

INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA – INPA

PROGRAMA DE PÓS GRADUAÇÃO EM ECOLOGIA

Utilizando Características Acústicas e Fisiológicas para Abordagens  
Ecológicas das Aves em uma Paisagem Fragmentada na Amazônia  
Central

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Manaus, Amazonas

Março, 2020

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Ecológicas das Aves em uma Paisagem Fragmentada na Amazônia  
Central

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Tese apresentada ao Instituto  
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Biologia (Ecologia)

Manaus, Amazonas

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## ATA DEFESA



## PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA



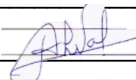

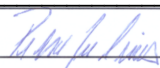
## ATA DA DEFESA PÚBLICA DA TESE DE DOUTORADO DO PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA DO INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA

Aos 27 dias do mês de março do ano de 2020, às 08:30 horas, reuniu-se virtualmente a Comissão Examinadora de Defesa Pública, composta pelos seguintes membros: o(a) Prof(a). Dr(a). **Igor Luis Kaefer**, da Universidade Federal do Amazonas - UFAM, Dr(a). **Paulo Estefano Dineli Bobrowiec**, do Instituto Nacional de Pesquisas da Amazônia – INPA, o(a) Prof(a). Dr(a). **Adalberto Luis Val**, do Instituto Nacional de Pesquisas da Amazônia - INPA, o(a) Prof(a). Dr(a). **Marconi Campos Cerqueira**, da Universidade de Porto Rico e o(a) Prof(a). Dr(a). **Pedro Ivo Simões**, da Universidade Federal de Pernambuco-UFPE. Tendo como suplentes o(a) Prof(a). Dr(a). Sérgio Henrique Borges, do Instituto Nacional de Pesquisas da Amazônia – INPA e o(a) Prof(a). Dr(a). Fernanda de Pinho Werneck, do Instituto Nacional de Pesquisas da Amazônia – INPA, sob a presidência do(a) primeiro(a), a fim de proceder a arguição pública do trabalho de **TESE DE DOUTORADO** de **THIAGO BICUDO KREMPEL SANTANA**, intitulado “**Utilizando Características Acústicas e Fisiológicas para Abordagens Ecológicas das Aves em uma Paisagem Fragmentada na Amazônia Central**”, orientado pela(o) Prof(a). Dr(a). Marina Anciães, do Instituto Nacional de Pesquisas da Amazônia – INPA e coorientado pelo(a) Prof(a). Dr(a). Diego Gil, do Museo Nacional de Ciencias Naturais, Madri-ES.

Após a exposição, o(a) discente foi argüido(a) oralmente pelos membros da Comissão Examinadora, tendo recebido o conceito final:

APROVADO(A)                       REPROVADO(A)  
 POR UNANIMIDADE                       POR MAIORIA

Nada mais havendo, foi lavrada a presente ata, que, após lida e aprovada, foi assinada pelos membros da Comissão Examinadora.

Prof(a). Dr(a). IGOR LUIS KAEFER	
Prof(a). Dr(a). PAULO ESTEFANO DINELI BOBROWIEC	
Prof(a). Dr(a). ADALBERTO LUIS VAL	
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**Sinopse :**

Estudou-se os efeitos da fragmentação florestal em aspectos da paisagem acústica e características fisiológicas de assembleias de aves nas ilhas do reservatório da UHE de Balbina. Foi testado se os atuais índices acústicos propostos realmente refletem a riqueza real observada de aves em florestas tropicais e como estes índices variam com relação ao tamanho da área. Adicionalmente testamos se os níveis de corticosterona nas penas das aves variam com relação ao tamanho da ilha, analisando a nível de comunidade e também a nível espécie-específico.

**Palavras-Chave:** Soundscape, Corticosterona, Balbina, Índices acústicos, Ilhas, Glicocorticoides

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Viver no centro da Amazônia me colocou distante fisicamente de alguns amigos da vida e me trouxe amigos pra vida. Ramon, Rafael, Juliba, Bruno, Ka, Mari, Lis, Rafa, Raffaello, Dudu, Bia, Thai, Bernardo, Luna, Lila, Italo, Yuri, Marcelo “Urubas”, Laion, Maju, MacCrate, não importa onde estiverem, o sentimento verdadeiro da amizade sempre permanecerá. Gostaria de agradecer a todos os alunos e pesquisadores do PPG-ECO por todas as experiências compartilhadas. A todos do LABECA, em especial aos companheiros de batalha Mariane e Fernando. Gostaria de agradecer em especial aos meus orientadores Marina Anciães e Diego Gil por todos os ensinamentos científicos e pessoais. Muito bom termos a amizade em união com a orientação. Gostaria muito de agradecer a Paulinha, que com todo seu bom coração sempre estava ali para ajudar. Muito obrigado.

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E é claro, a você que está lendo. Muito Obrigado.

“Se não podes nomear os pássaros,  
se não sabes como reconhecer as  
folhas das árvores pelos sons que  
fazem, ouvir uma catarata no rio, ou  
reconhecer quando um vento de  
inverno está a trazer uma tempestade,  
a natureza está anestesiada, e a sua  
sobrevivência depende de forças que  
não são humanas”. Murray Schafer,  
*Open Ears*

## RESUMO

Na Amazônia, a construção de usinas hidrelétricas é uma ação que amplia a perda de biodiversidade. Nesses ambientes, fragmentos funcionam como ilhas verdadeiras, apresentando uma baixa riqueza de espécies em ilhas pequenas. Dessa maneira, ferramentas que possam monitorar mudanças nas comunidades estão se tornando cada vez mais importantes. No primeiro capítulo, avaliamos o desempenho de sete índices acústicos (índices de biodiversidade baseados na análise da paisagem acústica) em um ambiente fragmentado na Amazônia central, para acessar: (1) a relação entre riqueza de aves e índices acústicos; (2) os padrões da paisagem acústica associadas ao tamanho da ilha e (3) para caracterizar os padrões das paisagens acústicas nas ilhas ao longo de 24h. Em ambientes fragmentados também é necessário entender os mecanismos fisiológicos fundamentais ao sucesso dos organismos que vivem nesses ambientes. No segundo capítulo, usamos os níveis de corticosterona presentes nas penas das aves como uma medida dos indicadores fisiológicos de estresse, avaliando se os níveis de corticosterona refletem os efeitos da fragmentação do ambiente relacionados ao tamanho da ilha. No primeiro capítulo, foram amostradas 12 ilhas e duas áreas de floresta contínua. Utilizando dois gravadores autônomos por área, foram feitas gravações de um minuto com intervalos de cinco minutos, durante 24 horas, por três dias consecutivos. Os índices acústicos exibiram diferentes padrões, com mudanças marcantes entre dia e noite. Nossos resultados mostram que cinco índices (complexidade acústica, diversidade acústica, entropia acústica, mediana do envelope de amplitude e achatamento espectral) refletem a riqueza de espécies em cada área. Quando relacionamos os índices acústicos com o tamanho da ilha, apenas o índice de entropia acústica teve resultados significativos, apresentando valores mais altos com o aumento do tamanho da área. Para o segundo capítulo, capturamos aves em 13 ilhas de tamanho variável e duas áreas de floresta contínua e analisamos os níveis de corticosterona nas penas de 265 indivíduos de oito espécies diferentes. Nossos resultados não suportam a hipótese de que os níveis de corticosterona variam em relação ao tamanho da ilha, exceto para *Hypocnemis cantator*, que apresentou diminuição dos níveis de corticosterona com aumento do tamanho da ilha. No geral, concluímos que mesmo em um ambiente altamente diverso, como florestas tropicais, os índices acústicos fornecem uma excelente ferramenta complementar para a avaliação da biodiversidade quando fornecidos com um banco de dados fundamentado. Estudos adicionais são necessários para avaliar a eficiência do uso dos níveis de corticosterona como uma medida fisiológica do estresse e determinar quais parâmetros são úteis para entender como a insularização causada pelas atividades humanas pode influenciar a resistência das populações de aves a distúrbios no habitat.

## ABSTRACT

Using Acoustic and Physiological Characteristics for Ecological Approaches of Birds in a Fragmented Landscape in Central Amazonia

In Amazon, the construction of hydroelectric dams is a growing factor in the biodiversity loss. In such environments, fragments acts as true islands, presenting low species richness on small islands. Consequently, tools that can monitor changes in communities are becoming increasingly important. In the first chapter, we evaluated the performance of seven acoustic indices (biodiversity indices based on the soundscapes analysis) in forest islands within an anthropogenic archipelago of central Amazonia, to address the following questions: (1) is there a relationship between bird richness and acoustic indices? (2) what is the relationship between soundscape patterns and landscape features such as island size? (3) how do soundscapes change throughout the day in islands of different sizes? and finally; (4) how do birds and cicadas use their acoustic space on islands of different sizes? In fragmented environments it is also necessary uncover the physiological mechanisms underlying the success of organisms living in disturbed habitats. In the second chapter, we use the corticosterone levels present in bird feathers as a measure of physiological stress, assessing whether corticosterone levels reflect the effects of landscape fragmentation related to the island size. For the first chapter, 12 islands and two areas of continuous forest were sampled. Using two autonomous recorders per area, one-minute recordings were made at five-minute intervals, for 24 hours, for three consecutive days. The acoustic indices exhibited different patterns, with marked changes between day and night. Our results show that five indices (acoustic complexity, acoustic diversity, acoustic entropy, median of the amplitude envelope and spectral flatness) reflect the species richness in each area. When we correlate the acoustic indices with island size, only the acoustic entropy index had significant results, presenting higher values with increasing area size. For the second chapter, we captured birds in 13 islands of varying size and in two continuous forests and analysed feather corticosterone levels of 265 individuals from eight different species. Our findings did not support the hypothesis that corticosterone varies in relation to island size, except for *Hypocnemis cantator*, which presented the predicted pattern: decreasing feather corticosterone levels with increasing island size. Overall, we conclude that even in a highly diverse environment, such as tropical forests, acoustic indices provide an excellent complementary tool for biodiversity assessment when provided with a ground-truthed database. Further studies are necessary to assess the reliability of corticosterone levels as a physiological measurement of stress and to determine which parameters are useful to understand how insularization caused by human activities may influence the resistance of avian populations to habitat disturbances.



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### Capítulo II

Figura 1. Distribuição espacial das áreas amostradas no reservatório da UHE de Balbina, Amazonas, Brasil. Em destaque as 13 ilhas em preto escuro e as duas áreas de floresta contínua, triângulos brancos.

Figura 2. Relação entre os níveis de fCORT ( $\log_{10}$ ) e tamanho da ilha ( $\log_{10}$ ) para a espécie *Hypocnemis cantator* (n= 28 indivíduos). A área sombreada representa o intervalo de confiança de 95% e os triângulos representam os valores de fCORT nas áreas de floresta contínua.

## INTRODUÇÃO GERAL

A fragmentação e perda de habitat são reconhecidos como grandes ameaças à biodiversidade, levando mudanças a longo prazo na estrutura e funcionamento dos fragmentos (Haddad *et al.* 2015, Fahrig 2017). Em paisagens fragmentadas, a área do fragmento é um dos principais fatores que influenciam a riqueza de espécies, dinâmicas populacionais e a composição da comunidade (MacArthur & Wilson 1963, Fahrig 2017). Pequenos fragmentos (<100-ha) podem perder até metade das suas espécies em menos de 15 anos de isolamento, levando a um declínio ou extinção das espécies mais sensíveis ao processo de fragmentação (Ferraz *et al.* 2003, Dardanelli *et al.* 2006).

Na Amazônia, a construção de usinas hidrelétricas é um fator crescente da perda de biodiversidade, onde 191 barragens já foram construídas e outras 246 estão planejadas ou em fase de construção (Lees *et al.* 2016). Devido ao relevo relativamente plano da região, o barramento dos rios para o enchimento do reservatório leva à inundação de imensas áreas florestais, criando simultaneamente ilhas de diversos tamanhos e graus de isolamento (Benchimol & Peres 2015c, Lees *et al.* 2016). A matriz aquática resultante pode agir como uma barreira ao movimento das aves, dificultando dinâmicas de colonização e extinção nesses arquipélagos artificiais, onde os fragmentos passam a funcionar como ilhas verdadeiras (MacArthur & Wilson 1963, Aurélio-Silva *et al.* 2016, Palmeirim *et al.* 2017, Bueno & Peres 2019).

Assim como previsto pela Teoria de Biogeografia de Ilhas (McArthur & Wilson 1963), as ilhas pequenas destes arquipélagos também estão sujeitas a maiores taxas de extinção do que ilhas grandes (Wolfe *et al.* 2015, Aurélio-Silva *et al.* 2016, Palmeirim *et al.* 2017), devido a fortes efeitos de borda que alteram o microclima, a mortalidade de árvores e a diversidade faunística (Murcia 1995, Benchimol & Peres 2015c, a, Laurance *et al.* 2018). De forma geral, espera-se que haja um decréscimo na disponibilidade de recursos tróficos e estruturais com a diminuição do tamanho da ilha (Palmeirim *et al.* 2017). Na Usina hidrelétrica de Balbina, as assembleias de aves que vivem em ilhas variam previsivelmente, com ilhas pequenas e isoladas retendo um menor número de espécies (Benchimol and Peres 2015c; Aurélio-Silva *et al.* 2016). Nestas áreas, menos de 10% de todas as ilhas existentes ao longo do

reservatório possuem uma comunidade de aves que pode ser comparável com as áreas de floresta contínua adjacentes (Aurélio-Silva *et al.* 2016).

Com a constante perda de habitat e biodiversidade, os ambientes também estão perdendo seus sons naturais (Pijanowski *et al.* 2011a). O termo paisagem acústica foi definido por Pijanowski *et al.* (2011b) como todos os sons produzidos pelos animais (biofonia), pela natureza (geofonia; e.g. chuva, vento) e pelos humanos (antropofonia; e.g. carro, avião), variando no espaço e no tempo. Esse conceito reflete processos ecossistêmicos importantes, bem como atividades humanas, no qual alterações na paisagem acústica podem servir como um proxy para avaliar mudanças no funcionamento dos ecossistema (Dumyahn & Pijanowski 2011).

Em ambientes tropicais, análises da paisagem acústica vêm sendo utilizadas como forma de investigar a homogeneização da diversidade em florestas onde há o corte seletivo de árvores (Burivalova *et al.* 2019) e como ferramenta para identificar tipos de ambiente com diferentes graus de perturbação (Gómez *et al.* 2018). Além disso, pode ser utilizada como forma de explorar quais os impactos que a exploração de gás tem na biodiversidade local (Deichmann *et al.* 2017), servindo também para avaliar quais os impactos que os ruídos gerados por maquinários utilizados na mineração podem trazer para a biofonia (Duarte *et al.* 2015).

Os sons produzidos pelos animais são utilizados há muito tempo em métodos tradicionais de amostragem (e.g., ponto escuta para aves ; Ribeiro *et al.* 2017), em que por meio de cantos ou chamados, as espécies podem ser facilmente detectadas e gravadas (Sueur, Pavoine, *et al.* 2008). O desenvolvimento de métodos automatizados para coleta e processamento desses sinais acústicos oferece um grande potencial para monitoramentos rápidos e econômicos da biodiversidade faunística (Ribeiro *et al.* 2017, Sugai *et al.* 2018, Gibb *et al.* 2019). O monitoramento acústico passivo (PAM) oferece a vantagem de que os dispositivos de gravação podem ser instalados no campo por dias ou semanas, expandindo a amostragem temporal e espacial, evitando a necessidade de excursões a campo constantes e reduzindo o viés do observador (Ribeiro *et al.* 2017, Deichmann *et al.* 2018, Sugai *et al.* 2018, 2019, Gibb *et al.* 2019). Uma outra vantagem é a possibilidade de armazenar gravações de longo prazo, servindo como cápsulas bioacústicas do

tempo, documentando para gerações futuras como as paisagens acústicas eram no passado (Towsey, Wimmer, *et al.* 2014, Sugai & Llusia 2019).

Embora a coleta de dados seja relativamente fácil, a análise e extração de informações biológicas dos dados acústicos apresenta alguns desafios devido à grande quantidade de dados gerados, tornando inviável escutar todas as gravações (Towsey, Zhang, *et al.* 2014, Phillips *et al.* 2018). Portanto, se torna importante aplicar métodos que possam sumarizar as informações geradas, tornando a interpretação ecológica dos dados mais eficaz (Towsey, Zhang, *et al.* 2014, Zhang *et al.* 2016).

Seguindo a mesma lógica dos índices ecológicos de diversidade, nos últimos cinco anos, mais de 60 índices acústicos foram desenvolvidos, sendo utilizados como proxies de riqueza animal e como métricas para avaliação e monitoramento de fauna (Sueur, Pavoine, *et al.* 2008, Sueur *et al.* 2014, Towsey, Wimmer, *et al.* 2014, Buxton *et al.* 2018, Bradfer-Lawrence *et al.* 2019). Um índice acústico é uma estatística que resume informações sobre a distribuição de energia sonora em uma determinada gravação (Towsey, Wimmer, *et al.* 2014), levando em consideração a diversidade de sons produzidos (Towsey, Wimmer, *et al.* 2014). Portanto, comunidades que possuem uma diversidade alta de espécies vocalizando irão apresentar uma maior diversidade acústica, sendo que em alguns casos irá correlacionar-se com os índices acústicos testados (Sueur, Pavoine, *et al.* 2008, Machado *et al.* 2017, Moreno-Gómez *et al.* 2019). No entanto, para que um índice acústico seja útil, é importante ter conhecimento da precisão com que refletem os padrões e variações das comunidades que estão sendo investigadas (Gasc *et al.* 2013, Machado *et al.* 2017, Mammides *et al.* 2017, Gibb *et al.* 2019). Apesar dos resultados promissores que o uso dos índices acústicos podem trazer, muitos dos trabalhos que testam sua eficácia trazem resultados contraditórios até para o mesmo índice testado (e.g., Fuller *et al.* 2015a, Machado *et al.* 2017, Mammides *et al.* 2017, Eldridge *et al.* 2018). A partir do momento em que esses problemas práticos são resolvidos, o monitoramento de comunidades acústicas passa a ter uma aplicabilidade ecológica com um grande potencial para oferecer informações importantes sobre a biodiversidade local (Gibb *et al.* 2019).

Em áreas fragmentadas, as espécies estão sujeitas a viver sob níveis altos de estresse ambiental, uma vez que a fragmentação do habitat geralmente leva à formação de habitats subótimos (Mokross *et al.* 2018) e baixa disponibilidade de alimento (Zanette *et al.* 2000). Apesar dos efeitos da fragmentação sobre a diversidade de espécies e da qualidade do ambiente serem extensivamente estudados, pouco se sabe sobre o real efeito da fragmentação e da perda de habitat nos mecanismos fisiológicos responsáveis pela resposta dos organismos a essas alterações ambientais (Wingfield 2013, Messina *et al.* 2018). Uma das medidas mais relevantes de alterações fisiológicas é fornecida pelos níveis de corticosterona (CORT), o principal esteroide glicocorticoide de aves, anfíbios, répteis e alguns mamíferos (e.g., roedores) (Buchanan 2000, Sapolsky *et al.* 2000, Schoech *et al.* 2011).

Devido à CORT atuar em diferentes vias metabólicas e comportamentais, ajudando as aves a lidar com as mudanças ambientais (Wingfield 2002, Cockrem 2007), esse hormônio é considerado como bioindicador por alguns autores (Fairhurst *et al.*, 2011; Warne *et al.*, 2015; Harris *et al.*, 2017; veja Madliger *et al.*, 2015). Quando as espécies passam por situações com efeitos negativos (e.g., interações agonísticas), o eixo hipotálamo-hipófise-adrenal (HPA) é ativado, levando a um aumento da síntese de CORT pelo organismo (Romero *et al.* 1998, Sapolsky *et al.* 2000). Embora elevações de curto prazo nos níveis de CORT possam ter evoluído como um mecanismo de defesa adaptativo para regular a homeostase, níveis cronicamente elevados têm consequências negativas para a sobrevivência dos indivíduos (Sapolsky *et al.* 2000, Blas 2015). Em vertebrados, por exemplo, os principais efeitos de níveis crônicos de CORT estão principalmente relacionados a supressão do sistema imunológico e diminuição da massa corporal (Sapolsky *et al.* 2000, Romero *et al.* 2009, Blas 2015)

Os glicocorticoides são medidos em indivíduos de muitas espécies de vida livre, mas um importante problema metodológico é que os níveis de CORT aumentam rapidamente após a captura e, portanto, as amostras precisam ser coletadas dentro de 2-3 minutos pós captura (Romero & Reed 2005, Blas 2015). Isso levou ao desenvolvimento de técnicas menos invasivas que baseiam-se no acúmulo de CORT a longo prazo nos tecidos (Bortolotti *et al.* 2008, Sheriff *et al.* 2011, Romero & Fairhurst 2016). No caso das aves, atualmente as penas são

usadas para medir os níveis de corticosterona (fCORT), que são incorporados e acumulados durante o crescimento das penas, proporcionando uma resposta fisiológica retrospectiva e cumulativa aos desafios ecológicos enfrentados (Bortolotti *et al.*, 2008; Fairhurst *et al.*, 2013; Jenni-Eiermann *et al.*, 2015, López-Jiménez *et al.*, 2017; Ganz *et al.*, 2018).

Diante do aumento global da fragmentação e perda de habitats (Lucas *et al.* 2006), no presente estudo avaliamos os efeitos da fragmentação florestal causada por um reservatório hidrelétrico sobre características acústicas e fisiológicas em uma comunidade de aves na Amazônia central. Para a abordagem acústica, utilizamos novos métodos baseados em índices acústicos para responder as seguintes perguntas: (1) existe relação entre a riqueza de aves e os índices acústicos? (2) qual a relação entre padrões da paisagem acústica com o tamanho da ilha? (3) como a paisagem acústica muda ao longo do dia em ilhas de diferentes tamanhos? E por fim, (4) como aves e cigarras usam o espaço acústico em ilhas de diferentes tamanhos?

Para a abordagem fisiológica, avaliamos se os efeitos da fragmentação do habitat sobre os padrões de ocorrência das espécies são refletidos pelos níveis de corticosterona nas penas das aves. Como esperamos que ilhas menores apresentem disponibilidade reduzida ou alterada de recursos (Zanette *et al.* 2000, Suorsa *et al.* 2004), prevemos um aumento dos níveis de CORT de forma a restaurar a homeostase (Suorsa *et al.* 2004).

As conclusões deste estudo serão relevantes para estudos de conservação, relacionando os efeitos da fragmentação florestal nas paisagens acústicas e na fisiologia das aves. Além disso, contribuirá para o conhecimento sobre o uso de índices acústicos como forma de caracterizar a diversidade local e o papel da fragmentação do habitat nos níveis de corticosterona em espécies de aves.



## **OBJETIVOS**

### **Objetivo Geral**

Avaliar os efeitos da fragmentação florestal causada por um reservatório hidrelétrico sobre características acústicas e fisiológicas em uma comunidade de aves na Amazônia central.

### **Objetivos Específicos**

#### **Capítulo 1**

- Avaliar se os índices acústicos realmente refletem a diversidade real observada em um ambiente tropical;
- Avaliar como a paisagem acústica se relaciona com características ecológicas da paisagem, levando em consideração o tamanho da ilha;
- Avaliar como os índices variam ao longo do dia nas áreas amostradas;
- Avaliar como as aves e cigarras usam o espaço acústico nas ilhas.

#### **Capítulo 2**

- Avaliar os efeitos do tamanho da ilha nos níveis de corticosterona nas penas de uma comunidade de aves;
- Avaliar os efeitos espécie-específicos do tamanho da ilha sobre os níveis de corticosterona nas penas.

## Capítulo I.

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Bicudo T, Anciães M, Llusia D, Gil D. Using acoustic indices to access biodiversity in an Amazonian fragmented soundscape. (*Artigo formatado para a revista Ecological Indicators*)

## **Using acoustic indices to access biodiversity in a fragmented Amazonian soundscape**

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## **Abstract**

Due to the rapid biodiversity loss that is happening in the Amazon, it is important to apply methods that can be effective for rapid biodiversity assessment. This study aims to test the effectiveness of seven acoustic indices (biodiversity indices based in soundscape analysis) in forest islands within an anthropogenic fluvial archipelago in central Amazonia, to address the following questions: (1) is there a relationship between bird species richness and estimates of acoustic indices? (2) are soundscape patterns associated with island size? (3) how do soundscapes change throughout the day in islands of different sizes? and finally; (4) how do birds and cicadas use their acoustic space on islands of different sizes? We sampled 12 islands and two control areas of continuous forest. Using two automatic recorders per area, recordings of one minute were made every five minutes, over 24 hours, for three consecutive days. Manual inspection of recordings revealed the presence of 181 bird species. The acoustic indices exhibited distinct patterns over the 24-hour period, with marked shifts between day and night soundscapes. Five indices (acoustic complexity, acoustic diversity, acoustic entropy, median of the amplitude envelope and spectral flatness) reflected the species richness in each area. Tests of relationships among acoustic indices and island size indicated that acoustic entropy was higher in larger islands. The remaining indices were not associated with variation in island size. Overall, we conclude that even in a highly diverse environment such as rainforests, the acoustic indices provide a complementary tool to biodiversity assessment when provided with a ground-verified database. Additionally, we provide evidence that habitat fragmentation caused by damming of an Amazonian river leads to measurable modifications of the soundscape.

## 1. Introduction

Habitat loss caused by human modifications is one of the major threats to biodiversity (McLellan *et al.* 2014) and leads to fragmentation of native habitat and to long-term changes in the structure and function of ecosystems (Haddad *et al.* 2015, Fahrig 2017). In a fragmented landscape, small patches (<100-ha) can lose up to half of their species in less than two decades of isolation, with area-sensitive species often declining or becoming extinct (Ferraz *et al.* 2003, Dardanelli *et al.* 2006).

In the Amazon, the construction of hydroelectric dams is an emergent driver of biodiversity loss. In the last twenty years, 191 dams have been built and another 246 are planned or under construction (Lees *et al.*, 2016). Reservoir filling often leads to the submergence of large forested areas and creates numerous small land-bridge islands (Benchimol & Peres 2015c, Lees *et al.* 2016). This results in an inhospitable aquatic matrix that acts as a barrier to bird movement and hampers the metapopulational dynamics of colonization and extinction (Aurélio-Silva *et al.* 2016, Palmeirim *et al.* 2017, Bueno & Peres 2019). In mega hydroelectric dams, such as Balbina in Central Amazonia, bird assemblages occupying both land-bridge islands and forest remnants in terrestrial landscapes vary predictably, with smaller and more isolated islands retaining a smaller number of species (Benchimol and Peres 2015c; Aurélio-Silva *et al.* 2016).

With the loss of habitat and biodiversity, fragmented landscapes are losing their natural sounds (Pijanowski *et al.* 2011a). Soundscape is defined as the collection of biological, geophysical and anthropogenic sounds that emanate from a landscape and which vary over space and time, reflecting key ecosystem processes and human activities (Pijanowski, Farina, *et al.* 2011, Farina 2014). The study of soundscapes enables the visualization of acoustic activity patterns in a given environment at a given period of time (e.g., day, month, year) and the monitoring of differences in acoustic communities that can be attributed to heterogeneity of the environment (Furumo & Aide 2019). Therefore, stable and healthy ecosystems are characterized by complex soundscapes with a structured use of frequency and temporal acoustic niches. Alterations of the soundscape can be a useful tool for assessing changes in the functioning of ecosystems (Dumyahn & Pijanowski 2011, Farina 2014).

Sounds produced by animals have long been used to assess biodiversity (e.g., point counts for birds and standardized acoustic transect for amphibians; Ribeiro *et al.* 2017). A variety of species reveal their presence through acoustic signals that can be easily detected and recorded (Bradbury & Vehrencamp 1998, Obrist *et al.* 2010). Tropical forests are characterized by a high richness of vocally active species, potentially causing acoustic masking interference (Luther & Gentry 2013, Rodriguez *et al.* 2014). The combination of a restricted period of communication and high acoustic diversity provides an excellent scenario to understand the strategies used by animals to avoid masking of sound signals and to ensure effective communication (Stanley *et al.* 2016). During the day, birds and insects are the most acoustically active groups (Hart *et al.* 2015), and acoustic signals emitted by cicadas (Hemiptera; Cicadidae) may restrict the communication of some bird species (Stanley *et al.* 2016, Aide *et al.* 2017). Therefore, to avoid overlapping, several bird species adopt a series of strategies, including adjustment of temporal (Hart *et al.* 2015, Chen & Ding 2016, Stanley *et al.* 2016) or spectral (Chen & Ding 2016) characteristics of their songs.

Soundscapes are changing throughout the world reflecting an environment in constant modification (Marques & De Araújo 2014), suggesting that their study and analysis can become an optimal and effective way for rapid biodiversity assessment. Passive acoustic monitoring (PAM) allows automated sound collection (Ribeiro *et al.* 2017, Sugai *et al.* 2018) so that recording devices can be deployed in the field for long periods (weeks, months or years), expanding temporal and spatial sampling, and avoiding the need for regular field work, while reducing observer bias by means of objective calculations of sound spectra (Ribeiro *et al.* 2017, Deichmann *et al.* 2018, Sugai *et al.* 2018, 2019). Also, this non-invasive method may work as a bioacoustic time capsule that documents, for future generations, how the worldwide acoustic communities were in the past (Marques & De Araújo 2014, Towsey, Wimmer, *et al.* 2014, Sugai & Llusia 2019).

While data acquisition is fairly easy, data curation, analysis and extraction of biological information present considerable challenges, due the massive and complex data generated by this collection method (Towsey, Zhang, *et al.* 2014, Phillips *et al.* 2018). In a typical sound recording, a large proportion of the information overlaps to a great extent, and it becomes essential to apply methods that can

retrieve the information collected, allowing an ecological interpretation of the data (Towsey, Zhang, *et al.* 2014, Zhang *et al.* 2016).

Over the last five years, more than 60 acoustic indices have been developed, allowing the rapid classification of soundscapes based on their acoustic properties and serving as estimates of species diversity (Sueur *et al.* 2014, Towsey, Wimmer, *et al.* 2014, Buxton *et al.* 2018, Bradfer-Lawrence *et al.* 2019). An acoustic index is a statistic that summarizes some aspect of the distribution of energy and information in a sound recording (Towsey, Wimmer, *et al.* 2014). These indices take into account the sound diversity produced in natural or urban environments, where sounds take the place of species, so that the acoustic community or the entire soundscape will be accessed by their sounds (Towsey, Wimmer, *et al.* 2014). Despite the promising results that the use of acoustic indices can bring, many of the studies that test their effectiveness as proxies for biodiversity have brought contradictory results, even when the same index was tested (e.g., Fuller *et al.* 2015a, Machado *et al.* 2017, Mammides *et al.* 2017, Eldridge *et al.* 2018). Differences in organism composition between biomes are a likely cause of these discrepancies, and thus it is important to test the reliabilities of these methods in the diversity of earth biomes. Therefore, for an acoustic index to be efficient it is important to know how accurately it reflects variations and patterns of the biodiversity that is being investigated, validating it as an useful, non-invasive tool for monitoring and conservation (Gasc *et al.* 2013, Machado *et al.* 2017, Mammides *et al.* 2017).

In this study, we analyzed the soundscape in forest islands in a mega dam reservoir in central Amazonia. More precisely, we used 12 acoustic indices (see Materials and methods below) to address the following questions: (1) is there a relationship between bird species richness and acoustic indices? (2) what is the relationship between soundscape patterns and island size? (3) how do soundscapes change throughout the day in islands of different sizes? and finally; (4) how do birds and cicadas use their acoustic space on islands of different sizes? To date, this is the first study testing the efficiency of acoustic indices to measure biodiversity in forest islands within an anthropogenic archipelago in the Amazon, testing how biodiversity and soundscapes change along a continuous level of fragmentation. We provided empirical evidence that acoustic indices should be used with caution due to their

inconsistency among habitat patches and that habitat fragmentation causes modifications in the soundscapes.

## **2. Materials and methods**

### ***Study Area***

The study was conducted in the Balbina mega hydroelectric reservoir, within the state of Amazonas, Brazil. The reservoir was filled in 1986 by the damming of the Uatumã River, a northern tributary of the Amazon River. Given the flat topography of the area, the resulting lake covered an area of approximately 312,900 ha, including more than 3,500 land-bridge islands ranging widely in size (0.2–4878 ha) and shape (Benchimol & Peres 2015a).

According to the Köppen classification, the climate is equatorial fully humid (Af), with annual mean temperature and precipitation of 26.5 °C and 2464 mm, respectively (Alvares *et al.* 2013). The vegetation of most islands is characterized by dense closed-canopy *terra-firme* forest. The islands are within, or adjacent to, areas of the Uatumã Biological Reserve (1°13'38"S, 59°27'28"W), the largest biological reserve in Brazil, encompassing both the fluvial island landscape and the contiguous adjacent upland areas on the east bank of the Uatumã River.

In this study, we selected 13 islands of the Balbina reservoir, varying in size from 13.6 to 673.4 ha, with a minimum distance of 2 km between them. Additionally, two continuous forest areas (i.e. areas of intact forest, not subject to fragmentation) located on both sides of the lake were included as control sites (Fig 1).

### ***Passive acoustic recordings***

To characterize the soundscape of the control area and island sites, we conducted passive acoustic recordings (Sugai *et al.* 2019) between June and September 2016, using four Song Meter Digital Field Recorders (model SM1 and SM2, Wildlife Acoustics, Maynard, MA, USA), with omnidirectional microphones model SMX-II (sensitivity:  $-36 \pm 4$  dB; frequency response: 20 Hz – 20,000 Hz; signal-noise-to-ratio:  $> 62$  dB). For each study site, we used two recording points to better capture spatial variability of soundscapes. These points were placed at a distance of approximately 200 m from each other to ensure that recorders were independent sampling points.



We used previously established trails within each area to deploy the devices, avoiding island edges by sampling at a minimum distance of 250 m from the island boundaries. Recorders were installed in the understory and fixed in trees at approximately 1.5 m from the ground.

Automated recorders were programmed to register sounds during one minute every five minutes, 24h per day, for three consecutive days along. Thus, we collected 14.4 hours of recordings per sampling point (12 min/h \* 24h \* three days), totaling 432 hours of recording (15 sites \* 2 points) equivalent to 25,920 one-minute recordings. Recording on a temporal schedule, rather than continuously, is a common practice in acoustic monitoring, reducing data storage and retaining most of the soundscape information (Pieretti *et al.* 2015). All recordings were made in mono using a sampling rate of 44,100 Hz set at 16 bits and stored in .wav format.

To assess possible variations in the dawn chorus activity, we used recordings from 05:30 to 08:00 am, encompassing the peak of vocal activity in the study area. Since most of the acoustic indices are sensitive to background noise, all recordings were first manually examined in order to remove those with presence of heavy rainfall. This resulted in a selection of 2,604 one-minute files, totalizing 43.4 h of dawn chorus recordings for all sampled areas.

This study was carried out in strict accordance with the recommendations of the Chico Mendes Institute for Biodiversity Conservation under license number: 51536-5.

### ***Acoustic richness***

In order to quantify the richness of vocal animal species and biological sound types within each acoustic recording, all selected dawn chorus recordings were manually scanned by a researcher skilled in acoustic identification of Amazon birds (TB). This procedure consists of listening to all files while simultaneously inspecting spectrograms created with Raven pro 1.5 (Bioacoustics Research Program 2014). Thus, presence/absence of bird species vocalizing and insect call types in each recording were recorded. When species identification was uncertain, vocalizations were reviewed and identified by additional researchers (see Acknowledgments).

Although all biological sounds were classified, only avian sounds were identified to the species level. Biological sounds other than birds were classified as insects (mainly Cicadas), primates and anurans. To insects, we used the term sonotypes (i.e. equivalent to “acoustic morphospecies”), defined as a note or series of notes representing a single acoustic signal (Aide *et al.* 2017, Ferreira *et al.* 2018). Each insect sonotype was classified according to their specific frequency range, and was counted only once per recording, representing a measure of richness (Ferreira *et al.* 2018). As the sampled sites are in remote locations with minimal human presence, anthropogenic sounds are rare and therefore were not taken into account.

### **Acoustic indices**

Using the R packages *seewave* (Sueur, Aubin, *et al.* 2008) and *soundecology* (Villanueva-Rivera & Pijanowski 2018), we computed 12 acoustic indices for all files recorded along the 24h: Acoustic complexity (ACI); Acoustic Diversity (ADI); Acoustic evenness (AEI); Median of the amplitude envelope (M); Bioacoustic Index (BI); Spectral flatness (SFM); Spectral entropy (SH); Temporal entropy (TH) and Total entropy (H). We also computed the Root Mean Square (RMS) of sound amplitude to different frequency bands, splitting in RMS.birds (from 200 to 5000 Hz), RMS.cicadas (from 5000 to 14000 Hz), and RMS.total, which is considered the mean amplitude of the whole sound recording (i.e. estimate of the amount of energy in that band). A description of each index is summarized in Table 1. Raw estimates of the M index were multiplied by  $10^6$  in order to allow interpretation of lower values. Both ADI and AEI were calculated using 500 steps and a decibel threshold of  $-50$  (default). A useful feature of TH, SH, H, SFM and M is that their values are normalized (0-1) and can therefore be used to compare recordings of quite different content and amplitude (Towsey, Wimmer, *et al.* 2014). A single pure tone, for example, has values near 0, representing a low acoustic diversity. On the other hand, a more diverse and noisier soundscape will have values close to 1 (Sueur, Pavoine, *et al.* 2008, Sueur *et al.* 2014).

Prior to the analyses, we performed a band-pass filter (from 200 to 14000 Hz) to remove low and high frequency noise. Since different types of organisms use different frequency ranges, we calculated indices for birds within the frequency range 200-5000Hz, which covers 92% of bird species (n=131), and 36% of cicadas (n=87).

For the frequency range used by cicadas (5000 – 14000 Hz) we only applied the RMS index, as 63% of the acoustic space at this range is occupied by cicadas and only 8% by birds.

### ***Soundscape characterization***

For an overview of the acoustic frequencies used by bird species, we used high quality recordings from the open-access on-line sound archive Xeno-canto ([www.xeno-canto.org](http://www.xeno-canto.org)). Using the R package *warbleR* (Araya-Salas & Smith-Vidaurre 2017), we downloaded two recordings of each bird species that was often present in our data (n=130), representing 72% of all species acoustically present (n=181), totalizing 260 recordings. When species had no recordings available in Xeno-canto, manual recordings made by TB were used (recordings made with unidirectional microphone Sennheiser, model ME66 with a recorder Tascam DR-100, using a sampling rate of 48000 Hz at 24 bits). For each recording, we selected 20 seconds of time resolution using the freeware Audacity 2.3.2 (<https://www.audacityteam.org>), where we manually tagged all songs through the spectrogram (window size 1024, type Hann) for posterior extraction of acoustic parameters. All recordings were filtered to reduce background noise outside the frequency range used by the species. For birds we used species-specific low-pass and high-pass filters according to spectral characteristics of their songs (Supplementary material appendix A, Table A1). For cicadas we used a low-pass and high-pass filter set at 2000 and 16000 Hz, respectively.

The acoustic measurements of bird songs were performed using R 3.4.0 (R Core Team 2018). First, using the package *seewave* (Sueur, Aubin, *et al.* 2008), we calculated the power spectrum (window type Hann, window size of 1024 samples, overlap 80%, hop size 205 samples, grid spacing 43.1 Hz) of each tagged species song, and then we calculated the minimum and maximum frequency of each song using 20 dB below the peak frequency (Podos 1997).

To define the acoustic space used by cicadas, we chose a subsample of four recordings per area (two recordings per recording point) in which we manually detected presence of cicada chorus, totalizing 56 recordings of one minute. Using Raven Pro 1.5 (Bioacoustics Research Program 2014), we calculated, for the whole chorus, the minimum and maximum frequencies from the power spectrum (window

type Hann, window size of 1024 samples, overlap 50%, hop size 512 samples,) using the same threshold that we used for bird songs (20 dB below the peak frequency).

In order to account for species-specific variation, we calculated a mean minimum and maximum frequency for each bird species and for all cicada choruses recorded.

### **Statistical analysis**

To test whether the acoustic indices predict dawn chorus bird richness, we performed general linear mixed models (GLMM) for each index separately, considering the acoustic indices as response variable and species richness as predictor and recording point, day and hour as random factors, with recording point nested in island. Since the RMS.cicadas index was calculated only for the cicadas frequency range, this variable was not tested in relation to bird richness.

Not all species sing at the same time, and thus it is interesting to investigate if averaged acoustic indices can help us predict the absolute richness of a location, rather than the specific richness that is recorded in a given time point. To investigate whether the accumulated hourly species richness per recording point can be predicted by the acoustic indices averaged per hour, we used GLMM using each averaged index as our response variable and the interaction between species richness and hour as predictor. As we have multiple days of sampling, using two independent recorders per site, we used day, recording point and island as random factors, with day and recording point nested in island.

Before running the mixed models, we tested variables for multicollinearity using the Variance Inflation Factor (VIF), through the *corvif* function (Zuur *et al.* 2010) in R. We sequentially dropped each acoustic index for which the VIF value was higher than 3 until all remaining VIFs were below that level. As a consequence, the following five indices were excluded from our analysis: AEI, RMS.birds, RMS.total, SH and TH. This implies that these indices were actually closely correlated with one or some of the remaining that were kept in the analyses, and were thus redundant. Despite the acoustic diversity index (ADI) is derived from the entropy index (H) (Sueur *et al.*, 2008), based in our data, no collinearity between them was found, and therefore both indices were kept.

A rich literature in island biogeography predicts strong relationships between species richness and composition and island size. In this study, we tested for a possible relationship between acoustic indices and island size using general linear models (GLM) for each index, considering the acoustic indices averaged per island as the response variables and island size ( $\log_{10}$ ) as the predictor. In addition, we tested for a possible species-area relationship using a GLMM, considering the bird and sonotype richness separately as response variable and island size ( $\log_{10}$ ) as predictor. Recording point, day and hour were considered as random factors.

As the soundscapes differ between day and night, due to different activity patterns of the species, we compared the acoustic indices averaged per hour, between islands of different sizes and areas of continuous forest over the 24h period to describe how soundscapes differed throughout the day in these areas.

All statistical analyses were conducted using R 3.4.0 (R Core Team 2018). GLMM were fitted using restricted maximum likelihood (lmer function in the lme4 package, Bates *et al.* 2015). The goodness of fit of GLMM were based on the marginal  $R^2$  and a conditional  $R^2$  (based on maximum likelihood) giving the variance explained by fixed effects, and both fixed and random effects, respectively, using the r.squaredGLMM function implemented in the *MuMIn* package (Nakagawa & Schielzeth 2013, Barton 2019). In our mixed models, p-values were based on Satterthwaite approximation to degrees of freedom (lmerTest package, Kuznetsova *et al.* 2017).

### 3. Results

A high diverse bird community inhabiting islands and continuous forest areas was identified by passive monitoring. Acoustic analysis of 2,604 one-minute recordings of the dawn chorus revealed the presence of 181 bird species distributed in 45 families and 18 orders (species list available in Supplementary material appendix A, Table A2). In addition to the bird species, we identified five monkey species and categorized 39 sonotypes belonging to unidentified anuran and insect species. Bird song frequencies ranged from 215 to 10,949 Hz. Cicadas called from 2,211 to 14,463 Hz. For the analyzed species ( $n = 131$ ), 38% emitted acoustic signals between 2,000

and 7,000 Hz, representing a possible range of frequency overlap (Fig 2). The most frequent species recorded was *Lipaugus vociferans*, which occupied a wide frequency range (2.0-6.0 kHz), with song notes of high amplitude and extreme frequency modulation.

There was no significant difference in species and sonotypes richness between the sampled sites (Supplementary material appendix A, Table A3) as shown by GLMM results (Table 2). On average, the islands had the same number of species ( $n = 12$ ; Birds:  $X \pm SD 54.3 \pm 8.6$ ; Sonotype:  $X \pm SD 29.4 \pm 20.7$ ) as the continuous forest areas ( $n = 2$ ; Birds:  $55.5 \pm 7.7$ ; Sonotype:  $X \pm SD 19.5 \pm 6.5$ ). Among all identified bird species, 92 were present only in islands, 12 only in continuous forest areas and 77 species were recorded in both environments (Supplementary material appendix A, Fig. A1).

Relationships between acoustic indices and bird species richness varied depending on the index examined (Table 3), ranging from positive (ADI and H), negative (M) and no relationship (ACI, BI, SFM) (Fig 3). Taking into account the conditional  $r$  squared, the H index performed better ( $R^2_c = 0.32$ ) than the other indices. Using indices averaged by hour, only ACI and SFM hour averages were related to accumulated species richness, showing a significant effect of the interaction term Richness:Hour. This implies that the relationship between richness and the index score changed with time (Table 4).

When we tested for a possible area effect in the acoustic indices, only H was related to island size (Table 5), presenting higher values with the increase in island size (Fig 4), with island size explaining 35% of the variation in the acoustic entropy.

The acoustic indices exhibited distinct patterns over the 24-hour period, with marked shifts between day and night soundscapes (Fig 5). The acoustic entropy index (H), and the acoustic diversity index (ADI) were high during the day and lower during the night. In contrast, the Amplitude index (M) values were lower during the day and higher during the night. Diel patterns in ACI and RMS.cicadas were not much evident, although there were high values at dusk. SFM showed a concentration of energy during the morning and night, with low levels in the middle of the day. The Bioacoustic Index (BI) exhibited different values during the day between islands, with a marked peak at dusk.

#### 4. Discussion

Soundscape recordings and different types of automated analyzes have great potential to be used as a powerful conservation tool to access changes in the acoustic landscape due to processes of habitat loss and consequent decline in biodiversity. Passive acoustic monitoring of a fragmented Amazonian landscape in a mega hydroelectric reservoir revealed distinct patterns in soundscapes, with clear diel patterns for some indices and high values for areas of continuous forest. Although studies testing the efficacy of acoustic indices are increasing, much of them do not test possible collinearities that might occur (but see Machado *et al.* 2017). Our results show that five of 12 tested indices presented collinearities and had to be dropped from the final models. Therefore, we suggest that collinearity between acoustic indices should be tested, in order to strength the results.

To find a single index that summarizes all biodiversity or soundscape facets is challenging and possibly illusory. This attempt probably came from the need to have a unique value that can be easy to understand for conservation decision-making (Magurran *et al.* 2010, Sueur *et al.* 2014). Given that each index is based on different characteristics of the soundscape, reflecting different spatio-temporal features (Eldridge *et al.* 2016, Bradfer-Lawrence *et al.* 2019), considering several indices in concert may give a much better representation of the soundscape rather than any one individual index (Towsey, Wimmer, *et al.* 2014, Buxton *et al.* 2018, Bradfer-Lawrence *et al.* 2019). On the other hand, it is possible that different indices perform differently in different biomes.

##### ***Acoustic indices and Bird richness***

The acoustic indices that best predict dawn chorus bird richness were ACI, ADI, H, M, SFM in all areas sampled. Although these indices present a significant relationship with species richness, the variance explained by each one is low, demonstrating a limitation in the use of acoustic indices as predictors of the number of species present.

In Brazil, studies relating acoustic indices to bird richness in areas of savanna

forest demonstrated that ADI and H could be used as proxies for richness, and that ADI may have different values depending on the tested environment (Machado *et al.* 2017, Ferreira *et al.* 2018). Despite other studies demonstrating that ADI is relatively more robust with regard to bird species richness (Zhao *et al.* 2019), in our study, the variance explained by ADI was very low. Although the M index can be used as a measure of the number of species calling, our results are contrary to expectations since M and species richness had an opposite relationship, i.e., with the increase in species richness, M had lower values. In the meantime, BI that was found to correlate positively with the number of bird species in an Atlantic rainforest fragment (Jorge *et al.*, 2018) was not related to bird richness in our study. This is also consistent with findings of studies that tested this index (Machado *et al.* 2017, Mammides *et al.* 2017, Zhao *et al.* 2019), suggesting that this index is not robust for bird monitoring.

### ***Acoustic indices and Island size***

The relationship between acoustic indices and the type of environment has been the subject of some studies (e.g. Tucker *et al.* 2014, Fuller *et al.* 2015a, Gómez *et al.* 2018) where their effectiveness in characterizing differences between these environments has been demonstrated. In our study, the entropy acoustic index (H) was the only index related to island size, where higher values were found on larger islands. Similar results were found in a study that evaluated acoustic indices in 19 forest sites in eastern Australia, with varying levels of landscape fragmentation (Fuller *et al.* 2015b). They found that the H index is not limited strictly to patch size, but rather they differentiate between similar sized sites being suitable for identifying environments. Although ADI, ACI, BI, M have demonstrated effectiveness in discriminating different types of environments (Gómez *et al.* 2018), in our study area they were unable to capture the different patterns of the acoustic landscape between the islands.

The fact that there is no difference in the number of species between the sampled islands and that the turnover of species between islands is greater than the loss of species, may have caused the tested indices to have different results when



related to the island size. This may have been due to the fact that the indices are possibly not only be sensitive to the number of species present but also to which species are present, since different acoustic signals would have different influences on the indices. This result is in line with what was found by Tucker et al. (2014), where the authors suggest that bird species compositional differences are strongly associated with landscape features and these differences are reflected in acoustic biophony measurements.

### ***Soundscapes Diel Patterns***

We found clear diel patterns to all indices, where almost all indices exhibited a marked peak at dusk. This could be because insects and anurans could be present in the frequency range that each index was measured (200-5000Hz), adding “noise” to acoustic indices. In fact, Ferreira *et al.* (2018) showed that most insect and anuran sonotypes occupied frequencies below 7000 Hz in a tropical savanna. Since these organisms are more active during dusk-night period, this may be the reason for the peaks at dusk.

For ACI, we did not have very clear patterns throughout the day, but one of the islands (Neto island) peaked at 17:00 pm, showing higher values than the other islands. High values of this index are usually indicative of thunderstorms, intermittent rainfall, insect stridulation or high bird activity. As for our study, rain recordings were previously removed, high values of this index probably represent a greater activity of bird species, more precisely *Lipaugus vociferans*, within a lek mating arena, where there are several individuals of the species vocalizing at the same time, using a modulated amplitude and high intensity song, spanning a wide spectral range (2000-6000 hz).

The index BI showed clear differences between islands during the morning period, where small islands presented higher values than large islands. However, during the night the values between areas are similar with the continuous forest areas presenting higher values. The high values found represent a great disparity between noisy and silent signals with high amplitude and minimal variation among frequency bands, characteristic of nocturnal insect noise and cicada choruses. This

pattern is opposite to what was found in fragmented areas in Australia, where BI show lower values during the night (Fuller *et al.* 2015b).

The pattern shown by ADI was very different to the other indices, except H. The low nighttime pattern presented by H and ADI may indicate that nocturnal soundscapes were more uneven with less frequency bands occupied. As ADI is related to bird richness, and can vary according to species activity (Machado *et al.* 2017), high bird activity during the day, could be causing higher index values, indicating that the daytime is noisier than the nighttime. This is opposite to what was found in studies conducted in a tropical savanna (Ferreira *et al.* 2018), fragmented areas in Australia (Fuller *et al.* 2015b) and temperate sites (Villanueva-Rivera *et al.* 2011), with peaks corresponding to dawn and dusk choruses. Another reason for the opposite results found in our study is perhaps due to the fact that the recordings were made outside the reproductive period of frogs, where there is not much vocal activity.

In general, the acoustic entropy index (H) showed clear patterns throughout the day. The decay during the early morning in Moita island is due to the fact that at this particular time there was a chorus of cicadas, presenting a constant sound and therefore lower values of the index. For the M index, high values found during the day come from the vocalization of specific bird species (*Monasa atra*), occupying a wide spectral range with large amplitude levels, which leads to higher index values.

## 5. Conclusions

This study shows the potential application of acoustic indices for the management of protected areas, mainly due to its relatively rapid assessment of avian communities. Although our results demonstrate that there is a relationship between acoustic indices and species richness, even in a highly diverse environment such as rainforests, there are some limitations to be overcome before they can be widely adopted, where the sensitivity to insect noise and acoustically dominant bird species, are one of those. The fact that the activity of single bird species influences so strongly a series of indices cautions about their utility as indicators of avian biodiversity. In this kind of biome, dense and loud social groups such as leks of very vocal species (e.g. *Lipaugus vociferans*) can strongly change the soundscape. This

difficulty may be absent in other biomes in which the contributions of most species are much more homogeneous. Therefore, if acoustic indices are going to be a useful environmental monitoring tool, they need to take in account the heterogeneity between species and if possible, have a component that allows the recognition of acoustic patterns, such as those used in automatic species recognition.

We do not propose that soundscapes measures should replace traditional biodiversity assessment but provides an excellent complementary tool when provided with a ground-truthed database. For decision-making, knowing which species are calling is as relevant as knowing how biodiverse an area is.

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## Figure legends

Figure 1. Spatial distribution of the study area and the 13 islands and two continuous forest sites (Control areas) surveyed at the Balbina Hydroelectric Reservoir, state of Amazonas, Brazil.

Figure 2. Proportion of usage of the acoustic space by birds and cicadas in the sampled areas from the Balbina reservoir, Amazonas, Brazil.

Figure 3. Acoustic indices that were related to bird richness in islands (n=12) and continuous forest (n=2) in the Balbina Hydroelectric reservoir, Amazonas, Brazil.

Figure 4. Relation between the Acoustic Entropy index (H), averaged per island (n=12), and Island size in log. The shaded area represents the 95% confidence interval and the black triangles represent H values in areas of continuous forest (n=2).

Figure 5. Diel patterns in mean acoustic indices per hour, for each sampled area. Values calculated for each 1-min recording over 24h, from 28-point recorders.

## Tables

Table 1. Summary of the acoustic indices used in this study, with description of general soundscape patterns they reflect

Index and reference	Index description
Acoustic Complexity Index (ACI) Pieretti et al. (2011)	Calculates the difference in amplitude between two time samples within a frequency band. Designed to give more importance to sounds that are modulated in amplitude reducing the importance of sound with constant amplitude.
Acoustic Diversity Index (ADI) (Villanueva-Rivera et al. 2011)	Calculated by dividing the spectrogram into bins, taking the proportion of the signals above a threshold (-50 dBFS) and calculating the Shannon index to each bin. A noisy or silent signal will give a high value and a pure tone signal will be closer to 0.
Acoustic evenness (AEI) (Villanueva-Rivera et al. 2011)	Calculated as the ADI, but using Gini-Simpson index, instead of Shannon index.
Bioacoustic Index (BI) (Boelman, et al. 2007)	Designed to assess relative avian abundance. The index consists in computing the dB mean spectrum and in calculating the area under the curve between two frequency limits. Higher values indicate greater disparity between noisy and silent signals.
Median of the amplitude envelope (M) (Depraetere et al., 2012)	Computes an acoustic index based on the median of the amplitude envelope, serving as an indicator of the number of animal vocalizations. It is a value between 0 and 1, where noisier soundscapes will tend towards 1.
Root Mean Square (RMS)	Calculated for the whole recording, which is considered the mean amplitude of the sound recording.
Root Mean Square.Birds (RMS.Birds)	Calculated only for the Birds frequency band (from 200 to 5000 Hz).
Root Mean Square.Cicadas (RMS.Cicada)	Calculated only for the Cicadas frequency band (from 5000 to 14000 Hz).
Spectral flatness (SFM)	Calculated as the ratio between the geometric mean and the arithmetic mean of a frequency spectrum. The SFM of a noisy signal will tend towards 1 whereas the SFM of a pure tone signal will tend towards 0.
Spectral entropy (SH) (Sueur 2008)	Calculated as the Shannon entropy of a frequency spectrum. The Shannon spectral entropy of a noisy signal will tend towards 1 whereas

	the Shannon spectral entropy of a pure tone signal will tend towards 0.
Temporal entropy (TH) (Sueur 2008)	Calculated as the entropy of a temporal envelope.  Note, that a sustained sound with an almost flat envelope will also show very high temporal entropy
Total entropy (H) (Sueur 2008)	The product of (SH*TH) that gives a global (time and frequency) estimation of signal entropy (i.e. the total entropy of a time wave).  The entropy of a noisy signal will tend towards 1 whereas the entropy of a pure tone signal will tend towards 0

Table 2. GLMM results to species richness (birds and sonotypes) and island size (log10) to all sampled areas (14) in the Balbina mega dam reservoir.

	<b>Parameters</b>	<b>Estimate</b>	<b>SE</b>	<b>t-Value</b>	<b>p-Value</b>
Bird Richness	Intercept	3.95	0.67	5.85	<0.001
	Log10(size)	-0.12	0.26	-0.47	0.63
Sonotype Richness	Intercept	4.20	1.02	4.12	<0.001
	Log10(size)	-0.36	0.38	-0.96	0.34

Table 3 - Linear mixed models between acoustic indices and bird species richness. Bold numbers represent variables that reached statistical significance.  $R^2m$  and  $R^2c$  represent the marginal and conditional  $R^2$  respectively.

Acoustic index	Parameters	Estimate	SE	t-Value	p-Value	$R^2m$	$R^2c$
ACI	Intercept	53.117	0.46	114.41	<0.001	0.00	0.30
	Richness	-0.05	0.03	-1.72	0.08		
ADI	Intercept	2.28e+0	2.99e-03	761.13	<0.001	0.00	0.08
	Richness	1.31e-03	4.56e-04	2.88	<b>&lt;0.01</b>		
BI	Intercept	8.36e+00	5.65e-01	14.79	<0.001	0.00	0.39
	Richness	4.11e-03	4.15e-02	0.09	0.92		
H	Intercept	7.67e-01	3.54e-03	216.30	<0.001	0.00	0.32
	Richness	1.26e-03	2.89e-04	4.38	<b>&lt;0.001</b>		
M	Intercept	5.38	0.42	12.53	<0.001	0.01	0.14
	Richness	-0.24	0.04	-4.92	<b>&lt;0.001</b>		
SFM	Intercept	5.57e-06	6.98e-07	7.99	<0.001	0.00	0.49
	Richness	-4.46e-09	3.01e-08	-0.14	0.88		

Model= lmer(Indices~richness + (1|island/recorder) + (1|hour) + (1|day))

Table 4 - Linear mixed models between acoustic indices averaged per hour and bird species richness accumulated per recorder with interaction Richness:Hour. Bold number represent statistical significance.

Acoustic Indices	Parameters	Estimate	SE	t-Value	p-Value
ACI	Intercept	52.50	1.50	34.81	<0.001
	Hour 6	1.77	1.42	1.24	0.21
	Hour 7	4.10	1.42	2.88	<b>0.00</b>
	Hour 8	4.65	1.42	3.26	<b>0.00</b>
	Richness	-0.01	0.03	-0.18	0.85
	Hour 6: Richness	-0.03	0.03	-1.02	0.30
	Hour 7: Richness	-0.07	0.03	-2.10	<b>0.03</b>
	Hour 8: Richness	-0.08	0.03	-2.55	<b>0.01</b>
ADI	Intercept	2.26	0.02	135.86	<0.001
	Hour 6	0.01	0.02	0.76	0.45
	Hour 7	0.01	0.02	0.71	0.48
	Hour 8	0.01	0.02	0.57	0.57
	Richness	0.00	0.00	1.51	0.13
	Hour 6: Richness	0.00	0.00	-0.73	0.47
	Hour 7: Richness	0.00	0.00	-0.63	0.53
	Hour 8: Richness	0.00	0.00	-0.48	0.63
BI	Intercept	12.24	2.57	4.77	<0.001
	Hour 6	2.59	2.40	1.08	0.28
	Hour 7	1.05	2.40	0.44	0.66
	Hour 8	2.48	2.40	1.03	0.30
	Richness	-0.09	0.06	-1.45	0.15
	Hour 6: Richness	-0.06	0.06	-1.12	0.26
	Hour 7: Richness	-0.03	0.06	-0.61	0.55
	Hour 8: Richness	-0.06	0.06	-1.09	0.28
H	Intercept	0.76	0.02	42.94	<0.001
	Hour 6	-0.01	0.02	-0.58	0.57
	Hour 7	0.01	0.02	0.36	0.72
	Hour 8	-0.02	0.02	-0.96	0.34

	Richness	0.00	0.00	0.76	0.45
	Hour 6: Richness	0.00	0.00	0.76	0.45
	Hour 7: Richness	0.00	0.00	-0.35	0.73
	Hour 8: Richness	0.00	0.00	0.82	0.42
M	Intercept	6.81	1.95	3.48	<0.001
	Hour 6	-1.12	2.42	-0.46	0.64
	Hour 7	-1.17	2.42	-0.48	0.63
	Hour 8	-1.27	2.42	-0.52	0.60
	Richness	-0.06	0.05	-1.39	0.16
	Hour 6: Richness	0.04	0.06	0.69	0.49
	Hour 7: Richness	0.04	0.06	0.72	0.47
	Hour 8: Richness	0.05	0.06	0.85	0.40
SFM	Intercept	0.00	0.00	2.92	0.02
	Hour 6	0.00	0.00	1.99	0.08
	Hour 7	0.00	0.00	2.47	<b>0.04</b>
	Hour 8	0.00	0.00	3.90	<b>0.01</b>
	Richness	0.00	0.00	-0.34	0.74
	Hour 6: Richness	0.00	0.00	-1.36	0.21
	Hour 7: Richness	0.00	0.00	-1.47	0.18
	Hour 8: Richness	0.00	0.00	-3.12	<b>0.02</b>

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Table 5 – Results of mixed models between acoustic indices averaged per island and island size in log. Bold numbers represent statistical significance.

Acoustic index	Parameters	Estimate	SE	t-Value	p-Value	R <sup>2</sup>
ACI	Intercept	51.75	0.53	96.86	<0.001	0.05
	log10(size)	0.30	0.23	1.31	0.21	
ADI	Intercept	2.26	0.00	245.15	<0.001	-0.07
	log10(size)	0.00	0.00	0.37	0.71	
BI	Intercept	11.23	1.27	8.81	<0.001	0.10
	log10(size)	-0.90	0.55	-1.61	0.13	
H	Intercept	0.72	0.00	80.99	<0.001	0.35
	log10(size)	0.01	0.00	2.82	<b>0.01</b>	
M	Intercept	8.58	1.56	5.47	<0.001	-0.04
	log10(size)	-0.46	0.68	-0.68	0.50	
SFM	Intercept	0.00	0.00	5.25	<0.001	-0.04
	log10(size)	0.00	0.00	0.65	0.52	



## Figures

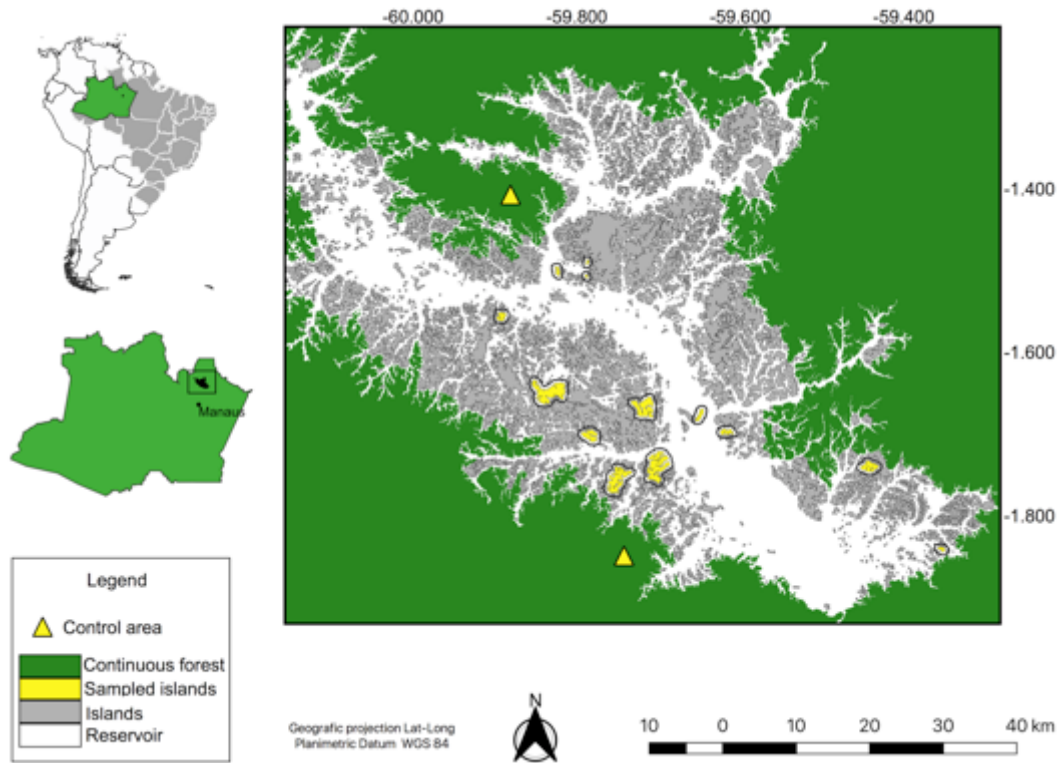


Figure 1. Spatial distribution of the study area and the 12 islands and two continuous forest sites (Control areas) surveyed at the Balbina Hydroelectric Reservoir, state of Amazonas, Brazil.

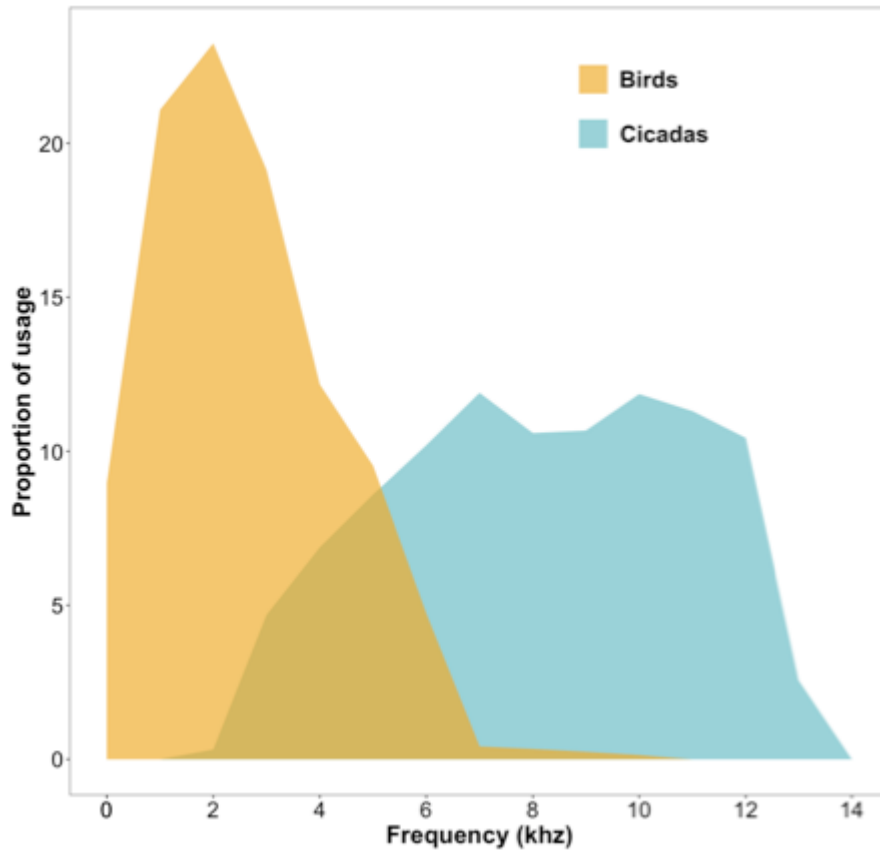


Figure 2. Proportion of usage of the acoustic space by birds and cicadas in the sampled areas from the Balbina reservoir, Amazonas, Brazil.

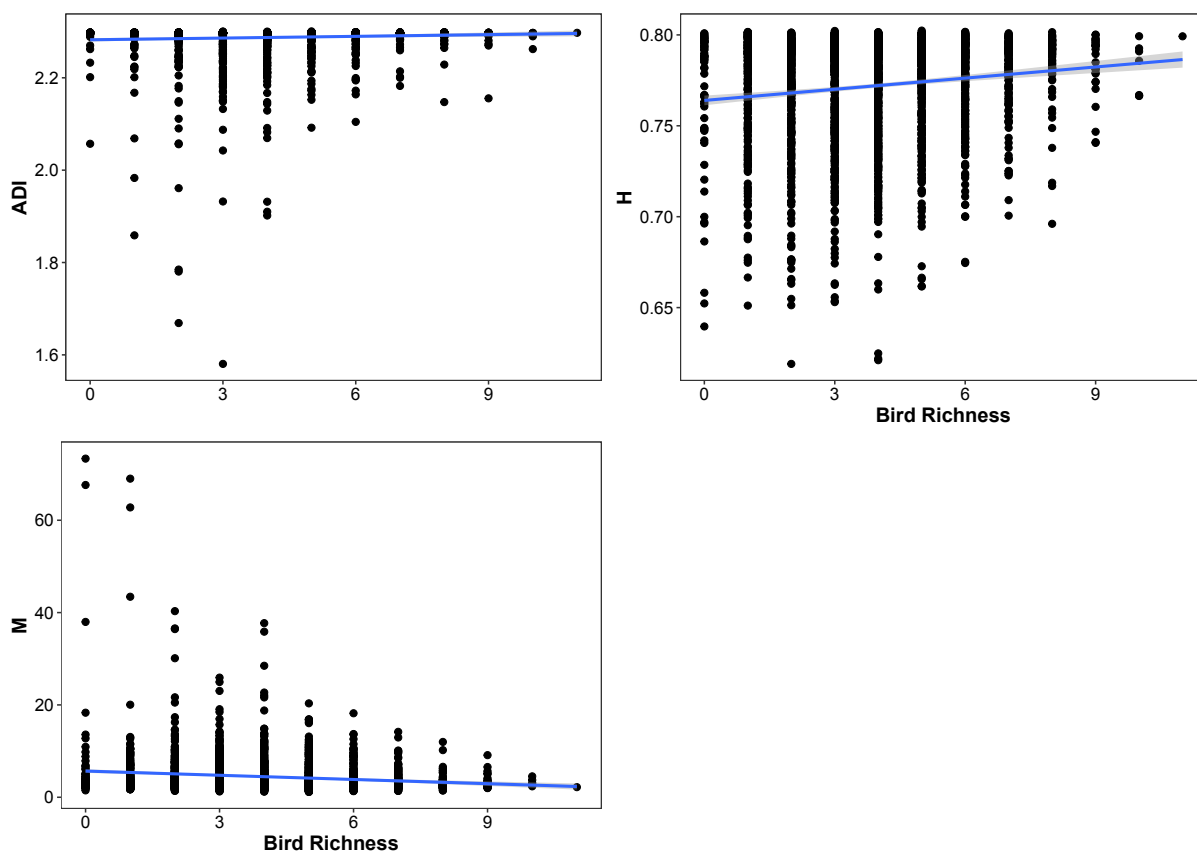


Figure 3 – Acoustic indices that were related to bird richness in islands (n=12) and continuous forest (n=2) in the Balbina Hydroelectric reservoir, Amazonas, Brazil.

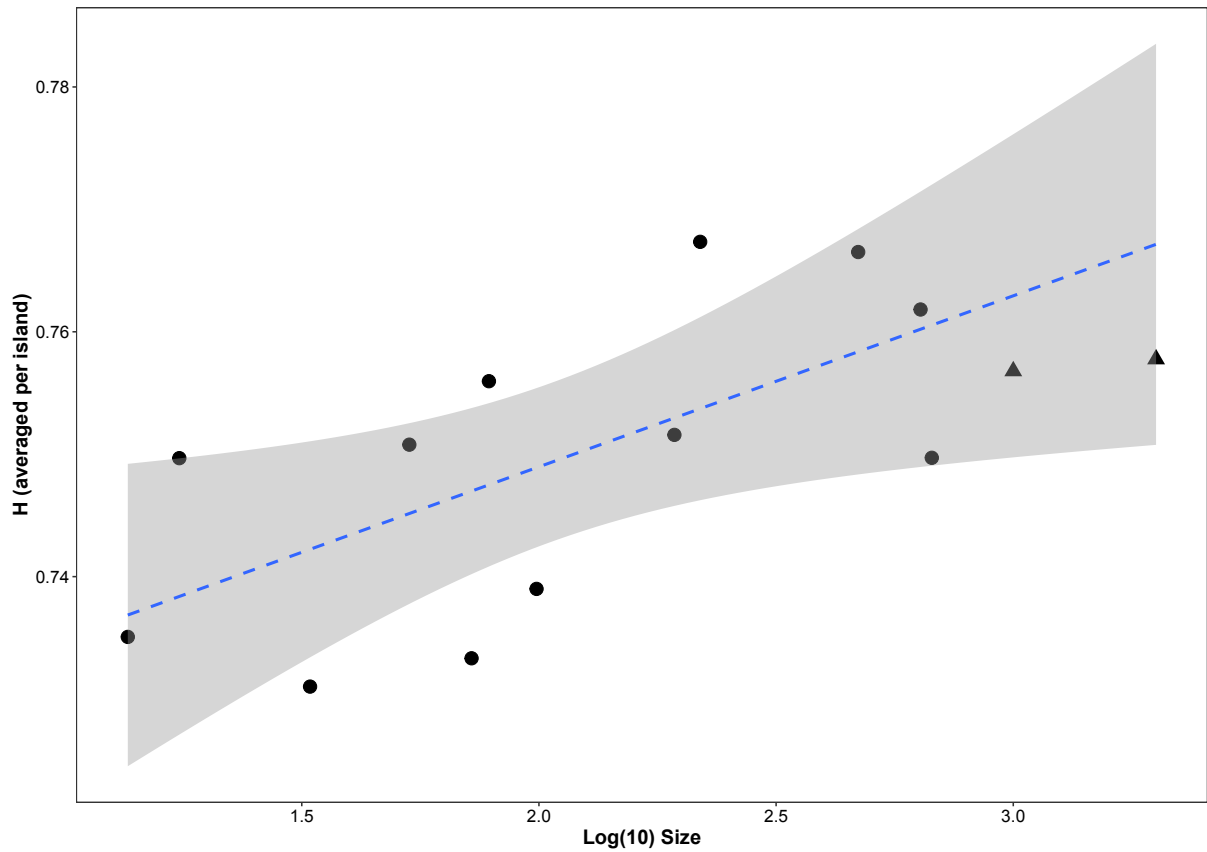


Figure 4 – Relation between the Acoustic Entropy index (H), averaged per island (n=12), and Island size in log. The shaded area represents the 95% confidence interval and the black triangles represent H values in areas of continuous forest (n=2).

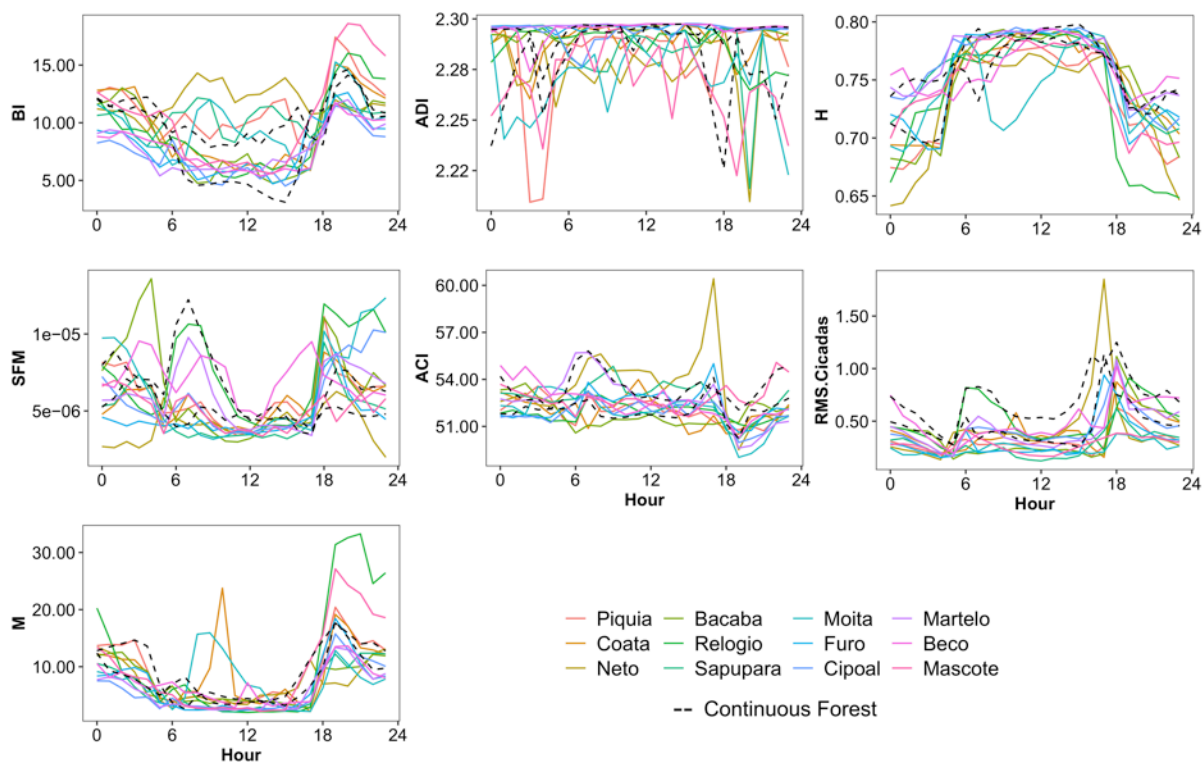


Figure 5. Diel patterns in mean acoustic indices per hour, for each sampled area. Values calculated for each 1-min recording over 24h, from 28-point recorders.

## Capítulo II.

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Bicudo T, Anciães M, Arregui L, Gil D. Effects of forest fragmentation on feather corticosterone levels in an Amazonian avian community. (Publicado em Ardeola: DOI: 10.13157/arla.67.2.2020.ra1)

## EFFECTS OF FOREST FRAGMENTATION ON FEATHER CORTICOSTERONE LEVELS IN AN AMAZONIAN AVIAN COMMUNITY

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Type of article: Research paper

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Author Contributions: TB, MA and DG designed the study. TB conducted data collection. TB and DG performed data analyses. TB and LA performed the corticosterone measurement. TB, MA and DG drafted the manuscript.

**Abstract**

In the Amazon, the construction of hydroelectric dams is an emergent driver of biodiversity loss, creating numerous land-bridge islands, most of them unable to sustain an assemblage of bird species comparable to the intact forest. Although we understand the effects of forest fragmentation on species richness and distribution, we still need to uncover the physiological mechanisms underlying the success of organisms living in disturbed habitats. In this study, we used feather corticosterone levels as a measurement of physiological indicators of stress, evaluating whether corticosterone levels mirror the effects of habitat fragmentation on species occurrence. Since data suggest that smaller islands can reduce habitat suitability, increasing stress in birds that live within them, we predicted that birds living in smaller islands would present increased feather corticosterone levels. We captured birds in 13 islands of varying size and in two continuous forests and analysed feather corticosterone levels of 265 individuals from eight different species. Overall, our findings did not support the hypothesis that corticosterone varies in relation to island size, except for *Hypocnemis cantator*, which presented the predicted pattern: decreasing feather corticosterone levels with increasing island size. These differences suggest that species respond differently to stressors driven by fragmentation. Further studies are necessary to assess the reliability of corticosterone levels as a physiological measurement of stress and to determine which parameters are useful to understand how insularization caused by human activities may influence the resistance of avian populations to habitat disturbances.



## Resumen

En la Amazonía, la construcción de represas hidroeléctricas es un factor emergente en la pérdida de biodiversidad debido a la formación de numerosas islas, la mayoría incapaces de sostener comunidades de aves comparables a las de los bosques intactos. Si bien entendemos los efectos de la fragmentación de los bosques en la riqueza y distribución de las especies, aún necesitamos descubrir los mecanismos fisiológicos relacionados con el éxito de los organismos que viven en hábitats perturbados. En este estudio, utilizamos los niveles de corticosterona en las plumas como una medida del estrés fisiológico, evaluando si estos niveles reflejan los efectos de la fragmentación del hábitat sobre las especies. Dado que los datos previos sugieren que las islas más pequeñas pueden reducir la adecuación del hábitat y aumentar así el estrés en las aves que viven en ellas, predecimos que las aves en las islas más pequeñas tendrían niveles más altos de corticosterona en las plumas. Capturamos aves en 13 islas de diferentes tamaños y dos bosques continuos y estimamos los niveles de corticosterona en plumas de 265 individuos de ocho especies distintas. En general, nuestros resultados no respaldaron la hipótesis de que la corticosterona varía con el tamaño de la isla, excepto para *Hypocnemis cantator*, que presentó el patrón esperado: disminución de los niveles de corticosterona en las plumas con el aumento del tamaño de la isla. Estas diferencias sugieren que las especies responden de manera diferente a los estresores causados por la fragmentación. Se necesitan más estudios para evaluar la fiabilidad de los niveles de corticosterona como una medida del estrés fisiológico y para determinar qué parámetros son útiles para comprender cómo la insularización causada por las actividades humanas puede influir en la resistencia de las poblaciones de aves a las alteraciones del hábitat.

## Introduction

Habitat fragmentation is recognised as a major threat to biodiversity, leading to long-term changes in the structure and function of fragments (Haddad *et al.* 2015, Fahrig 2017). In fragmented landscapes, patch area is one of the key factors influencing species richness, population abundance and community composition (MacArthur & Wilson 1963, Fahrig 2017). Small fragments (<100-ha), can lose up to half of their species in less than 15 years of isolation, although species strongly differ in their probabilities of extinction (Ferraz *et al.* 2003). The occurrence and survival of species is probably changed upon fragmentation, and area-sensitive species may decline or become extinct in small fragments (Dardanelli *et al.* 2006).

Most of the studies on the impact of fragmentation in Amazonian birds have analysed a particular type of fragmentation, namely habitat patches within a semi-permeable matrix of cattle pastures and abandoned cropland (Laurance *et al.* 2002, 2011, Ferraz *et al.* 2003, 2007, Wolfe *et al.* 2015). A different and emergent driver of habitat fragmentation is the construction of hydroelectric dams, which can induce the flooding of huge forest surfaces that creates simultaneously numerous small land-bridge islands surrounded by water (Benchimol & Peres 2015c, Lees *et al.* 2016). The resulting non-forest matrix may act as a barrier to birds with low flight ability, hampering the dynamics of colonisation and extinction in this system (Aurélio-Silva *et al.* 2016, Palmeirim *et al.* 2017, Bueno & Peres 2019).

In such archipelagos, habitat patches act like true islands, small ones being subject to a greater extinction of bird assemblages (Wolfe *et al.* 2015, Aurélio-Silva *et al.* 2016) and showing greater environmental degradation than larger ones due to strong edge effects that alter forest microclimate, tree mortality and faunal diversity (Murcia 1995, Benchimol & Peres 2015c, a, Laurance *et al.* 2018). Overall, the availability of trophic and structural resources is expected to decrease with diminishing island area (Palmeirim *et al.* 2017).

At Balbina Dam, a major hydroelectric dam in Central Amazonia, bird assemblages that occupy islands and areas of intact forest vary predictably, with smaller and more isolated islands retaining a smaller number of species (Lees & Peres 2006, Benchimol & Peres 2015b, Aurélio-Silva *et al.* 2016). For instance, fewer than 10% of all the islands created could hold a full assemblage of bird species

comparable to the intact forest (Aurélio-Silva *et al.* 2016). Similar patterns have been found in other taxonomic groups, including large terrestrial and arboreal vertebrates (Benchimol & Peres 2015a), lizards (Palmeirim *et al.* 2017), small mammals (Palmeirim *et al.* 2018) and trees (Benchimol & Peres 2015a), making forest fragmentation induced by hydroelectric dams a major threat to Amazonian biodiversity (Laurance *et al.* 2002, 2011, 2018, Ferraz *et al.* 2003, 2007, Stouffer *et al.* 2009, Wolfe *et al.* 2015).

Although we understand the effects of fragmentation on species richness and ecological-functional traits (Edwards *et al.* 2013, Hamer *et al.* 2015, Bicudo *et al.* 2016, Pryde *et al.* 2016, Bueno *et al.* 2018), we still need to explore in depth the physiological mechanisms responsible for how organisms respond to such environmental alterations (Wingfield 2013, Messina *et al.* 2018). Physiological changes due to habitat fragmentation can be less obvious than those indicated by species presence/absence data or density. Nevertheless, they have been shown to be a useful way to provide immediate measures of the effects of human-induced environmental disturbance (Lucas *et al.* 2006).

One of the most significant measures of physiological changes is provided by levels of corticosterone (CORT), the main glucocorticoid steroid of amphibians, reptiles, birds and some mammals (e.g. rodents), secreted by the cortex of the adrenal gland (Buchanan 2000, Sapolsky *et al.* 2000, Schoech *et al.* 2011). When vertebrates experience situations with negative effects (e.g. agonistic interactions), the hypothalamic–pituitary–adrenal axis (HPA) is activated resulting in increased CORT synthesis (Romero *et al.* 1998, Sapolsky *et al.* 2000). Because CORT acts on several metabolic and behavioural pathways that help birds cope with environmental changes (Wingfield 2002, Cockrem 2007), this hormone is considered a biomarker by some authors (Fairhurst *et al.*, 2011; Warne *et al.*, 2015; Harris *et al.*, 2017; but see Madliger *et al.*, 2015). Although short-term elevations in CORT levels may have evolved as an adaptive defence mechanism to regulate homeostasis, chronically elevated levels have negative consequences for the survival of individuals (Sapolsky *et al.* 2000, Blas 2015).

In fragmented landscapes, birds may survive despite high levels of environmental stress caused by the use of suboptimal habitats (Mokross *et al.* 2018)

and lower food availability induced by edge effects (Zanette *et al.* 2000). For instance, higher levels of CORT, due to habitat fragmentation, led to decreased quality and offspring survival in the Eurasian Treecreeper *Certhia familiaris* (Suorsa *et al.* 2004). In addition, some studies have shown that even generalist bird species inhabiting recently logged forests may present higher levels of CORT than those living in undisturbed areas (Lucas *et al.* 2006), suggesting that the stress response to habitat fragmentation is not exclusive to specialist birds (e.g., Suorsa *et al.*, 2003, 2004).

Glucocorticoids have been measured in free-living individuals of many species but an important methodological constraint is that CORT levels increase very rapidly upon capture, and thus samples need to be taken in a very short time (Romero & Reed 2005, Blas 2015). This limitation has led to the development of less invasive techniques that rely on the long-term accumulation of CORT in tissues (Bortolotti *et al.* 2008, Sheriff *et al.* 2011, Romero & Fairhurst 2016). In the case of birds, feathers are currently used for measuring levels of corticosterone (fCORT), which is incorporated and accumulated in growing feathers, when feather structures are being irrigated by blood (Fairhurst *et al.* 2013, Jenni-Eiermann *et al.* 2015). The use of fCORT was pioneered by Bortolotti and colleagues (Bortolotti *et al.* 2008), since when there has been a great increase in the number of studies employing this way of assessing CORT levels (e.g., Legagneux *et al.*, 2013; Patterson *et al.*, 2015; Freeman & Newman, 2018; Ganz *et al.*, 2018; Bosholn *et al.*, 2019). This is because fCORT reflects an integrative measure of plasma CORT levels (Lattin *et al.* 2011, Fairhurst *et al.* 2013), providing a retrospective and cumulative record of the physiological response to ecological challenges (Bortolotti *et al.* 2008, López-Jiménez *et al.* 2017, Ganz *et al.* 2018). In addition, feather collection is minimally invasive and samples can be easily stored for long periods of time (Fairhurst *et al.* 2013, Hansen *et al.* 2016, Romero & Fairhurst 2016).

In the face of increased global habitat loss and fragmentation (Lucas *et al.* 2006), we evaluated whether the well documented effects of habitat fragmentation upon species occurrence patterns are also mirrored by feather corticosterone levels. Since smaller islands are expected to present reduced or altered availability of food resources (Zanette *et al.* 2000, Suorsa *et al.* 2004), we predicted that this would lead

to an increase of CORT levels to restore homeostasis (Suorsa *et al.* 2004) and that this could be assessed in feather CORT (fCORT) levels.

With this less invasive method, we can evaluate physiological traits operating over an ecologically meaningful time scale, including exposure and response to environmental disturbances (Blas 2015), enabling us to assess the hidden effects of environment changes on animals (Berk *et al.* 2016).

## **Materials and methods**

### **Study Area**

The study was conducted at the Balbina Hydroelectric Reservoir, within Amazonas state, Brazil, which was filled in 1986 by damming the Uatumã River, a left-bank tributary of the Amazon. Given the flat topography of the area, the resulting lake covers an area of approximately 312,900ha, including over 3,500 land-bridge islands ranging widely in size and shape, surrounded by freshwater (Benchimol & Peres 2015a).

The vegetation of most islands consists of dense, closed-canopy, terra-firme forest with some small islands affected by edge-related windfalls and wildfires, that occurred during a late-1997 to early-1998 El Niño drought (Benchimol & Peres 2015c). This has led to major environmental changes caused by fire, which further increases the adverse situations that the species living on these islands have suffered.

The annual mean temperature is 28°C and mean rainfall 2,376mm, with the wet season spanning November to April (IBAMA 1997). The islands are within or adjacent to areas of the Uatumã Biological Reserve (1°13'38"S, 59°27'28"W), a federal conservation unit that covers both the island landscape and the contiguous adjacent upland areas on the east bank of the Uatumã River.

## Sampling design and feather collection

For our study, we selected 13 islands from the Balbina reservoir, ranging in size from 13.6 to 673.4ha and situated at least 2km from two continuous forest areas (CF) located on both sides of the lake (Control areas; Fig 1). We assigned area values of one order of magnitude greater than our two largest islands (i.e. 6,730.4ha, 3.83 in log) to the CF areas, following the logic of another study in the same area (Benchimol & Peres 2015a).

To test the possibility of spatial correlation in fCORT data, we performed Mantel tests (mantel function of the vegan package for R, Oksanen *et al.*, 2015) for each species by constructing distance matrices for fCORT values and geographic locations of the forest areas (islands or CF). The results showed non-significant spatial correlation patterns for all species (Supplementary material appendix 1B, Table B1), and thus the forest areas sampled (islands or CF) were assumed to be spatially-independent units.

Although we lack data on dispersion and movement of birds between islands in our study area, we assumed a very low level of dispersion between islands due to the typically low dispersion and flight capacity observed for Amazonian understory birds (Moore *et al.* 2008, Ibarra-Macias *et al.* 2011).

Birds were routinely captured by a team of 2–3 people, using 14 Ecotone nylon mist-nets of 12 x 2.5 m, mesh size: 32 mm. Mist-nets were placed in the understory along a continuous linear net-line (c. 200m) using previously established trails within each area, avoiding island edges by sampling at least 250m from the island boundaries. Mist-nets were left open for three consecutive days in each area (three sampling days \* 15 areas, 45 days of work), from 06:00h to 12:00h and inspected every half hour. The fieldwork was conducted from June to September 2016 and March to July 2017. Captured birds were identified to species level and ringed with standard metallic rings provided by CEMAVE (Centro Nacional de Pesquisa para Conservação das Aves Silvestres).

Feathers were only collected from adult birds (i.e. those that had reached sexual maturity in which the feather was produced through a moult process). We strived to sample the same feather types from each specimen to avoid possible bias

due to differences in fCORT deposition (Harris *et al.* 2016). Hence, assayed feathers were fully-grown and from the same locations in all birds captured: the eighth primary from both wings (numbered descendantly: innermost primary = 1) and the two outermost rectrices (tail feathers: right R6 and left R6). Feather numbering and classification follow Pyle (1997). In addition, we only collected feathers that showed no signs of abrasion. Feathers were stored in individual paper envelopes until fCORT levels were assayed in the laboratory.

We were unable to collect feather samples from all study sites for all species. Therefore, assays included a subsample of eight species for which we obtained sample sizes large enough for analyses at species level (12–58 individuals across forest areas of different sizes, either on islands or CF). The eight species represent understory birds (Del Hoyo *et al.* 2014), regarded *a priori* to be susceptible to habitat fragmentation (Stouffer *et al.* 2009, Aurélio-Silva *et al.* 2016, Bueno *et al.* 2018, Laurance *et al.* 2018) and that exhibit low dispersal ability through non-forest matrix habitats (Sekercioglu *et al.* 2002, Laurance *et al.* 2004, Moore *et al.* 2008): Plain-brown Woodcreeper *Dendrocincla fuliginosa*, Wedge-billed Woodcreeper *Glyphorhynchus spirurus*, Chestnut-rumped Woodcreeper *Xiphorhynchus pardalotus*, White-crowned Manakin *Pseudopipra pipra*, Guianan Antwren *Hypocnemis cantator*, White-flanked Antwren *Myrmotherula axillaris*, Black-headed Antbird *Percnostola subcristata* and White-necked Thrush *Turdus albicollis*.

Except for the *Pseudopipra pipra*, which is primarily frugivorous (but see, Fair *et al.*, 2013) the other species are insectivorous, the group most affected by habitat fragmentation (Sekercioglu *et al.* 2002, Aurélio-Silva *et al.* 2016, Laurance *et al.* 2018). None of the selected species exhibit a well-marked annual moulting season and instead show peaks of moulting usually at the end of the dry season (December) and in the middle of wet season (March), generally following the peaks of the breeding cycle (Johnson *et al.* 2012). It is not uncommon to find species moulting during each month, with substantial interspecific variation in both moult timing and duration (Johnson *et al.* 2012, Stouffer *et al.* 2013). Due to their limited dispersal ability (Laurance *et al.* 2004, Van Houtan *et al.* 2007, Moore *et al.* 2008), and the fact that the species were present in the study area since the creation of the dam reservoir (Willis & Oniki 1988), we expect that birds were equally exposed to stressors independent of age or timing of moult.

This study was approved by the Chico Mendes Institute for Biodiversity Conservation (license to TB, number 51536-5).

### **Feather corticosterone measurement**

All fCORT measurements followed a slight modification of a published protocol (Bortolotti *et al.* 2008). In brief, a methanol-based extraction technique was used to extract CORT from feathers, since steroid hormones are generally soluble in polar alcohols such as methanol (Pötsch & Moeller 1996).

All feather samples were equally processed, removing the calamus and mincing the rachis and vanes into pieces of less than 5mm, resulting in a highly homogeneous mixture. All feathers per individual were pooled and weighed on an analytical balance (A&D instruments, model FX-40) with 0.0001g of precision, to determine the feather mass available for fCORT extraction, and then transferred to a silanized glass tube (to increase hormone recovery).

We strived to obtain similar feather masses for each species, so that variation in the assay response due to feather mass would not bias within-species analyses, allowing for the representation of fCORT levels per unit mass (pg/mg) (Freeman & Newman 2018). Six millilitres of methanol were added to the sample, and tubes were placed in a sonicating water bath at room temperature for 30min, followed by overnight incubation (19 hours) in a shaking 50°C water bath. Subsequently, the methanol was separated from the feather remains using a nylon syringe filter (0.45µm). Feather debris was washed twice with 2ml of methanol to recover residual extracts, which were added to the previous methanol extract. The pooled methanol extract was then placed in a 50°C water bath and evaporated under a stream of nitrogen. Extract residues were resuspended in 150µl steroid-free serum (DRG, Germany) and then vigorously vortexed for 10min in a multi-tube vortexer. Reconstituted samples were frozen at -20°C until assays were conducted to quantify CORT levels. Recovery of cold-spiked samples was 107% (SD = 5,75, n = 6). Linearity of the spiked sample was parallel to the expected dilution (expected dilution in logs:  $y = -0.1931x + 2.6743$ , spiked sample:  $y = -0.198x + 2.7149$ ).



To measure fCORT concentrations we used an enzyme immunoassay (DRG, Germany). Inter- and intra-assay variability was assessed using the coefficient of variation (CV) of known standards. Samples and standards were run in duplicate across nine assays with a mean intra-assay CV of 9.17% and inter-assay CV of 12.88%. Serial dilutions revealed parallel displacement to the standard curves. All samples were above detection limits (1.1 pg/ml). All feather samples were randomised during the extraction and assay processes to avoid differences due to variation between extraction batches or assay plates. All fCORT analyses were performed at the Laboratory of Ecophysiology at the National Museum of Natural Sciences (MNCN) in Madrid (Spain).

### **Statistical analysis**

Before testing specific effects of island size on fCORT levels, we ran a linear mixed model (LMM) to test for expected effects of feather mass on the amount of hormone present in feathers, as this variable may affect CORT levels (Lattin *et al.* 2011, Jenni-Eiermann *et al.* 2015, Berk *et al.* 2016). We used feather mass as predictor of fCORT levels. Because this variable is generally species-specific, we also included species as random effects. In order to control for the effects of feather mass in fCORT levels, the residuals obtained from this analysis (see below) were used in all subsequent analyses involving fCORT. Given the highly skewed distribution of fCORT concentration and the irregular distribution of forest size (island and CF), these variables were log-transformed prior to analyses.

To test the hypothesis that fCORT levels increase with decreasing island size, we used a linear mixed model (LMM) pooling all species together, where the residuals obtained in the prior analysis (expected effects of feather mass in fCORT levels) were used as response variable and log-transformed forest size as predictor. Since we expected the response to fragmentation to show species-specific differences, we used species and island as a random effect. Visual inspection of residual plots did not reveal deviations from homoscedasticity or normality.

Finally, in order to analyse effects of fragmentation on species-specific fCORT levels, we conducted LMMs for individual species, using fCORT ( $\mu\text{g mg}^{-1}$ ; log-

transformed) as response variable, log-transformed forest size (islands or CF) and feather mass as fixed predictors, and island identity as random factor.

All statistical analyses were conducted using R 3.4.0 (R Development Core Team, Vienna). Linear mixed models were fitted using restricted maximum likelihood, lmer function in the lme4 package (Bates *et al.* 2015). The goodness of fit of linear mixed models was based on the marginal  $R^2$  and a conditional  $R^2$  (based on maximum likelihood) giving the variance explained by fixed effects, and both fixed and random effects, respectively, using the r.squaredGLMM function in the MuMIn package (Nakagawa & Schielzeth 2013, Barton 2019). In our models, p-values were based on the Satterthwaite approximation to degrees of freedom, lmerTest package (Kuznetsova *et al.* 2017).

## Results

We measured the fCORT levels of 265 individuals (225 from islands and 40 from CF) from eight different species ( $X \pm SD = 33.0 \pm 14.7$ ; Supplementary material appendix 1, Table B2). The fCORT concentrations ( $\text{pg mg}^{-1}$ ; log-transformed) were negatively correlated with feather mass when all species were pooled in the analysis (t-Student test:  $t_{6.66} = -4.96$ ,  $P = 0.01$ ). However, individual models per species showed that feather mass did not affect fCORT levels at the species level, possibly because of the reduced variation in assayed feather mass due to our laboratory protocol (Table 1).

Our hypothesis that fCORT would vary among islands according to their size was not met for the overall dataset (Table 2). The response in fCORT of each species to forest size was heterogeneous. Thus, we ran individual models for each species (Table 1), finding a significant island size-related estimate for only one species, the *Hypocnemis cantator*: which showed increasing levels of fCORT in smaller forests (Table 1; Fig 2), with a marginal and conditional  $R^2$  of 0.13. All the other species showed no significant association between fCORT levels and forest size.

## Discussion

Habitat fragmentation may induce reductions in species diversity that may in turn affect the individual fitness of the individuals that survive in this area. Using a true island system to examine the effects of forest habitat fragmentation on levels of feather corticosterone (fCORT) of Amazonian birds, we tested the hypothesis that fCORT levels would be affected by island size, expecting fCORT to increase with decreasing island size.

Our results did not support our expectations of overall higher fCORT levels in smaller forests, when all species were pooled in the same analysis. As such, fCORT was not affected by island size across species. This is in contrast to previous analyses in other species where habitat fragmentation was found to lead to elevated stress levels, as measured by increased CORT concentration within fragments (Suorsa *et al.* 2003, 2004, Lucas *et al.* 2006, Leshyk *et al.* 2012).

Birds living in habitat fragments are expected to experience stressful situations, such as lower food supply and greater exposure to predators, more frequently, which can lead to chronic stress (Romero 2004, Rich 2005, Blas 2015). However, contrary effects may occur, as found in a study comparing island and mainland populations of the Eurasian Blue Tit *Cyanistes caeruleus* where the results suggest that islands may select for lower CORT levels, perhaps because of lower rates of intraspecific aggression (Müller *et al.* 2007).

The lack of observed effects in our general analysis can be due to several reasons. First, since the Balbina archipelago was formed 28 years ago, birds may have adapted to these new conditions, i.e. individuals may have already responded to the new biotic and abiotic settings on islands. It is known that fragmentation leads to the extinction of highly area-sensitive species (Ferraz *et al.* 2003, Stouffer *et al.* 2009, Bregman *et al.* 2014, Aurélio-Silva *et al.* 2016). Thus, those species that we still find in smaller fragments may be those with a higher level of resilience. A second possibility is that birds are still stressed in the fragmented landscape, but they show a downregulation of glucocorticoids, with a return to baseline levels as a strategy to avoid the deleterious effects of chronic CORT elevation (Romero 2004, Rich 2005, Blas 2015). Indeed, it has been shown that chronic stress may result in both

increasing and decreasing patterns of glucocorticoid secretion according to species (Dickens & Romero 2013).

Alternative explanations that may help to interpret the observed lack of association between forest size and fCORT levels include the consequences of the variation in moulting dynamics among species in our fCORT data, given that individuals may moult at different periods within the year, thus potentially presenting seasonal variation in fCORT. In this regard, asynchronous moulting periods in tropical environments may make fCORT-based studies less reliable in these regions than in temperate areas, where birds have more synchronised moulting periods. Another possibility is the relatively small sample size. Despite the large sampling effort, only a relatively small number of individuals of each species could be captured. Although we do not have *a priori* expectations of effect sizes, given the moderate effects that are typical of biological processes (Møller & Jennions 2002), a larger sample size would have been desirable to avoid potential type-II errors.

In spite of the lack of relationship between fCORT levels and forest size for the data pooled across species, we did find the expected association in the *Hypocnemis cantator* (Thamnophilidae). A small percentage of the population of this species showed increasing fCORT levels with decreasing forest size. This raises the question of what is special about this species in comparison to the others analysed, in particular, the other two antbird species (*Percnostola subcristata* and *Myrmotherula axillaris*), for which we found no relationship between CORT and forest size. Decreased food availability in smaller islands is a first possibility. Antbirds typically feed on small insects and other arthropods taken from twigs and foliage in the understory of the forest, and some species specialise in following army ants (Del Hoyo *et al.* 2014). However, we could not find any clues in the scant bibliography on this species. For instance, a study in French Guiana showed that the prey size taken by the *Hypocnemis cantator* is very similar to that of other antbirds of similar size, such as the *Myrmotherula axillaris* (Thiollay 1988). In addition, we found no differentiation of this species in its isotopic nitrogen and carbon signatures (authors' unpublished data). Other aspects of its life history traits, such as the existence of male-female duets in this species (Seddon & Tobias 2006), may set this species apart in terms of social requirements. However, song duets are also present in the other antbirds that we studied. Finally, a previous study of area sensitivity in these

islands showed that, although species richness was lower in small islands, the *Hypocnemis cantator* was not particularly affected by island size (Aurélio-Silva *et al.* 2016). This runs counter our finding and suggests that apparent population resistance may hide reductions in individual physiological condition.

The strong differences in fCORT levels found among species were largely due to the negative relationship between feather mass and fCORT levels, by which larger species had larger feathers, and thus lower fCORT levels. After removing the effect of feather mass, differences among species in fCORT levels remained but they were no longer related to body mass, but may rather have been due to differences in ecological and life-history related attributes (Hau *et al.* 2010). Lastly, given that fCORT is an indirect measure of hormonal loads, which may be affected by a complex suite of factors, including feather abrasion and sunlight exposure (Freeman & Newman 2018), it would be premature to try to use this measurement as a *prima facie* indication of differences between species in their responses to environmental changes.

To conclude, we found weak evidence for the hypothesis that habitat fragmentation leads to increased levels of glucocorticosteroid production in this set of Amazonian species. Out of the eight species studied, only one, the *Hypocnemis cantator*, showed the expected pattern. This pattern shows that different species respond differently to stressors, perceiving the same stimulus to be more or less of a threat (Cockrem 2013), thus generating different physiological responses by species to the same stimulus, as some species are more area-sensitive than others (Sekercioglu *et al.* 2002, Anjos 2006, Dickens & Romero 2013, Bueno *et al.* 2018). Further studies are necessary to assess the reliability of this technique, and to determine which parameters are useful to understand how human-driven insularization affects avian population resilience.

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## SÍNTESE

Neste trabalho, propomos o uso de dois indicadores ecológicos para estudos com aves em áreas insularizadas que foram formadas a partir da construção da usina hidrelétrica de Balbina, na Amazônia central.

No primeiro capítulo, utilizamos o monitoramento acústico passivo para avaliar o desempenho de sete índices acústicos (índices de biodiversidade baseados na análise da paisagem acústica) nas ilhas do reservatório da usina hidrelétrica. Com isso, buscamos acessar: (1) a relação entre riqueza de aves e índices acústicos; (2) os padrões da paisagem acústica associadas ao tamanho da ilha e (3) para caracterizar os padrões das paisagens acústicas nas ilhas ao longo de 24h. A partir dos nossos resultados, concluímos que os índices acústicos apresentam diferentes padrões, com mudanças marcantes entre dia e noite, refletindo a riqueza real de espécies de aves observadas relacionadas com o tamanho da área da ilha. No geral, concluímos que mesmo em um ambiente altamente diverso, como florestas tropicais, os índices acústicos são uma excelente ferramenta complementar para a avaliação da biodiversidade quando fornecidos com um banco de dados fundamentado, mas que não devem substituir métodos tradicionais de levantamento da biodiversidade. Adicionalmente, nós apresentamos evidências que a insularização artificial de ambientes pela construção de barragens hidrelétricas, leva também a modificações na paisagem acústica desses ambientes.

No segundo capítulo, nós procuramos entender os mecanismos fisiológicos que estão por trás da capacidade de alguns organismos viverem em ambientes insularizados. Para isso, utilizamos os níveis de corticosterona, principal hormônio glicocorticoide das aves, como um indicador fisiológico do estresse, avaliando se os níveis de corticosterona refletem os efeitos que a insularização do habitat tem na ocorrência das espécies. Partindo do princípio que ilhas menores podem ter uma redução na qualidade do ambiente remanescente, podendo levar a um aumento do estresse nos organismos que vivem nesses locais, prevemos que aves que vivem em ilhas menores apresentariam níveis mais altos de corticosterona quando comparado com aves que vivem em ilhas maiores ou áreas de floresta contínua. No entanto, nossos resultados não apoiaram essa hipótese, exceto para a espécie *Hypocnemis cantator*, que apresentou o padrão esperado: diminuição dos níveis de

corticosterona nas penas com o aumento do tamanho da ilha. Essas diferenças sugerem que as espécies respondem de maneira diferente aos estressores causados pela fragmentação e mais estudos são sugeridos.

Em geral, podemos concluir que o uso desses indicadores ecológicos são promissores para estudos e monitoramentos em ambientes que sofreram distúrbios antrópicos. Porém, salientamos que cada espécie reage de maneira diferente ao mesmo distúrbio e assim, características espécie-específicas devem ser levadas em consideração para uma interpretação mais acurada das respostas retornadas pelos indicadores utilizados.



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### Figure legends

Fig 1. Spatial distribution of the study area and the 13 islands and two continuous forest site (Control areas) surveyed at the Balbina Hydroelectric Reservoir, state of Amazonas, Brazil.

Fig 2. Relationships between log fCORT levels and log island size in *Hypocnemis cantator* (N=28) sampled in the Balbina reservoir, Amazonas state, Brazil. The shaded area represents the 95% confidence interval and triangles represent the continuous forest (CF) fCORT values.



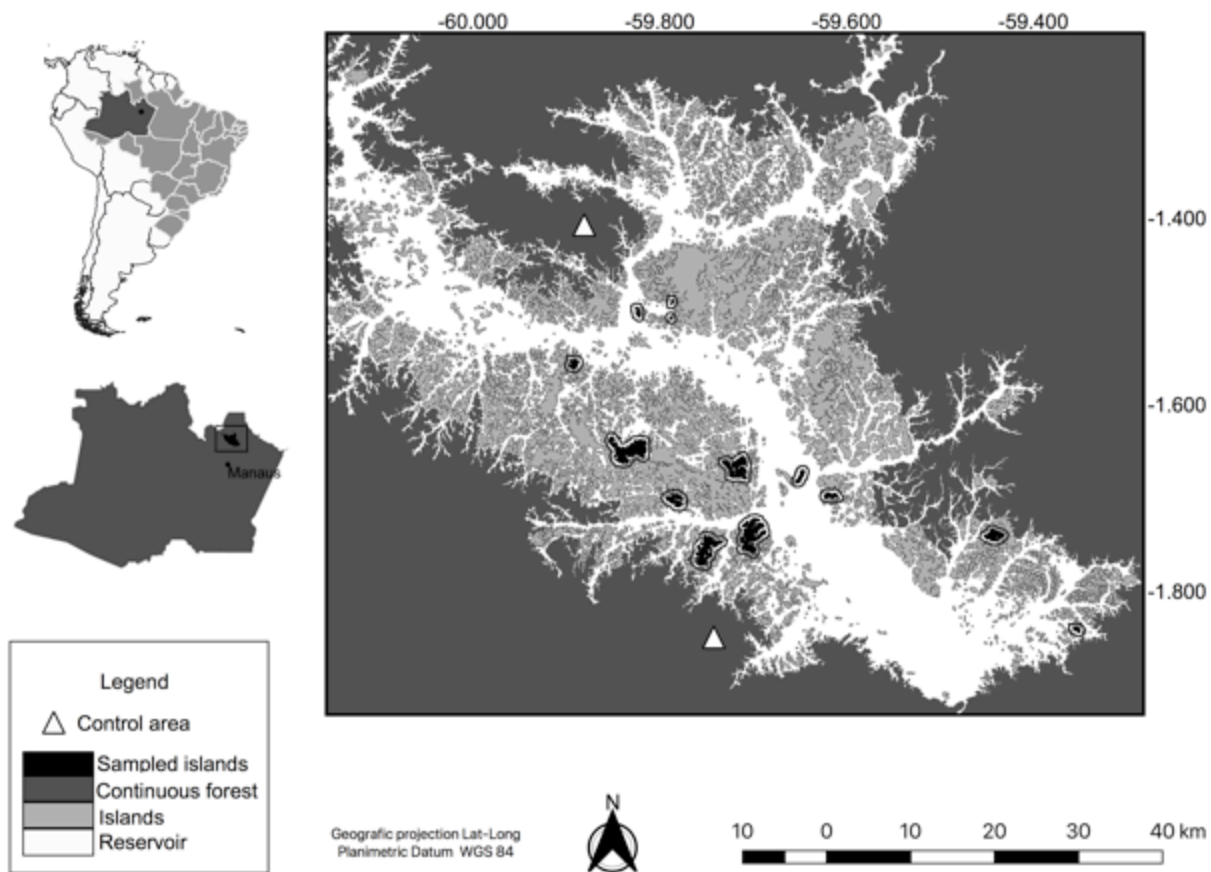


Fig. 1

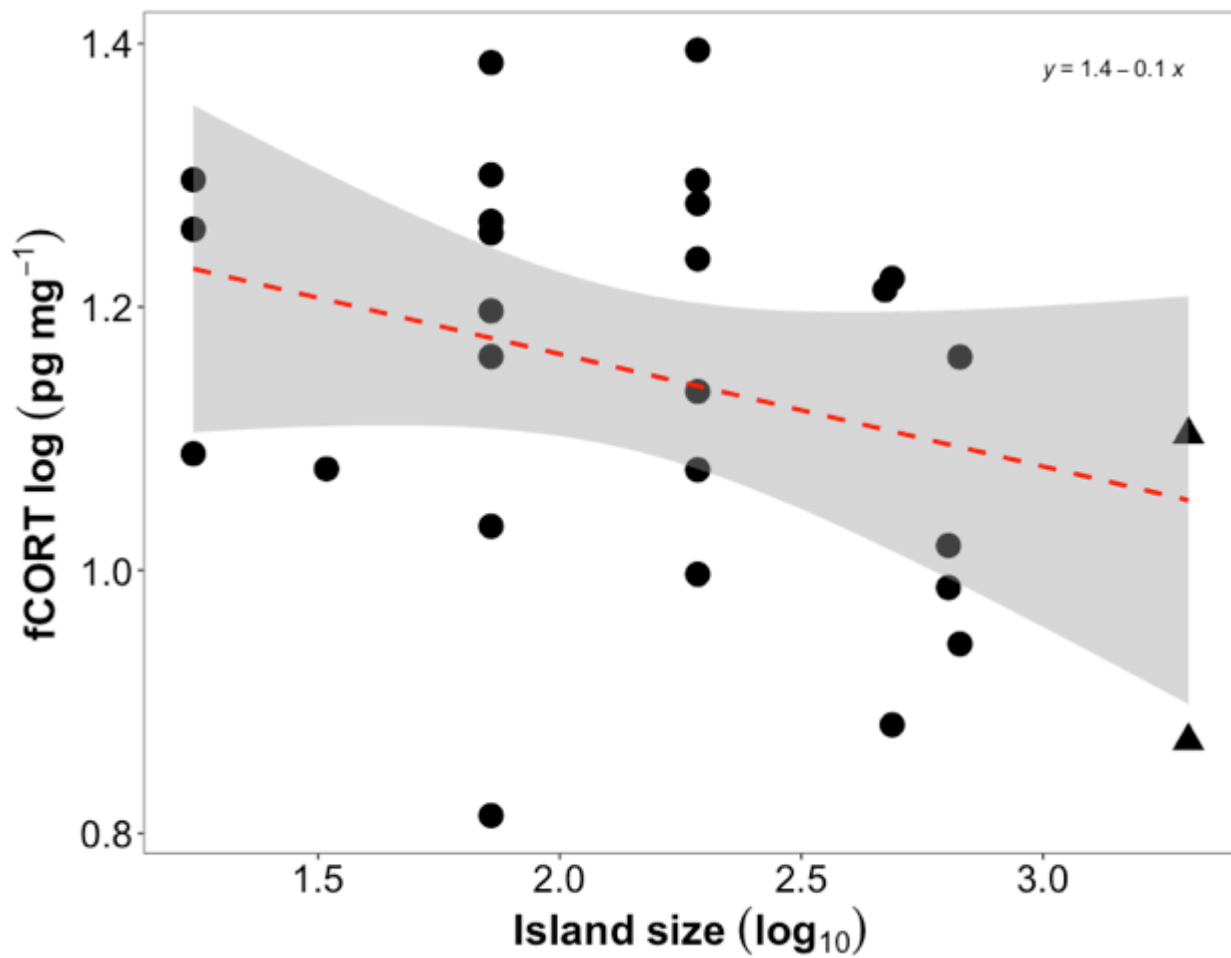


Fig. 2

Table 1. Table containing the species-specific statistics of linear mixed models, for each species sampled in the Balbina reservoir. Bold numbers represent probability values that reached statistical significance.

Species	N	fCORT (Average)	fCORT (SD)	Parameters	Estimated	SE	t value	Pr(> t )
<i>Myrmotherula axillaris</i>	37	18.43	5.46	Island size (log <sub>10</sub> )	-0.06	0.04	-1.50	0.14
				Feather mass	-0.04	0.02	-1.55	0.13
<i>Dendrocincla fuliginosa</i>	20	3.73	1.80	Island size (log <sub>10</sub> )	0.10	0.07	1.37	0.20
				Feather mass	-0.01	0.06	-0.15	0.88
<i>Pseudopipra pipra</i>	58	10.01	4.64	Island size (log <sub>10</sub> )	0.04	0.03	1.15	0.25
				Feather mass	-0.00	0.02	-0.27	0.78
<i>Glyphorhynchus spirurus</i>	43	5.82	1.90	Island size (log <sub>10</sub> )	-0.01	0.04	-0.27	0.78
				Feather mass	-0.02	0.03	-0.86	0.39
<i>Hypocnemis cantator</i>	28	14.68	4.93	Island size (log <sub>10</sub> )	-0.10	0.05	-2.01	<b>0.05</b>
				Feather mass	-0.00	0.04	-0.02	0.97
<i>Percnostola subcristata</i>	12	6.79	3.60	Island size (log <sub>10</sub> )	0.09	0.08	1.19	0.34
				Feather mass	0.05	0.04	1.16	0.28
<i>Turdus albicollis</i>	25	3.85	1.21	Island size (log <sub>10</sub> )	-0.05	0.05	-1.12	0.30
				Feather mass	-0.00	0.01	-0.44	0.65
<i>Xiphorhynchus pardalotus</i>	42	5.36	2.79	Island size (log <sub>10</sub> )	0.01	0.05	0.29	0.76
				Feather mass	-0.01	0.02	-0.49	0.62

Table 2. Linear mixed model statistics for all eight species pooled (265 individuals) to explain residual fCORT levels with island size.

Parameters	Estimate	Std. Error	t value	Pr(> t )
Intercept	-0.01	0.09	-0.13	0.89
Island Size ( $\log_{10}$ )	0.00	0.03	0.13	0.89

# ICES

## VOLUME I - APÊNDICE A

List of the bird species most found in the acoustic records, and the respective filters used in the acoustic

Species	High pass Filter (Hz)	Low pass Filter (Hz)
<i>arsicolor</i>	2000	13000
<i>autumnalis</i>	340	7500
<i>arinosa</i>	500	10000
<i>na</i>	300	6000
<i>optera</i>	300	6000
	300	6000
<i>iceus</i>	1600	4000
<i>chrysoptera</i>	2000	10000

Species	High pass Filter (Hz)	Low pass Filter (Hz)
<i>Bucco capensis</i>	940	23000
<i>Bucco tamatia</i>	1200	35000
<i>Cacicus cela</i>	600	10000
<i>Cacicus haemorrhous</i>	600	10000
<i>Campephilus melanoleucos</i>	600	50000
<i>Campylorhamphus procurvoides</i>	1500	60000
<i>Caryothraustes canadensis</i>	1150	10000
<i>Celeus torquatus</i>	1500	50000
<i>Cercomacra cinerascens</i>	1100	35000
<i>Cercomacroides tyrannina</i>	950	62000
<i>Coereba flaveola</i>	3600	15000
<i>Conopias parvus</i>	1200	65000

Species	High pass Filter (Hz)	Low pass Filter (Hz)
<i>s variegatus</i>	1300	2600
<i>nitidus</i>	4200	12000
<i>rujanensis</i>	1800	4000
<i>us lineatus</i>	1300	2200
<i>ira stictolaema</i>	850	5000
<i>astes rufigula</i>	1060	3000
<i>cla fuliginosa</i>	650	4000
<i>aptes certhia</i>	1600	5000
<i>aptes picumnus</i>	1200	6000
<i>ora</i>	3800	15000
<i>lineatus</i>	650	9000
<i>cayennensis</i>	3000	7000
<i>chrysopasta</i>	1700	10000
<i>s analis</i>	1400	2400
<i>s colma</i>	1800	4000
<i>irostris</i>	2200	5000
<i>hardyi</i>	900	2000

Species	High pass Filter (Hz)	Low pass Filter (Hz)
<i>Glyphorynchus spirurus</i>	1800	6000
<i>Hemitriccus zosterops</i>	1200	6000
<i>Hylexetastes perrotii</i>	1000	6000
<i>Hylopezus macularius</i>	540	5000
<i>Hylophilus muscicapinus</i>	2100	6000
<i>Hylophilus semicinereus</i>	1700	10000
<i>Hypocnemis cantator</i>	1300	7000
<i>Ibycter americanus</i>	600	6000
<i>Isleria guttata</i>	3400	6000
<i>Jacamerops aureus</i>	1900	4000
<i>Lamprospiza melanoleuca</i>	3000	10000
<i>Laniocera hypopyrra</i>	2300	5000
<i>Legatus leucophaeus</i>	2000	10000
<i>Lepidocolaptes albolineatus</i>	2300	7000
<i>Lepidothrix serena</i>	1500	4000
<i>Leptotila rufaxilla</i>	420	7000
<i>Leucopternis melanops</i>	2500	15000

Species	High pass Filter (Hz)	Low pass Filter (Hz)
<i>ociferans</i>	600	7000
<i>us vitiosus</i>	1000	6500
<i>s cruentatus</i>	600	11000
<i>ilvicollis</i>	350	2900
<i>nirandollei</i>	520	2500
<i>uficollis</i>	340	6000
<i>semitorquatus</i>	800	2000
<i>nomota</i>	240	500
<i>ra</i>	800	5000
<i>tuberculifer</i>	1750	6000
<i>gaimardii</i>	2500	10000
<i>caudatus</i>	3500	10000
<i>us ferrugineus</i>	2200	6000
<i>a campanisona</i>	600	2000
<i>ula axillaris</i>	1200	4000
<i>ula brachyura</i>	1600	4500
<i>ula menetriesii</i>	1600	4000

Species	High pass Filter (Hz)	Low pass Filter (Hz)
<i>Nonnula rubecula</i>	1600	4000
<i>Notharchus macrorhynchos</i>	1400	4000
<i>Nyctibius griseus</i>	320	2000
<i>Ortalis motmot</i>	270	1000
<i>Pachyramphus marginatus</i>	2200	5000
<i>Pachyramphus surinamus</i>	1500	5000
<i>Patagioenas plumbea</i>	200	1200
<i>Patagioenas subvinacea</i>	400	1400
<i>Penelope marail</i>	300	2700
<i>Percnostola subcristata</i>	1750	4000
<i>Perissocephalus tricolor</i>	200	2000
<i>Phaethornis ruber</i>	5500	15000
<i>Piaya cayana</i>	1500	5000
<i>Piculus flavigula</i>	1200	7000
<i>Pionus fuscus</i>	800	6000
<i>Pionus menstruus</i>	1000	10000
<i>Piprites chloris</i>	1400	3000

Species	High pass Filter (Hz)	Low pass Filter (Hz)
<i>Trons</i>	2750	5000
<i>alybea</i>	1500	6000
<i>s viridis</i>	350	15000
<i>us aracari</i>	800	8000
<i>erspicillata</i>	180	900
<i>a</i>	1500	6000
<i>icta</i>	800	11000
<i>irpurata</i>	850	2200
<i>os tucanus</i>	800	7000
<i>os vitellinus</i>	1500	4000
<i>enus melanurus</i>	2200	4000
<i>lus carbo</i>	2000	10000
<i>gon ruficauda</i>	1250	7000
<i>a simplex</i>	1150	5000
<i>ossus</i>	1500	3000
<i>olivacea</i>	2600	5000
<i>piperivora</i>	850	3000

Species	High pass Filter (Hz)	Low pass Filter (Hz)
<i>Spizaetus ornatus</i>	1150	3000
<i>Tachyphonus surinamus</i>	2000	15000
<i>Tangara punctata</i>	4500	13000
<i>Thamnomanes caesius</i>	800	8000
<i>Thamnophilus murinus</i>	550	4000
<i>Tityra cayana</i>	1200	7000
<i>Todirostrum pictum</i>	2300	15000
<i>Tolmomyias assimilis</i>	1500	6000
<i>Tolmomyias poliocephalus</i>	2000	7000
<i>Touit huetii</i>	1150	6200
<i>Trogon melanurus</i>	500	2400
<i>Trogon rufus</i>	700	3000
<i>Trogon violaceus</i>	750	4000
<i>Trogon viridis</i>	650	2400
<i>Turdus albicollis</i>	1300	2600
<i>Tyranneutes virescens</i>	2000	5500
<i>Tyrannulus elatus</i>	1600	5000



Species	High pass Filter (Hz)	Low pass Filter (Hz)
<i>melancholicus</i>	3200	15000
<i>cassini</i>	1300	5000
<i>ceus</i>	2000	6000
<i>poecilinotus</i>	1700	7000
<i>nutus</i>	2800	10000
<i>chus pardalotus</i>	1300	6000
<i>acer</i>	2500	4400

List of all species identified in the dawn chorus  
 (05:30-08:30 am) from the sampled areas (n=14) in

	Family	Order
<i>versicolor</i>	Trochilidae	Apodiformes
<i>autumnalis</i>	Psittacidae	Psittaciformes
<i>varinosa</i>	Psittacidae	Psittaciformes
<i>ucophthalma</i>	Psittacidae	Psittaciformes
<i>nomomeus</i>	Tyrannidae	Passeriformes
<i>viceus</i>	Tyrannidae	Passeriformes
<i>ochrolaemus</i>	Furnariidae	Passeriformes
<i>chrysoptera</i>	Psittacidae	Psittaciformes
<i>ensis</i>	Bucconidae	Galbuliformes
<i>atia</i>	Bucconidae	Galbuliformes
<i>la</i>	Icteridae	Passeriformes
<i>hemorrhous</i>	Icteridae	Passeriformes
<i>us</i> <i>cus</i>	Picidae	Piciformes
<i>us rubricollis</i>	Picidae	Piciformes
<i>ma obsoletum</i>	Tyrannidae	Passeriformes

the Balbina reservoir, Amazonas, Brazil.

Species	Family	Order
<i>Campylorhamphus</i> <i>procurvoides</i>	Dendrocolaptidae	Passeriformes
<i>Cantorchilus leucotis</i>	Troglodytidae	Passeriformes
<i>Caryothraustes canadensis</i>	Cardinalidae	Passeriformes
<i>Celeus elegans</i>	Picidae	Piciformes
<i>Celeus torquatus</i>	Picidae	Piciformes
<i>Celeus undatus</i>	Picidae	Piciformes
<i>Cercomacra cinerascens</i>	Thamnophilidae	Passeriformes
<i>Cercomacroides tyrannina</i>	Thamnophilidae	Passeriformes
<i>Certhiasomus stictolaemus</i>	Dendrocolaptidae	Passeriformes
<i>Chelidoptera tenebrosa</i>	Bucconidae	Galbuliformes
<i>Coereba flaveola</i>	Thraupidae	Passeriformes
<i>Conopias parvus</i>	Tyrannidae	Passeriformes
<i>Corythopsis torquatus</i>	Rhynchocyclidae	Passeriformes
<i>Cotinga Cayana</i>	Cotingidae	Passeriformes
<i>Crypturellus variegatus</i>	Tinamidae	Tinamiformes

	Family	Order
<i>caeruleus</i>	Thraupidae	Passeriformes
<i>nitidus</i>	Thraupidae	Passeriformes
<i>rujanensis</i>	Vireonidae	Passeriformes
<i>us lineatus</i>	Thamnophilidae	Passeriformes
<i>us arada</i>	Troglodytidae	Passeriformes
<i>rana</i>	Thraupidae	Passeriformes
<i>ra stictolaema</i>	Dendrocolaptidae	Passeriformes
<i>astes rufigula</i>	Dendrocolaptidae	Passeriformes
<i>cla fuliginosa</i>	Dendrocolaptidae	Passeriformes
<i>cla merula</i>	Dendrocolaptidae	Passeriformes
<i>aptus certhia</i>	Dendrocolaptidae	Passeriformes
<i>aptus picumnus</i>	Dendrocolaptidae	Passeriformes
<i>x picus</i>	Dendrocolaptidae	Passeriformes
<i>ora</i>	Pipridae	Passeriformes
<i>lineatus</i>	Picidae	Piciformes
<i>cayennensis</i>	Fringillidae	Passeriformes
<i>chrysopasta</i>	Fringillidae	Passeriformes
<i>ularis</i>	Falconidae	Falconiformes

Species	Family	Order
<i>Formicarius analis</i>	Formicariidae	Passeriformes
<i>Formicarius colma</i>	Formicariidae	Passeriformes
<i>Frederickena viridis</i>	Thamnophilidae	Passeriformes
<i>Galbula albirostris</i>	Galbulidae	Galbuliformes
<i>Galbula cyanicollis</i>	Galbulidae	Galbuliformes
<i>Galbula dea</i>	Galbulidae	Galbuliformes
<i>Galbula leucogastra</i>	Galbulidae	Galbuliformes
<i>Glaucidium hardyi</i>	Strigidae	Strigiformes
<i>Glyphorhynchus spirurus</i>	Dendrocolaptidae	Passeriformes
<i>Gymnopithys rufigula</i>	Thamnophilidae	Passeriformes
<i>Hemithraupis flavicollis</i>	Thraupidae	Passeriformes
<i>Hemitriccus zosterops</i>	Rhynchocyclidae	Passeriformes
<i>Hylexetastes perrotii</i>	Dendrocolaptidae	Passeriformes
<i>Hylopezus macularius</i>	Grallariidae	Passeriformes
<i>Hylophilus muscicapinus</i>	Vireonidae	Passeriformes
<i>Hylophilus ochraceiceps</i>	Vireonidae	Passeriformes
<i>Hylophilus semicinereus</i>	Vireonidae	Passeriformes
<i>Hylophilus thoracicus</i>	Vireonidae	Passeriformes

	Family	Order
<i>naevius</i>	Thamnophilidae	Passeriformes
<i>is cantator</i>	Thamnophilidae	Passeriformes
<i>ericanus</i>	Falconidae	Falconiformes
<i>ata</i>	Thamnophilidae	Passeriformes
<i>is aureus</i>	Galbulidae	Galbuliformes
<i>za melanoleuca</i>	Mitrospingidae	Passeriformes
<i>namus</i>	Thraupidae	Passeriformes
<i>hypopyrra</i>	Tityridae	Passeriformes
<i>ucophaius</i>	Tyrannidae	Passeriformes
<i>ptes</i> <i>'s</i>	Dendrocolaptidae	Passeriformes
<i>serena</i>	Pipridae	Passeriformes
<i>ifaxilla</i>	Columbidae	Columbiformes
<i>is melanops</i>	Accipitridae	Accipitriformes
<i>ociferans</i>	Cotingidae	Passeriformes
<i>us galeatus</i>	Rhynchocyclidae	Passeriformes
<i>us vitiosus</i>	Rhynchocyclidae	Passeriformes
<i>emitorquatus</i>	Caprimulgidae	Caprimulgiformes

Species	Family	Order
<i>Malacoptila fusca</i>	Bucconidae	Galbuliformes
<i>Megaceryle torquata</i>	Alcedinidae	Coraciiformes
<i>Megascops watsoni</i>	Strigidae	Strigiformes
<i>Melanerpes cruentatus</i>	Picidae	Piciformes
<i>Micrastur gilvicollis</i>	Falconidae	Falconiformes
<i>Micrastur mirandolei</i>	Falconidae	Falconiformes
<i>Micrastur ruficollis</i>	Falconidae	Falconiformes
<i>Micrastur semitorquatus</i>	Falconidae	Falconiformes
<i>Microbates collaris</i>	Poliotilidae	Passeriformes
<i>Momotus momota</i>	Momotidae	Coraciiformes
<i>Monasa atra</i>	Bucconidae	Galbuliformes
<i>Myiarchus tuberculifer</i>	Tyrannidae	Passeriformes
<i>Myiopagis gaimardii</i>	Tyrannidae	Passeriformes
<i>Myiornis ecaudatus</i>	Rhynchocyclidae	Passeriformes
<i>Myrmoderus ferrugineus</i>	Thamnophilidae	Passeriformes
<i>Myrmothera campanisona</i>	Grallariidae	Passeriformes
<i>Myrmotherula axillaris</i>	Thamnophilidae	Passeriformes
<i>Myrmotherula brachyura</i>	Thamnophilidae	Passeriformes

	Family	Order
<i>ula menetriesii</i>	Thamnophilidae	Passeriformes
<i>becula</i>	Bucconidae	Galbuliformes
<i>s chus</i>	Bucconidae	Galbuliformes
<i>riseus</i>	Nyctibiidae	Nyctibiiformes
<i>mot</i>	Cracidae	Galliformes
<i>phus marginatus</i>	Tityridae	Passeriformes
<i>phus surinamus</i>	Tityridae	Passeriformes
<i>as plumbea</i>	Columbidae	Columbiformes
<i>as subvinacea</i>	Columbidae	Columbiformes
<i>marail</i>	Cracidae	Galliformes
<i>a subcristata</i>	Thamnophilidae	Passeriformes
<i>phalus tricolor</i>	Cotingidae	Passeriformes
<i>is ruber</i>	Trochilidae	Apodiformes
<i>is superciliosus</i>	Trochilidae	Apodiformes
<i>rrhodes</i>	Furnariidae	Passeriformes
<i>us carnifex</i>	Cotingidae	Passeriformes
<i>na</i>	Cuculidae	Cuculiformes

Species	Family	Order
<i>Piaya melanogaster</i>	Cuculidae	Cucu
<i>Piculus capistratus</i>	Picidae	Picifo
<i>Piculus flavigula</i>	Picidae	Picifo
<i>Pionus fuscus</i>	Psittacidae	Psitta
<i>Pionus menstruus</i>	Psittacidae	Psitta
<i>Pipra erythrocephala</i>	Pipridae	Pass
<i>Piprites chloris</i>	Pipritidae	Pass
<i>Pithys albifrons</i>	Thamnophilidae	Pass
<i>Platyrinchus coronatus</i>	Platyrinchidae	Pass
<i>Platyrinchus platyrinchus</i>	Platyrinchidae	Pass
<i>Progne chalybea</i>	Hirundinidae	Pass
<i>Psarocolius viridis</i>	Icteridae	Pass
<i>Psophia viridis</i>	Psophiidae	Gruifo
<i>Pteroglossus aracari</i>	Ramphastidae	Picifo
<i>Pteroglossus viridis</i>	Ramphastidae	Picifo
<i>Pulsatrix perspicillata</i>	Strigidae	Strigit
<i>Pygoptila stellaris</i>	Thamnophilidae	Pass
<i>Pyrrhula caica</i>	Psittacidae	Psitta

	Family	Order
<i>icta</i>	Psittacidae	Psittaciformes
<i>irpurata</i>	Cotingidae	Passeriformes
<i>os tucanus</i>	Ramphastidae	Piciformes
<i>os vitellinus</i>	Ramphastidae	Piciformes
<i>enus melanurus</i>	Poliophtilidae	Passeriformes
<i>lus carbo</i>	Thraupidae	Passeriformes
<i>gon ruficauda</i>	Tyrannidae	Passeriformes
<i>clus olivaceus</i>	Rhynchocyclidae	Passeriformes
<i>a simplex</i>	Tyrannidae	Passeriformes
<i>ossus</i>	Thraupidae	Passeriformes
<i>turdinus</i>	Tityridae	Passeriformes
<i>piperivora</i>	Ramphastidae	Piciformes
<i>s griseicapillus</i>	Dendrocolaptidae	Passeriformes
<i>ornatus</i>	Accipitridae	Accipitriformes
<i>us surinamus</i>	Thraupidae	Passeriformes
<i>almarum</i>	Thraupidae	Passeriformes
<i>unctata</i>	Thraupidae	Passeriformes
<i>elia</i>	Thraupidae	Passeriformes

Species	Family	Order
<i>Terenotriccus erythrurus</i>	Onychorhynchidae	Passeriformes
<i>Thamnomanes caesius</i>	Thamnophilidae	Passeriformes
<i>Thamnophilus murinus</i>	Thamnophilidae	Passeriformes
<i>Tangara palmarum</i>	Thraupidae	Passeriformes
<i>Tityra cayana</i>	Tityridae	Passeriformes
<i>Todirostrum pictum</i>	Rhynchocyclidae	Passeriformes
<i>Tolmomyias assimilis</i>	Rhynchocyclidae	Passeriformes
<i>Tolmomyias poliocephalus</i>	Rhynchocyclidae	Passeriformes
<i>Tolmomyias sulphurescens</i>	Rhynchocyclidae	Passeriformes
<i>Touit huetii</i>	Psittacidae	Psittaciformes
<i>Touit purpuratus</i>	Psittacidae	Psittaciformes
<i>Trogon melanurus</i>	Trogonidae	Trogoniformes
<i>Trogon rufus</i>	Trogonidae	Trogoniformes
<i>Trogon violaceus</i>	Trogonidae	Trogoniformes
<i>Trogon viridis</i>	Trogonidae	Trogoniformes
<i>Turdus albicollis</i>	Turdidae	Passeriformes
<i>Tyranneutes virescens</i>	Pipridae	Passeriformes
<i>Tyrannulus elatus</i>	Tyrannidae	Passeriformes

	<b>Family</b>	<b>Order</b>
<i>melancholicus</i>	Tyrannidae	Passeriformes
<i>cassini</i>	Picidae	Piciformes
<i>ceus</i>	Vireonidae	Passeriformes
<i>poecilinotus</i>	Thamnophilidae	Passeriformes
<i>nutus</i>	Xenopidae	Passeriformes
<i>stes</i> <i>hynchus</i>	Dendrocolaptidae	Passeriformes
<i>chus obsoletus</i>	Dendrocolaptidae	Passeriformes
<i>chus pardalotus</i>	Dendrocolaptidae	Passeriformes
<i>acer</i>	Tyrannidae	Passeriformes