maximiliani*** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Season | 4.20 | 2.74 | 5.52 | 505.5 | **p<0.05** |
| Habitat type | 1.98 | 0.20 | 3.75 | 844.7 | **p<0.05** |
| Habitat*Season | -2.77 | -4.64 | -0.62 | 946.7 | **p<0.05** |
| ***Emballonuridae I*** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Season | 2.41 | -1.99 | 6.18 | 173.73 | 0.246 |
| Habitat type | 5.01 | 1.18 | 8.93 | 176.85 | **p<0.05** |
| Habitat*Season | -1.65 | -6.15 | 2.86 | 199.29 | 0.49 |
| ***Vespertilionidae I*** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Season | 1.52 | 0.45 | 2.60 | 274.0 | **p<0.05** |
| Habitat type | 6.67 | 5.54 | 7.70 | 285.3 | **p<0.05** |
| Habitat*Season | -3.43 | -4.87 | -2.13 | 311.7 | **p<0.05** |
| ***Furipterus horrens*** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Season | -0.09 | -0.98 | 0.88 | 779.8 | 0.82 |
| Habitat type | -3.04 | -4.45 | -1.61 | 365.9 | **p<0.05** |
| Habitat*Season | -0.40 | -2.11 | 1.12 | 612.2 | 0.61 |
| ***Molossidae I*** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Season | 11.66 | 0.06 | 23.72 | 4.42 | 0.08 |
| Habitat type | 32.20 | 20.93 | 44.80 | 4.31 | **p<0.05** |
| Habitat*Season | -13.54 | -26.71 | -2.73 | 4.71 | 0.06 |
| ***Molossidae II*** | | | | | |
| Coefficients: | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Season | 0.49 | -1.02 | 1.86 | 30.40 | 0.48 |
| Habitat type | 6.87 | 5.67 | 8.06 | 36.50 | **p<0.05** |
| Habitat*Season | -0.80 | -2.44 | 0.80 | 33.56 | 0.30 |

| ***Molossidae III*** | | | | | |
|---|---|---|---|---|---|
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Season | -6.74 | -11.45 | -1.83 | 4.59 | **p<0.05** |
| Habitat type | 5.64 | 4.05 | 7.52 | 29.66 | **p<0.05** |
| Habitat*Season | 6.44 | 1.71 | 11.46 | 5.03 | **p<0.05** |
| ***Myotis nigricans*** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Season | -2.24 | -3.97 | -0.51 | 13.05 | **p<0.05** |
| Habitat type | 8.53 | 7.49 | 9.58 | 52.44 | **p<0.05** |
| Habitat*Season | 2.33 | 0.46 | 4.41 | 21.89 | **p<0.05** |
| ***Molossops I*** | | | | | |
| Coefficients: | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Season | -10.57 | -41.06 | 19.99 | 4.22 | **p<0.05** |
| Habitat type | 15.20 | 4.82 | 28.62 | 14.45 | **p<0.05** |
| Habitat*Season | 12.35 | -18.73 | 43.11 | 4.48 | **p<0.05** |
| ***Myotis riparius*** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Season | -0.66 | -1.77 | 0.41 | 556.6 | 0.21 |
| Habitat type | 1.99 | 0.82 | 2.99 | 458.4 | **p<0.05** |
| Habitat*Season | 0.67 | -0.85 | 2.06 | 807.8 | 0.34 |
| ***Pteronotus rubiginosus*** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Season | -0.29 | -0.75 | 0.12 | 1000 | 0.16 |
| Habitat type | -0.38 | -0.88 | 0.13 | 1000 | 0.12 |
| Habitat*Season | -0.74 | -1.39 | -0.09 | 1000 | **p<0.05** |
| ***Pteronotus* sp1** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Season | 0.02 | -0.28 | 0.35 | 1000 | 0.90 |
| Habitat type | -1.54 | -2.02 | -1.20 | 1236 | **p<0.05** |
| Habitat*Season | -0.47 | -0.98 | 0.10 | 1000 | 0.08 |
| ***Promops I*** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Season | -0.07 | -9.23 | 9.89 | 9.80 | **p<0.05** |
| Habitat type | 13.03 | 6.57 | 20.05 | 11.62 | **p<0.05** |
| Habitat*Season | -0.86 | -10.99 | 8.04 | 7.84 | **p<0.05** |
| ***Pteronotus gymnonotus*** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Season | -5.71 | -17.72 | 1.89 | 5.66 | 0.32 |
| Habitat type | 9.23 | 4.54 | 14.42 | 17.79 | **p<0.05** |
| Habitat*Season | 3.95 | -3.94 | 15.68 | 6.14 | 0.60 |
| ***Peropteryx kappleri*** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Season | 2.21 | -1.10 | 4.90 | 5.79 | 0.19 |
| Habitat type | 10.21 | 8.02 | 12.39 | 12.07 | **p<0.05** |
| Habitat*Season | -1.28 | -4.54 | 1.79 | 7.10 | **p<0.05** |

| ***Peropteryx macrotis*** | | | | | |
|---|---|---|---|---|---|
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Season | 1.439 | -3.381 | 6.774 | 17.63 | 0.552 |
| Habitat type | 11.565 | 7.292 | 15.837 | 16.48 | **p<0.05** |
| Habitat*Season | -1.432 | -7.370 | 3.710 | 19.30 | 0.614 |
| ***Pteronotus personatus*** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Season | -12.71 | -36.43 | 9.67 | 3.06 | 0.42 |
| Habitat type | 18.08 | 4.33 | 29.62 | 6.23 | **<0.001** |
| Habitat*Season | 10.76 | -12.00 | 34.83 | 3.18 | 0.56 |
| ***Rhynchonycteris naso*** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Season | -14.55 | -33.59 | 6.41 | 1.12 | 0.31 |
| Habitat type | 19.45 | 12.25 | 26.89 | 10.43 | **p<0.05** |
| Habitat*Season | 13.01 | -8.80 | 32.06 | 1.23 | 0.37 |
| ***Saccopteryx bilineata*** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Season | 0.24 | -0.61 | 1.17 | 350.6 | 0.60 |
| Habitat type | 1.85 | 0.83 | 3.04 | 545.7 | **p<0.05** |
| Habitat*Season | 0.31 | -1.06 | 1.65 | 365.0 | 0.66 |
| ***Saccopteryx leptura*** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Season | 0.95 | 0.02 | 1.97 | 346.9 | **p<0.05** |
| Habitat type | 1.49 | 0.41 | 2.53 | 505.9 | **p<0.05** |
| Habitat*Season | 0.05 | -1.22 | 1.43 | 441.7 | 0.94 |

Table 7.S4. Results of multiple comparison tests following MCMC-GLMMs assessing differences between bat sonotype richness and total bat activity and the three size categories of lakes. Significant p-values (<0.05) are marked in bold. “CI” stands for confidence intervals, “Eff. samp” for effective sample size and “pMCMC” for particle MCMC.

| **Model: Y ~ Size, family = "poisson"** | | | | | |
|---|---|---|---|---|---|
| **Richness** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Large-Medium | -0.10 | -0.15 | -0.05 | 13.34 | **0.02** |
| Medium-Small | 0.25 | 0.19 | 0.28 | 3.34 | <**0.001** |
| Small-Large | -0.30 | -0.37 | -0.22 | 8.97 | <**0.001** |
| **Total activity** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Large-Medium | -0.96 | -1.44 | -0.50 | 1172 | <**0.001** |
| Medium-Small | 0.38 | -0.05 | 0.79 | 1000 | 0.08 |
| Small-Large | -1.36 | -1.87 | -0.90 | 1000 | <**0.001** |

Table 7.S5. Variance for the confidence intervals on the obtained points in the Figure 5.

| Lake size | sd | se | ci |
|---|---|---|---|
| Large | 1.82 | 0.40 | 0.40 |
| Medium | 2.05 | 2.05 | 2.05 |
| Small | 1.83 | 1.83 | 1.83 |

Table 7.S6. Results of multiple comparison tests following MCMC-GLMMs, assessing differences in activity levels of individual bat species between lake size categories. Significant p-values (<0.05) are marked in bold. “CI” stands for confidence intervals, “Eff. samp” for effective sample size and “pMCMC” for particle MCMC.

| **Model: Y ~ Size, family = "poisson"** | | | | | |
|---|---|---|---|---|---|
| ***Cormura brevirostris*** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Large-Medium | 2.36 | -0.46 | 4.71 | 491.9 | 0.06 |
| Medium-Small | -0.22 | -2.30 | 1.68 | 712.6 | 0.80 |
| Small-Large | 2.59 | 0.14 | 5.81 | 494.1 | 0.07 |
| ***Centronycteris maximiliani*** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Large-Medium | 0.61 | -2.08 | 3.67 | 725.7 | 0.67 |
| Medium-Small | -3.74 | -6.03 | -1.23 | 877.2 | **0.002** |
| Small-Large | 4.34 | 1.53 | 7.38 | 768.4 | **0.006** |
| ***Emballonuridae I*** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Large-Medium | -1.82 | -3.82 | 0.22 | 1000 | 0.08 |
| Medium-Small | 23.91 | 6.81 | 43.75 | 6.13 | **<0.001** |
| Small-Large | -30.21 | -52.50 | -9.41 | 3.72 | **<0.001** |
| ***Vespertilionidae I*** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Large-Medium | -1.25 | -2.46 | -0.11 | 869.1 | **0.03** |
| Medium-Small | -0.52 | -1.51 | 0.47 | 876.4 | 0.34 |
| Small-Large | -0.71 | -1.94 | 0.48 | 1000 | 0.25 |
| ***Furipterus horrens*** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Large-Medium | -3.44 | -5.00 | -1.79 | 332.24 | **<0.001** |
| Medium-Small | 26.32 | 2.96 | 44.63 | 2.74 | **0.002** |
| Small-Large | -16.22 | -26.50 | -6.97 | 9.17 | **<0.001** |
| ***Myotis nigricans*** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Large-Medium | -1.96 | -2.76 | -1.12 | 1000 | **<0.001** |
| Medium-Small | 0.08 | -0.67 | 0.88 | 1000 | 0.88 |
| Small-Large | -2.04 | -2.92 | -1.10 | 1000 | **<0.001** |
| ***Myotis riparius*** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Large-Medium | -0.11 | -0.94 | 0.86 | 899.9 | 0.79 |
| Medium-Small | -3.21 | -4.05 | -2.45 | 1000 | **<0.001** |
| Small-Large | 3.07 | 2.07 | 4.05 | 1000 | **<0.001** |

| ***Pteronotus rubiginosus*** | | | | | |
|---|---|---|---|---|---|
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Large-Medium | -0.18 | -0.97 | 0.59 | 1000 | 0.65 |
| Medium-Small | -1.76 | -2.45 | -1.09 | 888.5 | **<0.001** |
| Small-Large | 1.57 | 0.77 | 2.44 | 1000 | **0.002** |
| ***Pteronotus* sp1** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Large-Medium | 0.49 | -0.48 | 1.43 | 581.7 | 0.29 |
| Medium-Small | -2.63 | -3.32 | -1.94 | 885.4 | **<0.001** |
| Small-Large | 3.14 | 2.17 | 4.11 | 629.3 | **<0.001** |
| ***Pteronotus gymnonotus*** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Large-Medium | -0.04 | -1.46 | 1.62 | 1000.0 | 0.91 |
| Medium-Small | 1.08 | -0.40 | 2.68 | 1000.0 | 0.17 |
| Small-Large | -1.11 | -2.97 | 0.63 | 892.0 | 0.22 |
| ***Peropteryx kappleri*** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Large-Medium | -0.49 | -1.30 | 0.47 | 885.79 | 0.26 |
| Medium-Small | 7.11 | 6.07 | 8.17 | 72.31 | **<0.001** |
| Small-Large | -7.83 | -9.12 | -6.47 | 70.45 | **<0.001** |
| ***Peropteryx macrotis*** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Large-Medium | -2.38 | -4.25 | -0.86 | 1000 | **0.008** |
| Medium-Small | 7.98 | 6.04 | 10.32 | 212.4 | **<0.001** |
| Small-Large | -10.25 | -12.63 | -7.99 | 168 | **<0.001** |
| ***Pteronotus personatus*** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Large-Medium | 1.93 | -1.24 | 4.71 | 427.4 | 0.17 |
| Medium-Small | 0.16 | -2.29 | 2.37 | 868.6 | 0.93 |
| Small-Large | 1.66 | -1.31 | 4.83 | 775.5 | 0.29 |
| ***Rhynchonycteris naso*** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Large-Medium | -3.77 | -5.62 | -2.13 | 674.00 | **<0.001** |
| Medium-Small | 7.80 | 4.32 | 11.04 | 53.70 | **<0.001** |
| Small-Large | -11.26 | -15.74 | -7.82 | 47.55 | **<0.001** |
| ***Saccopteryx bilineata*** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Large-Medium | -0.73 | -1.86 | 0.46 | 849.2 | 0.21 |
| Medium-Small | 1.86 | 0.68 | 2.77 | 813.2 | **<0.001** |
| Small-Large | -2.61 | -3.86 | -1.41 | 833.0 | **<0.001** |
| ***Saccopteryx leptura*** | | | | | |
| Coefficients | Post.mean | l-95% CI | u-95% CI | Eff. samp | pMCMC |
| Large-Medium | -0.75 | -1.86 | 0.31 | 902.3 | 0.16 |
| Medium-Small | 1.88 | 0.72 | 2.81 | 426.9 | **<0.001** |
| Small-Large | -2.65 | -3.95 | -1.38 | 908.4 | **<0.001** |

# Chapter 8

## General discussion

Top: *Myotis riparius;* Bottom: Brazilian rainforest in Central Amazon

# CHAPTER 8

## General Discussion

Assessing the effects of habitat fragmentation in the tropics at ecosystem-, assemblage- or species- level is crucial to improve land management and biodiversity conservation (Haddad et al. 2015). Many studies have addressed this problem from different perspectives, in different habitats and for a variety of taxa (Fardila et al. 2017). Most bat studies in the Neotropics have focused on phyllostomid species (see Cortes-Delgado and Perez-Torres 2011, Cisneros et al. 2014, Meyer et al. 2016, Rocha et al. 2018), while aerial insectivorous species have been neglected. The scarcity of scientific knowledge about this ensemble results from the fact that this is a challenging group to study using traditional mist-netting (MacSwiney et al. 2008) and also because bat bioacoustics just started to emerge during the last decades. Although aerial insectivorous bats represent almost half of the bat diversity in the Neotropics (Jung and Kalko 2011), most of the species remain broadly understudied and their conservation threats poorly understood.

To make a contribution to filling the current knowledge gaps regarding the ecology and conservation of aerial insectivorous bats in the Neotropics, in this dissertation I addressed various questions from a broad array of topics. These ranged from the description of the echolocation calls of most of the aerial insectivorous bat species found in the Central Amazon and the publication of the first echolocation keys for the region, to a study on the effects of forest fragmentation on bat taxonomic, phylogenetic and functional diversity. In addition, I assessed the use of machine learning algorithms for the classification of bat calls, evaluated different acoustic sampling schemes for both species inventories and ecological studies, and demonstrated the importance of Amazonian lakes as hotspots for bat diversity in the Amazonian rainforest. In this chapter, I summarise the most important findings of this thesis and its contribution to bat conservation in the context of the forest fragmentation literature. I also discuss study limitations and suggest new avenues for future research.

## The use of bioacoustics in bat studies in the Neotropics

Bioacoustics is a rapidly growing field of increasing significance in wildlife conservation. The recent expansion of bioacoustics in science might play a key role in filling knowledge gaps in remote or underexplored regions (Walters et al. 2013). Autonomous ultrasound detectors have substantially improved in quality and capabilities during the last decades, e.g. low-cost autonomous passive detectors with highly sensitive omnidirectional microphones in fully customizable devices are now available. Detectors can now record at high frequencies and detect even species that typically vocalize at very low intensity, and one can synchronize their recordings with thermal video-images or reconstruct flight patterns in 3-dimensional space. However, although recent advances in technology have provided scientists with powerful equipment, which was unimaginable a few years ago, baseline information such as comprehensive regional bat reference call libraries and sampling guidelines are currently lacking for much of the tropics, including the Amazon (Walters et al. 2013, Madhukumar Menon et al. 2018).

In chapters 2, 3 and 4 I used the largest acoustic dataset on bats available to date from the Central Amazon, collected during a 3-year-long fieldwork period. While the dataset was collected with the main aim to study the effects of forest fragmentation on aerial insectivorous bats, I used it to first provide a comprehensive description of bat echolocation calls of the region as well as to test the classification protocols. This allowed me to then explore bat responses towards forest fragmentation in the second part of the thesis. The first stage of the project was not only a necessary step to reliably classify all recordings, but also constitutes an important means to facilitate further research on bat acoustics, not only in the Amazon but across the Neotropics.

## Optimizing protocols, from sampling design to call classification

In Chapter 2 I presented the first Field Guide to the bats of the Amazon. The book was published online by INPA (Instituto Nacional de Pesquisas da Amazônia) in 2016 and two years later by Pelagic Publishing (UK) in paper, which was also made freely available online as a pdf in http://www.tropicalconservation.net. These keys have been used in many courses, workshops and studies (M. Tuttle & R. Medellin pers. comm.) and have been adopted as a key reference for acoustic studies in the Amazon (Appel et al. 2017, Torrent et al. 2018). Three reviews of the book have already been published, one by Douglas (2018) in the *Journal of Mammalogy*, as well as another in Mammal Watching by Hall (2018), and by the US National Speleological Society (NSS) (in press). The book is comprehensive, easy to use in the field, and can be used to identify all species in hand that are known to occur in the region (i.e. mist-net captures) as well as to identify aerial insectivorous bats based on their echolocation calls. In the digital version, the book was designed to be interactive, with all the species directly hyperlinked to the IUCN webpage (https://www.iucnredlist.org/). After the publication of this work, similar initiatives emerged in Brazil (see for example Arias-Aguilar et al. 2018), some extending beyond the boundaries of the Amazon. Additionally, an acoustic committee of the Brazilian Bat Society (SBEQ) has recently been founded, which promotes the use of bioacoustics in the study of Neotropical bats.

In Chapter 3 I focused on the echolocation call classification process. Specifically, my analyses targeted the trade-off between choosing automatic algorithms or visual identification to classify bat recordings (Russo and Voigt 2016). Many studies have addressed this problem, and the performance of different algorithms has been thoroughly evaluated (see for example Ross and Allen 2014, Zamora-Gutiérrez et al. 2016, Botto Nuñez et al. 2018). However, no study so far has assessed quantitatively the extent to which classification accuracy could be improved by combining an automatic classifier with manual validation. I trained a custom-built classifier that was able to automatically identify a large proportion of files to species/sonotype level (with accuracy above 90%), leaving the rest to be manually classified. This means that workload could be reduced by up to ~75% compared to using only manual analysis. Therefore, I evaluated the reliability of using self-built automatic classifiers followed by visual post-validation in order to analyse large acoustic datasets that would be very difficult to classify only manually. Using this approach, I optimized the process by making it quicker and easier, but equally reliable. In fact, the combination of both methods overcomes some of the risks and biases that are commonly associated with commercially available classifiers (Russo and Voigt 2016). I also assessed the suitability of using recordings from free-flying bats instead of reference calls from hand-released bats (which are more difficult to obtain) to train the algorithms. Although this could be identified as an important source of error due to the risk of using misidentified calls to train the algorithm, I overcame this limitation by strictly restricting the identifications to easily distinguishable species/sonotypes, therefore avoiding misidentifications (Russo and Voigt 2016), a practice that I recommend for future studies.

In Chapter 4 I focused on the fieldwork protocol and how to optimise acoustic sampling design. I tested a combination of 20 different temporal sampling schemes, including different numbers of hours per night, different numbers of nights per site, and including either only the wet or the dry season, or both. I tested all of them under two different scenarios: in forest fragments embedded in a matrix of secondary forest, and in the same forest fragments after they had been re-isolated through clearing of the secondary forest. I proposed guidelines to improve the efficiency of acoustic studies by optimizing the trade-off between accuracy and workload. Similar guidelines have been proposed for other regions such as Europe (Froidevaux et al. 2014) and Australia (Law et al. 2015). The former assessed the reliability of using three different recording schemes (only dusk, dusk + dawn and the entire night) in forest ground, canopy, and forest gap sites and found that to achieve the best results, sampling the whole night in the three habitats was crucial. Law et al. (2015), however, evaluated the importance of using multiple detectors within each locality to ensure occupancy detection, and suggested that optimum sampling schemes included either three detectors for two nights or two detectors for three nights. Considering the financial crisis that some countries in the Neotropics such as Brazil are experiencing, these findings can help optimizing the use of the scarce financial research funds. Measuring the minimum effort required to achieve a high degree (e.g. 90%) of inventory completeness is crucial to understand the reliability of scientific studies. The results of this chapter suggested a minimum of 10 full sampling nights in high-contrast landscapes (e.g. open areas vs dense forest), and 80 nights in low-contrast landscapes (e.g. homogeneously forested areas). In terms of hours, the first four hours after sunset were always required to achieve 90% inventory completeness.

One of the novel aspects of this study is the fact that I quantified for the first time the trade-off between accuracy and workload when assessing the effects of forest fragmentation. I found that to model the effects of fragmentation, the minimum effort required was different to that required for inventories and also differed between high- and low- contrast landscapes. Before the re-isolation, when the forest fragments were embedded in a matrix of secondary forest (low fragment-matrix contrast), the number of hours and nights per site were important to detect patterns that could otherwise have gone unnoticed. However, after the re-isolation situation (high fragment-matrix contrast), bat responses were consistent independently of the sampling scheme.

Working with bioacoustics in some regions is logistically challenging because of the high costs of automatic detectors and commercially available automatic classifiers. In developing countries where most projects are financially limited by reduced funding availability, developing cost-effective solutions is crucial to overcome these limitations. Self-built classification algorithms that can be run with open-source software such as R as well as optimizing the sampling design represent potential solutions to keep project costs and investment in equipment low, especially where no classifier is commercially available. Results from chapters two, three and four broadly contribute to optimizing acoustic protocols. These improvements, together with the ongoing development of more user-friendly detectors and automatic classifiers (Whytock et al. 2017, Hill et al. 2018), promise a rapid increase in bioacoustic studies in the tropics in the near future.

## Insectivorous bats in a fragmented landscape

Forest fragmentation and habitat loss have been reported to be the main drivers of local species extinctions in isolated remnants of natural habitat (Harris and Silva-Lopez 1992, Brook et al. 2008, Haddad et al. 2015). The theory of island biogeography (IBT) by MacArthur and Wilson (1967) is a well-known framework to predict patterns of species loss in small isolated habitat fragments. There is plenty of evidence supporting the usefulness of MacArthur and Wilson’s theory for high-contrast systems, for example forested islands that became isolated as a result of dam construction (Benchimol and Peres 2015a, b, Jones et al. 2016). However, this theory has now been superseded in some contexts by Countryside Biogeography (Daily 1997, Mendenhall et al. 2013) due to the fact that IBT does not take into consideration changes in ecosystems following changes in the matrix habitat. Countryside Biogeography suggests that species might become extinct more easily when forest fragments are isolated by a “hard matrix” such as water or open areas, less permeable to animals moving between fragments, than in fragments surrounded by soft matrices such as secondary forest. Thus, this approach is more adequate for dynamic landscapes in which the permeability of the matrix changes over time (Mendenhall et al. 2014, Wolfe et al. 2015), which is often the case of terrestrial human-modified landscapes.

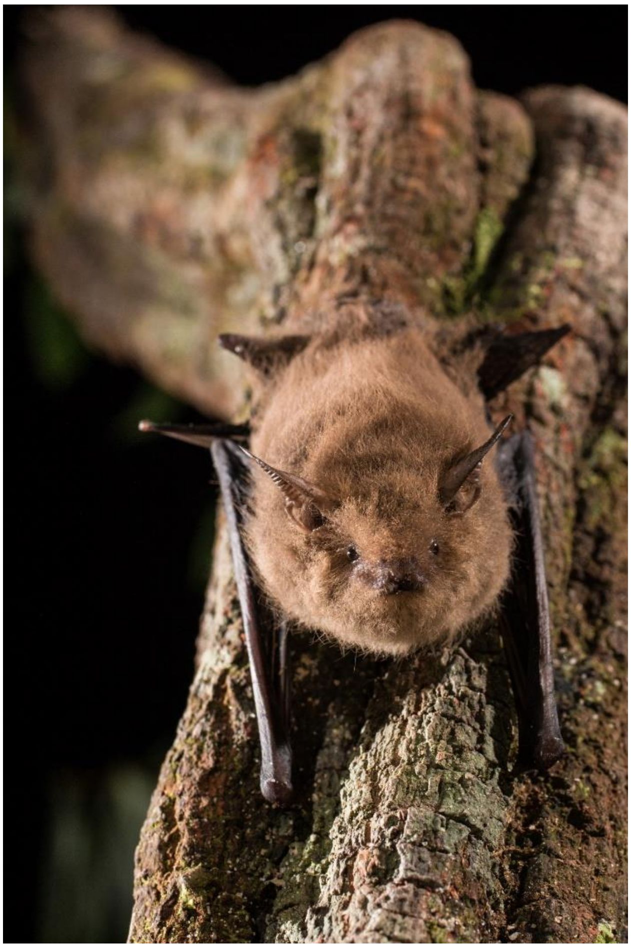
Neotropical bats are widespread, species-rich, and highly mobile, apart from being responsive to habitat change and disturbance and being potentially important bioindicators (Jones et al. 2009, De Conno et al. 2018). The high mobility of bats allows many species to move between patches in fragmented landscapes and to select the most appropriate habitats to forage. Thus, they have sometimes been considered as less vulnerable to forest fragmentation than other taxa (Lumsden and Bennett 2000, Lumsden and Bennett 2005). However, the number of bat studies greatly varies amongst families or groups and their responses towards habitat fragmentation are yet to be fully understood. Studies on the effects of forest fragmentation on aerial insectivorous bats are scarce (Grindal and Brigham 1999, Law et al. 1999, Lumsden and Bennett 2005, Ethier and Fahrig 2011, Rodríguez-San Pedro and Simonetti 2015), particularly in the Neotropics (Estrada-Villegas et al. 2010), where the consequences of habitat loss and modification on this ensemble remain poorly investigated. The increase in bioacoustic studies in the Neotropics, and their suitability to survey this bat ensemble are encouraging an increasing number of scientists to undertake similar projects.

In a mosaic landscape of natural, semi-natural and newly-established habitats, insectivorous bats are generally distributed according to their functional traits that make them more or less adapted to specific foraging habitats, usually mostly depending on vegetation clutter. Some studies on insectivorous bats have also stressed the relevance of vegetation strips as commuting routes (Krull et al. 1991, Walsh and Harris 1996b, a) and the importance of ecotones, which might increase the resilience of some species in highly disturbed habitats. In terms of habitat selection, although in the Neotropics species were classified as uncluttered, background cluttered, and highly cluttered space foragers by Schnitzler and Kalko (2001) based on their echolocation, the importance of vegetation clutter and microhabitat still remains quite unexplored for several less-known species.

Because the landscape and all their components are dynamic, diversity and structure of bat assemblages in forest fragments and the matrix of secondary regrowth need to be considered jointly for a better understanding of the ecological processes that take place during forest succession (Lu et al. 2002). Therefore, it is important to take into consideration: i) the role of the forest fragments (including the size, shape, edges or configuration) to retain certain forest-specialist species and ii) the role of the matrix habitat (in this case, a relatively old secondary forest) buffering the effects of forest fragmentation.

In Chapters 5, 6 and 7 of this dissertation I provided a thorough analysis of different processes linked to forest fragmentation that affect aerial insectivorous bats at the BDFFP: I studied a) the effect of forest fragment size on bat taxonomic, functional and phylogenetic diversity; b) whether periods of about ~30 years are sufficient for forest regrowth to reach conditions to harbour bat assemblages similar to those of primary forests; c) species vulnerability to forest fragmentation from the functional perspective. Additionally, I studied d) the role of Amazonian lakes for bat conservation, as sparse bat diversity hotspots that might need to be protected in a scenario of habitat loss and fragmentation.

## Resilience in forest fragments



*Rhynchonycteris naso*

During the study period, I did not register any local extinctions in the surveyed fragments, as all aerial insectivorous bat species present in continuous forest also occurred in the forest fragments. The species assemblages in the interior of forest fragments were generally not significantly different from those of continuous forest interiors, and sometimes even slightly more diverse (usually in the large fragments). This differs from the results obtained by Rocha et al. (2017b), who found lower diversity in the same fragment interiors for phyllostomids. Total bat activity did not significantly vary amongst forest fragment sizes or between interiors and edges, although I also did find some significant species-specific responses. However, the role of fragment size buffering the effects of habitat fragmentation was stronger for taxonomic, functional and phylogenetic α diversity than for activity. The fact that some fragment interiors were slightly more diverse than the interior of continuous forest was probably due to habitat heterogeneity. Almost all the edges of the fragments (or the interface between primary and secondary forest) peaked in terms of bat diversity (taxonomic, functional and phylogenetic). Microhabitat complexity allows the simultaneous presence of edge- and interior-specialists, probably due to habitat diversity, and the fact that highest plant diversity causes greater abundances of insects, as it has been reported by Law and Dickman (1998) in Australia. Edges can also be used as corridors, increasing the detectability of rare species that may be more difficult to detect in the habitat interiors (Zurita et al. 2012, Zurita and Bellocq 2012, Fonderflick et al. 2013, Kalcounis-Rueppell et al. 2013).

Larger fragments were also confirmed to act as important species sources, as secondary forest next to the largest fragments (10 and 100 ha) were taxonomically, functionally and phylogenetically more diverse than those adjacent to the smaller fragments. Similar patterns have been found for phyllostomid bats in the same area (Rocha et al. 2017b, Farneda et al. 2018a, Farneda et al. 2018b), and for aerial insectivorous bats in a land-bridge island system (Estrada-Villegas et al. 2010), where small islands harboured high taxonomic diversity of bat species. Similar patterns have also been reported for insectivorous birds (Stouffer et al. 2009).

The fact that forest edges and altered habitats peaked in species diversity might be mistakenly understood as a positive consequence of human-induced changes and may even lead land managers to promote edges and mosaic landscapes. This sudden upsurge in diversity has been described by Connell (1978) as the Intermediate Disturbance Hypothesis. It suggests that intermediate intensity of disturbance can locally boost species richness (favouring some species and disfavouring others) due to the fact that newly arrived species coexist for a certain time with the native species. However, if disturbance pressure remains for too long or at increasing intensity, richness would markedly decrease. It is therefore essential to understand which habitats and species are most sensitive to forest fragmentation, evaluate the status of their populations and design management policies focusing on those species, considering the potential bias resulting from intermediate disturbances (Vieira et al. 2014).

In this dissertation I have expanded the traditional taxonomic approach with the functional and phylogenetic component. These analyses provided strong evidence that diversity loss in small fragments is driven by the disappearance of specific functional traits and species, coming from phylogenetically distant origins, potentially undermining the provision of local ecosystem services.

## 30 years of forest regeneration are not enough for full diversity recovery

Vast areas of abandoned farmland in the Neotropics are now covered by secondary forest (Haddad et al. 2015), thus constituting one of the most common habitats in tropical fragmented landscapes (Chazdon et al. 2016). Despite the fact that ~720,000 $km^2$ in the Brazilian Amazon are covered by vegetation regrowth, the capacity of these secondary forests to harbour species-rich communities has been widely underestimated (Arroyo-Rodríguez et al. 2016). In Chapter 5 of this dissertation I explored species diversity in old secondary forests and showed that they usually have lower taxonomic, functional and phylogenetic diversity than continuous forest. Although some species seemed to select secondary forest, assemblage diversities were almost always lower. Possibly due to a lower vegetation clutter, primary forest was adequate and permeable to a greater number of species than the more cluttered and restrictive secondary forest.

One of the most frequently asked questions in conservation and restoration science is how long it takes for a secondary forest to mature and harbour species assemblages similar to those of pristine habitats (Chazdon et al. 2016). In this thesis, I followed the *recovery* definition suggested by Guariguata and Ostertag (2001) who describe it by comparing it with old-growth conditions, including structure, function and composition of the original forest before conversion. Some studies have addressed this in temperate areas (Dornelas et al. 2018), but very few have done so in the tropics (Finegan 1996, Rodríguez-San Pedro and Simonetti 2015). In my case, primary forest and secondary forest were not markedly different in vegetation structure and complexity and vegetation regrowth in the secondary forest matrix increased connectivity and facilitated species turnover. However, the time lapse of ~30 years of secondary regeneration was insufficient for full bat diversity recovery. In fact, during this time span secondary forest recovered 94% of taxonomic diversity, but only 84 and 87% of functional and phylogenetic diversity, respectively.

The time required for such recoveries is highly variable, and depends on landscape structure and composition, degree of fragment isolation, size and shape, proximity to pristine habitats, urbanization pressure, road construction, or environmental pollution, amongst many other factors (Brudvig 2011, Brudvig 2017, Rydgren et al. 2018). In general, species richness recovers relatively quickly, whereas recovery of species composition takes longer (Chazdon et al. 2009a, Chazdon et al. 2009b). It has been reported that 50 to 80 years are needed to recover tree species richness (Derroire et al. 2016) and 30 years to restore bird assemblages at the BDFFP (Powell et al. 2013, Powell et al. 2015). Before this thesis, for aerial insectivorous bats, no study in the Neotropics had evaluated their occurrence in secondary forests after decades of ecosystem recovery, and therefore, these are the first illustrative results available about minimum recovery times required to restore this bat ensemble.

Primary rainforest in central Amazon

The BDFFP has greatly contributed to the literature on forest fragmentation and to highlighting the importance of secondary forests for conservation (Laurance et al. 2017). Since the isolation of the fragments in the late 80s, the cleared areas have gradually turned into fairly old secondary forest with a structure somewhat similar to that of continuous primary forest. This vegetation regrowth has buffered the effects of forest fragmentation on several taxa including birds (Stouffer et al. 2011), dung beetles (Quintero and Roslin 2005), primates (Boyle and Smith 2010) and phyllostomid bats (Rocha et al. 2018). The fact that I did not have a baseline dataset for aerial insectivorous bats present in the forest fragments at the time of fragment isolation did not allow me to carry out long-term temporal analyses to understand the role of the secondary forest in terms of ecosystem recovery. However, I provide evidence that assemblages in secondary forest are influenced by the fragments, rather than the other way around.

## Which traits make species more vulnerable?

Functional traits are being increasingly used to understand why some species are more vulnerable than others to threats like habitat fragmentation or habitat loss (Duchamp and Swihart 2008, Meyer et al. 2008, Threlfall et al. 2011, Cisneros et al. 2015, Farneda et al. 2015, Farneda et al. 2018a, Farneda et al. 2018b). Functional diversity can be understood as the variation in traits that affect a species' performance, fitness and ecological function within a community (Violle et al. 2007). Understanding how functional traits influence species vulnerability to forest fragmentation is crucial to identify the most sensitive species and improve their management.

In Chapter 5 I showed how changes in β diversity between continuous forest and secondary forest or forest fragments were mainly related to species and traits loss rather than replacement (i.e. turnover). In order to further explore this, in the following Chapter 6 I studied, using a before-after-control experimental design, which functional traits make aerial insectivorous bats more vulnerable to deforestation. I hypothesised that the decrease in β diversity could be directly influenced by each families' flight performance and echolocation type, which are usually highly adapted to a species preferred foraging habitat (Norberg and Rayner 1987, Fraixedas 2017). I found that high duty-cycle echolocating bats (commonly with low aspect ratio and low wing loading) and those species adapted to forage in the understorey were more sensitive to habitat fragmentation, avoided cleared and open areas, mainly remaining in forest fragments and continuous forest. In contrast, species with high aspect ratio wings were linked to forest edges and clearings. These results, at species- and trait- level, together with those of the previous chapter at assemblage-level, contribute to the baseline knowledge required to design species and habitat management policies. In areas with extensive forest clearing activities, with little remaining forest cover, priority should be given to protect species with high duty-cycle echolocation, as well as other sensitive species such as *Furipterus horrens* by providing, for example, green corridors connecting their most important roosts to potential foraging areas such as lakes, ponds or swamps. For phyllostomid bats, animalivorous gleaners seem to be the most sensitive to deforestation and uncommon in secondary forests (Farneda et al. 2015, Rocha et al. 2017b). In that case, mobility, body mass, wing morphology and trophic level were the more relevant traits for phyllostomids that make them most vulnerable.

A loss of functional traits is commonly linked to an impoverishment of the ecosystem services provided (Luck et al. 2012). In my case, although the ecosystem services loss is not as extreme as those reported in habitats such as farmlands (Puig-Montserrat et al. 2015) the reduction of certain insectivorous species may also compromise the suppression of insect populations. By combining species functional traits with environmental characteristics, an approach recently developed by Dray et al. (2014), I detected the traits that made aerial insectivorous bats more vulnerable towards forest fragmentation. Likewise, my results reinforce the view that preserving structurally complex tropical forests is crucial to maintain functionally diverse assemblages. In the case of phyllostomid bats at the BDFFP, functional diversity has been found to recover in forest fragments and secondary forests through matrix regeneration (Farneda et al. 2018b, Farneda et al. 2018a), and therefore I would expect functional diversity of aerial insectivorous bats to also increase over time.

## The importance of Amazonian lakes for bat conservation

Riparian habitats and water bodies are commonly considered to be good foraging areas for bats because they are characterized by high insect availability (Fukui et al. 2006) and because they provide water for bats to drink (Seibold et al. 2013). However, studies on bats in riparian ecosystems or inundated areas in the tropics are rare (MacSwiney et al. 2009, Costa et al. 2011, Hagen and Sabo 2012). In the Brazilian Amazon, only Marques et al. (2015) studied their foraging activity in flooded forests and reported the importance of *varzea* habitats for insectivorous bats, highlighting their rich and diverse assemblages.

The predicted changes induced by climate change entail dramatic shifts in water availability as well as large decreases in the water level of ecosystems for the Amazon (Beaumont et al. 2011, Marengo and Espinoza 2016). A diminution of rainfall and rising temperatures might cause shrinking water levels in lakes, or even drying up of some of them. This would be detrimental for water-dependent species such as bats. In Chapter 7 I found that lakes embedded in Amazonian *terra firme* forest were important landscape structures for the conservation of bats. Eighteen out of 20 species were more active over lakes than in continuous forest, with a disproportionately high number of feeding buzzes (four times higher). These final results suggest that most aerial insectivorous bat species in my study area might be to some extent dependent on lakes, where I assessed for the first time seasonal differences in bat activity. In fact, I confirmed that they are most dependent on the studied lakes during the dry season.

## Conservation implications

Current trends indicate that forest fragmentation in the tropics is exponentially increasing, threatening the remaining natural habitats that still can be found in these regions and some of the most important biodiversity hotspots on the planet. In the Amazon, deforestation continues, although at a lower rate than in the past (Rosa et al. 2017), a trend that is mostly due to a better law enforcement. Notwithstanding some recent positive trends, this might be reverting in the near future because of the expansion of oil palm, soy and other similar threats (Butler and Laurance 2009, FAO 2016, Rosa et al. 2017) and upcoming changes in environmental politics. This would severely put at risk the recovery that has been taking place in some forest regions during the last decades due to the development of secondary forest and resulting increase in habitat connectivity (Chazdon 2014).

The results of this thesis suggest that the time required for full ecosystem recovery extends beyond 30 years and also that at this age secondary forests, although resembling primary forest in terms of structure and complexity, are only intermediate steps in the middle of a very long process of ecosystem regeneration. Unfortunately, as reported by Reid et al. (2018), secondary forests usually have relatively short lifespans as they often become recleared before they recover to predisturbance conditions. In Costa Rica, for instance, 50% of secondary forests are recleared before 20 years of regeneration, and 85% before 54 years, while only large forest fragments and riparian forests persist for longer periods. Ecosystem restoration and recovery are young fields in conservation science, bounded by big knowledge gaps, which will inevitably have a key role to restore natural habitats in the near future (Brudvig 2011, Brudvig 2017, Rydgren et al. 2018). The value of pristine primary forest (even if it is highly fragmented) cannot be underestimated (Barlow et al. 2007, Laurance et al. 2017, Rocha 2017) and these undisturbed forests will need stronger protection through an intense educational effort of our future generations.

However, responses to habitat fragmentation or deforestation are very species-specific (Rodríguez-San Pedro and Simonetti 2015) and therefore any current policy regarding habitat management must be designed taking into consideration the conservation status of the species, especially those that are more vulnerable to anthropogenic changes. I demonstrated that the functional approach is useful to help selecting species that need to be targeted by conservation measures. When developing conservation strategies, special attention should be given to landscape components that play important roles for bats, such as commuting paths or wooded corridors connecting roosts with important foraging habitats like rivers and lakes in terra firme rainforest.

I highlight the fact that climate change might affect terra firme lake water levels due to a forecast reduction of rainfall and longer drought periods (Marengo and Espinoza 2016). Although some of these lakes are temporary, they deserve special attention for conservation as their disappearance could result in a decline of some aerial insectivorous bat species. Therefore, I suggest that protection of floodplains, riparian forest along rivers and lakes should be encouraged across the Amazon Basin.

## Study limitations and future research

This thesis has expanded the understanding of Neotropical aerial insectivorous bat bioacoustics, as well as of the effects of tropical forest fragmentation on this ensemble. However, important questions on both topics remain unanswered, offering opportunities for lines of research that could be explored in the future.

The intrinsic limitations of bioacoustic techniques did not allow me to acquire information about sex, reproductive status and age of the recorded individuals, thus greatly limiting our ability to study topics such as species' phenology. Filling this information gap could reveal potential migration patterns or even sex-specific behaviours, as has been reported for phyllostomid bats (Rocha et al. 2017a). To gather this kind of information I would have had to use mist-nets, which, as previously mentioned, are very inefficient to capture aerial insectivores. The use of bat lures may increase the captures of insectivorous bats in mist-nets and this may help studying aspects of their biology that require the examination of the individuals.

Weather conditions and moonlight are known to affect bat activity (Appel et al. 2017), as bats tend to avoid light to minimize predation risk, and because high humidity attenuates echolocation, hindering prey location and foraging (Griffin 1971). Proper data on weather conditions were not available for my study area and I did not include the effect of moonlight in my analyses. Although this might not have affected the results too much due to the large dataset, taking these factors into account would certainly improve the accuracy of further scientific studies.

In the self-built algorithm, I still relied on the automatic extraction of measurements of echolocation pulses that was carried out using the ScanR software (Binary Technology, USA). This was the best option available when I analysed the recording dataset. However, ScanR is not an open source software and had an approximate cost of 195US$ in 2018. This was obviously a limitation as I were proposing a cost-effective solution for the analysis of massive acoustic datasets. Fortunately, a new completely open source software (*Tadarida*) has now been developed by Bas et al. (2017), which is capable of not only extracting the acoustic parameters of a large number of files, but also creating personal reference libraries with reference calls, and training machine learning algorithms (including random forest) to classify acoustic datasets according to one's reference data.

Almost all commercial classifiers and even several open source options that have emerged during the last years use supervised machine learning to classify bat sounds. I believe that the way to go for future research includes addressing acoustic analyses with unsupervised machine learning, that does not need any reference calls, and groups all recordings using a specified number of clusters based on their pulse similarity. This approach would be particularly useful in countries where libraries of reference calls are unavailable or very incomplete. Its results would be repeatable, robust and helpful for further acoustic studies, because it can be used directly on the raw data and the classification results will not depend on the quality of any reference call library.

Last but not least, Bernie Krause, a musician and soundscape ecologist considered one of the fathers of the field of soundscape ecology, opened this new research field that could improve our understanding of ecosystem health. The term “soundscape” was first used by Truax (1978), but in the late 1990s Krause introduced a number of concepts in this discipline such as geophony, biophony and anthropophony, as well as the concept of acoustic niche hypothesis. However, only recently papers about the soundscape and its use for biodiversity conservation started to be published (Farina and Gage 2017, Ozga 2017, Brown et al. 2018). Soundscape ecology is the study of the acoustic component of an ecosystem, with special emphasis on the relationship between living organisms (Pijanowski et al. 2011a, Pijanowski et al. 2011b). With a dataset such as ours, with recordings that cover from 12,000Hz to 192,000Hz, including bats but also insects and frogs, this new approach could be easily applied in order to quantify the effect of forest fragmentation upon the acoustic component of the landscape (Pekin et al. 2012, Brown et al. 2018).

Since this project has been carried out in the BDFFP, where only 11 forest fragments are available, some of the greatest limitations of my sampling design are the small number of replicates of fragments, as well as the fairly small distance of the fragments to continuous forest, which results in limited isolation for highly mobile species. This prevented me to explore different spatial sampling schemes in Chapter 2, as other authors such as Froidevaux et al. (2014) or Law et al. (2015) did in Europe and Australia respectively. Also, due to the proximity of some of the fragments, I decided to not include a landscape cover analysis to avoid spatial autocorrelation resulting from overlapping buffers. If possible, it would be great to enlarge the sampling size including more experimental fragments or, if this unlikely in the BDFFP, replicate the effort in other similar tropical fragmentation experiments such as the SAFE project in Borneo (Ewers et al. 2011).

Insect availability in the tropics can markedly oscillate between nights, depending on wind intensity and isolated resource blooms. There is plenty of evidence that bat activity is highly correlated with insect availability (e.g. Verboom and Spoelstra 1999, Wickramasinghe et al. 2004, Hagen and Sabo 2012), and also that bats can move from one foraging habitat to another very quickly depending on food availability. Understanding these movements in fragmented landscapes would help to better understand an important consequence of forest fragmentation.

Since Sampaio (2001) collected data at the BDFFP on phyllostomid bats using mistnets for her thesis in the late 1990s, Rocha et al. (2018) was able to undertake a long-term study comparing bat assemblages within the same landscape but with samples separated by about 15 years. Sampaio also collected acoustic data, but the difficulties in comparing both datasets, make a long-term comparison for aerial insectivorous bats too difficult to be included in this thesis. The technological and sampling differences between the two periods (1990s vs 2011/13) were very difficult to overcome. However, I strongly believe that long-term comparisons are crucial to improve land management and, due to the impressive results that can be acquired in this kind of projects, I would encourage this type of research.

The Amazonian rainforest remains quite well-conserved compared to the Atlantic forest, where less than 15% of natural habitats remain (Ribeiro et al. 2009). Thus, I encourage that to the extent possible, the research summarised in this dissertation was combined with similar projects in more disturbed areas. Understanding the effects of forest fragmentation requires a holistic view which considers different degrees of deforestation. The final aim of this thesis is to provide results that could be combined with similar studies carried out in the BDFFP on other organisms. By assembling a common knowledge database including not only bats, but a vast diversity of taxa, I will be able to increase our knowledge on the effects of forest fragmentation and improve scientific conservation outputs. Only by combining all available information can I provide a complete image of the ecological processes that are shaping the ecosystems in fragmented Neotropical forests, expand our understanding of the consequences of forest fragmentation at all levels, and consequently, improve our land management strategies and conservation policies. Although this has partially been done in the latest published reviews (Laurance et al. 2011, Laurance et al. 2017), I consider that a bigger review, including all taxa, projects and contributors is still needed.

## Parallel side-projects

During the realization of this project, I have had the opportunity to carry out and collaborate on several side projects on bat research and conservation. These projects have mostly resulted in scientific publications that I have authored or co-authored, which include: a) studies about the effect of moonlight on the activity of insectivorous bats and about geographical variation in echolocation (Appel et al. 2017, López-Baucells et al. 2017c), b) natural history notes (Alberdi et al. 2012, López-Baucells et al. 2013, Treitler et al. 2013, Rocha et al. 2014, Rocha and López-Baucells 2014b, Rocha and López-Baucells 2014a, Mas et al. 2015, Rocha et al. 2016, Gonçálvez et al. 2017), c) description of the echolocation calls of some Amazonian species (López-Baucells et al. 2014, López-Baucells et al. 2018), d) projects on bat conservation in Kenya and Madagascar (López-Baucells et al. 2016b, López-Baucells et al. 2017b, Kemp et al. 2019), e) studies on bat conservation in the Mediterranean (Flaquer et al. 2014, Puig-Montserrat et al. 2015, López-Baucells et al. 2016a, López-Baucells et al.

2017a, Coronado et al. 2018, Martin Bideguren et al. 2018), f) the works carried out by R. Rocha and F. Farneda in their parallel PhD projects (Ferreira et al. 2017, Rocha et al. 2017a, Rocha et al. 2017b, Rocha et al. 2017c, Farneda et al. 2018a, Farneda et al. 2018b, Rocha et al. 2018) as well as g) some international collaborations in meta-analyses and reviews (Lucati and López-Baucells 2017, Dornelas et al. 2018).

*Carollia perspicillata*

## References

Alberdi, A., J. Aihartza, J. C. Albero, O. Aizpurua, A. López-Baucells, L. Freixas, X. Puig, C. Flaquer, and I. Garin. 2012. First records of the parti-coloured bat *Vespertilio murinus* (Chiroptera:Vespertilionidae) in the Pyrenees. Mammalia **76**:109-111.

Appel, G., A. López-Baucells, E. Magnusson, and P. E. D. Bobrowiec. 2017. Aerial insectivorous bat activity in relation to moonlight intensity. Mammalian Biology **85**:37-46.

Arias-Aguilar, A., F. Hintze, L. M. S. Aguiar, V. Rufray, E. Bernard, and M. J. R. Pereira. 2018. Who’s calling? Acoustic identification of Brazilian bats. Mammal Research **63**:231-253.

Arroyo-Rodríguez, V., C. Rojasa, R. A. Saldaña-Vázqueza, and K. E. Stonerb. 2016. Landscape composition is more important than landscape configuration for phyllostomid bat assemblages in a fragmented biodiversity hotspot. Biological Conservation **198**:84-92.

Barlow, J., L. A. Mestre, T. A. Gardner, and C. A. Peres. 2007. The value of primary, secondary and plantation forests for Amazonian birds. Biological Conservation **136**:212-231.

Bas, Y., D. Bas, and J.-F. Julien. 2017. Tadarida: A toolbox for animal detection on acoustic recordings. Journal of Open Research Software **5**:6.

Beaumont, L. J., A. Pitman, S. Perkins, N. E. Zimmermann, N. G. Yoccoz, and W. Thuiller. 2011. Impacts of climate change on the world's most exceptional ecoregions. Proceedings of the National Academy of Sciences **108**:2306-2311.

Benchimol, M., and C. A. Peres. 2015a. Edge-mediated compositional and functional decay of tree assemblages in Amazonian forest islands after 26 years of isolation. Journal of Ecology **103**:408-420.

Benchimol, M., and C. A. Peres. 2015b. Predicting local extinctions of Amazonian vertebrates in forest islands created by a mega dam. Biological Conservation **187**:61-72.

Botto Nuñez, G., G. Lemus, M. Muñoz Wolf, A. L. Rodales, E. M. González, and C. Crisci. 2018. The first artificial intelligence algorithm for identification of bat species in Uruguay. Ecological Informatics **46**:97-102.

Boyle, S. A., and A. T. Smith. 2010. Can landscape and species characteristics predict primate presence in forest fragments in the Brazilian Amazon? Biological Conservation **143**:1134-1143.

Brook, B. W., N. S. Sodhi, and C. J. Bradshaw. 2008. Synergies among extinction drivers under global change. Trends in Ecology & Evolution **23**:453-460.

Brown, K., M. McCann, E. Biggar, S. Ziatek, J. Pumilio, and A. Jimenez. 2018. The utilization of soundscape ecology to measure and track changes to biodiversity in the forests surrounding Colgate University. Page 17 *in* Integrative and Comparative Biology. Oxford University Press, Cary, United States of America.

Brudvig, L. A. 2011. The restoration of biodiversity: where has research been and where does it need to go? American Journal of Botany **98**:549-558.

Brudvig, L. A. 2017. Toward prediction in the restoration of biodiversity. Journal of Applied Ecology **54**:1013-1017.

Butler, R. A., and W. F. Laurance. 2009. Is oil palm the next emerging threat to the Amazon? Tropical Conservation Science **2**:1-10.

Cisneros, L. M., M. E. Fagan, and M. R. Willig. 2014. Season-specific and guild-specific effects of anthropogenic landscape modification on metacommunity structure of tropical bats. Journal of Animal Ecology **84**:373-385.

Cisneros, L. M., M. E. Fagan, and M. R. Willig. 2015. Effects of humans-modified landscapes on taxonomic, functional and phylogenetic dimensions of bat biodiversity. Diversity and distributions **21**:523-533.

Connell, J. H. 1978. Diversity in tropical rain forests and coral reefs. Science **199**:1302-1310.

Coronado, A., C. Flaquer, X. Puig-Montserrat, E. Barthe, M. Mas, A. Arrizabalaga, and A. López-Baucells. 2018. The role of secondary trees in Mediterranean mature forests for the conservation of the forest-dwelling bat *Myotis alcathoe*. Are current logging guidelines appropriate? Hystrix, the Italian Journal of Mammalogy **28**:240–246.

Cortes-Delgado, N., and J. Perez-Torres. 2011. Habitat edge context and the distribution of phyllostomid bats in the Andean forest and anthropogenic matrix in the Central Andes of Colombia. Biodiversity and Conservation **20**:987-999.

Costa, L. D., E. C. Lourenco, J. L. Luz, A. P. F. de Carvalho, and C. E. L. Esberard. 2011. Activity of two species of free-tailed bats over a stream in southeastern Brazil. Acta Chiropterologica **13**:405-409.

Chazdon, R. L. 2014. Second growth: The promise of tropical forest regeneration in an age of deforestation. University of Chicago Press, Connecticut, United States of America.

Chazdon, R. L., P. H. Brancalion, L. Laestadius, A. Bennett-Curry, K. Buckingham, C. Kumar, J. Moll-Rocek, I. C. G. Vieira, and S. J. Wilson. 2016. When is a forest a forest? Forest concepts and definitions in the era of forest and landscape restoration. Ambio **45**:538-550.

Chazdon, R. L., C. A. Harvey, O. Komar, D. M. Griffith, B. G. Ferguson, M. Martínez-Ramos, H. Morales, R. Nigh, L. Soto-Pinto, M. Van Breugel, and S. M. Philpott. 2009a. Beyond Reserves: A Research Agenda for Conserving Biodiversity in Human-modified Tropical Landscapes. Biotropica **41**:142-153.

Chazdon, R. L., C. A. Peres, D. Dent, D. Sheil, A. E. Lugo, D. Lamb, N. E. Stork, and S. E. Miller. 2009b. The potential for species conservation in tropical secondary forests. Conservation Biology **23**:1406-1417.

Daily, G. C. 1997. Countryside biogeography and the provision of ecosystem services. Pages 104-113 *in* Raven, editor. Nature and human society: The quest for a sustainable world. National Academy Press, Washington, United States of America.

De Conno, C., V. Nardone, L. Ancillotto, S. De Bonis, M. Guida, I. Jorge, U. Scarpa, and D. Russo. 2018. Testing the performance of bats as indicators of riverine ecosystem quality. Ecological Indicators **95**:741-750.

Derroire, G., P. Balvanera, C. Castellanos-Castro, G. Decocq, D. K. Kennard, E. Lebrija-Trejos, J. A. Leiva, P.-C. Odén, J. S. Powers, V. Rico-Gray, M. Tigabu, and J. R. Healey. 2016. Resilience of tropical dry forests - a meta-analysis of changes in species diversity and composition during secondary succession. Oikos **125**:1386-1397.

Dornelas, M., L. H. Antao, F. Moyes, A. E. Bates, A. E. Magurran, D. Adam, A. A. Akhmetzhanova, W. Appeltans, J. M. Arcos, H. Arnold, and E. al. 2018. BioTIME: A database of biodiversity time series for the Anthropocene. Global Ecology and Biogeography **27**:760-786.

Douglas, W. 2018. Book Review: Amazonian Bats. Journal of Mammalogy **99**:1014-1015.

Dray, S., P. Choler, S. Dolédec, P. R. Peres-Neto, W. Thuiller, S. Pavoine, and C. J. F. ter Braak. 2014. Combining the fourth-corner and the RLQ methods for assessing trait responses to environmental variation. Ecology **95**:14-21.

Duchamp, J., and R. Swihart. 2008. Shifts in bat community structure related to evolved traits and features of human-altered landscapes. Landscape Ecology **23**:849-860.

Estrada-Villegas, S., C. F. Meyer, and E. K. Kalko. 2010. Effects of tropical forest fragmentation on aerial insectivorous bats in a land-bridge island system. Biological Conservation **143**:597-608.

Ethier, K., and L. Fahrig. 2011. Positive effects of forest fragmentation, independent of forest amount, on bat abundance in eastern Ontario, Canada. Landscape Ecology **26**:865-876.

Ewers, R. M., R. K. Didham, L. Fahrig, G. Ferraz, A. Hector, R. D. Holt, V. Kapos, G. Reynolds, W. Sinun, J. L. Snaddon, and E. C. Turner. 2011. A large-scale forest fragmentation experiment: the Stability of Altered Forest Ecosystems Project. Philosophical Transactions of the Royal Society B: Biological Sciences **366**:3292-3302.

FAO. 2016. State of the World's forests. Food and Agriculture Organization of the United Nations, Rome, Italy.

Fardila, D., L. T. Kelly, J. L. Moore, and M. A. McCarthy. 2017. A systematic review reveals changes in where and how we have studied habitat loss and fragmentation over 20 years. Biological Conservation **212**:130-138.

Farina, A., and S. H. Gage. 2017. Ecoacoustics: The ecological role of sounds. Wiley, Michigan, United States of America.

Farneda, F. Z., R. Rocha, A. López-Baucells, M. Groenenberg, I. Silva, J. M. Palmeirim, P. E. D. Bobrowiec, C. F. J. Meyer, and M. Pocock. 2015. Trait-related responses to habitat fragmentation in Amazonian bats. Journal of Applied Ecology **52**:1381-1391.

Farneda, F. Z., R. Rocha, A. López-Baucells, E. M. Sampaio, J. M. Palmeirim, P. E. D. Bobrowiec, C. E. V. Grelle, and C. F. J. Meyer. 2018a. Functional recovery of Amazonian bat assemblages following secondary forest succession. Biological Conservation **218**:192-199.

Farneda, F. Z., R. Rocha, A. López-Baucells, E. M. Sampaio, J. M. Palmeirim, P. E. D. Bobrowiec, C. E. V. Grelle, and C. F. J. Meyer. 2018b. The road to functional recovery: temporal effects of matrix regeneration on Amazonian bats. Tropical Conservation Science **11**:1-4.

Ferreira, D. F., R. Rocha, A. López-Baucells, F. Z. Farneda, J. M. B. Carreiras, J. M. Palmeirim, and C. F. J. Meyer. 2017. Season-modulated responses of Neotropical bats to forest fragmentation. Ecology and Evolution **7**:4059-4071.

Finegan, B. 1996. Pattern and process in neotropical secondary rain forests: the first 100 years of succession. Trends in Ecology & Evolution **11**:119-124.

Flaquer, C., X. Puig-Montserrat, A. López-Baucells, I. Torre, L. Freixas, M. Mas, X. Porres, and A. Arrizabalaga. 2014. Could overheating turn bat boxes into death traps? Barbastella, Journal of Bat Research **7**:39-46.

Fonderflick, J., A. Besnard, and J.-L. Martin. 2013. Species traits and the response of open-habitat species to forest edge in landscape mosaics. Oikos **122**:42-51.

Fraixedas, S. 2017. Which functional traits make Neotropical aerial insectivorous bats more vulnerable to forest fragmentation? MSc Thesis. Autonomous University of Barcelona, Barcelona, Spain.

Froidevaux, J. S. P., F. Zellweger, K. Bollmann, and M. K. Obrist. 2014. Optimizing passive acoustic sampling of bats in forests. Ecology and Evolution **4**:4690-4700.

Fukui, D., M. Murakami, S. Nakano, and T. Aoi. 2006. Effect of emergent aquatic insects on bat foraging in a riparian forest. Journal of Animal Ecology **75**:1252-1258.

Gonçálvez, C., A. López-Baucells, and R. Rocha. 2017. Opportunistic predation of a silky short-tailed bat (*Carollia brevicauda*) by a tawny-bellied screech-owl (*Megascops watsonii*), with a compilation of predation events upon bats entangled in mist-nets. Journal of Bat Research & Conservation **10**:41-46.

Griffin, D. R. 1971. The importance of atmospheric attenuation for the echolocation of bats (Chiroptera). Animal Behaviour **19**:55-61.

Grindal, S. D., and R. M. Brigham. 1999. Impacts of forest harvesting on habitat use by foraging insectivorous bats at different spatial scales. Ecoscience **6**:25-34.

Guariguata, M. R., and R. Ostertag. 2001. Neotropical secondary forest succession: changes in structural and functional characteristics. Forest Ecology and Management **148**:185-206.

Haddad, N. M., L. A. Brudvig, J. Clobert, K. F. Davies, A. Gonzalez, R. D. Holt, T. E. Lovejoy, J. O. Sexton, M. P. Austin, and C. D. Collins. 2015. Habitat fragmentation and its lasting impact on Earth's ecosystems. Science advances **1**:e1500052.

Hagen, E., and J. Sabo. 2012. Influence of river drying and insect availability on bat activity along the San Pedro River, Arizona (USA). Journal of Arid Environments **84**:1-8.

Hall, J. 2018. Book Review: Field Guide to the Bats of the Amazon. Mammal Watching.

Harris, L. D., and G. Silva-Lopez. 1992. Forest Fragmentation and the Conservation of Biological Diversity. Pages 197-237 *in* P. L. Fiedler and S. K. Jain, editors. Conservation Biology: The Theory and Practice of Nature Conservation Preservation and Management. Springer, Boston, United States of America.

Hill, A. P., P. Prince, E. P. Covarrubias, C. P. Doncaster, J. L. Snaddon, and A. Rogers. 2018. AudioMoth: Evaluation of a smart open acoustic device for monitoring biodiversity and the environment. Methods in Ecology and Evolution **9**:1199-1211.

Jones, G., D. S. Jacobs, T. Kunz, M. R. Willig, and P. A. Racey. 2009. Carpe noctem: the importance of bats as bioindicators. Endangered Species Research **8**:93-115.

Jones, I. L., N. Bunnefeld, A. S. Jump, C. A. Peres, and D. H. Dent. 2016. Extinction debt on reservoir land-bridge islands. Biological Conservation **199**:75-83.

Jung, K., and E. K. V. Kalko. 2011. Adaptability and vulnerability of high flying Neotropical aerial insectivorous bats to urbanization. Diversity and distributions **17**:262-274.

Kalcounis-Rueppell, M. C., K. M. Briones, J. A. Homyack, R. Petric, M. M. Marshall, and D. A. Miller. 2013. Hard forest edges act as conduits, not filters, for bats. Wildlife Society Bulletin **37**:571-576.

Kemp, J., A. López-Baucells, R. Rocha, O. S. Wangensteen, Z. Andriatafika, A. Nair, and M. Cabeza. 2019. Bats as potential suppressors of multiple agricultural pests: A case study from Madagascar. Agriculture, Ecosystems & Environment **269**:88-96.

Krull, D., A. Schumm, W. Metzner, and G. Neuweiler. 1991. Foraging areas and foraging behavior in the notch-eared bat, *Myotis emarginatus* (Vespertilionidae). Behavioral Ecology and Sociobiology **28**:247-253.

Laurance, W. F., J. L. C. Camargo, P. M. Fearnside, T. E. Lovejoy, G. B. Williamson, R. C. G. Mesquita, C. F. J. Meyer, P. E. D. Bobrowiec, and S. G. W. Laurance. 2017. An Amazonian rainforest and its fragments as a laboratory of global change. Biological Reviews of the Cambridge Philosophical Society **93**:223-247.

Laurance, W. F., J. L. C. Camargo, R. C. C. Luizao, S. G. Laurance, S. L. Pimm, E. M. Bruna, P. C. Stouffer, G. B. Williamson, J. Benitez-Malvido, H. L. Vasconcelos, K. S. Van Houtan, C. E. Zartman, S. A. Boyle, R. K. Didham, A. Andrade, and T. E. Lovejoy. 2011. The fate of Amazonian forest fragments: A 32-year investigation. Biological Conservation **144**:56–67.

Law, B., and C. Dickman. 1998. The use of habitat mosaics by terrestrial vertebrate fauna: implications for conservation and management. Biodiversity & Conservation **7**:323-333.

Law, B., L. Gonsalves, P. Tap, T. Penman, and M. Chidel. 2015. Optimizing ultrasonic sampling effort for monitoring forest bats. Austral Ecology **40**:886–897.

Law, B. S., J. Anderson, and M. Chidel. 1999. Bat communities in a fragmented forest landscape on the south-west slopes of New South Wales, Australia. Biological Conservation **88**:333-345.

López-Baucells, A., L. Casanova, X. Puig-Montserrat, A. Espinal, F. Páramo, and C. Flaquer. 2017a. Evaluating the use of *Myotis daubentonii* as an ecological indicator in Mediterranean riparian habitats. Ecological Indicators **74**:19-27

López-Baucells, A., M. Mas, X. Puig-Montserrat, and C. Flaquer. 2013. Hypopigmentation in vespertilionid bats: the first record of a leucistic soprano pipistrelle *Pipistrellus pygmaeus*. Barbastella, Journal of Bat Research **6**:66-72.

López-Baucells, A., X. Puig-Montserrat, I. Torre, L. Freixas, M. Mas, A. Arrizabalaga, and C. Flaquer. 2016a. Bat boxes in urban non-native forests: a popular practice that should be reconsidered. Urban Ecosystems **20**:217-225.

López-Baucells, A., R. Rocha, T. Andriatafika, T. Tojosoa, J. Kemp, K. Forbes, and M. Cabeza. 2017b. Roost selection by synanthropic bats in rural Madagascar: what makes non-traditional structures so tempting? Hystrix, the Italian Journal of Mammalogy **28**:28-35.

López-Baucells, A., R. Rocha, G. Fernández-Arellano, P. E. D. Bobrowiec, J. M. Palmeirim, and C. F. J. Meyer. 2014. Echolocation of the big red bat *Lasiurus egregius* (Chiroptera: Vespertilionidae) and first record from the Central Brazilian Amazon. Studies on Neotropical Fauna and Environment **49**:18-25.

López-Baucells, A., R. Rocha, V. C. Tavares, L. Mora, P. E. D. Bobrowiec, S. E. Silva, and C. Meyer. 2018. Molecular, morphological and acoustic identification of *Eumops maurus* and *E. hansae* (Chiroptera: Molossidae) with new reports from Central Amazonia. Tropical Zoology **31**:1-20.

López-Baucells, A., R. Rocha, P. Webala, A. Nair, R. Uusitalo, T. Sironen, and K. M. Forbes. 2016b. Rapid assessment of bat diversity in the Taita Hills Afromontane cloud forests, southeastern Kenya. Barbastella, Journal of Bat Research **9**:16-27.

López-Baucells, A., L. Torrent, R. Rocha, A. C. Pavan, P. E. D. Bobrowiec, and C. F. J. Meyer. 2017c. Geographical variation in the high-duty cycle echolocation of the cryptic common mustached bat *Pteronotus* cf. *rubiginosus* (Mormoopidae). Bioacoustics Journal **27**:341-357.

Lu, D., E. Moran, and P. Mausel. 2002. Linking Amazonian secondary succession forest growth to soil properties. Land Degradation & Development **13**:331-343.

Lucati, F., and A. López-Baucells. 2017. Chromatic disorders in bats: a review of pigmentation anomalies and the misuse of terms to describe them. Mammal Review **47**:112-123.

Luck, G. W., S. Lavorel, S. McIntyre, and K. Lumb. 2012. Improving the application of vertebrate trait-based frameworks to the study of ecosystem services. Journal of Animal Ecology **81**:1065-1076.

Lumsden, L., and A. Bennett. 2000. Bats in rural landscapes: a significant but largely unknown faunal component. Pages 42-50 *in* T. Barlow and R. Thorburn, editors. Balancing conservation and production in grassy landscapes. Environment Australia, Canberra, Australia.

Lumsden, L. F., and A. F. Bennett. 2005. Scattered trees in rural landscapes: foraging habitat for insectivorous bats in south-eastern Australia. Biological Conservation **122**:205-222.

MacArthur, R. H., and E. O. Wilson. 1967. Theory of Island Biogeography. Princeton University Press, Princeton, United States of America.

MacSwiney, G. C., F. M. Clarke, and P. A. Racey. 2008. What you see is not what you get: the role of ultrasonic detectors in increasing inventory completeness in Neotropical bat assemblages. Journal of Applied Ecology **45**:1364-1371.

MacSwiney, M. C., B. B. Cime, F. M. Clarke, and P. A. Racey. 2009. Insectivorous bat activity at cenotes in the Yucatan Peninsula, Mexico. Acta Chiropterologica **11**:139-147.

Madhukumar Menon, A., M. João Ramos Pereira, and L. Moura de Souza Aguiar. 2018. Are automated acoustic identification software reliable for bat surveys in the Neotropical region? PeerJ:(in press).

Marengo, J. A., and J. Espinoza. 2016. Extreme seasonal droughts and floods in Amazonia: causes, trends and impacts. International Journal of Climatology **36**:1033-1050.

Marques, J. T., M. J. Ramos Pereira, and J. M. Palmeirim. 2015. Patterns in the use of rainforest vertical space by Neotropical aerial insectivorous bats: all the action is up in the canopy. Ecography **39**:476-486.

Martin Bideguren, G., A. López-Baucells, X. Puig-Montserrat, M. Mas, X. Porres, and C. Flaquer. 2018. Bat boxes and climate change: testing the risk of over-heating in the Mediterranean region. Biodiversity and Conservation:(in press).

Mas, M., A. López-Baucells, and A. Arrizabalaga. 2015. Predation on bats by genets *Genetta genetta* (Linneaus, 1758): a review. Barbastella, Journal of Bat Research **8**:5-11.

Mendenhall, C. D., C. V. Kappel, and P. R. Ehrlich. 2013. Countryside Biogeography. Pages 347-360 *in* S. A. Levin, editor. Encyclopedia of Biodiversity. Academic Press, Waltham, United States of America.

Mendenhall, C. D., D. S. Karp, C. F. J. Meyer, E. A. Hadly, and G. C. Daily. 2014. Predicting biodiversity change and averting collapse in agricultural landscapes. Nature **509**:213-217.

Meyer, C. F. J., J. Fruend, W. P. Lizano, and E. K. V. Kalko. 2008. Ecological correlates of vulnerability to fragmentation in Neotropical bats. Journal of Applied Ecology **45**:381-391.

Meyer, C. F. J., M. J. Struebig, and M. R. Willig. 2016. Responses of tropical bats to habitat fragmentation, logging, and deforestation. Pages 63-103 *in* C. C. Voigt and T. Kingston, editors. Bats in the Anthropocene: Conservation of bats in a changing world. Springer, New York, United States of America.

Norberg, U. M., and J. M. V. Rayner. 1987. Ecological morphology and flight in bats (Mammalia, Chiroptera) - wing adaptations, flight performance, foraging strategy and echolocation. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences **316**:337-419.

Ozga, A. 2017. Scientific Ideas Included in the Concepts of Bioacoustics, Acoustic Ecology, Ecoacoustics, Soundscape Ecology, and Vibroacoustics. Archives of Acoustics **42**:415-421.

Pekin, B. K., J. Jung, L. J. Villanueva-Rivera, B. C. Pijanowski, and J. A. Ahumada. 2012. Modeling acoustic diversity using soundscape recordings and LIDAR-derived metrics of vertical forest structure in a neotropical rainforest. Landscape Ecology **27**:1513-1522.

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

Pijanowski, B. C., L. J. Villanueva-Rivera, S. L. Dumyahn, A. Farina, B. L. Krause, B. M. Napoletano, S. H. Gage, and N. Pieretti. 2011b. Soundscape ecology: the science of sound in the landscape. Bioscience **61**:203-216.

Powell, L. L., P. C. Stouffer, and E. I. Johnson. 2013. Recovery of understory bird movement across the interface of primary and secondary amazon rainforest. The Auk **130**:459-468.

Powell, L. L., J. D. Wolfe, E. I. Johnson, J. E. Hines, J. D. Nichols, and P. C. Stouffer. 2015. Heterogeneous movement of insectivorous Amazonian birds through primary and secondary forest: A case study using multistate models with radiotelemetry data. Biological Conservation **188**:100-108.

Puig-Montserrat, X., I. Torre, A. López-Baucells, E. Guerrieri, M. M. Monti, R. Rafols-Garcia, X. Ferrer, D. Gisbert, and C. Flaquer. 2015. Pest control service provided by bats in Mediterranean rice paddies: linking agroecosystems structure to ecological functions. Mammalian Biology **80**:237-245.

Quintero, I., and T. Roslin. 2005. Rapid recovery of dung beetle communities following habitat fragmentation in Central Amazonia. Ecology **86**:3303-3311.

Reid, J. L., M. E. Fagan, J. Lucas, J. Slaughter, and R. A. Zahawi. 2018. The ephemerality of secondary forests in southern Costa Rica. Conservation Letters:e12607.

Ribeiro, M. C., J. P. Metzger, A. C. Martensen, F. J. Ponzoni, and M. M. Hirota. 2009. The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. Biological Conservation **142**:1141-1153.

Rocha, R. 2017. Tropical forest fragmentation: effects on the spatio-temporal dynamics of its bat communities. PhD Thesis. University of Lisbon, Lisbon, Portugal.

Rocha, R., T. Almeida, and A. López-Baucells. 2014. Field observation of an adult Lesser treefrog *Dendropsophus minutus* (Anura: Hylidae) being consumed by a neotropical *Lethocerus* sp.(Hemiptera: Belostomatidae) nymph. Alytes **31**:37-39.

Rocha, R., D. F. Ferreira, A. López-Baucells, F. Z. Farneda, J. Carreiras, J. M. Palmeirim, and C. F. Meyer. 2017a. Does sex matter? Gender-specific responses to forest fragmentation in Neotropical bats. Biotropica **49**:881-890.

Rocha, R., M. Gordo, and A. López-Baucells. 2016. Completing the menu: addition of *Scinax cruentommus* and *Scinax* cf. *garbei* (Anura: Hylidae) to the diet of *Trachops cirrhosus* (Chiroptera: Phyllostomidae) in Central Amazon. North-Western Journal of Zoology **12**:199-201.

Rocha, R., and A. López-Baucells. 2014a. Opportunistic predation by Crested owl *Lophostrix cristata* upon Seba’s Short-tailed Bat *Carollia perspicillata*. Revista Brasileira de Ornitologia-Brazilian Journal of Ornithology **22**:35-37.

Rocha, R., and A. López-Baucells. 2014b. Predation attempt of *Hypsiboas boans* (Anura: Hylidae) by *Helicops angulatus* (Squamata: Dipsadidae) with notes on defensive behavior. Alytes **30**:78-81.

Rocha, R., A. López-Baucells, F. Z. Farneda, M. Groenenberg, P. E. D. Bobrowiec, M. Cabeza, J. M. Palmeirim, and C. F. J. Meyer. 2017b. Consequences of a large-scale fragmentation experiment for Neotropical bats: disentangling the relative importance of local and landscape-scale effects. Landscape Ecology **32**:31-45.

Rocha, R., O. Ovaskainen, A. López-Baucells, F. Z. Farneda, D. F. Ferreira, P. E. D. Bobrowiec, M. Cabeza, J. M. Palmeirim, and C. F. J. Meyer. 2017c. Design matters: An evaluation of the impact of small man-made forest clearings on tropical bats using a before-after-control-impact design. Forest Ecology and Management **401**:8-16.

Rocha, R., O. Ovaskainen, A. López-Baucells, F. Z. Farneda, E. M. Sampaio, P. E. D. Bobrowiec, M. Cabeza, J. M. Palmeirim, and C. F. J. Meyer. 2018. Secondary forest regeneration benefits old-growth specialist bats in a fragmented tropical landscape. Scientific Reports **8**:3819.

Rodríguez-San Pedro, A., and J. A. Simonetti. 2015. The relative influence of forest loss and fragmentation on insectivorous bats: does the type of matrix matter? Landscape Ecology **30**:1561-1572.

Rosa, I. M. D., C. Gabriel, and J. M. B. Carreiras. 2017. Spatial and temporal dimensions of landscape fragmentation across the Brazilian Amazon. Regional Environmental Change **17**:1687-1699.

Ross, J. C., and P. E. Allen. 2014. Random Forest for improved analysis efficiency in passive acoustic monitoring. Ecological Informatics **21**:34-39.

Russo, D., and C. C. Voigt. 2016. The use of automated identification of bat echolocation calls in acoustic monitoring: A cautionary note for a sound analysis. Ecological Indicators **66**:598-602.

Rydgren, K., R. Halvorsen, J. P. Töpper, I. Auestad, L. N. Hamre, E. Jongejans, J. Sulavik, and L. Brudvig. 2018. Advancing restoration ecology: A new approach to predict time to recovery. Journal of Applied Ecology:(in press).

Sampaio, E. 2001. Effects of the forest fragmentation on the diversity and abundance patterns of central Amazonian bats. PhD Thesis. University of Tübingen, Berlin, Germany.

Schnitzler, H. U., and E. K. V. Kalko. 2001. Echolocation by insect-eating bats: We define four distinct functional groups of bats and find differences in signal structure that correlate with the typical echolocation tasks faced by each group Bioscience **51**:557-569.

Seibold, S., J. Buchner, C. Bässler, and J. Müller. 2013. Ponds in acidic mountains are more important for bats in providing drinking water than insect prey. Journal of Zoology **290**:302-308.

Stouffer, P. C., E. I. Johnson, R. O. Bierregaard, Jr., and T. E. Lovejoy. 2011. Understory bird communities in Amazonian rainforest fragments: species turnover through 25 years post-isolation in recovering landscapes. PLoS ONE **6**:e20543.

Stouffer, P. C., C. Strong, and L. N. Naka. 2009. Twenty years of understorey bird extinctions from Amazonian rain forest fragments: consistent trends and landscape-mediated dynamics. Diversity and distributions **15**:88-97.

Threlfall, C., B. Law, T. Penman, and P. B. Banks. 2011. Ecological processes in urban landscapes: mechanisms influencing the distribution and activity of insectivorous bats. Ecography **34**:814-826.

Torrent, L., A. López-Baucells, R. Rocha, P. E. D. Bobrowiec, and C. F. J. Meyer. 2018. The importance of lakes for bat conservation in Amazonian rainforests: an assessment using autonomous recorders. Remote Sensing in Ecology and Conservation:(in press).

Treitler, J. T., A. López-Baucells, S. Gomes Farias, J. F. Tenaçol Jr, and R. Rocha. 2013. First record of a leucistic piebald *Phyllostomus discolor* (Chiroptera: Phyllostomidae). Chiroptera Neotropical **19**:1179-1181.

Truax, B. 1978. The world soundscape project's handbook for acoustic ecology. Aesthetic Research Centre Publications, Vancouver, Canada.

Verboom, B., and K. Spoelstra. 1999. Effects of food abundance and wind on the use of tree lines by an insectivorous bat, *Pipistrellus pipistrellus*. Canadian Journal of Zoology **77**:1393-1401.

Vieira, I., T. Gardner, J. Ferreira, A. Lees, and J. Barlow. 2014. Challenges of governing second-growth forests: a case study from the Brazilian Amazonian State of Para. Forests **5**:1737-1752.

Violle, C., M. L. Navas, D. Vile, E. Kazakou, C. Fortunel, I. Hummel, and E. Garnier. 2007. Let the concept of trait be functional! Oikos **116**:882-892.

Walsh, A. L., and S. Harris. 1996a. Factors determining the abundance of vespertilionid bats in Britain: geographical, land class and local habitat relationships. Journal of Applied Ecology **33**:519-529.

Walsh, A. L., and S. Harris. 1996b. Foraging habitat preferences of vespertilionid bats in Britain. Journal of Applied Ecology **33**:508-518.

Walters, C. L., A. Collen, T. Lucas, K. Mroz, C. A. Sayer, and K. E. Jones. 2013. Challenges of using bioacoustics to globally monitor bats. Pages 479-499 *in* S. C. Pedersen and R. A. Adams, editors. Bat Evolution, Ecology, and Conservation. Springer, New York, United States of America.

Whytock, R. C., J. Christie, and K. Jones. 2017. Solo: an open source, customizable and inexpensive audio recorder for bioacoustic research. Methods in Ecology and Evolution **8**:308-312.

Wickramasinghe, L. P., S. Harris, G. Jones, and N. V. Jennings. 2004. Abundance and species richness of nocturnal insects on organic and conventional farms: Effects of agricultural intensification on bat foraging. Conservation Biology **18**:1283-1292.

Wolfe, J. D., P. C. Stouffer, K. Mokross, L. L. Powell, and M. M. Anciães. 2015. Island vs. countryside biogeography: an examination of how Amazonian birds respond to forest clearing and fragmentation. Ecosphere **6**:1-14.

Zamora-Gutiérrez, V., C. López-Gonzalez, M. C. MacSwiney González, B. Fenton, G. Jones, E. K. V. Kalko, S. J. Puechmaille, V. Stathopoulos, K. E. Jones, and R. Freckleton. 2016. Acoustic identification of Mexican bats based on taxonomic and ecological constraints on call design. Methods in Ecology and Evolution **7**:1082-1091.

Zurita, G., G. Pe’er, M. I. Bellocq, and M. M. Hansbauer. 2012. Edge effects and their influence on habitat suitability calculations: a continuous approach applied to birds of the Atlantic forest. Journal of Applied Ecology **49**:503-512.

Zurita, G. A., and M. I. Bellocq. 2012. Bird assemblages in anthropogenic habitats: identifying a suitability gradient for native species in the Atlantic Forest. Biotropica **44**:412-419.