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MAMÍFEROS EM AGROECOSSISTEMAS: ABORDAGENS PARA AVALIAÇÃO DA DIVERSIDADE E EXPOSIÇÃO A CONTAMINANTES

ÉRICA FERNANDA GONÇALVES GOMES DE SÁ

João Pessoa, 28 de junho de 2024

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Tese apresentada ao Programa de Pós-Graduação em Ciências Biológicas (Zoologia) da Universidade Federal da Paraíba, como requisito parcial para a obtenção do título de Doutor em Ciências Biológicas.

Orientador: Prof. Dr. Pedro Cordeiro Estrela de Andrade Pinto

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Ata da 175ª Apresentação e Banca de Defesa de Doutorado de Érica Fernanda Gonçalves Gomes de Sá

Ao(s) vinte e oito dias do mês de junho de dois mil e vinte e quatro, às 13:00 horas, no(a) ambiente virtual, da Universidade Federal da Paraíba, reuniram-se, em caráter de solenidade pública, membros da banca examinadora para avaliar a tese de doutorado de **Érica Fernanda Gonçalves Gomes de Sá**, candidato(a) ao grau de Doutor(a) em Ciências Biológicas. A banca examinadora foi composta pelos seguintes membros: **Dr. Pedro Cordeiro Estrela (Orientador - UFPB-PB); Dr. Fábio de Oliveira Roque (UFMS/MS); Dr. Marcelo Magioli (Instituto Pró - Carnívoros/SP); Dr. Ricardo Siqueira Bovendorp (UESC/BA) e o Dr. Helder Farias de Araújo (UFPB/PB).** Compareceram à solenidade, além do(a) candidato(a) e membros da banca examinadora, alunos e professores do PPGCB. Dando início à sessão, a coordenação fez a abertura dos trabalhos, apresentando o(a) discente e os membros da banca. Foi passada a palavra ao(à) orientador(a), para que assumisse a posição de presidente da sessão. A partir de então, o(a) presidente, após declarar o objeto da solenidade, concedeu a palavra a **Érica Fernanda Gonçalves Gomes de Sá**, para que dissertasse, oral e sucintamente, a respeito de seu trabalho intitulado **"MAMÍFEROS EM AGROECOSSISTEMAS: ABORDAGENS PARA AVALIAÇÃO DA DIVERSIDADE E EXPOSIÇÃO A CONTAMINANTES".** Passando então a discorrer sobre o aludido tema, dentro do prazo legal, a candidata foi a seguir arguida pelos examinadores na forma regimental. Em seguida, passou a Comissão, em caráter secreto, a proceder à avaliação e julgamento do trabalho, concluindo por atribuir-lhe o conceito APROVADA. Perante o resultado proclamado, os documentos da banca foram preparados para trâmites seguintes. Encerrados os trabalhos, nada mais havendo a tratar, eu, orientador(a), como presidente, lavrei a presente ata que, lida e aprovada, assino juntamente com os demais membros da banca examinadora.

João Pessoa, 28/06/2024.

Examinador Dr. Ricardo Siqueira Bovendorp

Orientador Dr. Pedro Cordeiro Estrela de A. Pinto

Examinador Dr. Fábio de Oliveira Roque

Examinador Dr. Marcelo Magioli

Examinador Dr. Helder Farias P. de Araújo

Érica Fernanda Gonçalves Gomes de Sá
(discente ciente do resultado)

(Em modo de webconferência, as assinaturas digitalizadas são certificadas pelo presidente da banca)

Dedicatória

Dedico esta tese a minha mãe, Eliana Gomes, a quem eu sou grata pelo presente da vida e apoio incondicional em toda a minha trajetória. Eliana não é uma mãe "comum", ela é mãe, pai, irmã e minha melhor amiga. Por incontáveis vezes, ela também foi meu maior suporte no campo e durante os percalços da vida acadêmica. Eliana me criou com o apoio dos meus avós e como uma mãe solteira enfrentou muitos obstáculos, abdicando dos seus sonhos para que os meus pudessem ser realizados. Obrigada mãe, por me mostrar desde cedo que com esforço e dedicação podemos alcançar grandes coisas. Você é meu maior exemplo e inspiração. Dedico esta tese a senhora não somente como uma homenagem, mas também como uma promessa que chegarei muito mais longe, por nós. Te amo mãe!

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É quase desnecessário mencionar, mas é bom lembrar que um trabalho de tese não se realiza isoladamente. Uma tese possui tantas nuances que é difícil imaginar alguém que ousaria afirmar tê-la realizado completamente sozinho. Entre as muitas pessoas que desejo agradecer, agradeço em primeiro lugar ao meu orientador Pedro Estrela, a quem tenho uma grande admiração como pessoa e profissional. Obrigada por me aceitar como sua orientanda, pelas infinitas discussões sobre a tese, as ideias geniais, pelo imenso apoio profissional e pessoal que me ofereceu nos últimos anos e pela amizade que construímos. Aproveito para agradecer a mais duas estrelas que o doutorado me presenteou, Malu e Fabiana Rocha. Maluzinha, com seus cachinhos e sorriso inocente, mesmo sem saber, trouxe leveza para os momentos de lazer e nas datas comemorativas quando as saudades de casa batiam mais forte. E a Bia? O que dizer? Um exemplo de mulher, mãe e pesquisadora. Obrigada Pedro, Bia e Malu, por me acolherem e me trazerem a sensação de que mesmo longe da família sul-matogrossense eu ainda podia me sentir "em casa".

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"Era uma primavera sem vozes. Nas manhãs que antes pulsavam com o coro do amanhecer de tordos, pássaros-gatos, pombas, gaios, carriças e dezenas de outras vozes de pássaros, agora não havia nenhum som; apenas o silêncio pairava sobre os campos e florestas e pântanos."

- "Uma fábula para o amanhã" de Rachel Carson em seu livro Silent Spring (1962)

RESUMO

A expansão e intensificação da agricultura ocasionam a redução da biodiversidade em decorrência da fragmentação e perda de habitat e a exposição a contaminantes. Os impactos são refletidos em todos os níveis da diversidade biológica, incluindo níveis de comunidade, população e individual. Nosso objetivo foi investigar esses impactos utilizando mamíferos como biomonitores. No Cap. I; o objetivo foi avaliar a exposição dos mamíferos no cenário agrícola brasileiro. Para tal, conduzimos uma revisão sistemática sobre a ocorrência de mamíferos em agroecossistemas e baseados na avaliação de risco ambiental de agrotóxicos para organismos não-alvo (ARA), avaliamos os traços funcionais dos mamíferos nos principais cultivos. Revisamos 200 estudos que reportam a ocorrência de 319 espécies, das quais 205 no cultivo (in crop). Trinta e cinco espécies ameaçadas de extinção ocorrem em cultivos, sugerindo potencial exposição a contaminantes. O espaço funcional de mamíferos mostra que são necessárias duas a três espécies modelo genéricas para avaliar corretamente a exposição. Baseado na probabilidade de ocorrência dos grupos funcionais nos tipos de agroecossistemas, espécies modelo genéricas podem ser utilizadas para sistemas de pastagem, plantações de árvores e cultivos anuais, com as características funcionais terrestre, crepuscular, de tamanho médio a grande e dieta de vertebrados. Nas culturas agroflorestais e perenes, são indicadas espécies modelo genéricas com as características funcionais arbóreos e dietas frugívora e nectarívora. No Cap. II e III, o objetivo foi avaliar os impactos no sistema arroz-Pantanal, nas comunidades e populações de pequenos mamíferos como estudo de caso. No Cap. II, através de um inventário completo da comunidade no arrozal e na reserva adjacente (12.774 armadilhas/noite), encontramos alta diferenciação (beta=0.95) quando estimada com abundância. A reserva apresentou uma maior diversidade taxonômica. Métricas de diversidade funcional e filogenética foram maiores no arrozal do que na reserva quando baseadas em incidência, enquanto o contrário foi observado quando baseado em abundância. A inclusão da abundância é essencial para avaliar com precisão o impacto de agroecossistemas no nível de comunidade. No cap. III, avaliamos a exposição dos pequenos mamíferos através da quantificação de metais em tecidos críticos e no solo em dois ambientes: arrozal e reserva legal. Encontramos cádmio no fígado e rim em elevadas concentrações nos animais de ambos os habitats. No geral, as concentrações mais elevadas de metais não essenciais, alumínio (605,86 ± 298,10 mg/kg) e chumbo (74,09 ± 57,71 mg/kg), foram observadas nos rins dos roedores da reserva legal. Para o solo, também observamos maiores concentrações dos metais não essenciais,

alumínio (4.881,48 ± 1.034,69) e cádmio (0,33 ± 0,20) na reserva legal. Os resultados desta tese orientam o setor público regulatório e a academia na avaliação do risco ambiental de contaminantes para organismos não-alvo. Através da avaliação do sistema modelo arroz-Pantanal, fornecemos um protocolo inédito para acessar os impactos de agroecossistemas na comunidade de pequenos mamíferos, utilizando múltiplas métricas de diversidade e quantificação de contaminantes. Este protocolo pode ser replicado em outros sistemas agrícolas para ampliar o conhecimento sobre a ocorrência e saúde de mamíferos em agroecossistemas, direcionar futuras pesquisas de conservação e auxiliar na criação de legislações para o monitoramento de contaminantes no meio ambiente.
Palavras-chave: índices de diversidade, metais pesados, agrotóxicos

ABSTRACT

Agricultural expansion and intensification result in reduced biodiversity due to habitat fragmentation, habitat loss, and exposure to contaminants. These impacts are observed across all levels of biological diversity, including community, population, and individual levels. Our aim was to investigate these impacts using mammals as biomonitors. In Chapter I, the objective was to evaluate mammal exposure in the Brazilian agricultural scenario. We conducted a systematic review on the occurrence of mammals in agroecosystems and based on the environmental risk assessment of pesticides for non-target organisms (ERA), we evaluated the functional traits of mammals in the main crops. We reviewed 200 studies reporting the occurrence of 319 species, 205 of which were found in crops. Thirty-five endangered species occur in crop, suggesting potential exposure to contaminants. The functional space of mammals indicates that two or three generic model species are required to accurately assess exposure. Based on the probability of occurrence of functional groups across different agroecosystem types, generic model species can be used for pasture systems, tree plantations, and annual crops, mammals with terrestrial, crepuscular traits, medium to large size, and vertebrate diets. In agroforestry and perennial crops, generic model species with arboreal characteristics and frugivorous or nectarivorous diets are recommended. In Chapters II and III, we evaluate a rice-Pantanal system using small mammals as a case study. In Chapter II, we sampled the small mammal community in the rice paddy and legal reserve until reaching saturation on the species accumulation curve over 12,774 trap nights. We found a high level of differentiation (beta=0.95) when abundance was estimated. The reserve exhibited greater taxonomic diversity. Functional and phylogenetic diversity metrics were higher in the rice paddy than in the reserve when based on incidence, whereas the opposite was observed when based on abundance. Abundance-based metrics are essential for accurately assessing the impact of agroecosystems at the community level. In Chapter III, we assessed the small mammals exposure by quantifying metals in critical tissues and in the soil in rice paddy and reserve. We found high concentrations of cadmium in the liver and kidney of animals from both habitats. Overall, the highest concentrations of non-essential metals, aluminum (605.86 ± 298.10 mg/kg) and lead (74.09 ± 57.71 mg/kg), were observed in the kidneys of rodents from the legal reserve. For the soil, we also observed higher concentrations of the non-essential metals aluminum (4,881.48 ± 1,034.69) and cadmium (0.33 ± 0.20) in the legal reserve. The results of this thesis guide the public regulatory sector and academia in assessing the environmental risk of contaminants to non-target organisms. Through the evaluation of the rice-Pantanal model system, we provide an unprecedented protocol to assess the impacts of agroecosystems on the small mammal community, using multiple diversity metrics and contaminant quantification. This protocol can be replicated in other agricultural systems to expand knowledge about the occurrence and health of mammals in agroecosystems, direct future conservation research, and assist in creating legislation for monitoring contaminants in the environment.

Key words: diversity indices, heavy metals, pesticides

Sumário

INTRODUÇÃO GERAL

Panorama geral da problemática dos agroecossistemas e seus impactos

A Organização das Nações Unidas (ONU) aponta como principais ameaças para a biodiversidade a agricultura e a urbanização (ONU, 2008). As áreas agrícolas representam 40% da cobertura da terra (FOLEY ET AL. 2011; ELLIS ET AL. 2020), de maneira que os agroecossistemas tornaram-se os ecossistemas mais comuns do Antropoceno (GIBSON & QUINN 2017; REISMAN & FAIRBAIRN 2021). Em decorrência do crescimento exponencial da população e ao aumento substancial da demanda por alimentos até 2050, as áreas agrícolas tendem a aumentar, tornando a segurança alimentar uma questão global critica (FAO 2019). A expansão e intensificação da agricultura estão associadas a inúmeras ameaças ambientais, como as alterações climáticas, a perda da biodiversidade em decorrência da fragmentação e perda de habitat, a degradação do lençol freático e do solo e, a redução dos serviços ecossistêmicos (FOLEY ET AL. 2005; POWER 2010; FAHRIG ET AL. 2011; LAURANCE ET AL. 2014; ZINGG ET AL. 2019). As mudanças no uso da terra podem deixar alguns serviços de provisão inalterados, enquanto melhora serviços de provisão agrícola, como a produção de alimentos e combustíveis (BARRAL ET AL. 2015). Ao mesmo tempo, a agricultura reduz a quantidade de terra disponível para fornecer serviços ecossistêmicos de suporte, regulamentação e culturais fornecidos pela biodiversidade (BULLOCK ET AL. 2011; PILGRIM ET AL. 2010; RAUDSEPP-HEARNE ET AL. 2010).

Por outro lado, as matrizes agrícolas podem não ser completamente hostis a biodiversidade (TSCHARNTKE ET AL. 2012), sendo capazes de fornecer recursos utilizados pela vida selvagem e permitir a ocupação de diferentes habitats através da complementação da paisagem ou do suprimento dos requisitos ecológicos das espécies (DUNNING ET AL. 1992). De fato, certos tipos de agroecossistema são considerados "amigos da vida selvagem", através da manutenção de bordas ao longo das plantações, preservando fragmentos de habitats semi-naturais e adotando práticas sustentáveis de manejo (KREMSA 2021; MARREC ET AL. 2022; MONDIÈRE ET AL. 2024). Entretanto, efeitos a longo prazo das práticas agrícolas podem representar um desafio de conservação para as espécies que ocorrem em agroecossistemas, especialmente devido à aplicação intensiva de pesticidas e fertilizantes, em grande parte resultante da expansão da monocultura em larga escala (SOTO-GÓMEZ & PÉREZ-RODRÍGUEZ 2022; DHULDHAJ ET AL. 2023; MICHALKO ET AL. 2024), tais como a pecuária industrial, juntamente com a monocultura de soja e cana-de-açúcar, que transformam grandes áreas em “desertos alimentares” (GLOBAL FOREST COALITION, 2023). Em contraste, a agricultura familiar desempenha um papel crucial na garantia da segurança alimentar da população brasileira, sendo responsável por 70% dos alimentos consumidos no país (AZADI ET AL. 2023). A utilização de agrotóxicos é uma ameaça sem precedentes para a biodiversidade (GODFRAY ET AL. 2010; REZENDE-TEIXEIRA ET AL. 2022). Os agrotóxicos ficaram conhecidos com os incidentes de envenenamento agudo no final do século XIX, mas somente após a publicação do livro de Rachel Carson – Silent Spring, na década de 1960 é que o público tomou consciência dos efeitos nocivos dos produtos químicos no ambiente e os pesquisadores direcionaram seus esforços no campo da ecotoxicologia selvagem (KÖHLER & TRIEBSKORN 2013).

Assim, os impactos dos agroecossistemas na biodiversidade não são aspectos isolados e ocorrem simultaneamente em diferentes níveis da biodiversidade. No nível individual os impactos são mais frequentemente observados em decorrência dos efeitos toxicológicos de metais pesados e agrotóxicos na saúde individual, tais como neurotoxicidade, disrupção endócrina e imuno toxicidade (STANSLEY & ROSCOE 1996; GIBBONS ET AL. 2015; LUSHCHAK ET AL. 2018; RIVEROS ET AL. 2021; ZÚÑIGA-VENEGAS ET AL. 2022; POWOLNY ET AL. 2023). Vastas evidências também apontam para os impactos ecológicos ao nível de população, incluindo bioacumulação em mamíferos e declínios populacionais em abelhas e aves (BENNETT & THIES 2007; BRITTAIN ET AL. 2010; HALLMANN ET AL. 2014; SÁNCHEZ-BAYO & WYCKHUYS 2019; DA COSTA ET AL. 2023). Em um último nível, efeitos a nível de comunidade também são observados em decorrência do filtro ambiental proveniente das mudanças e alterações no uso do solo, podendo alterar as abundâncias, distribuição e riqueza de espécies (PRUGH ET AL. 2008). Impactos da atividade agrícola são particularmente preocupantes nos países tropicais, que são extremamente ricos em biodiversidade, mas que vêm sofrendo alterações sem precedentes na cobertura do solo (ZÚÑIGA-VENEGAS ET AL. 2022). No Brasil, a área destinada a agricultura e pecuária é estimada em 282,5 milhões de hectares, o que representa um terço do território nacional (MAPBIOMAS 2024). Estima-se que a safra de grãos 2023/24 atinja 294,1 milhões de toneladas (CONAB 2024). O Brasil também é o maior consumidor mundial de agrotóxicos, sendo que em 2021 atingiu a marca de 720.000 toneladas de aplicações de pesticidas para uso agrícola (FAO 2023), com um mercado avaliado em 20 mil bilhões de dólares entre 2022-23 (KYNETEC 2024).

Nesse contexto, dada a sua vasta expansão e crescente uso de agrotóxicos, é necessário que cada vez mais os esforços de pesquisa direcionem-se para o entendimento e proposição de mitigação dos impactos das mudanças no uso da terra e produção agrícola na biodiversidade brasileira. No Brasil, no que diz respeito ao monitoramento ambiental dos metais pesados e agrotóxicos, são utilizados monitoramentos periódicos de água, solo e ar a fim de apontar as concentrações desses poluentes no ambiente, tendo como base limites pré-estabelecidos pela legislação ambiental (por exemplo, CONAMA 357/2005; CONAMA 420/2009; CETESB 2001). Mas esse monitoramento por si é inadequado para avaliar a disponibilidade e toxicidade potencial de contaminantes na vida selvagem (TALMAGE & WALTON 1991). Sendo que o Brasil, até o momento, não detém de legislação reguladora e limites para os níveis de contaminantes na vida selvagem. A exceção é o manual de avaliação de risco de agrotóxicos para abelhas (CHAM ET AL. 2017), e uma avaliação de risco de pesticidas para mamíferos silvestres que está em andamento (PROJETO IBAMA/FDD 2022). Neste sentido, o **Capítulo I** da tese visa fornecer respaldo científico para a elaboração da avaliação de risco de agrotóxicos para mamíferos no Brasil, mapeando os registros de ocorrência de mamíferos em agroecossistemas brasileiros a partir de uma revisão sistemática da literatura. Os capítulos II e III serão tratados a seguir.

Integrando métricas de diversidade e exposição em um sistema modelo para avaliação de impactos

Uma abordagem integradora e unificada para equilibrar e otimizar a produção agrícola sustentável é a proposta de *UMA SÓ SÁUDE*, que reconhece a interconexão entre saúde humana, dos animais domésticos e selvagens, das plantas e do ambiente em geral (incluindo o ecossistema) (OMS 2021). Como resultado, uma visão de saúde única quando aplicada ao se investigar agroecossistemas pode proporcionar uma interpretação mais abrangente dos impactos positivos e negativos da atividade agrícola nos diferentes níveis de biodiversidade.

Com o objetivo de avaliar os impactos de um agroecossistema através de métricas de diversidade e avaliação de contaminantes (metais pesados), o **Capítulo II** e **Capítulo III** da tese abordam esses aspectos. Sendo que nosso sistema modelo de investigação está situado em uma área de agro-ecoturismo de arroz irrigado, pecuária e silvicultura no Pantanal Sul, Mato Grosso do Sul. Devido ao seu estado relativamente intacto em comparação com biomas muito degradados, como a Mata Atlântica ou a Caatinga, nosso sistema modelo de avaliação de impactos apresenta ainda, uma alta densidade de mamíferos de médio porte, destacando-se principalmente carnívoros como a onça-pintada (*Panthera onca*), a jaguatirica (*Leopardus pardalis*), o cachorro-do-mato (*Cerdocyon thous*), o lobo-guará (*Chrysocyon brachyurus*) e o mão-pelada (*Procyon cancrivorus*). A presença desses animais impulsionou o ecoturismo, que constitui atualmente a principal fonte de renda da fazenda, juntamente com a criação de gado e o cultivo de arroz irrigado.

Além disso, observações de (CONCONE 2004) e (TERIBELE 2007) indicaram que a frequência de avistamentos de mamíferos de médio e grande porte no arrozal foi maior do que em áreas de vegetação natural, como a reserva legal dentro da fazenda, protegida pelo Código Florestal Lei nº 12.651/2012 (BRASIL 2012). Embora o arroz seja considerado uma das três culturas mais importantes para a segurança alimentar em todo o mundo (YUAN ET AL. 2021), sua produção envolve o uso de fertilizantes e agrotóxicos (SATPATHY ET AL. 2014), levantando questões significativas sobre a exposição dos mamíferos a metais pesados devido às atividades agrícolas, tornando as espécies vulneráveis a contaminação indireta através da bioacumulação e biomagnificação. Nesse contexto, os pequenos mamíferos que ocorrem na área agrícola e reserva legal adjacente da fazenda podem fornecem indicadores realistas da exposição desses animais a contaminação ambiental (TALMAGE & WALTON 1991).

Uma vez que a escala espacial das respostas das espécies a diferentes usos da terra e tipos de vegetação está fortemente relacionado a alguns atributos ecológicos da comunidade, como frequência, riqueza de espécies e diversidade local, bem como processos de dispersão, padrões de movimento, intensidades de uso do habitat e fluxos gênicos (ZURLINI & GIRARDIN 2008; WEIHER ET AL. 2011; BENEDEK & SÎRBU 2018) a avaliação da diversidade da comunidade de pequenos mamíferos através de múltiplas métricas de diversidade pode fornecer informações importantes quanto ao efeito do arroz na comunidade de pequenos mamíferos. Sendo que (JARZYNA & JETZ 2016) discutem que a integração da diversidade em três componentes - diversidade taxonômica, diversidade funcional e a diversidade filogenética – são uma ferramenta poderosa para capturar respostas da biodiversidade nos agroecossistemas numa análise abrangente. Assim, o sistema modelo de agro-ecoturismo arroz-Pantanal, torna-se um cenário potencial para avaliação dos impactos na diversidade de espécies e de contaminantes.

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ESTRUTURA DA TESE

A tese está dividida em três capítulos e três anexos:

Capítulo 1: Através de uma meta-análise abrangente sobre ocorrência de mamíferos em agroecossistemas brasileiros, buscamos responder as seguintes questões: (1) Em quais cultivos e em quais tipos de agroecossistemas os mamíferos ocorrem? (2) A distribuição taxonômica difere entre as ocorrências de mamíferos registadas fora e dentro das culturas? (3) Quantas espécies de mamíferos ameaçados ocorrem nas culturas? Os pesticidas são identificados como ameaças a estas espécies? (4) Quais são as dez principais culturas com o maior número de ocorrências de mamíferos nas culturas? Além disso, que espécies de mamíferos ocorrem nestas dez principais culturas e com que frequência? (5) Quais são as características funcionais dos mamíferos nessas dez principais culturas de ocorrência de mamíferos e como o espaço funcional pode ser usado para discutir os riscos de exposição em tipos de agroecossistemas? Nossos resultados foram discutidos embasados na avaliação de risco de agrotóxicos para organismos não-alvo no Brasil.

Capítulo 2: Nosso objetivo foi avaliar as múltiplas métricas de diversidade na comunidade de pequenos mamíferos em um agroecossistema arroz-Pantanal. Mais especificamente, objetivamos: (i) comparar a diversidade taxonômica, funcional e filogenética de pequenos mamíferos entre os arrozais e a área natural adjacente de reserva legal florestada; (ii) examinar como os estimadores baseados em abundância influenciam as métricas de diversidade múltipla entre os locais de amostragem quando comparados com estimadores baseados em incidência mais comumente usados.

Capítulo 3: O objetivo foi avaliar a extensão da contaminação por metais nos tecidos (fígado e rim) dos pequenos mamíferos e avaliar potenciais vias de exposição através da quantificação de contaminantes no solo do agroecossistema arroz-Pantanal. Mais especificamente, nossos objetivos foram: i) determinar as concentrações de metais (Al, Cd, Cr, Cu, Fe, Pb e Zn) em amostras de solo em dois habitats (reserva legal e arrozal); ii) quantificar as concentrações desses metais em amostras de fígado e rim dos pequenos mamíferos em ambos os habitats; iii) comparar as concentrações no solo e nos tecidos críticos de pequenos mamíferos entre os habitats para avaliar a exposição dos pequenos mamíferos em nossa área de estudo.

Anexo 1: Este anexo refere-se à apresentação de um banco de dados sobre a ocorrência de mamíferos em paisagens agrícolas brasileiras, chamado *MamInAgro*. Este documento detalha o desenvolvimento e a criação do banco de dados, realizado em parceria com um projeto de TCC na Universidade Federal do Rio Grande do Sul. O objetivo foi desenvolver um sistema para reunir as ocorrências de mamíferos em agroecossistemas. O sistema é composto por um banco de dados, inicialmente alimentado pelos dados da revisão sistemática **(Cap. I)**, modelado para permitir o armazenamento do maior número possível de informações; um backend e uma interface, com diversas opções de processos de inserção de dados, para popular o banco e, posteriormente, servir de base para construir visualizações que auxiliem na análise dos dados e permitam seu uso em diferentes contextos relacionados à diversidade e exposição de espécies não-alvo em agroecossistemas no Brasil.

Anexo 2: Este anexo está relacionado a um artigo em preparação, que será submetido à revista Chemosphere (Qualis A1, Fator de Impacto 8.1). O estudo aborda a determinação de agrotóxicos no fígado e rim de roedores silvestres no Pantanal Sul. As amostras biológicas foram coletadas durante as campanhas de campo descritas nos Capítulos 1 e Capítulo 2. O protocolo de quantificação multiresíduo desenvolvido, em amostras de tecidos de roedores silvestres, é pioneiro no Brasil. Os resultados deste artigo serão discutidos em um artigo posterior à tese. Este artigo abrangerá a avaliação da comunidade de pequenos mamíferos (Capítulo 2) e a avaliação da saúde individual desses animais (Apêndice 3). Juntos, esses dados irão compor uma análise abrangente do risco ambiental de agrotóxicos no sistema agrícola arroz-pantanal, utilizando pequenos mamíferos como biomonitores.

Anexo 3: Este anexo refere-se a um artigo publicado (Qualis A2) que apresenta análises histopatológicas para a avaliação da saúde dos roedores capturados no sistema agrícola arroz-pantanal. As amostras biológicas foram coletadas durante a campanha de campo descrita no Capítulo 2. Este artigo também introduz uma matriz histopatológica, que será utilizada em um artigo de síntese pós-tese, onde serão discutidos os resultados da avaliação de saúde e contaminantes em pequenos mamíferos no contexto do sistema arroz-pantanal.

CAPÍTULO 1

Model mammal review

Conservation Blind Spots: Scenarios For Assessing the Exposure of Brazilian Mammals to Pesticides

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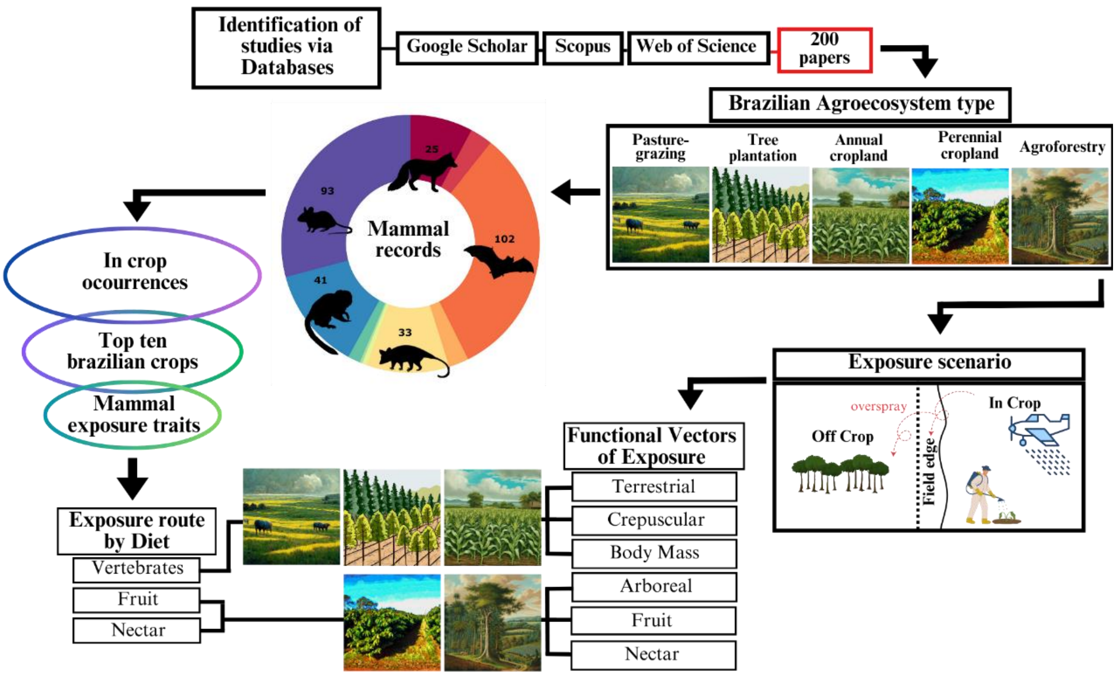
GRAPHCAL ABSTRACT



ABSTRACT

1. Brazil is one of the world's largest agricultural producers and a megadiverse country. The use of pesticides poses risks to non-target species, including wild mammals.
2. Due to the complexity of the adverse effects and the permanent risk to human health and wildlife, Environmental Risk Assessment (ERA) approaches for pesticide have been adopted by the European Food Safety Authority (EFSA) and the United States Environmental Protection Agency (EPA). However, Brazil has yet to present a pesticide risk assessment for vertebrates.
3. To apply ERAs for Brazilian mammals, data is needed on the occurrence and distribution of species within and outside agricultural fields, their biological characteristics and life history traits, as well as the crops and agroecosystem types where they are found.
4. Here, we conduct a comprehensive meta-analysis of mammal occurrences in Brazilian agroecosystems. We highlight the main crops, assess the national and global conservation status of endangered mammals and check if pesticides were identified as threats and, discuss mammal traits that lead exposure across agroecosystems.
5. We demonstrated that at least 44% of Brazilian terrestrial mammals occur in agroecosystems, with more than half (64.26%) found in crop. Mammal occurrences were most common in large-scale monoculture agroecosystems, including pasture-grazing, tree plantation and annual croplands showing important gaps of studies in family farming.
6. Regarding endangered species, we found many carnivores for which pesticides have not yet been identified as a threat. Pasture-grazing, tree plantations, and annual croplands, showed the exposure traits terrestrial, crepuscular, and body mass, with vertebrates as their main diet. Agroforestry and perennial cropland showed exposure traits: arboreal, with frugivorous and nectarivorous diet. We conclude that more than one indicator model species (IMS) is needed for mammals in the Neotropical region.

Keywords: mammals, crop, Brazilian agroecosystem, pesticide exposure
Word Count: 10.012

1. Introduction

The role of agroecosystems in the conservation of biodiversity can vary greatly due to the enormous variety of these systems, ranging from large-scale industrial monocultures to traditional silvopastoral systems (Altieri 1999). After the suppression of native vegetation, most agricultural management practices maintain chronic detrimental impacts on biodiversity through soil erosion, water depletion (Sud 2020) and the use of fertilizers and pesticides. The latter are both the most consistently recognized as detrimental to biodiversity (Berny 2007, López-Bao & Mateo-Tomás 2022).

The acceptance of the environmental impact of pesticides is commonly justified by the resulting increase crop yields over the past seven decades (Rezende-Teixeira et al. 2022), and the increase of the world’s livestock population to 1 billion, both of which should provide food security for a portion humankind (USDA 2023). Environmental costs are also nuanced by the development of a very profitable global agro-industrial complexes (Ioris 2018). Despite that, pesticides have been blamed for the world's insect decline a predicted collapse of pollination systems (Wagner et al. 2021). Other indirect ecosystem services have yet to be assessed for pesticide-induced disruptions.

Adverse effects have also affected the human population, with clear links to teratogenicity, mutagenicity, carcinogenicity, and hormonal alteration (Lushchak et al. 2018, Oliveira et al. 2021). However, these adverse effects on wildlife are mostly noted through acute effects such as massive deaths, population crashes, and embryonic malformation (Gibbons et al. 2015, Lushchak et al. 2018, Hoshi 2021, Zúñiga-Venegas et al. 2022), and thoroughly documented when affecting endangered species (Vicente & Guedes 2021). Despite this a large body of evidence, pesticides are seldom identified as threats to wildlife (Ducatez & Shine 2017), unlike other threats such as genomic erosion, diseases, climate change or invasive species (Díez-Del-Molino et al. 2018, Dueñas et al. 2021, Plowright et al. 2021, Jia et al. 2022). The chronic effects of pesticides can include a wide range of alterations that will often impact population dynamics in the long run such as immunotoxicity, endocrine system disruption, decrease in reproductive success, and alteration of behavioral patterns (Berny 2007).

The exposure of animals to pesticides occurs through inhalation, dermal contact, and the consumption of contaminated food, water, or soil (US 2017). Exposure levels often differ inter and intra species, as modulated by with behavioral traits, dietary habits, or habitat occupancy and preference (US 2017). Even forest-dwelling species can be exposed to pesticides through direct contact with overspray (i.e. from aerial spraying)

(Freemark 1995), to a greater extent on those that occur in wild habitats adjacent to crops. Therefore, species occurring within crop fields (in crop henceforth), are more likely to be exposed, particularly in fields that are treated more frequently and/or receive larger amounts of pesticides (EFSA et al. 2023), such as large-scale monocultures. Mobile species with large home ranges are also exposed through contact with contaminated vegetation or through the trophic chain when their prey are exposed in treated crops (Freemark 1995, US 2017). Due to the complexity of adverse effects and the ongoing risk to human health and wildlife, Environmental Risk Assessment (ERA) approaches for pesticide registration have been adopted by the European Food Safety Authority (EFSA et al. 2023) or the USA - United States Environmental Protection Agency (US-EPA) (US 2004, US 2017). Both agencies assess the potential exposure of non-target organisms and include the use of exposure models to predict environmental concentrations (exposure), in order to avoid approving compounds that may pose unacceptable risks to the environment or human health (Vryzas et al. 2020).

In Brazil, ERA has been under specific regulation since 2017 (IN nº 02/2017), resulting in a pesticide risk manual for bees (Cham et al. 2017), and a pesticide risk assessment for vertebrates underway. The Brazilian Institute of Environment and Renewable Natural Resources (IBAMA) opted for the EFSA approach to environmental risk assessment (Portaria Ibama nº 84/1996, Decreto nº 4.074/2002, Ibama/MMA 2023 - www.gov.br/ibama). Brazil faces significant challenges in implementing environmental risk assessment (ERA/EFSA) for vertebrates due to its high biodiversity and diverse landscape configuration. Moreover, the diversity of crops, agricultural practices, and environmental conditions ranging from tropical to subtropical climates, with humid, monsoonal, and semiarid variants, make way for numerous scenarios. In addition, Brazil was the largest user of pesticides in the world in 2021, with 720,000 tons of pesticide applications for agricultural use (FAO 2023), with a market reaching USD $20 billion in 2022-23 (Kynetec 2024). With more than 400 active ingredients in 2022, sales reached 800,652 tons, an increase of about 11% compared to the previous year - 2021 (Ibama/MMA 2022). In this context, for adequate implementation of risk assessment for Brazilian mammals, data is needed on the occurrence and distribution of species within and outside agricultural fields, their biological characteristics and life history traits, as well as the crops and agroecosystem types where they are found.

Scope of this review

This study aimed to map the occurrence records of mammals in Brazilian agroecosystems based on a systematic literature review. More specifically, we aimed to answer the following questions: (1) In which crops and in which agroecosystem types do mammals occur? (2) Does the taxonomic distribution differ between the occurrences of mammals registered off crop and in crop? (3) How many species of threatened mammals occur in crop? Are pesticides identified as threats to these species? (4) What are the top ten crops with the highest number of mammal occurrences in crop? Additionally, which mammal species occur in these top ten crops and how frequently do they occur? (5) What are the functional traits of mammals in these top ten crops of mammalian occurrences, and how can the functional space be used to discuss exposure risks in agroecosystem types? Finally, we used our findings to discuss the risk of mammal exposure in agroecosystems within the context of the Brazilian agricultural scenario. To achieve this, we examined our results regarding mammal occurrences in agroecosystem types and compared them with the regulatory guidelines for environmental risk assessment of pesticides.

2. Methods

2.1. Bibliographic search

We conducted our literature search following PRISMA (Preferred reporting items for systematic reviews and meta-analysis) guidelines (Page et al. 2021) across Google Scholar, Web of Science, and SCOPUS databases. We did not restrict our search using the year of publication, and collected references up to, and including, August 2022. We searched for the following keywords: “didelphimorphia” OR “cingulata” OR “pilosa” OR “primates” OR “lagomorpha*” OR “rodentia*” OR “chiroptera” OR “carnivor*” OR “perissodactyla” OR “artiodactyla” OR “mammal*” OR “bat*” OR “small mammal*” OR “medium and large sized mammal*” OR “carnivore*” OR “rodent*” OR “marsupial*” AND "human modified landscapes" OR "pastures" OR "farm" OR "mosaic" OR "matrix" OR "agriculture" OR "agro*" OR "crop*" OR "monoculture" OR "land use" OR "defaunation" AND “brazil*” OR “atlantic forest” OR “pantanal” OR “pampa” OR "campos sulinos" OR “amazon*” OR “cerrado” OR “caatinga”. The terms were searched in the title, abstract, keywords, and main text. Additionally, we searched in grey literature studies on the national database "Brazilian Digital Library of Theses and Dissertations" (https://bdtd.ibict.br/vufind/) using the same terms. The studies were excluded if: 1) the study was not carried out in Brazil; 2) the species that were recorded in a specific agroecosystem or crop type were not specified; and 3) the study was not based on primary empirical observational or experimental research. Our literature review retrieved 200 studies. Our selection process and dataset are presented in **Appendices S1 and S2**.

2.2. Data compilation

General information

The occurrences were summarized for the 200 studies that reported wild mammals in Brazilian agroecosystems. Nomenclature and taxonomy followed (Abreu et al. 2021). We assigned each species of mammals to one or more crop types, depending on whether its spatial occurrence was specifically associated with a single crop or with multiple crops in a study. Consequently, we quantified mammal occurrences by crop types. This step was necessary since many studies described the occurrence at the agroecosystem landscape level, which encompasses multiple crops and/or crop rotation, a pervasive practice in the tropics. For instance, when a mammal was sighted in an area with two or more cultivated species (such as sugarcane and pasture), the occurrence was attributed to both crops.

We grouped crop types into five Agroecosystem Types; (1) Agroforestry: tree and shrub crops shaded by native or exotic trees (e.g., coffee, cocoa); (2) Tree plantation: arborescent plantations allocated to the production of wood, pulp, and other timber products species (e.g. Eucalyptus, Pine, Oil Palm); (3) Perennial cropland: crops that are cultivated and live longer than two years without the need of being replanted each year, including perennial monocultures (e.g. coconut, banana); (4) Annual cropland: monoculture or polyculture lasting over a single or a few harvests within a year (e.g., soybean, corn, (Ferreira et al. 2018) and (5) Pasture-grazing systems: continuous grazing, simple rotational grazing, and intensive rotational grazing, or general classifications cited by the studies as grazing/pasture or livestock/cattle ranching. When the occurrence was reported in the landscape without information on the specific spatial occurrence, we classified it as off crop to be conservative. On the other hand, when a study identified a mammal within one or more crops, we classified this record as in crop. The terms “off crop” and “in crop” are usually used by the working groups in the environmental risk assessment. In crop refers to the area within the field where pesticides are applied, while off crop denotes occurrences outside the field, which may be in the agricultural landscape (SETAC 2012, Cham et al. 2017).

Occurrences of mammals in crop

A subset of our database comprises only in crop occurrences. We assessed nation and worldwide conservation status (ICMBio/MMA 2022, IUCN 2023) of the resulting list of species and reviewed their assessments to check if pesticides and/or contaminants were identified as threats. We considered a mammal species threatened by pesticides if its threat category 9.3.3. Herbicides and pesticides were listed in the IUCN assessment of the species (IUCN Threats Classification Scheme Version 3.2. Available at: https://www.iucnredlist.org/resources/threat-classification-scheme). Similarly, we searched for pesticide threats in the National Action Plans (PAN) for the Conservation of Endangered Species in Brazil (Available at: https://www.gov.br/icmbio/pt-br/assuntos/biodiversidade/pan). Finally, we compiled a list of the top ten crops with the highest occurrences of mammals in crop, categorizing these crops into one of the five agroecosystem types, and discussing exposure scenarios based on mammal functional space. We collected information on four natural history and behavioral traits (Wilman et al. 2014) that can increase or decrease pesticide exposure (EFSA et al. 2023): body mass, feeding guild, daily activity, and foraging class **(Appendix S3; Appendix S7).**

2.3. Data analysis

To visually represent the distribution of agricultural land in Brazil we used the Mapbiomas collection 8.0 (Mapbiomas, 2023). We calculated the area covered by each crop in each of the Brazilian biomes (through mapbiomas-user-toolkit-lulc.js): Atlantic Forest, Amazon, Cerrado, Pantanal, Caatinga, and Pampa. We removed natural land classes using the tidyterra (Hernangómez 2024) and terra (Hijmans 2024) packages in R (R Core Team, 2024), and then created the layout, maintaining only crop classes(with packages geobr (R package geobr version 1.8.2 2024), sf (Pebesma & Bivand 2023), and ggspatial (Dunnington 2023).

Species accumulation curve was calculated to extrapolate the number of unobserved species based on the number of species present in each of the five agroecosystem types, with 999 random subsampling iterations in the vegan package (Oksanen 2022). We used the non-parametric bootstrap method with a 95% confidence interval to estimate species richness. A Mann-Whitney U-test was used to statistically evaluate the difference between the observed (Sobs) and bootstrap estimated (Sexp) richness.

Top ten crops and exposure trait group for mammals

We log-converted body mass and z-transformed all traits to converge to data normality (Villéger et al. 2008, Cox et al. 2021) and estimate the space of functional characteristics by agroecosystem type. We reduced dimensionality with funspaceDim. The probability of occurrence of trait combinations within the functional space was estimated with the funspace library (Carmona et al. 2024) through multivariate kernel probability density. We evaluated the probability density functional quantiles as a proxy for functional exposure probability. In this context, species within with the highest probability quantile of functional exposure represents the maximum probability of occurrence or exposure (i.e., the expected total functional space). In this context, we chose to evaluate only groups of occurrences with quantiles greater than 0.5 (50% probability of occurrence of trait combinations). This refinement is necessary to cover species that are in the lower quantiles of the trait space distribution since once stratified by crop type, the sample size is limited. The variance explained by each of the principal component and trait loadings can help understanding how well bivariate projection in trait space represents the multidimensional functional space and how each trait contributes to this space (Carmona et al. 2024). We then built a categorical typology of exposure based on the loadings of the traits in the functional and diets in functional space and their correlation by agroecosystem type. For this step, the hotspots were ranked in decreasing order according to the probability gradient (A > B > C). For each high probability density hotspot, we evaluated where occurrences were grouped and combination of traits with higher loadings for which resulted in Exposure Traits Groups (ETG).

3. Results

3.1. Occurrence of mammals in Brazilian agroecosystems

The 200 studies selected were published between 1998 and 2022, with 60% published after 2015. We found a total of 3,085 records of mammals, documented in all six Brazilian biomes: Atlantic Forest (39.61%), Amazon (13.06%), Cerrado (12.8%), Pantanal (4.41%), Caatinga (0.62%), and Pampa (0.36%) **(Fig. 1).** Moreover, 26.86% of these records were found in ecotones, which are transitional areas between biomes. The crop species planted in the agroecosystem was identified in 3,038 records (98.48%), summing 39 species/genus distributed among the five agroecosystem types.

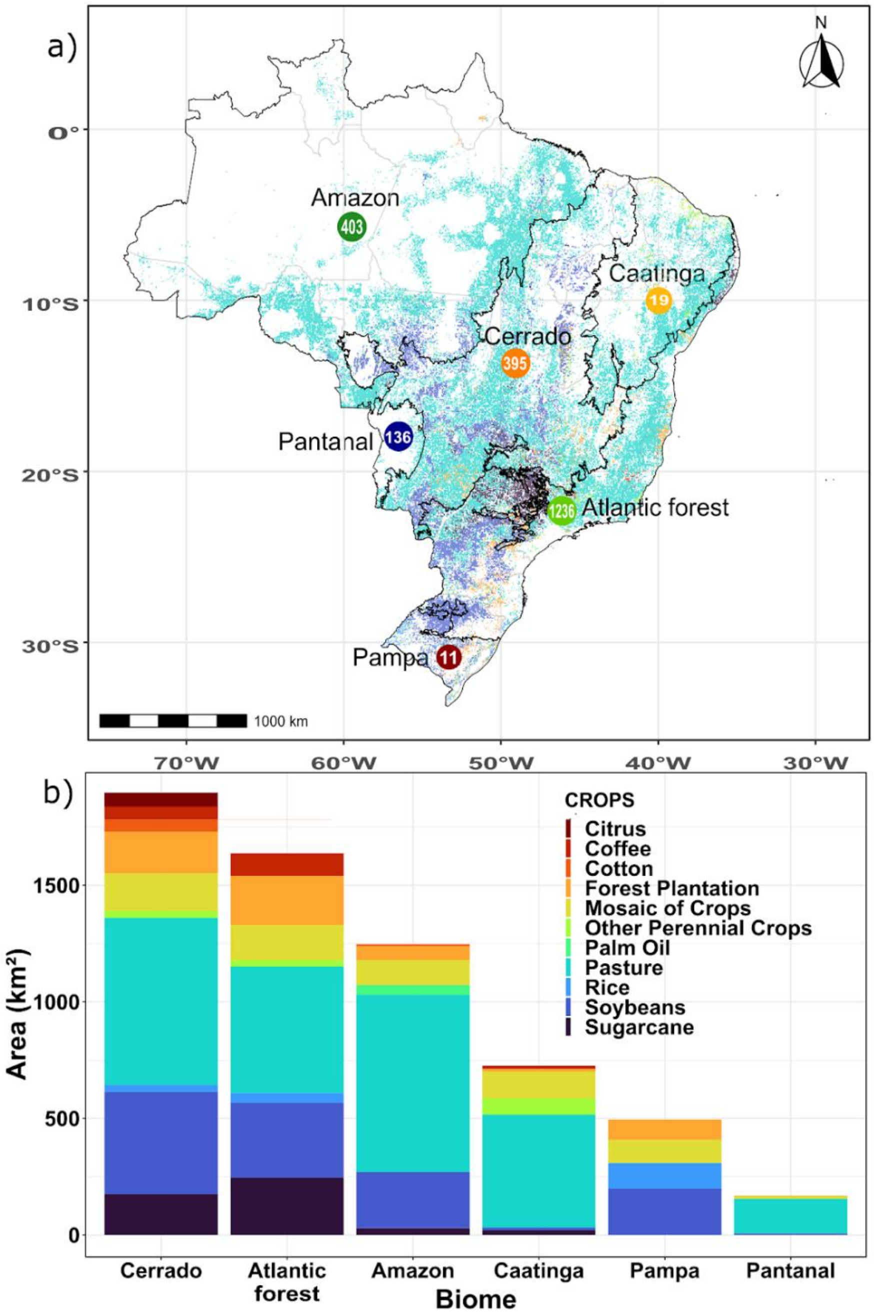


Fig. 1. (a) Number of mammal occurrences in agroecosystems in Brazilian biomes from 200 studies between 1998 and 2022 and current planted area by crops. State borders in light gray; **(b)** Planted area in km² in each biome stratified by crops extracted from mapBiomas (2024).

A total of 319 mammal species were reported in agroecosystems. Most of the recorded species (140 spp.; 60%) had at least five occurrence records in the agroecosystem (≥5). Conversely, mammal species with more than 20 occurrence records represented 13.48% (43 species) of the total mammal records **(Appendix S4)**. All orders of terrestrial mammals were found within Brazilian agroecosystems. The most recorded orders were Chiroptera (102 spp.; 31.97%) and Rodentia (93 spp.; 29.15%), followed by

Primates (41 spp.; 12.85%), Didelphimorphia (33 spp.; 10.34%) and Carnivora (25 spp.; 7.8%). The least represented orders were: Cetartiodactyla [Artiodactyla] (9 spp.; 2.82%), Cingulata (8 spp.; 2.50%), Pilosa (5 spp.; 1.56%), Lagomorpha (2 spp.; 0.62%) and Perissodactyla (1 spp.; 0.31%) as expected from their relative diversity**.** The agroecosystem types were ranked by their mammalian richness, with Pasture-grazing systems having the highest species count of 260 spp., followed by Tree plantations (221 spp.), Annual croplands (163 spp.), Agroforestry (150 spp.), and Perennial croplands (84 spp.).

The accumulation curves revealed significant differences between observed (incidence-based data) and expected (bootstrap) mammal richness within pasture-grazing system (Sobs=260; Sexp=305(±5.7); p<0.01), tree plantation (Sobs=224; Sexp=268.24(±7.24); p<0.01); agroforestry (Sobs=151; Sexp= 173.71(±6.68); p<0.01; and annual cropland (Sobs=167; Sexp=196(±3.89); p<0.01). In summary, there were less mammal species detected in these agroecosystem types than expected, whereas we found no differences in observed and expected richness in perennial croplands (Sobs=84; Sexp=107.26(±5.52); p= 0.16 **(Appendix S5.).** In terms of fine scale spatial occurrence, all mammalian orders are found in both off crop and in crop. But we found significantly more records of mammals off crop (n=1,931; 62.59%) than in crop (n=1,154; 37.41%; W=66923; p-value<0.01) as would be expected. Moreover, there was a significant reduction in richness in crop records (W=65076; p<0.01), particularly for primates (-70%) and rodents (- 50%) **(Fig. 3)**.

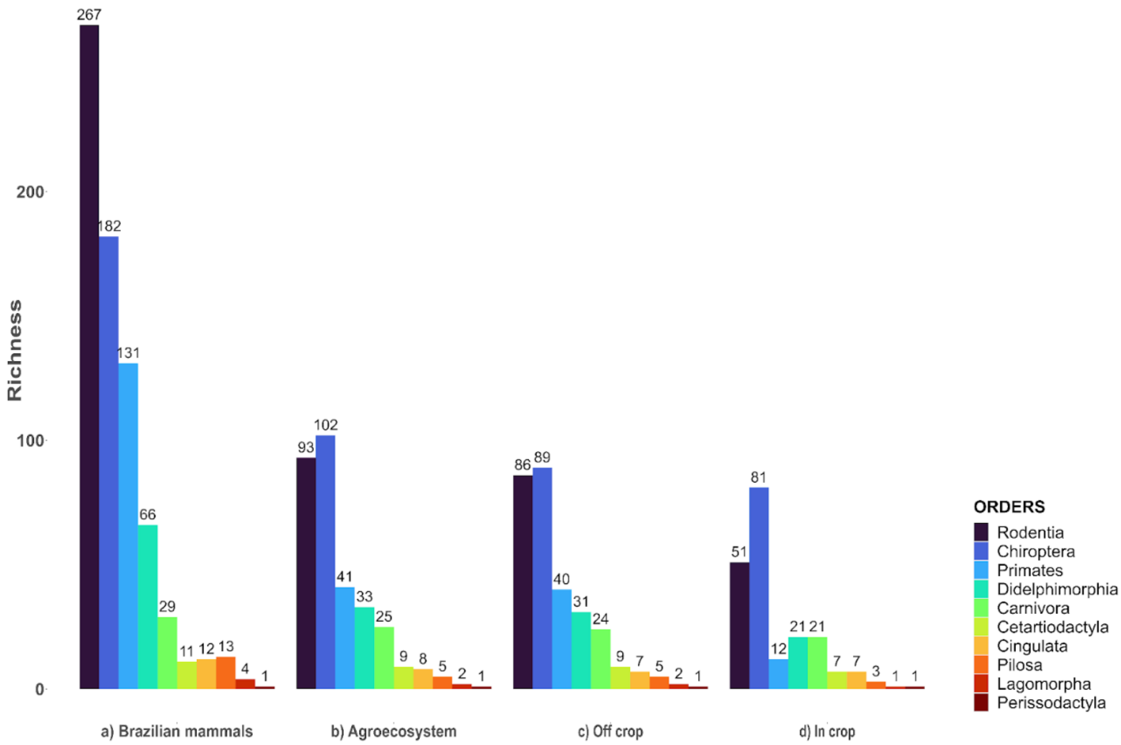


Fig. 3. Number of mammal species recorded by spatial category from 200 studies between 1998 and 2022 in Brazil (a) List of Brazilian Mammals (N=716 Continental Mammals - Non-strictly aquatic, Abreu Jr. et al. 2021) (b) Total agroecosystem (N= 319 spp.), (c) Off-crop (N= 294 spp.), (d) In-crop (N=205 spp.). The colors represent the orders of mammals recorded in each category.

3.2. Conservation status and threats to mammals occurring in crop

Of the 205 mammals occurring in-crop in Brazil, 31 species are listed as threatened (Critically Endangered - CR, Endangered - EN, Vulnerable - VU and Near Threatened - NT) in the IUCN Red List (IUCN 2023). The Golden-Bellied Capuchin (*Sapajus xanthosternos*) is the only Critically Endangered (CR) species registered in crop, in cocoa plantations in the Atlantic Forest of Bahia state (e.g Cassano et al. 2012, Cassano et al. 2014, de Almeida-Rocha et al. 2020, Suscke et al. 2021). Four species registered in crop are classified as Endangered (12.9%): two primates (*Leontopithecus chrysomelas* and *Sapajus robustus*), one lagomorph (*Sylvilagus brasiliensis*), and one bat (*Lonchophylla dekeyseri*). Fourteen species registered in crop are classified as Vulnerable, making it the category with the highest number of species threatened of extinction registered in crop in Brazil (N=14, 72%). Although not a threatened category, mammals listed as Near threatened had a significant number of species (38.7%, N=12). Furthermore, our search in the IUCN Red List showed that pesticides are listed as a threat only for the jaguar (*Panthera onca*) (Quigley et al. 2017) (**Appendix S6; Fig. 4a**).

Regarding the list of threatened mammals of Brazil (MMA, 2022), the majority of species registered in crop are classified as Vulnerable (72%, N=18) and seven species are classified as Endangered (28%). Searching through the National Action Plans for the Conservation of Endangered Mammals (PAN), we found that pesticide threats and/or contamination (even heavy metals) is a concern for 50% (N=9) of species listed in the Vulnerable category. These species were the bristle-spined rat (*Chaetomys subspinosus*), the giant anteater (*Myrmecophaga tridactyla*), the giant armadillo (*Priodontes maximus*), the tapir (*Tapirus terrestris*), the white-lipped Peccary (*Tayassu pecari*), the Marsh deer (*Blastocerus dichotomus*) and the Pampas deer (*Ozotocerus bezoarticus*). For the maned wolf (*Chrysocyon brachyurus*) and Geoffroy's Cat (*Leopardus geoffroyi*) assessments did not clearly associate pesticides to threats but indicated potential bioaccumulation threats. Among the mammals in the endangered category, only the three-banded armadillo (*Tolypeutes tricinctus*) had pesticides included as a possible threat in its assessment **(Appendix S6; Fig. 4b).**

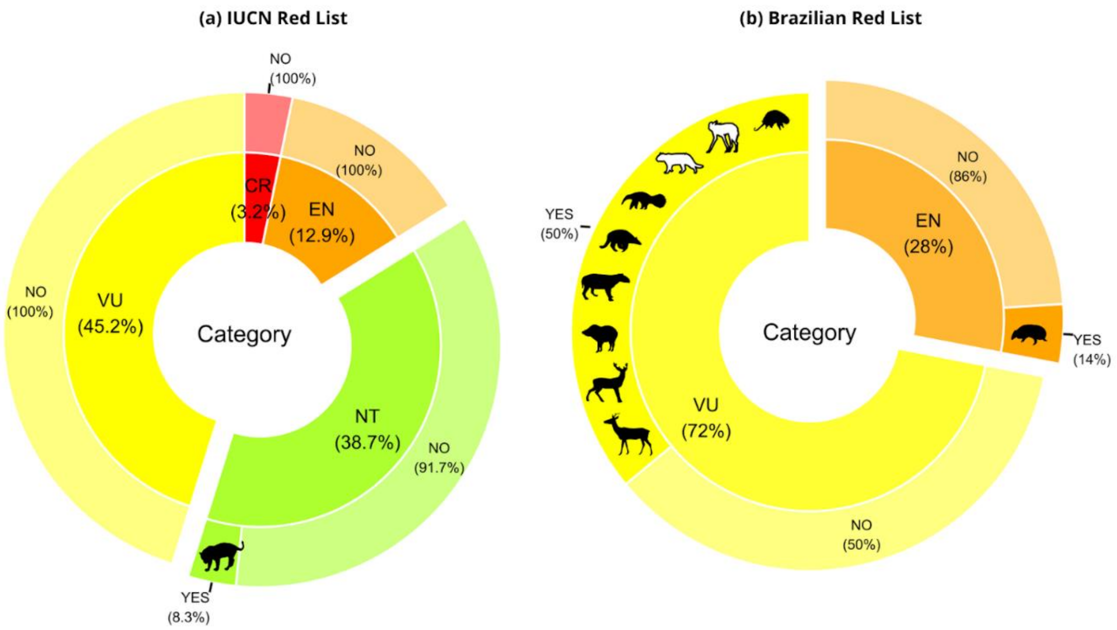


Fig. 4. The proportion of mammal species for which pesticides, either directly (black figures) or through bioaccumulation (white figures), are considered a threat (YES) for each threat category. Results are presented for the (a) IUCN Red List (2023); (b) Brazilian Red List (MMA, 2022). The proportion of species which are threatened by pesticides are shown in the external donut chart and the corresponding threat criteria (indicated by letters) in the central pie chart. CR, Critically Endangered (red); EN, Endangered (orange); VU, Vulnerable (yellow) and NT, Near Threatened (green); For clarity, the panels do not include the Not Applicable (NA), Data Deficient (DD) and Least Concern (LC) categories **(Appendix S6).**

3.3. Top ten crops, mammal richness and exposure trait group

The top ten crops with the highest number of mammal occurrences and their respective agroecosystem type were: undefined pasture and signal grass (*Urochloa* spp.) - Pasture-grazing system; eucalyptus (*Eucalyptus* spp.), pine (*Pinus* spp.) and rubberwood (*Hevea brasiliensis*) - Tree plantations; soybean (*Glycine max*), maize (*Zea mays*) and sugarcane (*Saccharum officinarum*) - Annual cropland; cocoa (*Theobroma cacao*) - Agroforestry; banana (*Musa paradisiaca*) - Perennial crop. Upon filtering the number of occurrences of each mammal, only 23 species had at least 20 occurrences **(Figure 5)**. These results are important for future studies selecting focal species in each crop.

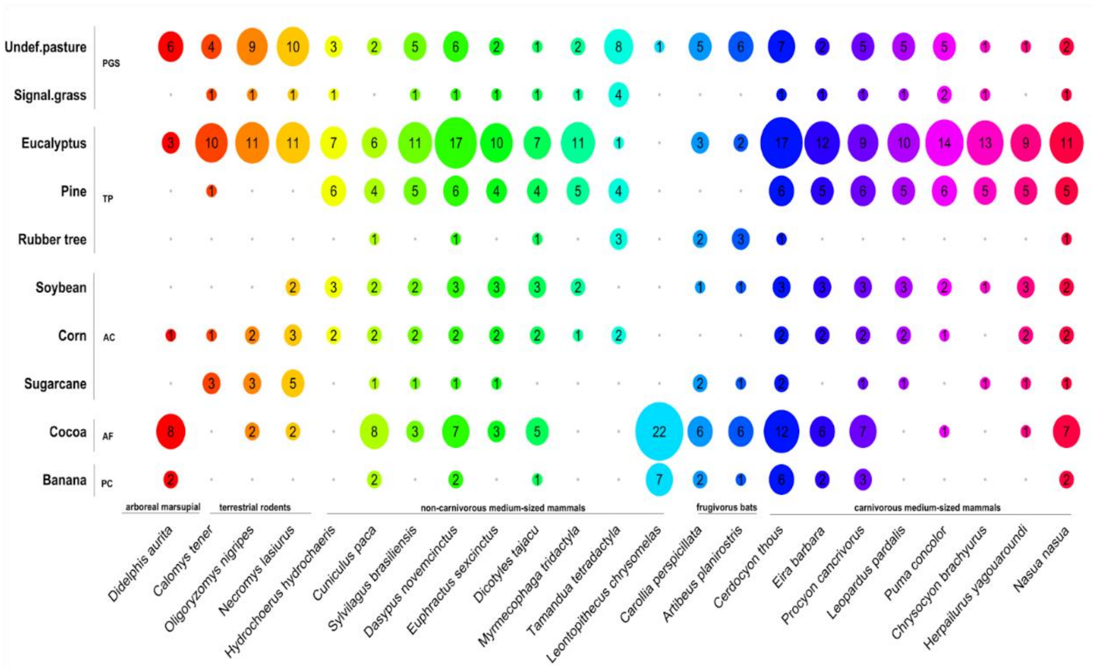


Figure 5. Frequency of occurrence of mammal species (>=20 records) in each of the top ten crops. Agroecosystem types: PGS - pasture grazing-systems, TP - tree plantation, AC - annual cropland, AF - agroforestry, PC - perennial cropland.

Concerning functional groups, all agroecosystem types presented three high density mammal functional groups, except annual cropland which presented only two mammal high density functional groups. For most functional spaces the functional groups are defined by correlated traits. Functional groups that are presents over all the agroecosystem types are defined by the following combination of functional traits: i) terrestrial carnivores and herbivores with a large body mass (pasture-grazing system, tree plantation and annual cropland); ii) arboreal frugivores and nectarivores (all agroecosystem types); ii) aerial with invertebrate diet (all agroecosystem types except annual croplands) **(Fig. 6)**. The main functional vectors of exposure for the pasture system, tree plantations and annual crops were terrestrial, crepuscular and body mass. On the other hand, the main vectors for agroforestry and perennial crops were arboreal, fruit and nectar. The exposure trait groups by agroecosystem type were summarized in **Table 1.**

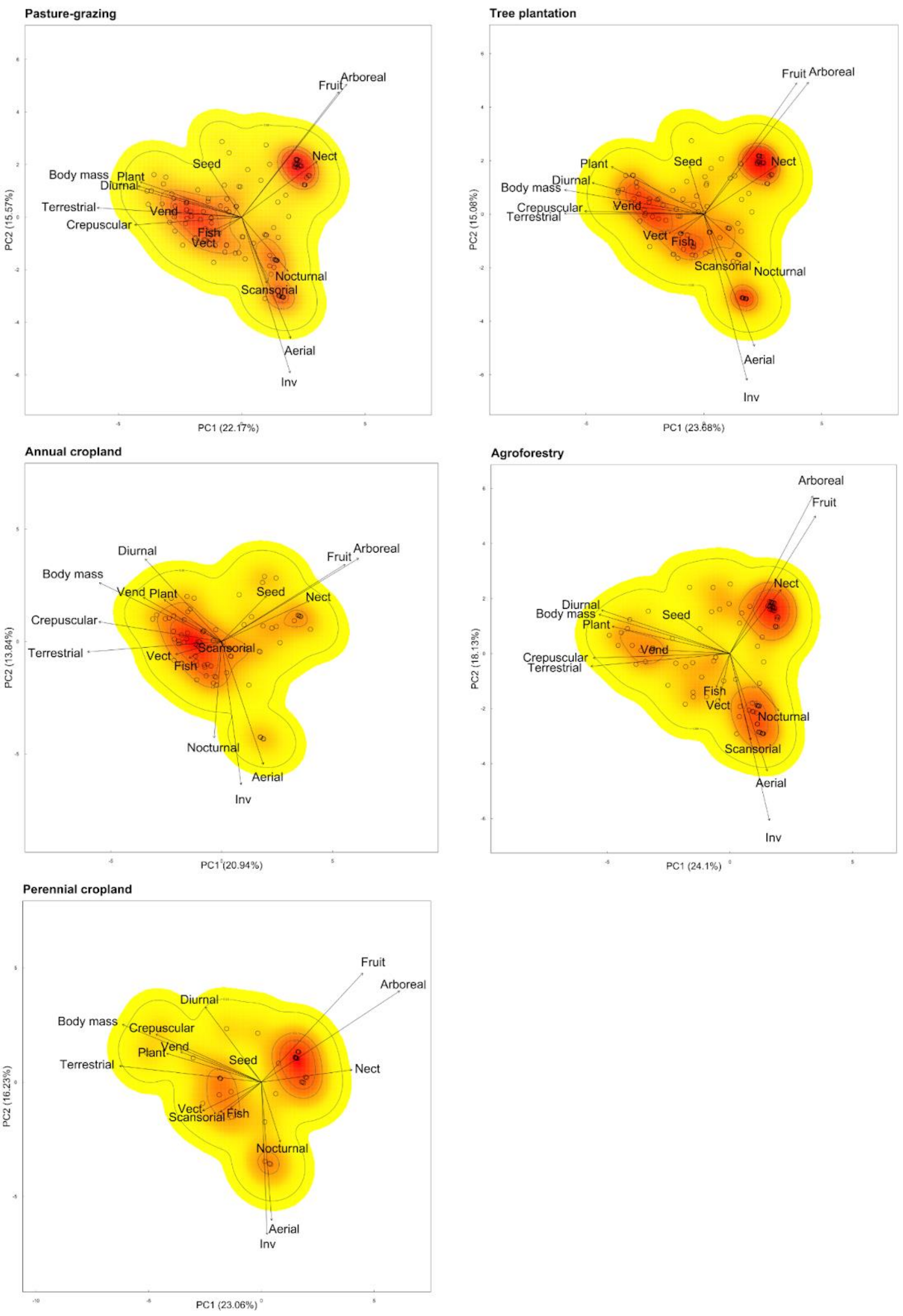


Fig. 6. Principal components of the functional space of occurrences of mammals in brazilian agroecosystems (dots) by agroecosystem type. The color gradient stands for the probability density distribution of the trait combinations in the functional trait space defined by Principal Component Analysis (PCA) (red = high probability, yellow = low probability). Contour lines represent the 0.99, 0.50, and 0.25 quantiles of the probability distribution. Additionally, the variance explained by each component and the loadings of the original traits are shown. Foraging stratum: Terrestrial (including semi-aquatic foraging); Scansorial; Arboreal; Aerial. Activity period: Crepuscular; Diurnal; Nocturnal. Diet: Fruit (fruits, drupes); Inv (invertebrates, insects, worms, gastropods, etc.); Nect (nectar, pollen, plant exudates); Plant (grass, leaves, sprouts); Seed (seed, maize, grains); Vect (vertebrates ectotherms, reptiles, amphibians); Vend (vertebrates endotherms, mammals, birds); Fish.

Table 1. Summary table of the mammal functional space by agroecosystem type showing exposure trait groups defined by the set of functional traits (main functional vectors of exposure) and by diet (exposure route by diet).

Agroecosystem type	Number of mammal functional groups	Exposure trait group*	Main Functional Vectors of Exposure	Exposure route by Diet
Pasture-grazing	3	A	Terrestrial, Crepuscular, Body Mass	Vertebrates (Vend, Vect), Plant
		B	Arboreal, Fruit	Fruit, Nectar
		C	Inv, Aerial	Invertebrates
Tree plantation	3	A	Terrestrial, Crepuscular, Body Mass	Vertebrates (Vend, Vect), Plant
		B	Arboreal, Fruit	Fruit, Nectar
		C	Inv, Aerial	Invertebrates
Annual cropland	2	A	Terrestrial, Crepuscular, Body Mass	Vertebrates (Vend, Vect), Plant
		B	Arboreal, Fruit, Nectar, Seed	Fruit, Nectar, Seed
Agroforestry	3	A	Arboreal, Fruit, Nectar	Fruit, Nectar
		B	Aerial, Invertebrates, Scansorial	Invertebrates
		C	Terrestrial, Crepuscular, Body Mass	Plant, Endothermic vertebrates
Perennial cropland	3	A	Arboreal, Fruit, Nectar	Fruit, Nectar
		B	Ectotherms vertebrates, Fish, Scansorial	Ectotherms vertebrates, Fish
		C	Invertebrates, Aerial	Invertebrates

*Trait groups of mammals within the quantiles with a probability greater than 50% (0.50 quantiles).

4. Discussion

Brazil is simultaneously the most megadiverse tropical country in the world (Convention on Biological Diversity 2024), the world's fourth largest producer of grains and has the largest cattle herd in the world (FAO 2024). Our systematic literature review provides a comprehensive overview of mammal records in Brazilian agroecosystems, offering unprecedented data and valuable insights for regulatory bodies and researchers involved in mammalian ecology and environmental risk assessment of pesticides.

We found that almost half of the species (44.55%) and all the orders of continental mammals (non-strictly aquatic) listed for Brazil occur across the five different agroecosystem types. Nevertheless, although there are occurrences in all five Brazilian biomes, the Atlantic Forest from the Southeastern region is overrepresented, as for most scientific data, because of the higher concentration of universities and research investments in this region (Geocapes 2022).

The literature regarding mammals in agroecosystems generally presents studies conducted at an agricultural landscape scale, with few studies focused on specific crops. Mammal occurrences were most common in large-scale monoculture agroecosystems, including pasture-grazing (notably managed pasture but also unidentified management systems), tree plantations (especially eucalyptus and its subspecies), and annual croplands (mainly sugarcane, soybeans, and corn). Moreover, our data also shows a lack of research focusing on small family farming properties, that occupy 23% of Brazil's territory with 80.9 million hectares (IBGE 2017) and which produces important crops, strategic to food security, like beans, cassava, sweet potato, bananas, pineapples and coffee (IBGE 2017). Except for coffee and bananas, the other crops were poorly represented in our data. For instance, despite cotton being one of Brazil's five main crops distributed throughout the country, with 2.60 million hectares (MapBiomas 2023) it is barely mentioned in the literature.

Regarding mammal occurrences in agroecosystems, the most represented orders reflected the species richness found in Brazil, with bats, small mammals (rodents and marsupials), carnivores, and primates among the most frequently recorded species. However, it is expected that mammal communities in agroecosystems are influenced by environmental filtering (Suárez-Tangil & Rodríguez 2023). We observed a significant reduction ($p<0.05$) in occurrences and species richness reported in crop areas, with primates and rodents showing the greatest reduction, -70% and -50%, respectively. As they are dependent on forest environments, the decline of primates in croplands represents a significant threat to primate populations (Estrada et al. 2012). Nevertheless, although our data indicate a clear reduction of primates in crop settings, studies conducted worldwide have demonstrated that primate species may exhibit dietary flexibility in forest-agricultural mosaics, with crops providing them with fallback foods (Freitas et al. 2008, Lins & Ferreira 2019, Bryson-Morrison et al. 2020). Indeed, the blonde capuchin (*Sapajus flavius*) registered in our data, occupies the highly deforested Pernambuco Endemism Center (Feijó et al. 2023), with frequent reports of animals raiding plantations, such as sugarcane (Lins & Ferreira 2019).

At the same time, while greater agricultural intensification may result in a decrease in rodent richness, the food supply in agricultural fields tends to lead to an increase in the abundance of generalist species (Gentili et al. 2014). Making generalist rodents more common and abundant in agricultural fields worldwide (Sullivan & Sullivan 2006, Caudill et al. 2014, Verdade et al. 2020, Pustika et al. 2024). Additionally, although bats did not exhibit greater differences between off crop and in crop assemblies, they remained the richest group in terms of species within crops **(Fig. 3)**, raising concerns about their exposure to pesticides. We encourage researchers to detail the specific occurrences within the agroecosystem (e.g., outside the crop, at the edge, or inside the crop) as this information was generally lacking, highlighting existing gaps in studies on biodiversity in agroecosystems (Prosser et al. 2016, Ferreira et al. 2018).

Understanding and assessing the off crop and in crop mammal communities is essential for a proper exposure risk evaluation. The direct effects of exposure to pesticides vary to a greater or lesser degree for species that occur off crop and in crop, or simultaneously in these two environments (Tew et al. 1992, Haughton et al. 1999). Moreover, it is expected that in crop species are more exposed to the direct effect of the application of pesticides. The direct exposure to pesticides can result in deaths, reproductive and behavioral disorders, bioaccumulation, and other negative effects (Köhler and Triebskorn 2013, Gibbons et al. 2015, Prosser et al. 2016). While off crop species are more susceptible to the indirect effects of exposure (EFSA et al. 2023). The indirect exposure can lead to a decrease in abundance or fitness due to the loss of habitat and food resources when non-target plant habitats are degraded or destroyed (Tew et al. 1992, Prosser et al. 2016).

4.1. Applications to risk assessments of extinction and exposure to pesticides

Our result shows that 41% of the threatened species (Critically Endangered, Endangered, and Vulnerable) found in Brazilian agroecosystems occurred in crop. Their occurrence and diversity within agroecosystems and within agricultural fields, suggest exposure to pesticides and detrimental consequences at unstudied biological levels, such as population, individual health, and molecular levels. This is particularly concerning for threatened species, which are already susceptible to other threats.

Evaluating pesticides as threats can enhance the effectiveness of pesticide risk assessments for endangered species, highlighting the significance of this data in IUCN assessments and National Action Plans for the Conservation of Endangered Mammals (PANs). Looking at the IUCN Red List, the only species for which pesticides were included as a threat was the Near Threatened jaguar (Quigley 2017). Counterintuitively, in Brazil, where the species is listed as Vulnerable, there is no mention of pesticides in the PAN Grandes Felinos. Furthermore, no threats of bioaccumulation or presence of heavy metals were found in the jaguar PAN, even though metal contamination has already been recorded for the jaguar in Brazilian Pantanal (May Júnior et al. 2018) and Amazon forest (Lopes et al. 2020).

Among Brazilian threatened mammals, pesticides were listed as threats to the target species of the PAN Anteaters and Armadillos, and to the PAN Ungalados. Recently, (Medici et al. 2021) demonstrated that the Brazilian tapir is exposed to contamination by Organophosphates, Carbamates, Pyrethroids, and metals in the Brazilian Cerrado. This study pioneered the identification of pesticides threats to ungulates in Brazil. This is an important issue for future research because for many threatened carnivores occurring in Brazil, like the small cats (e.g, *Herpailurus yagouaroundi*, *Leopardus guttulus*, *Leopardus wiedii*, *L. tigrinus*) pesticides are not even mentioned in national action plans (PAN Pequenos Felinos); for others species (e.g. *C. brachyurus* [PAN Canídeos] and *L. geoffroyi* [PAN Pequenos Felinos]), when it is mentioned, only potential threats of bioaccumulation due to contamination by heavy metals and pesticides are discussed. Unfortunately, there are still few studies focusing on contaminants in Brazilian carnivores (e.g. Brait et al. 2009, May Júnior et al. 2018, Lopes et al. 2020, Soresini et al. 2021).

Thus, given that there is little data available on the effects and exposure for most endangered species, relying on ecotoxicology studies to fill this gap is one of the priorities for advancing risk assessment and coverage of endangered species in ERA. It is recommended that non-lethal, non-invasive, or minimally invasive biomarkers of exposure be developed to enhance our comprehension of exposure pathways and impacts on terrestrial species that are threatened or endangered (Smith et al. 2007). In some cases, blood, hair, spines, feces, small skin and/or fat biopsies, or the collection of tissues from carcasses, can be obtained from threatened and endangered species and are suitable means of investigating exposure to pollutants (Rogival et al. 2006, Wang et al. 2020, Medici et al. 2021, van As et al. 2022, García-Muñoz et al. 2023). We emphasize that it is important that studies to expand knowledge about pesticides are carried out based on the quantification of pesticides in biological samples and in paired off crops and in crop designs, so that these data are reliable for use in ERA.

4.2. Top ten crops, mammal richness and exposure trait group

Categorizing all mammal occurrences across all Brazilian crops proved impossible, and as demonstrated, some records might have been underrepresented while others overrepresented. Therefore, we opted to list the top ten crops with the highest number of mammal occurrences. This approach enabled us to discuss focal species by crop and explore the functional traits that led to mammal exposure in agroecosystem types.

Focal species are ideally selected for their ecological relevance, occurrence and distribution in agricultural fields, sensitivity to potential stressors (different groups of pesticides) and ease of sampling (Vallon et al. 2018). In-depth knowledge of the ecotoxicology and exposure of these species can therefore provide reliable information on the effects on many other species (EFSA Scientific Committee 2016, Vallon et al. 2018). According to our data, 23 mammals had a frequency >=20 in the top ten crops and these could be potential focal species **(Fig. 5.)**. The rodents of family Cricetidae, subfamily Sigmodontinae - *Necromys lasiurus, Oligoryzomys nigripes* and *Calomys tener* - exhibited highest frequency in pasture grazing systems and tree plantation (Eucalyptus), showing in these systems, these rodents can be focal species in terms of frequency of occurrence. The high diversity and extensive distribution of cricetid rodents make them a suitable group for assessing pesticide exposure in realistic agricultural field scenarios (Prado & Percequillo 2013, Fritsch et al. 2022). Additionally, rodents as the main prey of several predators are important components of the food chain (Sousa & Bager 2008).

Insectivores small mammals and rodents are also of particular focus in post-registration surveys to assess pesticides impacts on mammals, being largely represented in the list of "mammal indicator species", "generic focal species" in the first levels of the ERA (EFSA et al. 2023). *Didelphis aurita* was the only marsupial among the most frequent mammals, mainly in pastures and cocoa fields. Recent reports have also indicated the presence of marsupials feeding on prey in sugarcane fields (de Camargo et al., 2022), which points to a potential trophic route of exposure for this group. Didelphimorphia marsupials are unique components of the Neotropical fauna, exhibiting a great diversity of feeding habits and locomotor patterns. Furthermore, due to their ancient divergence from placental mammals, known model organisms (mainly rodents such as *Mus musculus* and *Rattus sp.*) cannot be used to understand the pesticide exposure risks faced by marsupials. Ultimately, their presence in crops should be of great concern, as they may be intensely exposed to pesticides and the consequences of exposure are not covered by current models.

Concerning primates, the endangered golden-headed lion tamarin (*Leontopithecus chrysomelas*) emerges as a potential focal species for primates in cabrucas, given its high frequency within this crop. The *L. chrysomelas* mainly occupies landscapes shaded by agroforestry cocoa (Flesher 2015, Oliveira et al. 2011, de Almeida-Rocha et al. 2020) and rubber plantations (De Vleeschouwer & Oliveira 2017), as well they have been reported to cause damage to forest plantations (Mikich & Liebsch 2014).

Many carnivores showed higher frequency of occurrence in the top ten crops, among them two listed as vulnerable - *Chrysocyon brachyurus* and *Herpailurus yagouaroundi* - potential focal species, especially in tree plantation. As carnivore richness increases in higher quality agricultural patches (Prevedello & Vieira 2010), exposure may depend on this structural quality. Indeed, eucalyptus, pine and palm oil plantations are the most frequently used by carnivores (Ferreira et al. 2018), but foraging also occurs in annual crops because of the presence of abundant prey (Courtalon & Busch 2010, Gheler-Costa et al. 2012, Gomes de Sá et al., 2024). Their presence emphasizes the necessity of incorporating monitoring of these species as a fundamental component of the initial ERA assessment, rather than solely at higher tiers, as is presently practiced. In addition, the most frequent mammal in top ten crops is also a carnivore (*Cerdocyon thous*) and could be a potential focal species to cover other carnivores in Brazil, due to its generalist habits and large home range.

Finally, two bat species (*Carollia perspicillata* and *Artibeus planirostris*) were among the most frequent species. Bats exhibit an array of feeding adaptations (Kunz et al. 2011), using tree plantations like rubber trees as stopover sites or foraging habitats (Heer et al. 2015). Frugivorous and nectarivores species feed on fruit plants both in the canopy and in the herbaceous layers in shade plantations (Estrada & Coates-Estrada

2002), and are frequently recorded in agroforestry, like cocoa cultivation in Brazil (Faria et al. 2006, Faria & Baumgarten 2007). Due the services they provide in agroecosystems - pollination, seed dispersal and pest control – bats are an important group to consider in risk assessment, and are not yet covered, with regard to the protection goals of ecosystem services in the ERA assessment (Kunz et al. 2011, Ramírez-Fráncel et al. 2022, Buxton et al. 2022).

Moreover, as expected, the results indicate that the analysis of functional traits across agroecosystem types cannot be condensed into a single exposure trait group, given the high complexity of the functional space of mammals. Among mammals, there are considerable variations in behavior and functional traits that determine the occupation of specific niches (Cox et al. 2021). Each taxon possesses intrinsic ecological characteristics that influence its potential for exposure to contaminants. Therefore, the use of model organisms in risk assessment is limited and poses a challenge (Smith et al. 2007). The ERA scheme assumes that food exposure is the main route of exposure and considers a single diet type in the screening tier of the exposure assessment. Additionally, it utilizes Indicator Model Species (IMS). Although it is not a true species, IMS is considered to have a higher internal exposure than all wild species, and is therefore sufficiently protective for mammals in a conservative exposure scenario (EFSA et al. 2023). Given the diversity of crops and mammals found in this study, we do not consider that a single model indicator species approach is appropriate in the Brazilian screening tier. Our evaluation of exposure trait groups showed that at least three groups of species should be considered among agroecosystem types. The exception is annual cropland, where we found two exposure trait groups.

It's worth mentioning that for the three types of agroecosystems (pasture-grazing, tree plantation, and annual cropland), the primary functional vectors of exposure in group A were: terrestrial, crepuscular, and body mass, with vertebrates as their main diet. This suggested that for these agroecosystems, a single model indicator species might suffice to cover focal species at the screening tier level. Conversely, in agroforestry and perennial crop agroecosystems, it's recommended to consider at least two model indicator species with arboreal habits: one with a frugivorous diet and the other with a nectarivorous diet. The Tier 1 (steps in ERA) exposure assessment in the subsequent step relies on the oral exposure to generic model species (GMS). It is believed that GMS, which are identified by their distinct feeding guilds, serves as a representative sample for all focal mammal species within crop groupings characterized by comparable growth patterns (EFSA et al.

2023). Thus, it is assumed that in the pasture-grazing system, tree plantations and annual cropland should be considered as exposure routes for carnivorous and herbivorous mammals. In agroforests and perennial croplands, GMS that represent the diet of frugivorous and nectarivorous mammals should be prioritized. Furthermore, in three of the five agroecosystem types (pasture, tree plantation, and perennial crop), the primary functional vectors of exposure were Invertebrates and Aerial. Although these traits were clustered together in group C due to the low probability distribution of the trait combinations in the functional space, we recommend the inclusion of aerial insectivorous bats as a third GMS in Tier 1. Especially because the abundance of prey in agricultural areas serves as a source of food for bats (Wickramasinghe et al. 2004) and insectivorous bats are considered more susceptible to pesticide contamination (Bennett & Thies 2007).

4.3. Limitations of Study and Future Directions Regarding Mammalian Exposure

Brazil is in the early stages of assessing the risk of pesticides to non-target organisms. Data from the literature is a fundamental tool for understanding the occurrence and exposure of mammals in agroecosystems.

However, methodological limitations make it difficult to compare studies and groups of mammals, such as: i) Sample size - The accumulation curves showed no trend towards stabilization for the agroecosystems of pasture-grazing, tree plantation, annual cropland, and agroforestry. Suggesting that for these agroecosystems, more mammals are expected to occur than are currently detected, except for perennial plantation agroecosystems; ii) Mammal sampling methods - The diverse behaviors and evolutionary traits exhibited by Neotropical mammals pose challenges in sampling this group effectively. A range of methods are used, commonly separated into three main categories: nets and recorders for bats, live-traps for small mammals, and census, camera-trap for medium and large-sized mammals, which may introduce biases in species richness estimation, influencing our overarching interpretations; iii) Data availability and agroecosystem descriptors - We noted a significant omission in the studies reviewed related to the absence of agroecosystem involved in the study area descriptions. In numerous instances, articles were excluded from our review due to failure to meet the minimum inclusion criterion - specifically, the type of crop involved - highlighting the lack of description of the study area. The absence of standardized information on agroecosystem descriptors may have introduced potential biases or gaps in our analysis. Refer to Box 1 for detailed crop descriptors crucial for accurate exposure assessment.

Box 1. Minimum descriptors for mammal exposure assessment in agroecosystems.

Data	Description
Mammal ocurrence	· Outside the treated area, within remnants of natural vegetation (off crop) · At the edge of the field (edge - in crop) · Treated area (in crop)
Crop	· Scientific name · Plant cultivar or variety · first and last harvest times · Phenological stages of crop at the time of study · Agricultural area (ha)
Landscape	· Information about agroecosystem types and landscape detail, if: · planted pasture · perennial or annual agricultural land · agroforestry · tree plantation · or other types
Pesticides	· When feasible, collecting information on the types of pesticides, class of use, mode of application, and time of application in the field at the time of study implementation is valuable

The threat of mammal exposure in agroecosystems can manifest as acute or chronic impacts. The main research challenge in this regard lies in identifying the chronic effects of potential pesticides applied to crops, which are contingent upon the presence and duration of mammal exposure within the crop, as well as the potential biodynamics and exposure routes of the pesticide. Here, we outline several future directions for investigating mammal exposure: i) Field validation - Carrying out field studies to validate the effectiveness of the model and generic model species in predicting exposure in Brazilian agricultural scenarios, including incorporating more detailed ecological data and refining trait-based approaches; ii) Develop studies in biomes with gaps - according to our review, the knowledge gaps are more pronounced outside the southeast region, the Caatinga and the Pampa were the least represented and, more studies covering exposure in these environments are needed; iii) pesticides as a threat to endangered species - Beyond agroecosystems, exposure to pesticides is probably the most neglected threat in the National Action Plans for the Conservation of Endangered Species in Brazil. We advocate for a comprehensive examination of pesticide threats to these species and the initiation of research projects aimed at quantifying pesticide exposure in endangered species; iv) Development of quantification and monitoring protocols - To accurately assess exposure to pesticides in wild mammals, it is essential development of quantification protocols and implementing long-term monitoring programs to track changes in mammal populations and exposure patterns over time; vi) Create a database - Establish a comprehensive database on the occurrence of wild mammals Brazilian crops.

5. Conclusions

Our review addresses a pioneering topic on the occurrence and exposure of mammals in Brazilian agroecosystems and provides important information, applicable to research and the environmental regulatory sector, on the environmental risk assessment of pesticides for non-target organisms. We demonstrated that at least 44% of Brazilian terrestrial mammals occur in agricultural landscapes, and more than half of these species (64.26%) are found in crops. The presence of mammals in crops is an unequivocal indication of the need to consider pesticide threats in endangered species assessments. Furthermore, it is important to carry out more studies that focus on the gaps in our review, such as family farming and the Caatinga and Pampa biomes.

Finally, our functional traits approach revealed the main exposure traits by agroecosystem type, indicating that more than one indicator model species is needed for mammals in the Neotropical region. We emphasize that pesticide quantification and monitoring studies, especially on endangered species, should be carried out to fill these conservation blind spots.

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Supporting information

Conservation blind spot: exposure of mammals to pesticides in Brazilian agroecosystems

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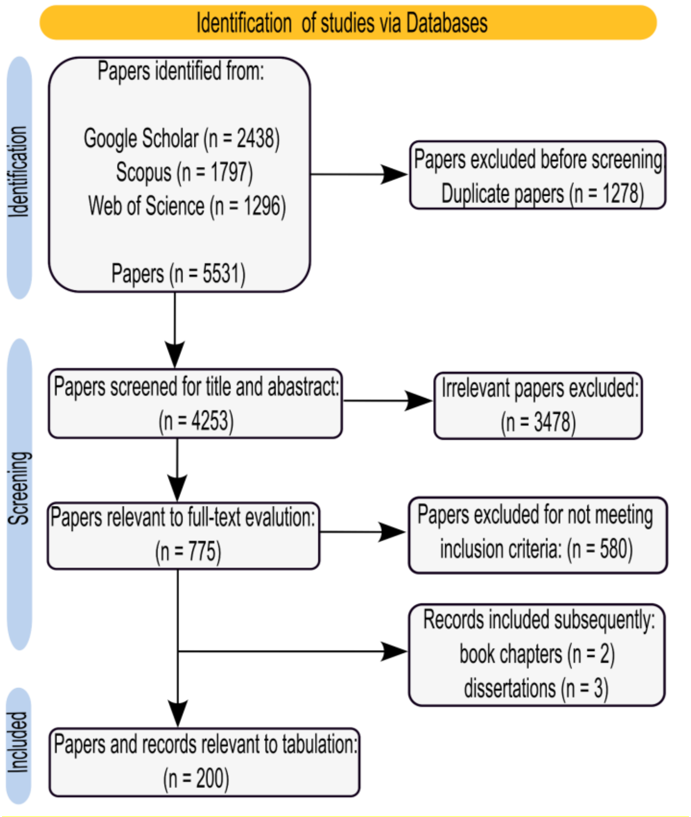
Appendix S1. Flowchart of the procedures of our literature review of occurrences of mammals in agroecosystems in Brazil, following PRISMA (Preferred reporting items for systematic reviews and meta-analysis) guidelines (Page et al. 2021).



Appendix S2. Selected Studies on the Occurrence of Mammals in Brazilian Agroecosystems

ID	Authors	Year	Title	DOI
1	Abra et al. A, F.D., CANENA, A.C., GARBINO, G.S.T., MEDICI, E.P.	2020	Use of unfenced highway underpasses by lowland tapirs and other medium and large mammals in central-western Brazil.	https://doi.org/10.1016/j.pecon.2020.10.006
2	ABRA, F.D., GARBINO, G.S., PRIST, P.R., NASCIMENTO, F.O., LEMOS, F.G.	2020	New occurrences of Hoary Fox, *Lycalopex vetulus* (Lund, 1842), and Pantanal Cat, *Leopardus braccatus* (Cope, 1889) (Mammalia, Carnivora), in a Cerrado-Caatinga-Atlantic Forest ecotone in northeastern Brazil.	https://doi.org/10.15560/16.6.1673
3	ABRAHAMS, M.I., PERES, C.A., COSTA, H.C.M.	2018	Manioc Losses by Terrestrial Vertebrates in Western Brazilian Amazonia	https://doi.org/10.1002/jwmg.21443
4	ADAM, M.L., PESSOA, L.A.R., LIMA, A.R.B., BERNARD, E.	2022	DNA damage as indicator of the environmental vulnerability of bats in Brazil's Caatinga drylands.	https://doi.org/10.1007/s10661-022-09906-9
5	ALMEIDA-MAUÉS, P.C.R., BUENO, A.S., PALMEIRIM, A.F., PERES, C.A., MENDES-OLIVEIRA, A.C.	2022	Assessing assemblage - wide mammal responses to different types of habitat modification in Amazonian forests.	https://doi.org/10.1038/s41598-022-05450-1
6	ALVES, T.S., ALVARADO, F., ARROYO-RODRÍGUEZ, V., SANTOS, B.A.	2020	Landscape-scale patterns and drivers of novel mammal communities in a human-modified protected area.	https://doi.org/10.1007/s10980-020-01040-6
7	ALVES, T.R., FONSECA, R.C.B., ENGEL, V.L.	2012	Mamíferos de médio e grande porte e sua relação com o mosaico de habitats na cuesta de Botucatu, Estado de São Paulo, Brasil.	https://doi.org/10.1590/S0073-47212012000200006
8	AMARAL, C.D., COSTA, G.B., DE SOUZA, W.M., ALVES, P.A., BORGES, I.A., TOLARDO, A.L., ROMEIRO, M.F., DRUMOND, B.P., ABRAHÃO, J.S., KROON, E.G., PAGLIA, A.P.,FIGUEIREDO, L.T.M., DE SOUZA TRINDADE, G.	2018	Orthohantavirus Circulation Among Humans and Small Mammals from Central Minas Gerais, Brazil.	https://doi.org/10.1007/s10393-018-1353-2
9	ASSIS, T.O., CARVALHO, É.C., CURI, N.H.A., PASSAMANI, M.	2020	Use of forest fragments and agricultural matrices by small mammals in southeastern Brazil.	https://doi.org/10.31687/saremMN.20.27.1.0.10
10	AZEVEDO, F.C.C., LEMOS, F.G., FREITAS-JUNIOR, M.C., ROCHA, D.G., AZEVEDO, F.C.C.	2018	Puma activity patterns and temporal overlap with prey in a human-modified landscape at Southeastern Brazil.	https://doi.org/10.1111/jzo.12558
11	AZEVEDO, F.C., LEMOS, F.G., ROCHA, D.G., COSTA, A.N., FREITAS-JÚNIOR, M.C.	2016	Novo registro do cachorro-vinagre *Speothos venaticus* em uma paisagem antropizada no oeste de Minas Gerais, Brasil.	https://doi.org/10.14393/BJ-v32n1a2016-33302
12	BARBOSA, R.A.P., OLIVEIRA, M.A.	2022	New records and range extension of *Euphractus sexcinctus* (Linnaeus, 1758) (Cingulata, Chlamyphoridae) in Rondônia state, Brazil.	https://doi.org/10.15560/18.2.265
13	BARROS, B.C.V., CHAGAS, E.N., BEZERRA, L.W., RIBEIRO, L.G., BARBOZA DUARTE, J.W., PEREIRA, D., DA PENHA, E.T., SILVA, J.R., MELO BEZERRA, D.A.,BANDEIRA, R.S., COSTA PINHEIRO, H.H., DOS SANTOS GUERRA, S.F., GUIMARÃES, R.J.P.S., MASCARENHAS, J.D.A.P.	2018	Rotavirus A in wild and domestic animals from areas with environmental degradation in the Brazilian Amazon.	https://doi.org/10.1371/journal.pone.0209005
14	BARROS, M.A.S., DE MAGALHÃES, R.G., RUI, A.M.	2015	Species composition and mortality of bats at the Osório Wind Farm, southern Brazil.	https://doi.org/10.1080/01650521.2014.1001595
15	BECA, G., VANCINE, M.H., CARVALHO, C.S., PEDROSA, F., ALVES, R.S.C., BUSCARIOL, D., PERES, C.A., RIBEIRO, M.C., GALETTI, M.	2017	High mammal species turnover in forest patches immersed in biofuel plantations.	https://doi.org/10.1016/j.biocon.2017.02.033
16	BEGOTTI, R.A., PACÍFICO, E.S., FERRAZ, S.F.B., GALETTI, M.	2018	Landscape context of plantation forests in the conservation of tropical mammals.	https://doi.org/10.1016/j.jnc.2017.11.009
17	BÉLLON, B.A.W., HENRY, D., RENAUD, P.C., ROQUE, F.O., SANTOS, C.C., MELO, I., ARVOR, D., VOS, A.	2022	Landscape drivers of mammal habitat use and richness in a protected area and its surrounding agricultural lands.	https://doi.org/10.1016/j.agee.2022.107989

18	BENVINDO-SOUZA, M., HOSOKAWA, A.V., SANTOS, C.G.A., ASSIS, R.A., PEDROSO, T.M.A., BORGES, R.E., PACHECO, S.M., SANTOS, L.R.S., SILVA, D.M.	2022	Evaluation of genotoxicity in bat species found on agricultural landscapes of the Cerrado savanna, central Brazil.	https://doi.org/10.1016/J.ENVPOL.2021.118579
19	BERTASSONI, A., MOURÃO, G., BIANCHI, R.C.	2020	Space use by giant anteaters (*Myrmecophaga tridactyla*) in a protected area within human-modified landscape.	https://doi.org/10.1002/ece3.5911
20	BERTRAND, A.	2016	Characterization and Conservation of the Iguaçu National Park, Brazil	https://search.proquest.com/openview/268ffc639becdfbff1bfe583a640b075/1?pq-origsite=gscholar&cbl=2026366&diss=y
21	BIANCHI, R., JENKINKS, J.M.A., LESMEISTER, D.B., GOUVEA, J.A., CESÁRIO, C.S., FORNITANO, L., OLIVEIRA, M.Y., MORAIS, K.D.R., RIBEIRO, R.L.A., GOMPPER, M.E.	2021	Tayra (*Eira barbara*) landscape use as a function of cover types, forest protection, and the presence of puma and free-ranging dogs.	https://doi.org/10.1111/btp.13005
22	BIANCONI, G.V., MIKICH, S.B., TEIXEIRA, S.D., MAIA, B.H.L.N.S.	2007	Attraction of fruit-eating bats with essential oils of fruits: A potential tool for forest restoration.	https://doi.org/10.1111/j.1744-7429.2006.00236.x
23	BIANCONI, G.V., SUCKOW, U.M.S., CRUZ-NETO, A.P., MIKICH, S.B.	2012	Use of Fruit Essential Oils to Assist Forest Regeneration by Bats.	https://doi.org/10.1111/j.1526-100X.2010.00751.x
24	BOCCHIGLIERI, A., MENDONÇA, A.F., HENRIQUES, R.P.B.	2010	Composição e diversidade de mamíferos de médio e grande porte no cerrado do Brasil central.	https://doi.org/10.1590/S1676-06032010000300019
25	BONECKER, S.T., PORTUGAL, L.G., COSTA-NETO, S.F., GENTILE, R.	2009	A long term study of small mammal populations in a Brazilian agricultural landscape.	https://doi.org/10.1016/j.mambio.2009.05.010
26	BOVO, A.A.A., MAGIOLI, M., PERCEQUILLO, A.R., KRUSZYNSKI, C., ALBERICI, V., MELLO, M.A.R., CORREA, L.S., GEBIN, J.C.Z., RIBEIRO, Y.G.G., COSTA, F.B., RAMOS, V.N., BENATTI, H.R., LOPES, B., MARTINS, M.Z.A., DINIZ-REIS, T.R., CAMARGO, P.B., LABRUNA, M.B., FERRAZ, K.M.P.M.B.	2018	Human-modified landscape acts as refuge for mammals in atlantic forest.	https://doi.org/10.1590/1676-0611-BN-2017-0395
27	BOYLE, S.A., SMITH, A.T.	2010	Can landscape and species characteristics predict primate presence in forest fragments in the Brazilian Amazon?	https://doi.org/10.1016/j.biocon.2010.02.008
28	BRAGA, C.A.C., PREVEDELLO, J.A., PIRES, M.R.S.	2015	Effects of Cornfields on Small Mammal Communities: A Test in the Atlantic Forest Hotspot.	https://doi.org/10.1093/jmammal/gyv094
29	BRANDÃO, E.M.V., XAVIER, S.C.C., CARVALHAES, J.G., D'ANDREA, P.S., LEMOS, F.G., AZEVEDO, F.C., CÁSSIA-PIRES, R., JANSEN, A.M., ROQUE, A.L.R.	2019	Trypanosomatids in small mammals of an agroecosystem in central brazil: Another piece in the puzzle of parasite transmission in an anthropogenic landscape.	https://doi.org/10.3390/pathogens8040190
30	BRUM, T.R., SANTOS-FILHO, M., CANALE, G.R., IGNÁCIO, A.R.A.	2017	Effects of roads on the vertebrates diversity of the Indigenous Territory Paresi and its surrounding.	https://doi.org/10.1590/1519-6984.08116
31	CÁCERES, N.C., NÁPOLI, R.P., CASELLA, J., HANNIBAL, W.	2010	Mammals in a fragmented savannah landscape in south-western Brazil.	https://doi.org/10.1080/00222930903477768
32	CÁCERES, N.C., NÁPOLI, R.P., LOPES, W.H., CASELLA, J., GAZETA, G.S.	2007	Natural history of the marsupial *Thylamys macrurus* (Mammalia, Didelphidae) in fragments of savannah in southwestern Brazil.	https://doi.org/10.1080/00222930701520835
33	CAJAIBA, R.L., PÉRICO, E., SILVA, W.B., VIEIRA, T.B., SANTOS, F.M.B., SANTOS, M.	2021	Are neotropical cave-bats good landscape integrity indicators? Some clues when exploring the cross-scale interactions between underground and above-ground ecosystems.	https://doi.org/10.1016/j.ecolind.2020.107258
34	CAMPOS, B.M., CHARTERS, J.D., VERDADE, L.M.	2018	Diversity and distribution patterns of medium to large mammals in a silvicultural landscape in south-eastern Brazil.	https://doi.org/10.3832/ifor2721-011

35	CARDOSO, T.S., SIMÕES, R.O., LUQUE, J.L.F., MALDONADO, A., GENTILE, R.	2016	The influence of habitat fragmentation on helminth communities in rodent populations from a Brazilian Mountain Atlantic Forest.	https://doi.org/10.1017/S0022149X15000589
36	CARVALHO, W.D., MEYER, C.F.J., XAVIER, B.S., MUSTIN, K., CASTRO, I.J., SILVESTRE, S.M., PATHEK, D.B., CAPAVERDE, U.D., HILÁRIO, R., TOLEDO, J.J.	2020	Consequences of Replacing Native Savannahs With Acacia Plantations for the Taxonomic, Functional, and Phylogenetic α- and β-Diversity of Bats in the Northern Brazilian Amazon.	https://doi.org/10.3389/fevo.2020.609214
37	CASAGRANDE, A.F., SANTOS-FILHO, M.	2019	Use of forest remnants and teak (*Tectona grandis*) plantations by small mammals in Mato Grosso, Brazil.	https://doi.org/10.1080/01650521.2019.1656520
38	CASANOVA, D.C., BERNARDO, R.	2017	New record of the jaguar, *Panthera onca* (Linnaeus, 1758) (Felidae), from a mosaic of Atlantic Forest in the Paraná state, Brazil	https://doi.org/10.15560/13.2.2075
39	CASSANO, C.R., BARLOW, J., PARDINI, R.	2012	Large Mammals in an Agroforestry Mosaic in the Brazilian Atlantic Forest.	https://doi.org/10.1111/j.1744-7429.2012.00870.x
40	CASSANO, C.R., BARLOW, J., PARDINI, R.	2014	Forest loss or management intensification? Identifying causes of mammal decline in cacao agroforests.	https://doi.org/10.1016/j.biocon.2013.10.006
41	CASSANO, C.R., KIERULFF, M.C.M., CHIARELLO, A.G.	2011	The cacao agroforests of the Brazilian Atlantic forest as habitat for the endangered maned sloth *Bradypus torquatus*.	https://doi.org/10.1016/j.mambio.2010.06.008
42	CASSANO, C.R., RIOS, V.A., GAIOTTO, F.A.	2021	Who ate my chocolate? Small mammals and fruit damage in cacao agroforestry.	https://doi.org/10.1007/s10457-021-00645-3
43	CAVALCANTI, S.M.C., GESE, E.M.	2010	Kill rates and predation patterns of jaguars (*Panthera onca*) in the southern Pantanal, Brazil.	https://doi.org/10.1644/09-MAMM-A-171.1
44	CERBONCINI, R.A.S., PASSAMANI, M., BRAGA, T.V.	2011	Use of space by the black-eared opossum *Didelphis aurita* in a rural area in southeastern Brazil.	https://doi.org/10.1515/mamm.2011.024
45	CHRISTIANINI, A.V., GALETTI, M.	2007	Spatial variation in post-dispersal seed removal in an Atlantic forest: Effects of habitat, location and guilds of seed predators.	https://doi.org/10.1016/j.actao.2007.06.004
46	COLLEVATTI, R.G., VITORINO, L.C., VIEIRA, T.B., OPREA, M., TELLES, M.P.C.	2020	Landscape changes decrease genetic diversity in the Pallas' long-tongued bat.	https://doi.org/10.1016/j.pecon.2020.06.006
47	CORRÊA, M.R.J., BELLGAMBA, Y.M., MAGALHÃES, A.P., MARTINS, J.P.V., CRUZ, A.J.D.R., KOZOVITZ, A.R., MESSIAS, M.C.T.B., AZEVEDO, C.S.	2018	Microhabitat structure and food availability modelling a small mammal assemblage in restored riparian forest remnants.	https://doi.org/10.1515/mammalia-2017-0026
48	COSTA, A.R.C., PASSAMANI, M., DA CUNHA, R.G.T.	2019	Survey of medium-sized and large mammals in semideciduous Atlantic Forest patches near Alfenas, southern Minas Gerais, Brazil.	https://doi.org/10.15560/15.1.209
49	COSTA, N.A., CARDOSO, T.S., NETO, S.F.C., JÚNIOR, A.M., GENTILE, R.	2019	Metacommunity Structure of Helminths of *Necromys Lasiurus* (Rodentia: Sigmodontinae) in Different Land Use Areas in the Brazilian Cerrado.	https://doi.org/10.1645/17-199
50	COSTA, T.S.O., NOGUEIRA-FILHO, S.L.G., VLEESCHOUWER, K.M., OLIVEIRA, L.C., SOUZA, M.B.C., MENDL, M., CATENACCI, L.S., NOGUEIRA, S.S.C.	2020	Individual behavioral differences and health of golden-headed lion tamarins (*Leontopithecus chrysomelas*).	https://doi.org/10.1002/ajp.23118
51	CULLEN, L., SANA, D.A., LIMA, F., ABREU, K.C., UEZU,A.	2013	Selection of habitat by the jaguar, *Panthera onca* (Carnivora: Felidae), in the upper Paraná River, Brazil.	https://doi.org/10.1590/S1984-46702013000400003
52	DA ROSA, C.A.	2018	New records of *Blastocerus dichotomus* Illiger, 1815 (Mammalia: Cervidae) in a monoculture area in the state of Minas Gerais, Brazil.	http://doi.org/10.1016/j.jsames.2016.06.013
53	DA ROSA, C.A., SECCO, H., CARVALHO, N., MAIA, A.C., BAGER, A.	2018	Edge effects on small mammals: Differences between arboreal and ground-dwelling species living near roads in Brazilian fragmented landscapes.	https://doi.org/10.1111/aec.12549
54	DA SILVA, L.C.N., VIEIRA, T.B., OLIVEIRA, A.S.Q.A., MENDES, P., PEIXOTO, F.P., OPREA, M., AGUIAR, L.M.D.S.	2022	Bat species of a karstic region in the Brazilian savanna and extension of the *Hsunycteris thomasi* (Phyllostomidae: Lonchophyllinae) distribution.	https://doi.org/10.1515/mammalia-2021-0019

55	DA SILVA, L.G., RIBEIRO, M.C., HASUI, É., DA COSTA, C.A., DA CUNHA, R.G.T.	2015	Patch size, functional isolation, visibility and matrix permeability influences neotropical primate occurrence within highly fragmented landscapes.	https://doi.org/10.1371/journal.pone.0114025
56	DALPONTE, J.C., LIMA, H.S., KLORFINE, S., DA LUZ, N.C.	2018	Home range and spatial organization by the Hoary Fox *Lycalopex vetulus* (Mammalia: Carnivora: Canidae): response to social disruption of two neighboring pairs.	https://doi.org/10.11609/jott.3082.10.6.11703-11709
57	D'ANDREA, P.S., GENTILE, R., MAROJA, L.S., FERNANDES, F.A., COURA, R., CERQUEIRA, R.	2007	Small mammal populations of an agroecosystem in the Atlantic Forest domain, southeastern Brazil.	https://doi.org/10.1590/s1519-69842007000100025
58	DE ALMEIDA-ROCHA, J.M., DE VLEESCHOUWER, K.M., REIS, P.P., CARLOS, C.E., OLIVEIRA, L.C.	2015	Do Habitat Use and Interspecific Association Reflect Predation Risk for the Golden-Headed Lion Tamarin (*Leontopithecus chrysomelas*)?	https://doi.org/10.1007/s10764-015-9885-6
59	DE ALMEIDA-ROCHA, J.M., PERES, C.A., MONSALVO, J.A.B., OLIVEIRA, L.D.C.	2020	Habitat determinants of golden-headed lion tamarin (*Leontopithecus chrysomelas*) occupancy of cacao agroforests: Gloomy conservation prospects for management intensification.	https://doi.org/10.1002/ajp.23179
60	DE BARROS FERRAZ, K.M.P.M., SIQUEIRA, M.F., MARTIN, P.S., ESTEVES, C.F., COUTO, H.T.Z.	2010	Assessment of *Cerdocyon thous* distribution in an agricultural mosaic, southeastern Brazil.	https://doi.org/10.1515/mamm.2010.036
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143	OLIVEIRA, M.B., BRANDÃO, M.L., CORDEIRO, J.L.P., DE OLIVEIRA, L.F.B., PERACCHI, A.L.	2020	Composition and frequency of capture of bat assemblages in a landscape mosaic in northern pantanal, mato grosso, brazil.	https://doi.org/10.31687/saremMN.20.27.1.0.25
144	PAISE, G., VIEIRA, E.M., PRADO, P.I.	2020	Small mammals respond to extreme habitat fragmentation in the Brazilian Atlantic Forest according to the landscape continuum model.	https://doi.org/10.1007/s13364-019-00464-z
145	PALMEIRA, F.B.L., CRAWSHAW, P.G., HADDAD, C.M., FERRAZ, K.M.P.MB., VERDADE, L.M.	2008	Cattle depredation by puma (*Puma concolor*) and jaguar (*Panthera onca*) in central-western Brazil.	https://doi.org/10.1016/j.biocon.2007.09.015
146	PALMEIRIM, A.F., SANTOS-FILHO, M., PERES, C.A.	2020	Marked decline in forest-dependent small mammals following habitat loss and fragmentation in an Amazonian deforestation frontier.	https://doi.org/10.1371/journal.pone.0230209
147	PAOLINO, R.M., ROYLE, J.A., VERSIANI, N.F., RODRIGUES, T.F., PASQUALOTTO, N., KREPSCHI, V.G., CHIARELLO, A.G.	2018	Importance of riparian forest corridors for the ocelot in agricultural landscapes.	https://doi.org/10.1093/jmammal/gyy075
148	PARDINI, R.	2004	Effects of forest fragmentation on small mammals in an Atlantic Forest landscape.	https://doi.org/10.1023/B:BIOC.0000048452.18878.2d
149	PASSAMANI, M., FERNANDEZ, F.A.S.	2011	Abundance and richness of small mammals in fragmented Atlantic Forest of southeastern Brazil.	https://doi.org/10.1080/00222933.2010.534561

150	PASSAMANI, M., RIBEIRO, D.	2009	Small mammals in a fragment and adjacent matrix in southeastern Brazil.	https://doi.org/10.1590/s1519-69842009000200010
151	PENA, S.A., MENDES-OLIVEIRA, A.C.	2019	Effect of oil palm (*Elaeis guineensis* jacq.) plantations on individual-level diet variation of *Hylaeamys megacephalus* (G. Fisher 1814) (Rodentia, Cricetidae) in eastern amazon forest.	https://doi.org/10.1590/1676-0611-bn-2018-0597
152	PINA, S.M.S., MEYER, C.F.J., ZORTÉA, M.	2014	A comparison of habitat use by phyllostomid bats (Chiroptera: Phyllostomidae) in natural forest fragments and Eucalyptus plantations in the Brazilian cerrado.	
153	PIÑA, T.E.N., CARVALHO, W.D., ROSALINO, L.M.C., HILÁRIO, R.R.	2019	Drivers of mammal richness, diversity and occurrence in heterogeneous landscapes composed by plantation forests and natural environments.	https://doi.org/10.1016/j.foreco.2019.117467
154	PIRES, A.S., LIRA, P.K., FERNANDEZ, F.A.S., SCHITTINI, G.M., OLIVEIRA, L.C.	2002	Frequency of movements of small mammals among Atlantic Coastal Forest fragments in Brazil.	https://doi.org/10.1016/S0006-3207(02)00109-X
155	PÔNZIO, M., ALBERICI, V., PASQUALOTTO, N., PAOLINO, R., RODRIGUES, T., CHIARELLO, A.	2022	Mammals of Cajuru State Forest and surroundings: a neglected but important Protected Area for the Cerrado conservation in the São Paulo state, Brazil.	https://doi.org/10.1590/1676-0611-BN-2021-1261
156	PÜTTKER, T., BUENO, A.A., DE BARROS, C.S., SOMMER, S., PARDINI, R.	2013	Habitat specialization interacts with habitat amount to determine dispersal success of rodents in fragmented landscapes.	https://doi.org/10.1644/12-MAMM-A-119.1
157	PÜTTKER, T., MEYER-LUCHT, Y., SOMMER, S.	2008	Effects of fragmentation on parasite burden (nematodes) of generalist and specialist small mammal species in secondary forest fragments of the coastal Atlantic Forest, Brazil.	https://doi.org/10.1007/s11284-007-0366-z
158	PÜTTKER, T., PARDINI, R., MEYER-LUCHT, Y., SOMMER, S.	2008	Responses of five small mammal species to micro-scale variations in vegetation structure in secondary Atlantic Forest remnants, Brazil.	https://doi.org/10.1186/1472-6785-8-9
159	RAGHUNATHAN, N., FRANÇOIS, L., CAZETTA, E., PITANCE, J.L., DE VLEESCHOUWER, K., HAMBUCKERS, A.	2020	Deterministic modelling of seed dispersal based on observed behaviours of an endemic primate in Brazil.	https://doi.org/10.1371/journal.pone.0244220
160	RAMOS, V.N., LEMOS, F.G., AZEVEDO, F.C., ARRAIS, R.C., LIMA, C.F.M., CANDEIAS, I.Z., MARTINS, M.M., SANDRIN, A.C.L.G., SIQUEIRA, S.M., SZABÓ, M.P.J.	2020	Wild carnivores, domestic dogs and ticks: Shared parasitism in the Brazilian Cerrado.	https://doi.org/10.1017/S0031182020000335
161	REALE, R., FONSECA, R.C.B., UIEDA, W.	2014	Medium and large-sized mammals in a private reserve of natural Heritage in the municipality of Jaú, São Paulo, Brazil.	https://doi.org/10.15560/10.5.997
162	REGOLIN, A.L., CHEREM, J.J., GRAIPEL, M.E., BOGONI, J.A., RIBEIRO, J.W., VANCINE, M.H., TORTATO, M.A., OLIVEIRA-SANTOS, L.G., FANTACINI, F.M., LUIZ, M.R., CASTILHO, P.V., RIBEIRO, M.C., CÁCERES, N.C.	2017	Forest cover influences occurrence of mammalian carnivores within Brazilian Atlantic Forest.	https://doi.org/10.1093/jmammal/gyx103
163	ROCHA, E.C., SILVA, E., FEIO, R.N., MARTINS, S.V., LESSA, G.	2008	Densidade populacional de raposa-do-campo *Lycalopex vetulus* (Carnivora, Canidae) em áreas de pastagem e campo sujo, Campinápolis, Mato Grosso, Brasil.	https://doi.org/10.1590/S0073-47212008000100011
164	ROCHA, M.F., PASSAMANI, M., LOUZADA, J.	2011	A small mammal community in a forest fragment, vegetation corridor and coffee matrix system in the brazilian atlantic forest.	https://doi.org/10.1371/journal.pone.0023312
165	ROCHA, R.G., FERREIRA, E., MARTINS, I.C.M., COSTA, L.P., FONSECA, C.	2014	Seasonally flooded stepping stones: Emerging diversity of small mammal assemblage in the Amazonia-Cerrado ecotone, central Brazil.	https://doi.org/10.1186/s40555-014-0060-0
166	ROCHA, V.J., AGUIAR, L.M., SILVA-PEREIRA, J.E., MORO-RIOS, R.F., PASSOS, F.C.	2008	Feeding habits of the crab-eating fox, *Cerdocyon thous* (Carnivora: Canidae), in a mosaic area with native and exotic vegetation in Southern Brazil.	https://doi.org/10.1590/s0101-81752008000400003
167	RODRIGUES, T.F., CERVEIRA, J.F., DUARTE, J.M.B.	2014	Uso de áreas agrícolas por *Mazama gouazoubira* (mammalia, cervidae) no estado de São Paulo.	https://doi.org/10.1590/1678-4766201410444439445
168	RODRIGUES, T.F., CHIARELLO, A.G.	2018	Native forests within and outside protected areas are key for nine-banded armadillo (*Dasypus novemcinctus*) occupancy in agricultural landscapes.	https://doi.org/10.1016/j.agee.2018.08.001

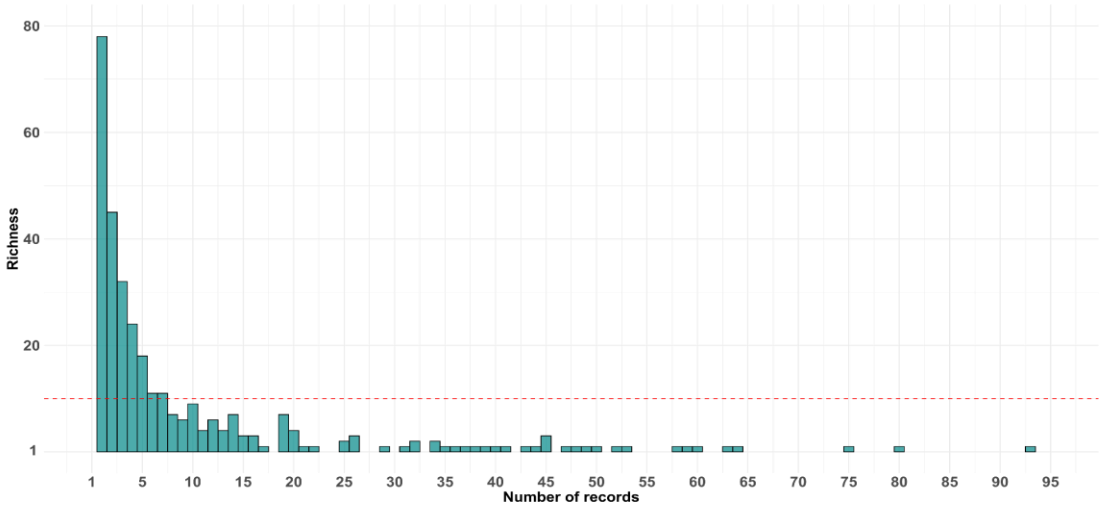
169	RODRIGUES, T.F., KAYS, R., PARSONS, A., VERSIANI, N.F., PAOLINO, R.M., PASQUALOTTO, N., KREPSCHI, V.G., CHIARELLO, A.G.	2017	Managed forest as habitat for gray brocket deer (*Mazama gouazoubira*) in agricultural landscapes of southeastern Brazil.	https://doi.org/10.1093/jmammal/gyx099
170	ROSALINO, L.M., MARTIN, P.S., GHELER-COSTA, C., LOPES, P.C., VERDADE, L.M.	2013	Allometric relations of neotropical small rodents (sigmodontinae) in anthropogenic environments.	https://doi.org/10.2108/zsj.30.585
171	ROSALINO, L.M., MARTIN, P.S., GHELER-COSTA, C., LOPES, P.C., VERDADE, L.M.	2014	Neotropical small mammals' diversity in the early cycle of commercial Eucalyptus plantations.	https://doi.org/10.1007/s10457-014-9702-9
172	SÁ, É.F.G.G., RODRIGUES, V.D.S., GARCIA, M.V., ZIMMERMANN, N.P., RAMOS, V.D.N., BLECHA, I.M.Z., DUARTE, P.D.O., MARTINS, T.F., BORDIGNON, M.O., ANDREOTTI, R.	2018	Ticks on *Didelphis albiventris* from a Cerrado area in the Midwestern Brazil.	https://doi.org/10.11158/saa.23.5.11
173	SANTOS, F.O.	2018	Ocorrência de *Necromys lasiurus* (Lund, 1841), em áreas abertas da mata atlântica e seu papel como potencial hospedeiro de hantavírus no estado do Rio de Janeiro, Brasil.	https://www.arca.fiocruz.br/handle/icict/39941
174	SANTOS, N.J.	2012	Efeito da porcentagem da cobertura florestada sobre o ectoparasitismo de pequenos mamíferos silvestres em quatro paisagens da Mata Atântica da Bahia, Brasil.	https://repositorio.ufba.br/ri/handle/ri/19560
175	SANTOS-FILHO, M., DA SILVA, D.J., SANAIOTTI, T.M.	2008	Edge effects and landscape matrix use by a small mammal community in fragments of semideciduous submontane forest in Mato Grosso, Brazil.	https://doi.org/10.1590/S1519-69842008000400004
176	SANTOS-FILHO, M., PERES, C.A., DA SILVA, D.J., SANAIOTTI, T.M.	2012	Habitat patch and matrix effects on small-mammal persistence in Amazonian forest fragments.	https://doi.org/10.1007/s10531-012-0248-8
177	SANHAROLI, B.H., CHÁVEZ-CONGRAINS, K., GALETTI, P.M.	2017	Evidence of recent fine-scale population structuring in South American *Puma concolor*.	https://doi.org/10.3390/d9040044
178	SCHEIBLER, D.R., CHRISTOFF, A.U.	2007	Habitat associations of small mammals in southern Brazil and use of regurgitated pellets of birds of prey for inventorying a local fauna.	https://doi.org/10.1590/S1519-69842007000400005
179	SILVA, A.A.S., ALVAREZ, M.R.D.V., MARIANO-NETO, E., CASSANO, C.R.	2020	Is shadier better? The effect of agroforestry management on small mammal diversity.	https://doi.org/10.1111/btp.12750
180	SILVA, C., GRACIOLLI, G.	2013	Prevalence, mean intensity of infestation and host specificity of *Spinturnicidae mites* (Acari: Mesostigmata) on bats (Mammalia: Chiroptera) in the Pantanal, Brazil.	https://doi.org/10.2478/s11686-013-0134-x
181	SILVEIRA, M., TOMAS, W.M., FISCHER, E., BORDIGNON, M.O.	2018	Habitat occupancy by *Artibeus planirostris* bats in the Pantanal wetland, Brazil.	https://doi.org/10.1016/j.mambio.2018.03.003
182	SOARES, C.S., FANECA, L.F., BARRETO, R.M.F., ALVAREZ, M.R.D.V.	2014	Levantamento de mamíferos de maior porte em seringais e florestas do sul da Bahia (Brasil) utilizando armadilhas fotográficas.	
183	STEVENS, S.M., HUSBAND, T.P.	1998	The influence of edge on small mammals: Evidence from Brazilian Atlantic forest fragments	
184	STONE, A.I., LIMA, E.M., AGUIAR, G.F.S., CAMARGO, C.C., FLORES, T.A., KELT, D.A., MARQUES-AGUIAR, S.A., QUEIROZ, J.A.L., RAMOS, R.M., SILVA JÚNIOR, J.S.	2009	Non-volant mammalian diversity in fragments in extreme eastern Amazonia.	https://doi.org/10.1007/s10531-008-9551-9
185	SUSCKE, P., PRESOTTO, A., IZAR, P.	2021	The role of hunting on *Sapajus xanthosternos*' landscape of fear in the Atlantic Forest, Brazil.	https://doi.org/10.1002/ajp.23243
186	TIMO, T., LYRA-JORGE, M., GHELER-COSTA, C., VERDADE, L.	2015	Effect of the plantation age on the use of Eucalyptus stands by medium to large-sized wild mammals in south-eastern Brazil.	https://doi.org/10.3832/ifor1237-008
187	TIRELLI, F.P., MAZIM, F.D., CRAWSHAW, P.G., ALBANO, A.P., ESPINOSA, C., QUEIROLO, D., LOPES ROCHA, F., BONIFÁCIO SOARES, J., TRIGO, T.C., MACDONALD, D.W., LUCHERINI, M., EIZIRIK, E.	2019	Density and spatio-temporal behaviour of Geoffroy's cats in a human-dominated landscape of southern Brazil.	https://doi.org/10.1016/j.mambio.2019.11.003

188	TISOVEC, K.C., CASSANO, C.R., BOUBLI, J.P., PARDINI, R.	2014	Mixed-species Groups of Marmosets and Tamarins Across a Gradient of Agroforestry Intensification.	https://doi.org/10.1111/btp.12098
189	TREVELIN, L.C., SILVEIRA, M., PORT-CARVALHO, M., HOMEM, D.H., CRUZ-NETO, A.P.	2013	Use of space by frugivorous bats (Chiroptera: Phyllostomidae) in a restored Atlantic forest fragment in Brazil.	https://doi.org/10.1016/j.foreco.2012.11.013
190	TROLLE, M., BISSARO, M.C., PRADO, H.M.	2007	Mammal survey at a ranch of the Brazilian Cerrado.	https://doi.org/10.1007/s10531-006-9106-x
191	TROLLE, M., NOSS, A.J., DE LIMA, E.S., DALPONTE, J.C.	2006	Camera-trap studies of maned wolf density in the Cerrado and the Pantanal of Brazil.	https://doi.org/10.1007/978-1-4020-6320-6_24
192	UMETSU, F., PARDINI, R.	2007	Small mammals in a mosaic of forest remnants and anthropogenic habitats - Evaluating matrix quality in an Atlantic forest landscape.	https://doi.org/10.1007/s10980-006-9041-y
193	VÁSQUEZ, L.C., MARQUES, T.S., DE ABREU, E.F., CIOCI, R., PIÑA, C.I., VERDADE, L.M.	2021	Diversity of small mammals on the early second commercial cycle of Eucalyptus plantations in southeast Brazil.	https://doi.org/10.1016/j.foreco.2021.119052
194	VERDADE, L.M., MORAL, R.A., CALABONI, A., DO AMARAL, M.V.S.G., MARTIN, P.S., AMORIM, L.S., GHELER-COSTA, C., PIÑA, C.I.	2020	Temporal dynamics of small mammals in Eucalyptus plantations in Southeast Brazil.	https://doi.org/10.1016/j.gecco.2020.e01217
195	VIEIRA, M.V., OLIFIERS, N., DELCIELLOS, A.C., ANTUNES, V.Z., BERNARDO, L.R., GRELLE, C.E.V., CERQUEIRA, R.	2009	Land use vs. fragment size and isolation as determinants of small mammal composition and richness in Atlantic Forest remnants.	https://doi.org/10.1016/j.biocon.2009.02.006
196	VILAS BOAS, A.H., VERÍSSIMO, I., NOVAES, R., CUPOLILLO, G., DE ANDREAZZI, C., COSTA-NETO, S., MORATELLI, R.	2022	Survey of medium- and large-sized mammals in Atlantic Forest remnants of Conceição dos Ouros, Minas Gerais, Brazil.	
197	WOLF, R.W., ROSSI, R.V., ARAGONA, M., AGUIAR, D.M.	2016	Primeiro registro de *Pseudoryzomys simplex* (Cricetidae, sigmodontinae) em área alagada do Pantanal, Brasil.	https://doi.org/10.1590/1519-6984.09015
198	YANG, S.G.N.S., DA SILVA, I.J.S., SOUZA, D.S., FONSECA, C.F., SANTIAGO, A.C.S., SOARES, P.C., OLIVEIRA, J.B.	2021	Multi-elemental exposure assessment through concentrations in hair of free-ranging capybaras (*Hydrochoerus hydrochaeris* Linnaeus, 1766) in the Atlantic Forest remnants, Northeast of Brazil.	https://doi.org/10.1016/j.chemosphere.2020.127800
199	ZANZINI, A.C.S., SILVA, A.A.N., PEREIRA, C.Z., SANTIAGO, W.T.V., ZANON, M.H.C.	2017	Composição de espécies de mamíferos de médio e grande porte em plantio de eucalipto e fragmentos de florestas nativas, no sudeste do Estado de Minas Gerais.	https://doi.org/10.18671/scifor.v45n116.08
200	ZIMBRES, B., PERES, C.A., MACHADO, R.B.	2017	Terrestrial mammal responses to habitat structure and quality of remnant riparian forests in an Amazonian cattle-ranching landscape.	https://doi.org/10.1016/j.biocon.2016.11.033

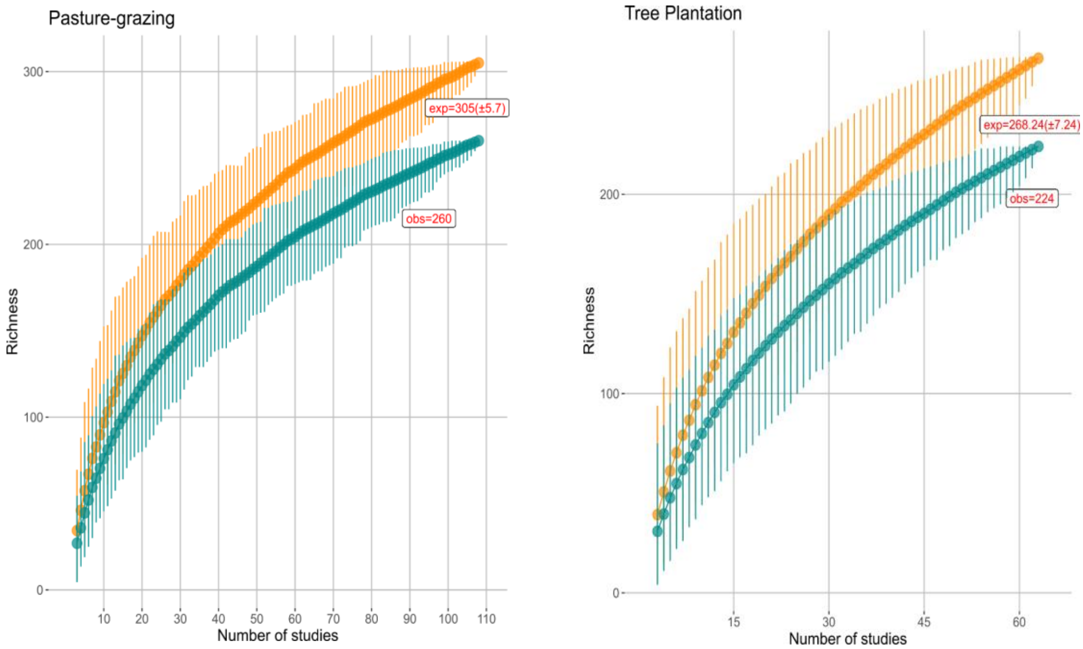
Appendix S3. Functional traits used to construct the functional space for five types of agroecosystems. The traits were extracted from Elton Traits 1.0 (Wilman et al., 2014).

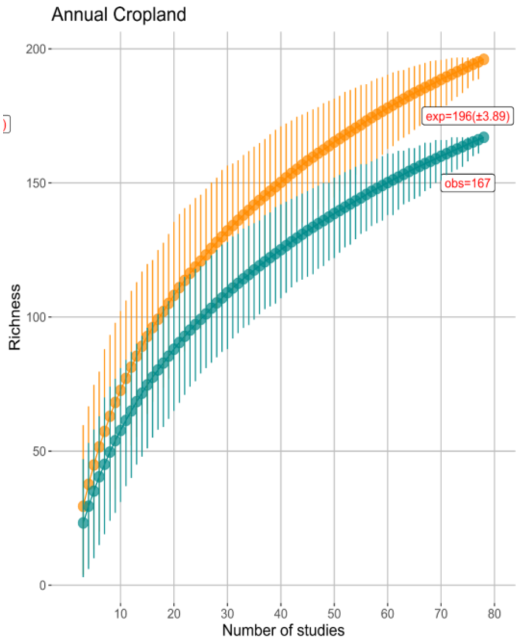
Functional component	**Attribute**	
Size	Body mass	average body mass (g)
Daily activity	Crepuscular	Foraging activity at twilight
	Diurnal	Foraging activity at day
	Nocturnal	Foraging activity at night
Diet	Fruit	Percent use of: Fruit, drupes
	Inv	Percent use of: Invertebrates-general, aquatic invertebrates, shrimp, krill, squid, crustacaeans, molluscs, cephalapod, polychaetes, gastropods, orthoptera, terrestrial Invertebrates, ground insects, insect larvae, worms, orthopterans, flying insects estimated % use (categories sum to 100% total)
	Nect	Percent use of: Nectar, pollen, plant exudates, gums
	Plant	Percent use of: Other plant material, Grass, ground vegetation, seedlings, weeds, lichen, moss, small plants, reeds, cultivated crops, forbs, vegetables, fungi, roots, tubers, legumes, bulbs, leaves, above ground vegetation, twigs, bark, shrubs, herbs, shoots, aquatic vegetation, aquatic plants
	Seed	Percent use of: Seed, maize, nuts, spores, wheat, grains
	Vect	Percent use of: Reptiles, snakes, amphibians, salamanders
	Vend	Percent use of: Mammals, Birds
	Vfish	Percent use of: Fish
Foraging stratum	Arboreal	mammals that live or spend a large part of their lives among trees
	Terrestrial	terrestrial mammals, including aquatic foraging
	Scansorial	refers to the ability or propensity to climb

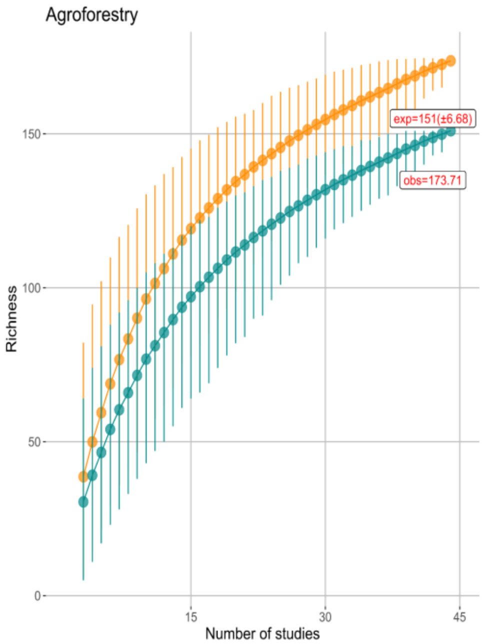
Appendix S4. Frequency distribution of mammal species recorded in Brazilian agroecosystems.

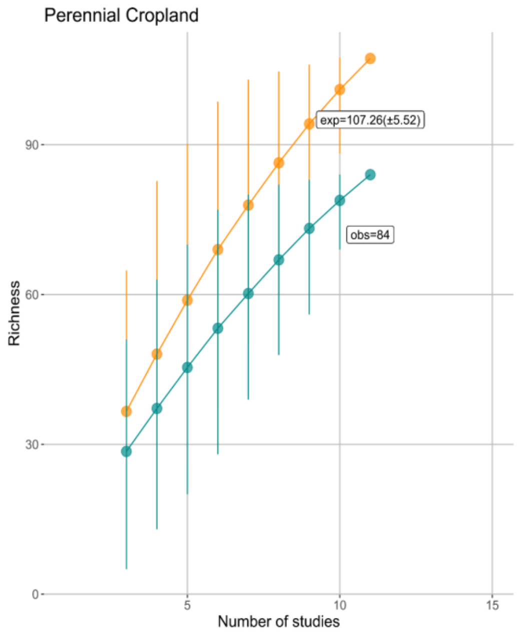


Appendix S5. Accumulation curves and 95% confidence intervals (gray lines) for mammal species richness based on the number of species that were either observed and expected (bootstrap method) within 200 agricultural sites across five agroecosystem types. The samples correspond to the number of studies for each type of agroecosystem. Significant differences [at $p < 0.05$] between observed and expected species richness in each agroecosystem type are highlighted in red.




Annual Cropland
exp=196(±3.89)
obs=167
Richness
Number of studies
200
150
100
50
0
10
20
30
40
50
60
70
80


Agroforestry
exp=151(±6.68)
obs=173.71
Richness
Number of studies
150
100
50
0
15
30
45


Perennial Cropland
exp=107.26(±5.52)
obs=84
Richness
Number of studies
90
60
30
0
5
10
15

Appendix S6. List of threatened mammal species in IUCN Red List (2023), Brazilian Red List (MMA, 2022) and pesticides threats.

Species	IUCN (2023)	IUCN.Herbicides &pesticides	MMA (2022)	MMA.Herbicides &pesticides	Brazilian Action Plans for Endangered Species	Endemic Brazil
Akodon cursor	LC	NO	LC	NA		NO
Akodon montensis	LC	NO	LC	NA		NO
Akodon paranaensis	LC	NO	LC	NA		NO
Anoura caudifer	LC	NO	LC	NO		NO
Anoura geoffroyi	LC	NO	LC	NO		NO
Artibeus cinereus	LC	NO	LC	NO		NO
Artibeus concolor	LC	NO	LC	NO		NO
Artibeus fimbriatus	LC	NO	LC	NO		NO
Artibeus gnoma	LC	NO	LC	NA		NO
Artibeus lituratus	LC	NO	LC	NO		NO
Artibeus obscurus	LC	NO	LC	NO		NO
Artibeus planirostris	LC	NO	LC	NO		NO
Bibimys labiosus	LC	NO	LC	NA		NO
Blarinomys breviceps	LC	NO	LC	NA		NO
Blastocerus dichotomus	VU	NO	VU	YES	Pan Ungulados	NO
Bradypus torquatus	VU	NO	VU	NO	PAN Primatas da Mata Atlântica e preguiça-de-coleira	YES
Brucepattersonius soricinus	DD	NO	LC	NA		YES
Cabassous tatouay	LC	NO	LC	NA		NO
Cabassous unicinctus	LC	NO	LC	NA		NO
Callithrix kuhlii	VU	NO	LC	NA		YES
Calomys callosus	LC	NO	LC	NA		NO
Calomys cerqueirai	NA	NA	LC	NA		YES
Calomys expulsus	LC	NO	LC	NA		YES
Calomys tener	LC	NO	LC	NA		NO
Calomys tocantinsi	LC	NO	LC	NA		YES
Caluromys philander	LC	NO	LC	NO		NO
Carollia brevicauda	LC	NO	LC	NO		NO
Carollia perspicillata	LC	NO	LC	NO		NO
Cavia aperea	LC	NO	LC	NA		NO
Cerdocyon thous	LC	NO	LC	NO		NO
Cerradomys scotti	LC	NO	LC	NA		NO
Cerradomys subflavus	LC	NO	LC	NA		NO
Cerradomys vivoi	NA	NA	LC	NA		YES
Chaetomys subspinosus	VU	NO	VU	YES	PAN Pequenos Mamíferos de Áreas Florestais	YES
Chiroderma villosum	LC	NO	LC	NO		NO
Chiropotes albinasus	VU	NO	LC	NA		YES
Choeroniscus minor	LC	NO	LC	NO		NO

Chrotopterus auritus	LC	NO	LC	NO		NO
Chrysocyon brachyurus	NT	NO	VU	BIACUM	PAN Canídeos	NO
Coendou insidiosus	LC	NO	LC	NA		YES
Coendou prehensilis	LC	NO	LC	NA		NO
Conepatus chinga	LC	NO	LC	YES		NO
Conepatus semistriatus	LC	YES	LC	YES		NO
Cryptonanus agricolai	DD	NO	LC	NO		YES
Cryptonanus chacoensis	LC	NO	LC	NO		NO
Cuniculus paca	LC	NO	LC	NA		NO
Cynomops planirostris	LC	NO	LC	YES		NO
Dasyprocta azarae	DD	NO	LC	NA		NO
Dasyprocta leporina	LC	NO	LC	NA		NO
Dasyprocta prymnolopha	LC	NO	LC	NA		YES
Dasypus novemcinctus	LC	NO	LC	NA		NO
Dasypus septemcinctus	LC	NO	LC	NA		NO
Desmodus rotundus	LC	NO	LC	NO		NO
Diaemus youngii	LC	NO	LC	NO		NO
Diclidurus albus	LC	NO	LC	NO		NO
Dicotyles tajacu	LC	NO	LC	NO		NO
Didelphis albiventris	LC	NO	LC	NO		NO
Didelphis aurita	LC	NO	LC	NO		NO
Didelphis marsupialis	LC	NO	LC	NO		NO
Diphylla ecaudata	LC	NO	LC	NO		NO
Eira barbara	LC	NO	LC	NO		NO
Eptesicus brasiliensis	LC	NO	LC	NO		NO
Eptesicus diminutus	LC	NO	LC	NO		NO
Eptesicus taddeii	DD	NO	LC	NO		YES
Euphractus sexcinctus	LC	NO	LC	NA		NO
Euryoryzomys nitidus	LC	NO	LC	NA		NO
Euryoryzomys russatus	LC	NO	LC	NA		NO
Galictis cuja	LC	NO	LC	NO		NO
Galictis vittata	LC	NO	LC	NO		NO
Gardnerycteris crenulatum	LC	NO	LC	NO		NO
Glossophaga soricina	LC	NO	LC	NO		NO
Gracilinanus agilis	LC	NO	LC	NO		NO
Gracilinanus microtarsus	LC	NO	LC	NO		NO
Gracilinanus peruanus	NA	NA	LC	NO		NO
Guerlinguetus aestuans	LC	NO	LC	NO		NO
Herpailurus yagouaroundi	LC	NO	VU	NO	PAN Pequenos Felinos	NO
Histiotus velatus	DD	NO	LC	NO		NO
Holochilus brasiliensis	LC	NO	LC	NA		NO
Holochilus sciureus	LC	NO	LC	NA		NO
Hsunycteris thomasi	NA	NO	LC	NO		NO

Hydrochoerus hydrochaeris	LC	NO	LC	NA		NO
Hylaeamys megacephalus	LC	NO	NA	NO		NO
Hylaeamys seuanezi	NA	NO	LC	NA		YES
Juliomys pictipes	LC	NO	LC	NA		NO
Kannabateomys amblyonyx	LC	NO	LC	NA		NO
Lampronycteris brachyotis	LC	NO	LC	NO		NO
Lasiurus blossevillii	LC	NO	LC	NO		NO
Leontopithecus chrysomelas	EN	NO	EN	NO	PAN dos Primatas Amazônicos	YES
Leopardus geoffroyi	LC	NO	VU	BIACUM	PAN Pequenos Felinos	NO
Leopardus guttulus	VU	NO	VU	NA	PAN Pequenos Felinos	NO
Leopardus pardalis	LC	NO	LC	NO		NO
Leopardus tigrinus	VU	NO	EN	NO	PAN Pequenos Felinos	NO
Leopardus wiedii	NT	NO	VU	NO	PAN Pequenos Felinos	NO
Lonchophylla dekeyseri	EN	NO	EN	NO	PAN Morceguinho-do-Cerrado	NO
Lonchophylla mordax	NT	NO	LC	NO		YES
Lonchophylla peracchii	LC	NO	LC	NO		YES
Lonchorhina aurita	LC	NO	LC	NO		NO
Lontra longicaudis	NT	NO	LC	BIACUMHV		NO
Lophostoma brasiliense	LC	NO	LC	NO		NO
Lophostoma carrikeri	LC	NO	LC	NO		NO
Lophostoma silvicola	LC	NA	LC	NO		NO
Lycalopex vetulus	NT	NO	VU	NA	PAN Canídeos	YES
Makalata didelphoides	LC	NO	LC	NA		NO
Marmosa demerarae	LC	NO	LC	NO		NO
Marmosa murina	LC	NO	LC	NO		NO
Marmosa paraguayana	LC	NO	LC	NO		NO
Marmosops incanus	LC	NO	LC	NO		YES
Marmosops noctivagus	LC	NO	LC	NO		NO
Mazama americana	DD	NO	LC	NO		NO
Mazama nemorivaga	LC	NO	LC	NO		NO
Mesophylla macconnelli	LC	NO	LC	NO		NO
Mico emiliae	LC	NO	LC	NA		YES
Micronycteris hirsuta	LC	NO	LC	NO		NO
Micronycteris megalotis	LC	NO	LC	NO		NO
Micronycteris microtis	LC	NO	LC	NO		NO
Micronycteris sanborni	LC	NO	LC	NO		YES
Micronycteris schmidtorum	LC	NO	LC	NO		NO
Molossops neglectus	DD	NO	LC	YES		NO
Molossops temminckii	LC	NO	LC	NO		NO

Molossus molossus	LC	NO	LC	NO		NO
Monodelphis americana	LC	NO	LC	NO		YES
Monodelphis dimidiata	LC	NO	LC	NO		NO
Monodelphis domestica	LC	NO	LC	NO		NO
Monodelphis iheringi	DD	NO	LC	NO		YES
Monodelphis kunsi	LC	NO	LC	NO		NO
Myotis albescens	LC	NO	LC	NO		NO
Myotis nigricans	LC	NO	LC	NO		NO
Myotis riparius	LC	NO	LC	NO		NO
Myotis ruber	NT	NO	LC	NO		NO
Myotis simus	DD	NO	LC	NO		NO
Myrmecophaga tridactyla	VU	NO	VU	YES	PAN Tamanduá-bandeira, Tatu-canastra e Tatu-bola	NO
Nasua nasua	LC	NO	LC	NO		NO
Natalus macrourus	NT	NO	VU	NO		NO
Neacomys amoenus	LC	NA	LC	NA		NO
Necromys lasiurus	LC	NO	LC	NA		NO
Nectomys squamipes	LC	NO	LC	NA		NO
Noctilio albiventris	LC	NO	LC	NO		NO
Noctilio leporinus	LC	NO	LC	YES		NO
Nyctinomops laticaudatus	LC	NO	LC	YES		NO
Oecomys bicolor	LC	NO	LC	NA		NO
Oecomys roberti	LC	NO	LC	NA		NO
Oligoryzomys chacoensis	LC	NO	LC	NA		NO
Oligoryzomys flavescens	LC	NO	LC	NA		NO
Oligoryzomys mattogrossae	NA	NA	LC	NA		YES
Oligoryzomys microtis	LC	NO	LC	NA		NO
Oligoryzomys nigripes	LC	NO	LC	NA		NO
Oxymycterus delator	LC	NO	LC	NA		NO
Ozotoceros bezoarticus	NT	NO	VU	YES	PAN Ungulados	NO
Panthera onca	NT	YES	VU	NO		NO
Peropteryx kappleri	LC	NO	LC	NO		NO
Peropteryx macrotis	LC	NO	LC	NO		NO
Philander opossum	LC	NO	LC	NO		NO
Philander quica	NA	NA	LC	NO		NO
Phylloderma stenops	LC	NO	LC	NO		NO
Phyllomys nigrispinus	LC	NO	LC	NA		YES
Phyllostomus discolor	LC	NO	LC	NO		NO
Phyllostomus elongatus	LC	NO	LC	NO		NO
Phyllostomus hastatus	LC	NO	LC	NO		NO
Platyrrhinus fusciventris	LC	NO	LC	NO		NO
Platyrrhinus incarum	LC	NO	LC	NO		NO
Platyrrhinus lineatus	LC	NO	LC	NO		NO

Platyrrhinus recifinus	LC	NO	LC	NO		YES
Potos flavus	LC	NO	LC	NO		NO
Priodontes maximus	VU	NO	VU	YES	PAN Tamanduá-bandeira, Tatu-canastra e Tatu-bola	NO
Procyon cancrivorus	LC	NO	LC	NO		NO
Proechimys roberti	LC	NO	LC	NA		YES
Pseudoryzomys simplex	LC	NO	LC	NA		NO
Pteronotus gymnonotus	LC	NO	LC	NO		NO
Pteronotus rubiginosus	LC	NO	LC	NO		NO
Puma concolor	LC	NO	LC	NO		NO
Pygoderma bilabiatum	LC	NO	LC	NO		NO
Rhinophylla pumilio	LC	NO	LC	NO		NO
Rhipidomys ipukensis	DD	NO	LC	NA		YES
Rhipidomys mastacalis	LC	NO	LC	NA		YES
Rhipidomys tribei	DD	NO	EN	NA		YES
Rhynchonycteris naso	LC	NO	LC	NO		NO
Saccopteryx bilineata	LC	NO	LC	NO		NO
Saccopteryx leptura	LC	NO	LC	NO		NO
Saguinus midas	LC	NO	LC	NA		NO
Saguinus ursula	VU	NO	LC	NA		YES
Saimiri ustus	NT	NO	LC	NA		YES
Sapajus apella	LC	NO	LC	NO		NO
Sapajus cay	VU	NO	VU	NO	PAN Cerpan	NO
Sapajus libidinosus	NT	NO	LC	NA		YES
Sapajus robustus	EN	NO	EN	NA		YES
Sapajus xanthosternos	CR	NO	EN	NO	PAN Primatas do Nordeste	YES
Speothos venaticus	NT	NO	VU	NO	PAN Canídeos	NO
Sturnira lilium	LC	NO	LC	NO		NO
Sturnira tildae	LC	NO	LC	NO		NO
Subulo gouazoubira	LC	NO	LC	NO		NO
Sylvilagus brasiliensis	EN	NO	LC	NA		YES
Tamandua tetradactyla	LC	NO	LC	NO		NO
Tapirus terrestris	VU	NO	VU	YES	PAN Ungulados	NO
Tayassu pecari	VU	NO	VU	YES	PAN Ungulados	NO
Thaptomys nigrita	LC	NO	LC	NA		NO
Thyroptera tricolor	LC	NO	LC	NO		NO
Tolypeutes tricinctus	VU	NO	EN	YES	PAN Tamanduá-bandeira, Tatu-canastra e Tatu-bola	YES
Tonatia bidens	DD	NO	LC	NO		NO
Tonatia maresi	NA	NA	NA	NA		NO
Trachops cirrhosus	LC	NO	LC	NO		NO

Trinycteris nicefori	LC	NO	LC	NO		NO
Uroderma bilobatum	LC	NO	LC	NO		NO
Vampyressa pusilla	DD	NO	LC	NO		NO
Vampyriscus bidens	LC	NO	LC	NO		NO
Vampyrodes caraccioli	LC	NO	LC	NO		NO

Appendix S7. List of traits for the mammals sampled in the study. The traits were extracted from the Elton Traits 1.0.

scientificName	Diet									Habitat type	Daili activity			
	Inv	Vend	Vect	Vfish	Scav	Fruit	Nect	Seed	Plant		Nocturnal	Crepuscular	Diurnal	BodyMass
Akodon cursor	80	0	0	0	0	0	0	0	20	G	1	1	1	39,85
Akodon montensis	70	0	0	0	0	0	0	0	30	G	1	1	1	31,32
Akodon paranaensis	70	0	0	0	0	0	0	0	30	G	1	1	1	31,32
Akodon toba	80	0	0	0	0	0	0	0	20	G	1	1	1	51,2
Alouatta belzebul	0	0	0	0	0	40	0	0	60	Ar	0	0	1	6400
Alouatta caraya	0	0	0	0	0	40	0	0	60	Ar	0	0	1	5862,46
Alouatta guariba	0	0	0	0	0	40	0	0	60	Ar	0	0	1	5188,33
Alouatta macconnelli	0	0	0	0	0	40	0	0	60	Ar	0	0	1	6320,06
Alouatta seniculus	0	0	0	0	0	40	0	0	60	Ar	0	0	1	6145,54
Anoura caudifer	30	0	0	0	0	30	40	0	0	Ar	1	0	0	10,81
Anoura geoffroyi	30	0	0	0	0	30	40	0	0	Ar	1	0	0	14,62
Aotus infulatus	20	0	0	0	0	20	10	20	20	Ar	1	0	0	929,57
Artibeus cinereus	10	0	0	0	0	80	10	0	0	Ar	1	0	0	12,63
Artibeus concolor	10	0	0	0	0	80	10	0	0	Ar	1	0	0	19,35
Artibeus fimbriatus	10	0	0	0	0	80	10	0	0	Ar	1	0	0	63,9
Artibeus gnoma	10	0	0	0	0	80	10	0	0	Ar	1	0	0	10,06
Artibeus lituratus	10	0	0	0	0	80	10	0	0	Ar	1	0	0	60,54
Artibeus obscurus	10	0	0	0	0	80	10	0	0	Ar	1	0	0	35,2
Artibeus planirostris	10	0	0	0	0	80	10	0	0	Ar	1	0	0	42,17
Ateles chamek	10	0	0	0	0	60	0	10	10	Ar	0	0	1	5999,98
Ateles marginatus	10	0	0	0	0	60	0	10	10	Ar	0	0	1	5999,98
Ateles paniscus	10	0	0	0	0	60	0	10	10	Ar	0	0	1	7900,05
Atelocynus microtis	0	80	0	0	0	0	0	0	20	G	1	0	0	7749,97
Bibimys labiosus	0	0	0	0	0	0	0	30	70	G	1	0	0	29
Blarinomys breviceps	20	0	0	0	0	10	0	40	30	G	1	0	0	36,8
Blastocerus dichotomus	0	0	0	0	0	0	0	0	100	G	1	1	0	86666,25
Brachyteles arachnoides	0	0	0	0	0	20	10	20	50	Ar	0	1	1	13499,88
Bradypus torquatus	0	0	0	0	0	0	0	0	100	Ar	1	1	1	3899,96
Bradypus variegatus	0	0	0	0	0	0	0	0	100	Ar	1	1	1	4335,01
Brucepattersonius iheringi	90	0	0	0	0	0	0	0	10	G	1	0	0	43
Brucepattersonius soricinus	90	0	0	0	0	0	0	0	10	G	1	0	0	30,87
Cabassous tatouay	100	0	0	0	0	0	0	0	0	G	1	1	0	5349,95
Cabassous unicinctus	100	0	0	0	0	0	0	0	0	G	1	1	0	4799,99
Callicebus coimbrai	20	0	0	0	0	50	0	0	30	Ar	0	0	1	1011,32
Callicebus melanochir	20	0	0	0	0	50	0	0	30	Ar	0	0	1	1370
Callicebus moloch	20	0	0	0	0	50	0	0	30	Ar	0	0	1	854,73
Callicebus nigrifrons	20	0	0	0	0	50	0	0	30	Ar	0	0	1	1011,32
Callithrix aurita	20	0	0	0	0	30	40	0	0	Ar	0	0	1	342
Callithrix geoffroyi	20	0	0	0	0	30	40	0	0	Ar	0	0	1	342
Callithrix jacchus	20	0	0	0	0	30	40	0	0	Ar	0	0	1	292
Callithrix kuhlii	20	0	0	0	0	30	40	0	0	Ar	0	0	1	342

Callithrix penicillata	20	0	0	0	0	30	40	0	0	Ar	0	0	1	342
Calomys callidus	0	0	0	0	0	0	0	0	100	G	1	1	0	27
Calomys callosus	0	0	0	0	0	0	0	0	100	G	1	1	0	44,95
Calomys cerqueirai	0	0	0	0	0	0	0	0	100	G	1	1	0	39,86
Calomys expulsus	0	0	0	0	0	0	0	0	100	G	1	1	0	39,86
Calomys tener	0	0	0	0	0	0	0	0	100	G	1	1	0	13,8
Calomys tocantinsi	0	0	0	0	0	0	0	0	100	G	1	1	0	25,3
Caluromys lanatus	20	0	0	0	0	20	0	10	40	Ar	1	1	0	325
Caluromys philander	20	0	0	0	0	20	0	10	40	Ar	1	1	0	229,25
Carollia brevicauda	0	0	0	0	0	100	0	0	0	Ar	1	0	0	14,4
Carollia perspicillata	0	0	0	0	0	100	0	0	0	Ar	1	0	0	18
Castoria angustidens	80	0	0	0	0	0	0	0	20	G	1	1	1	28,3
Cavia aperea	0	0	0	0	0	0	0	0	100	G	0	1	0	549
Cebus kaapori	10	0	0	0	0	70	0	10	10	Ar	0	0	1	3000
Cebus olivaceus	20	0	0	0	0	20	10	20	20	Ar	0	0	1	2599,98
Cerdocyon thous	50	0	0	0	10	0	0	0	0	G	1	0	0	5239,98
Cerradomys maracajuensis	20	0	0	0	0	20	0	20	40	G	1	1	1	57,93
Cerradomys scotti	20	0	0	0	0	20	0	20	40	G	1	1	1	57,93
Cerradomys subflavus	20	0	0	0	0	20	0	20	40	G	1	1	1	50
Cerradomys vivoi	20	0	0	0	0	20	0	20	40	G	1	1	1	50
Chaetomys subspinosus	0	0	0	0	0	40	0	60	0	Ar	1	0	0	1299,99
Chiroderma doriae	0	0	0	0	0	100	0	0	0	Ar	1	0	0	19,9
Chiroderma villosum	0	0	0	0	0	100	0	0	0	Ar	1	0	0	23,12
Chironectes minimus	50	0	0	30	0	0	0	0	20	G	1	0	0	946
Chiropotes albinasus	10	0	0	0	0	40	0	20	30	Ar	0	0	1	2900,01
Chiropotes satanas	10	0	0	0	0	40	0	20	30	Ar	0	0	1	2999,99
Choeroniscus minor	30	0	0	0	0	40	30	0	0	Ar	1	0	0	8,73
Choloepus didactylus	0	0	0	0	0	30	0	0	70	Ar	1	0	0	5160
Chrotopterus auritus	30	0	20	20	0	30	0	0	0	S	1	0	0	77,86
Chrysocyon brachyurus	10	50	20	0	0	10	0	0	10	G	1	1	0	23249,84
Coendou insidiosus	0	0	0	0	0	30	0	0	70	Ar	1	0	0	4399,97
Coendou prehensilis	0	0	0	0	0	30	0	0	70	Ar	1	0	0	4399,97
Coendou spinosus	0	0	0	0	0	30	0	0	70	Ar	1	0	0	750,79
Conepatus chinga	80	0	10	0	0	10	0	0	0	G	1	0	0	1917,52
Conepatus semistriatus	80	0	10	0	0	10	0	0	0	G	1	0	0	1200
Cormura brevirostris	100	0	0	0	0	0	0	0	0	A	1	0	0	9,18
Cryptonanus agricolai	50	0	0	0	0	30	20	0	0	S	1	1	0	18,9
Cryptonanus chacoensis	50	0	0	0	0	30	20	0	0	S	1	1	0	22
Cuniculus paca	0	0	0	0	0	20	0	30	50	G	1	0	0	8172,55
Cynomops planirostris	100	0	0	0	0	0	0	0	0	A	1	0	0	12,84
Dasyprocta azarae	10	0	0	0	0	40	0	0	50	G	0	1	1	2309,99
Dasyprocta iacki	10	0	0	0	0	40	0	0	50	G	0	1	1	3020,02
Dasyprocta leporina	10	0	0	0	0	40	0	0	50	G	0	1	1	3020,02
Dasyprocta prymnolopha	10	0	0	0	0	40	0	0	50	G	0	1	1	2900,01
Dasypus kappleri	100	0	0	0	0	0	0	0	0	G	1	0	0	9500
Dasypus novemcinctus	100	0	0	0	0	0	0	0	0	G	1	0	0	4203,78
Dasypus septemcinctus	100	0	0	0	0	0	0	0	0	G	1	0	0	1526,65
Delomys dorsalis	20	0	0	0	0	10	0	40	30	G	1	0	0	67,5
Delomys sublineatus	20	0	0	0	0	10	0	40	30	G	1	0	0	90
Desmodus rotundus	0	100	0	0	0	0	0	0	0	G	1	0	0	31,21
Diaemus youngii	0	100	0	0	0	0	0	0	0	G	1	0	0	36,31
Diclidurus albus	100	0	0	0	0	0	0	0	0	A	1	0	0	15,6
Dicotyles tajacu	10	0	20	0	0	10	0	0	60	G	1	1	0	21266,69
Didelphis albiventris	20	0	0	0	30	0	0	0	20	S	1	0	0	904

Didelphis aurita	20	0	0	0	30	0	0	0	20	S	1	0	0	1163,99
Didelphis marsupialis	20	0	0	0	30	0	0	0	20	S	1	0	0	1091,16
Diphylla ecaudata	0	100	0	0	0	0	0	0	0	G	1	0	0	29,3
Eira barbara	0	90	0	0	0	10	0	0	0	G	1	1	1	3910,03
Eptesicus brasiliensis	100	0	0	0	0	0	0	0	0	A	1	0	0	9,47
Eptesicus chiriquinus	100	0	0	0	0	0	0	0	0	A	1	0	0	11,25
Eptesicus diminutus	100	0	0	0	0	0	0	0	0	A	1	0	0	6
Eptesicus furinalis	100	0	0	0	0	0	0	0	0	A	1	0	0	7,74
Eptesicus taddeii	100	0	0	0	0	0	0	0	0	A	1	0	0	9,47
Eumops perotis	100	0	0	0	0	0	0	0	0	A	1	0	0	51,13
Euphractus sexcinctus	50	0	0	0	0	0	0	0	50	G	1	1	1	4782,89
Euryoryzomys nitidus	20	0	0	0	0	20	0	20	40	G	1	1	1	55,2
Euryoryzomys russatus	20	0	0	0	0	20	0	20	40	G	1	1	1	60,5
Euryzygomatomys spinosus	0	0	0	0	0	30	0	20	50	G	1	0	0	187,5
Galea spixii	0	0	0	0	0	0	0	0	100	G	0	0	1	326,2
Galictis cuja	20	30	20	0	0	20	0	0	0	G	1	1	1	1000
Galictis vittata	20	30	20	0	0	20	0	0	0	G	1	1	1	3200
Gardnerycteris crenulatum	50	0	0	0	0	50	0	0	0	S	1	0	0	13,75
Glironia venusta	40	0	0	0	0	20	0	20	0	Ar	1	0	0	114
Glossophaga soricina	40	0	0	0	0	30	30	0	0	Ar	1	0	0	9,43
Glyphonycteris sylvestris	80	0	0	0	0	20	0	0	0	S	1	0	0	8,91
Gracilinanus agilis	50	0	0	0	0	30	20	0	0	S	1	1	0	22
Gracilinanus microtarsus	50	0	0	0	0	30	20	0	0	S	1	1	0	31
Gracilinanus peruanus	50	0	0	0	0	30	20	0	0	S	1	1	0	22
Guerlinguetus aestuans	0	0	0	0	0	20	0	50	30	Ar	0	0	1	185
Guerlinguetus brasiliensis	0	0	0	0	0	20	0	50	30	Ar	0	0	1	185
Hadrosciurus ignitus	0	0	0	0	0	20	0	50	30	Ar	0	0	1	190
Hadrosciurus spadiceus	0	0	0	0	0	20	0	50	30	Ar	0	0	1	403,33
Herpailurus yagouaroundi	10	80	0	0	0	10	0	0	0	G	0	1	1	6875
Histiotus velatus	100	0	0	0	0	0	0	0	0	A	1	0	0	11,2
Holochilus brasiliensis	10	0	0	0	0	0	0	0	90	G	1	0	0	155
Holochilus sciureus	10	0	0	0	0	0	0	0	90	G	1	0	0	170,96
Hsunycteris thomasi	40	0	0	0	0	30	30	0	0	Ar	1	0	0	6,85
Hydrochoerus hydrochaeris	0	0	0	0	0	10	0	0	90	G	0	0	1	48144,91
Hylaeamys megacephalus	20	0	0	0	0	20	0	20	40	G	1	1	1	57
Hylaeamys seuanezi	20	0	0	0	0	20	0	20	40	G	1	1	1	57,93
Juliomys pictipes	0	0	0	0	0	40	0	0	60	G	1	0	0	22,9
Kannabateomys amblyonyx	0	0	0	0	0	0	0	0	100	Ar	1	0	0	600
Kerodon acrobata	0	0	0	0	0	0	0	0	100	G	0	1	0	800
Lagothrix cana	10	0	0	0	0	70	0	10	10	Ar	0	0	1	6299,99
Lampronycteris brachyotis	80	0	0	0	0	20	0	0	0	S	1	0	0	10,39
Lasiurus blossevillii	100	0	0	0	0	0	0	0	0	A	1	0	0	9,5
Lasiurus ega	100	0	0	0	0	0	0	0	0	A	1	0	0	13,2
Lasiurus villosissimus	100	0	0	0	0	0	0	0	0	A	1	0	0	25,28
Leontopithecus chrysomelas	50	0	0	0	0	50	0	0	0	Ar	0	0	1	572,8
Leopardus braccatus	0	100	0	0	0	0	0	0	0	G	1	1	1	5157,94
Leopardus emiliae	0	100	0	0	0	0	0	0	0	S	1	1	1	2250

Leopardus geoffroyi	0	80	10	10	0	0	0	0	0	G	1	0	0	5157,94
Leopardus guttulus	0	100	0	0	0	0	0	0	0	S	1	1	1	2250
Leopardus pardalis	0	70	20	10	0	0	0	0	0	G	1	0	0	11900,08
Leopardus tigrinus	0	100	0	0	0	0	0	0	0	S	1	1	1	2250
Leopardus wiedii	0	80	0	0	0	20	0	0	0	S	1	1	0	3249,97
Lichonycteris degener	0	0	0	0	0	50	50	0	0	Ar	1	0	0	5,6
Lonchophylla dekeyseri	40	0	0	0	0	30	30	0	0	Ar	1	0	0	11,8
Lonchophylla mordax	40	0	0	0	0	30	30	0	0	Ar	1	0	0	10,7
Lonchophylla peracchii	40	0	0	0	0	30	30	0	0	Ar	1	0	0	11,8
Lonchorhina aurita	100	0	0	0	0	0	0	0	0	S	1	0	0	15,7
Lontra longicaudis	10	0	0	80	0	0	0	0	0	G	1	1	0	6554,96
Lophostoma brasiliense	90	0	0	0	0	10	0	0	0	S	1	0	0	9,76
Lophostoma carrikeri	90	0	0	0	0	10	0	0	0	S	1	0	0	22,35
Lophostoma silvicola	90	0	0	0	0	10	0	0	0	S	1	0	0	32,29
Lutreolina crassicaudata	20	40	20	20	0	0	0	0	0	S	1	0	0	537,3
Lycalopex gymnocercus	10	30	30	0	0	20	0	0	10	G	1	0	0	5146,58
Lycalopex vetulus	10	30	30	0	0	20	0	0	10	G	0	0	1	4233,47
Macrophyllum macrophyllum	100	0	0	0	0	0	0	0	0	A	1	0	0	8,1
Makalata didelphoides	0	0	0	0	0	50	0	50	0	S	1	0	0	398,66
Marmosa constantiae	90	0	0	0	0	10	0	0	0	S	1	0	0	90
Marmosa demerarae	90	0	0	0	0	10	0	0	0	S	1	0	0	75,75
Marmosa murina	40	10	10	0	0	30	0	0	0	Ar	1	0	0	26
Marmosa paraguayana	30	0	0	0	0	20	20	0	0	S	1	0	0	112,43
Marmosops bishopi	50	0	0	0	0	50	0	0	0	Ar	1	0	0	19,5
Marmosops incanus	50	0	0	0	0	50	0	0	0	Ar	1	0	0	62,3
Marmosops noctivagus	50	0	0	0	0	50	0	0	0	Ar	1	0	0	21
Marmosops parvidens	50	0	0	0	0	50	0	0	0	Ar	1	0	0	15
Mazama americana	0	0	0	0	0	0	0	20	80	G	1	1	1	22799,75
Mazama nana	0	0	0	0	0	0	0	20	80	G	1	1	1	16499,85
Mazama nemorivaga	0	0	0	0	0	30	0	20	50	G	1	1	1	16633,17
Mesomys hispidus	0	0	0	0	0	30	0	20	50	Ar	1	0	0	175
Mesomys occultus	0	0	0	0	0	30	0	20	50	Ar	1	0	0	126,85
Mesophylla macconnelli	10	0	0	0	0	90	0	0	0	Ar	1	0	0	6,92
Metachirus nudicaudatus	30	20	20	0	0	20	0	0	0	S	1	0	0	375
Mico argentatus	20	0	0	0	0	60	0	0	0	Ar	0	0	1	440
Mico emiliae	20	0	0	0	0	30	40	0	0	Ar	0	0	1	309,58
Micronycteris hirsuta	80	0	0	0	0	20	0	0	0	S	1	0	0	12,01
Micronycteris megalotis	80	0	0	0	0	20	0	0	0	S	1	0	0	6,11
Micronycteris microtis	80	0	0	0	0	20	0	0	0	S	1	0	0	6,5
Micronycteris minuta	80	0	0	0	0	20	0	0	0	S	1	0	0	6,5
Micronycteris sanborni	80	0	0	0	0	20	0	0	0	S	1	0	0	6,3
Micronycteris schmidtorum	80	0	0	0	0	20	0	0	0	S	1	0	0	7,53
Mimon bennettii	30	0	20	20	0	30	0	0	0	S	1	0	0	25,2
Molossops neglectus	100	0	0	0	0	0	0	0	0	A	1	0	0	11
Molossops temminckii	100	0	0	0	0	0	0	0	0	A	1	0	0	5,9
Molossus aztecus	100	0	0	0	0	0	0	0	0	A	1	0	0	14,86
Molossus molossus	100	0	0	0	0	0	0	0	0	A	1	0	0	13,58
Molossus pretiosus	100	0	0	0	0	0	0	0	0	A	1	0	0	27
Molossus rufus	100	0	0	0	0	0	0	0	0	A	1	0	0	25
Monodelphis americana	20	20	0	0	20	20	0	20	0	G	1	0	0	19,5
Monodelphis brevicaudata	20	20	0	0	20	20	0	20	0	G	1	0	0	69,63
Monodelphis dimidiata	20	20	0	0	20	20	0	20	0	G	1	0	0	58

Monodelphis domestica	20	20	0	0	20	20	0	20	0	G	1	0	0	71,4
Monodelphis glirina	20	20	0	0	20	20	0	20	0	G	1	0	0	61,54
Monodelphis iheringi	20	20	0	0	20	20	0	20	0	G	1	0	0	112
Monodelphis kunsi	20	20	0	0	20	20	0	20	0	G	1	0	0	11,3
Monodelphis scalops	20	20	0	0	20	20	0	20	0	G	1	0	0	741
Myocastor coypus	0	0	0	0	0	0	0	0	100	G	1	0	0	6937,45
Myoprocta acouchy	10	0	0	0	0	40	0	0	50	G	0	0	1	600
Myotis albescens	100	0	0	0	0	0	0	0	0	A	1	0	0	5,3
Myotis izecksohni	100	0	0	0	0	0	0	0	0	A	1	0	0	4,24
Myotis lavali	100	0	0	0	0	0	0	0	0	A	1	0	0	4,24
Myotis nigricans	100	0	0	0	0	0	0	0	0	A	1	0	0	4,24
Myotis riparius	100	0	0	0	0	0	0	0	0	A	1	0	0	5,55
Myotis ruber	100	0	0	0	0	0	0	0	0	A	1	0	0	5
Myotis simus	100	0	0	0	0	0	0	0	0	A	1	0	0	8,2
Myrmecophaga tridactyla	100	0	0	0	0	0	0	0	0	G	1	1	1	22333,15
Nasua nasua	10	20	0	0	0	70	0	0	0	S	0	0	1	3793,85
Natalus macrourus	100	0	0	0	0	0	0	0	0	A	1	0	0	5,48
Neacomys amoenus	20	0	0	0	0	20	0	20	40	G	1	0	0	19
Necromys lasiurus	70	0	0	0	0	0	0	0	30	G	0	0	1	39,93
Nectomys rattus	30	0	20	20	0	0	0	0	30	G	1	0	0	248,8
Nectomys squamipes	30	0	20	20	0	0	0	0	30	G	1	0	0	190,75
Noctilio albiventris	80	0	0	20	0	0	0	0	0	G	1	0	0	30,85
Noctilio leporinus	50	0	0	50	0	0	0	0	0	G	1	0	0	58,32
Nyctinomops laticaudatus	100	0	0	0	0	0	0	0	0	A	1	0	0	12,22
Nyctinomops macrotis	100	0	0	0	0	0	0	0	0	A	1	0	0	18,4
Odocoileus virginianus	0	0	0	0	0	0	0	10	90	G	1	1	0	55508,56
Oecomys bicolor	0	0	0	0	0	50	0	50	0	G	1	0	0	34
Oecomys catherinae	0	0	0	0	0	50	0	50	0	G	1	0	0	67,41
Oecomys cleberi	0	0	0	0	0	50	0	50	0	G	1	0	0	73,4
Oecomys concolor	0	0	0	0	0	50	0	50	0	G	1	0	0	61,55
Oecomys mamorae	0	0	0	0	0	50	0	50	0	G	1	0	0	62,5
Oecomys roberti	0	0	0	0	0	50	0	50	0	G	1	0	0	34
Oligoryzomys chacoensis	30	0	0	0	0	30	0	40	0	G	1	0	0	23
Oligoryzomys flavescens	30	0	0	0	0	30	0	40	0	G	1	0	0	21,3
Oligoryzomys mattogrossae	30	0	0	0	0	30	0	40	0	G	1	0	0	22,5
Oligoryzomys microtis	30	0	0	0	0	30	0	40	0	G	1	0	0	22,5
Oligoryzomys moojeni	30	0	0	0	0	30	0	40	0	G	1	0	0	20,5
Oligoryzomys nigripes	30	0	0	0	0	30	0	40	0	G	1	0	0	20,5
Oxymycterus amazonicus	90	0	0	0	0	0	0	0	10	G	0	0	1	60,57
Oxymycterus dasytrichus	90	0	0	0	0	0	0	0	10	G	0	0	1	60,57
Oxymycterus delator	90	0	0	0	0	0	0	0	10	G	0	0	1	81,5
Oxymycterus nasutus	90	0	0	0	0	0	0	0	10	G	0	0	1	68
Oxymycterus rufus	90	0	0	0	0	0	0	0	10	G	0	0	1	75,4
Ozotoceros bezoarticus	0	0	0	0	0	0	0	0	100	G	1	1	1	40000
Panthera onca	0	80	10	10	0	0	0	0	0	G	1	1	1	100000
Peropteryx kappleri	100	0	0	0	0	0	0	0	0	A	1	0	0	9,42
Peropteryx macrotis	100	0	0	0	0	0	0	0	0	A	1	0	0	5,42
Philander opossum	30	30	20	0	0	10	0	0	0	S	1	1	1	585,5
Philander quica	30	30	20	0	0	10	0	0	0	S	1	1	1	395,45

Phylloderma stenops	10	0	0	0	0	20	10	60	0	Ar	1	0	0	53,68
Phyllomys nigrispinus	0	0	0	0	0	80	0	0	20	G	1	0	0	224,3
Phyllomys pattoni	0	0	0	0	0	60	0	0	40	G	1	0	0	298,33
Phyllostomus discolor	30	0	0	0	0	40	30	0	0	S	1	0	0	34,13
Phyllostomus elongatus	70	0	10	0	0	10	10	0	0	S	1	0	0	40,43
Phyllostomus hastatus	40	0	50	0	0	10	0	0	0	S	1	0	0	87,21
Pithecia pithecia	0	30	0	0	0	30	10	0	30	Ar	0	0	1	1375,5
Platyrrhinus fusciventris	10	0	0	0	0	90	0	0	0	Ar	1	0	0	13,5
Platyrrhinus incarum	10	0	0	0	0	90	0	0	0	Ar	1	0	0	13,5
Platyrrhinus lineatus	10	0	0	0	0	90	0	0	0	Ar	1	0	0	24,36
Platyrrhinus recifinus	10	0	0	0	0	90	0	0	0	Ar	1	0	0	22
Potos flavus	10	0	0	0	0	80	0	0	0	Ar	1	0	0	3000
Priodontes maximus	90	0	0	0	0	0	0	0	0	G	1	0	0	45359,68
Procyon cancrivorus	50	0	10	20	0	10	0	10	0	G	1	0	0	6949,92
Proechimys longicaudatus	0	0	0	0	0	20	0	10	70	G	1	0	0	205
Proechimys roberti	0	0	0	0	0	20	0	10	70	G	1	0	0	284,99
Promops centralis	100	0	0	0	0	0	0	0	0	A	1	0	0	26,37
Promops nasutus	100	0	0	0	0	0	0	0	0	A	1	0	0	15,2
Pseudoryzomys simplex	20	0	0	0	0	20	0	20	40	G	1	0	0	51,25
Pteronotus gymnonotus	100	0	0	0	0	0	0	0	0	A	1	0	0	14,43
Pteronotus rubiginosus	100	0	0	0	0	0	0	0	0	A	1	0	0	19,67
Puma concolor	0	100	0	0	0	0	0	0	0	G	1	1	1	51600,04
Pygoderma bilabiatum	0	0	0	0	0	100	0	0	0	Ar	1	0	0	18,5
Rhagomys rufescens	20	0	0	0	0	10	0	40	30	Ar	1	0	0	21,2
Rhinophylla pumilio	10	0	0	0	0	90	0	0	0	Ar	1	0	0	9,55
Rhipidomys ipukensis	20	0	0	0	0	30	0	30	20	Ar	1	0	0	41,6
Rhipidomys itoan	20	0	0	0	0	30	0	30	20	Ar	1	0	0	80
Rhipidomys macrurus	20	0	0	0	0	20	0	30	30	Ar	1	0	0	79,72
Rhipidomys mastacalis	20	0	0	0	0	30	0	30	20	Ar	1	0	0	77,5
Rhipidomys nitela	20	0	0	0	0	30	0	30	20	Ar	1	0	0	89
Rhipidomys tribei	20	0	0	0	0	30	0	30	20	Ar	1	0	0	80
Rhynchonycteris naso	100	0	0	0	0	0	0	0	0	A	1	0	0	3,83
Saccopteryx bilineata	100	0	0	0	0	0	0	0	0	A	1	1	0	7,8
Saccopteryx leptura	100	0	0	0	0	0	0	0	0	A	1	1	0	4,72
Saguinus midas	40	0	0	0	0	30	30	0	0	Ar	0	0	1	540
Saguinus niger	10	0	0	0	0	30	20	0	20	Ar	0	0	1	462,13
Saguinus ursula	10	0	0	0	0	30	20	0	20	Ar	0	0	1	462,13
Saimiri collinsi	20	0	0	0	0	20	0	20	20	Ar	0	0	1	743,24
Saimiri sciureus	20	0	0	0	0	20	0	20	20	Ar	0	0	1	743,24
Saimiri ustus	20	0	0	0	0	20	0	20	20	Ar	0	0	1	1000
Sapajus apella	20	0	0	0	0	20	10	20	20	Ar	0	0	1	2500
Sapajus cay	20	0	0	0	0	20	10	20	20	Ar	0	0	1	2500
Sapajus flavius	20	0	0	0	0	20	10	20	20	Ar	0	0	1	2500
Sapajus libidinosus	20	0	0	0	0	40	20	0	10	Ar	0	0	1	2687,21
Sapajus nigritus	20	0	0	0	0	40	20	0	10	Ar	0	0	1	2687,21
Sapajus robustus	20	0	0	0	0	20	10	20	20	Ar	0	0	1	2500
Sapajus xanthosternos	20	0	0	0	0	40	20	0	10	Ar	0	0	1	2687,21
Sooretamys angouya	20	0	0	0	0	20	0	20	40	G	1	1	1	120,17
Speothos venaticus	0	100	0	0	0	0	0	0	0	G	0	1	1	5999,98
Sturnira lilium	0	0	0	0	0	100	0	0	0	Ar	1	0	0	19,1
Sturnira tildae	0	0	0	0	0	100	0	0	0	Ar	1	0	0	24,04
Subulo gouazoubira	0	0	0	0	0	30	0	20	50	G	1	1	1	16633,17
Sylvilagus brasiliensis	0	0	0	0	0	0	0	0	100	G	1	1	0	949,99
Sylvilagus minensis	0	0	0	0	0	0	0	0	100	G	1	1	0	949,99

Tadarida brasiliensis	10 0	0	0	0	0	0	0	0	0	A	1	0	0	12,49
Tamandua tetradactyla	10 0	0	0	0	0	0	0	0	0	S	1	1	1	5515,06
Tapirus terrestris	0	0	0	0	0	0	0	0	100	G	1	0	0	207500,9 1
Tayassu pecari	10	0	20	0	0	10	0	0	60	G	1	1	0	21266,69
Thaptomys nigrita	20	0	0	0	0	10	0	40	30	G	1	1	1	19,9
Thrichomys laurentius	0	0	0	0	0	60	0	40	0	G	1	0	0	339
Thrichomys pachyurus	0	0	0	0	0	60	0	40	0	G	1	0	0	339
Thylamys macrurus	80	0	0	0	0	10	0	0	0	S	1	0	0	34,43
Thyroptera tricolor	10 0	0	0	0	0	0	0	0	0	A	1	0	0	4,36
Tolypeutes tricinctus	80	0	0	0	0	20	0	0	0	G	1	1	1	1487
Tonatia bidens	90	0	0	0	0	10	0	0	0	A	1	0	0	27,21
Tonatia maresi	90	0	0	0	0	10	0	0	0	A	1	0	0	30
Trachops cirrhosus	50	0	40	0	0	10	0	0	0	G	1	0	0	37,67
Trinomys dimidiatus	0	0	0	0	0	20	0	10	70	G	1	0	0	167,6
Trinomys iheringi	0	0	0	0	0	20	0	10	70	G	1	0	0	220,39
Trinomys paratus	0	0	0	0	0	20	0	10	70	G	1	0	0	235,59
Trinomys setosus	0	0	0	0	0	20	0	10	70	G	1	0	0	284,99
Trinycteris nicefori	80	0	0	0	0	20	0	0	0	S	1	0	0	8,25
Uroderma bilobatum	10	0	0	0	0	80	10	0	0	Ar	1	0	0	16,69
Vampyressa pusilla	0	0	0	0	0	100	0	0	0	Ar	1	0	0	8,63
Vampyriscus bidens	0	0	0	0	0	100	0	0	0	Ar	1	0	0	11,6
Vampyrodes caraccioli	0	0	0	0	0	100	0	0	0	Ar	1	0	0	34,7

CAPÍTULO 2

Ecological Indicators

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The curious case of small mammal community in a rice-Pantanal agroecosystem of Brazil: A tale of multiple diversity metrics

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ABSTRACT

To understand how certain types of agroecosystem management provide resources for biodiversity and conservation opportunities beyond protected areas, it is necessary to consider the cumulative effects on a local scale. Our study focuses on a rice-Pantanal wetland agricultural system within Brazil, where our goals were: i) to quantify the impact of rice paddies on taxonomic, functional, and phylogenetic diversity metrics of small mammals as biodiversity indicators, ii) to compare diversity indices based on incidence and abundance data. We carried out a thorough assessment to sample small mammal species in both the rice paddy and the adjacent legal reserve by employing a multiple diversity metrics approach. We captured the small mammals until reaching saturation on the species accumulation curve over 12,774 trap nights, resulting in 447 individuals, 10 species, including seven rodents and three marsupial species. Abundance-based beta-diversity between rice-paddy and forest reserve were higher (0.95) than incidence-based estimate (0.33). The species composition differed between the legal reserve (8 spp.) and the rice paddy (7 spp.), with the reserve exhibiting higher taxonomic diversity values compared to the rice paddy. In terms of functional and phylogenetic diversity, overall, the rice paddy exhibited higher values for the incidence-based metrics and the abundance-based metrics indicated that the reserve was more diverse. We conclude that Neotropical Pantanal rice fields retain substantial functional and phylogenetic diversities, providing suitable habitats for species thriving in open and semi-aquatic environments. Simultaneously, the legal reserve retains an essential aspect of functional diversity, when considering abundance, needed to maintain ecosystem resilience and stability. Proper abundance estimation is thus a key parameter for accurately evaluating the role of agricultural landscapes as biodiversity conservation opportunities beyond protected areas. Abundance-based indices should be used to correctly evaluate the contribution of each habitat to agroecosystem sustainability, especially in seasonal or extreme event-prone environments.

Keywords: rice paddy, biodiversity-friendly agriculture, diversity metrics, small mammals

1. Introduction

Land use changes are the primary drivers of habitat fragmentation and the decline of large vertebrate populations (Foley et al., 2005), resulting in ecosystem impoverishment and defaunation, commonly referred to as the "empty forest" (Redford, 1992; Powers and Jetz, 2019); Pires and Galetti, 2022). As a result, agricultural anthromes encompass approximately 20% of the Earth's land surface (Ellis and Ramankutty, 2008), comprising a mosaic of agricultural crop matrices and patches of natural habitats. Biodiversity research on agroecosystems focused mainly on assessing biodiversity in its semi-natural habitats (hedgerows, field boundaries) (Morelli, 2013; Regos et al., 2016), compared to crops, to understand their role as refuges for wildlife (Le Cœur et al., 2002; Vasseur et al., 2013). While these areas pose challenges to biodiversity conservation and management goals outside protected areas, they may also present new conservation opportunities (Quinn et al., 2014). In fact, certain agroecosystems can offer resources for wildlife (Fahrig et al., 2015). Still, their availability depends on our comprehension of the agroecosystem type (Fahrig et al., 2011) and its management practices (Vasseur et al., 2013). Notably, substantial research emphasis has been placed on the potential of crop edges to serve as habitats for a wide array of species, and in certain instances, to function as connecting corridors between the remaining patches of natural vegetation within the landscape (Benton et al., 2003; Morelli, 2013; Broughton et al., 2014).

Habitats with remnants of natural vegetation can be the focus of rewilding initiatives aimed at restoring diverse biomes (Galetti et al., 2017). In Brazil, the Forest Code protects natural vegetation areas, which includes Areas of Permanent Protection (APPs) along rivers and hilltops and Legal Reserves (LRs), an obligatory reserve area percentage of the property (Law Nº 12 651/2012 - (Brazil, 2012). Similarly, the role of APPs as forest corridors has been suggested and acknowledged for various taxa (Galetti et al., 2010). Unfortunately, for rural registration, the law dictates that APPs and LRs are evaluated solely based on the presence of vegetation and water bodies, completely disregarding wildlife aspects and overlooking the value of animal biodiversity within the agroecosystem. The overlooked fauna within these habitats is vital in balancing sustainable production and biodiversity conservation. This relation is notable when it comes to the provision of ecosystem services by insects, birds, and small mammals in pest control and crop pollination, as semi-natural habitats, particularly those bordering crops, can exert a substantial influence in promoting natural enemies of pests and parasitoids, thus offering essential ecosystem services (Power, 2010; Morizet Davis et al., 2023).

Furthermore, the majority of studies investigating the effects of land use changes on crops have primarily focused on landscape-level impacts (Dross et al., 2017; González Maya et al., 2017; Magioli et al., 2021; Rurangwa et al., 2022). However, understanding the biodiversity responses to these changes and implementing management practices requires considering the cumulative effects at the farm level and its impacts on local food systems. On-farm wildlife-friendly recommendations encompass maintaining hedgerows along field edges, preserving patches of semi-natural habitats, and adopting sustainable land use practices (Kremsa, 2021). This recommendation poses a challenge to comprehending diversity in agricultural areas, especially for small-scale farmers who require more accessible site-scale data despite their significant contribution to global food production, accounting for approximately one-third of the world's food (Lowder et al., 2021). Likewise, the Food and Agriculture Organization of the United Nations (FAO) highlights the role of cereals as staple foods worldwide, with rice holding the third position, serving as a dietary mainstay for over 3 billion individuals (FAO et al., 2022). Additionally, the ecological function of rice paddies in providing habitats for wetland wildlife (Fasola and Ruiz, 1996) plays a crucial role in mitigating adverse effects of rice production, such as high water, pesticide, and fertilizer demands (Yuan et al., 2021), as well as their negative role with an utterly high Global Warming Potential in terms Green House Gas emission of CH4 and NO2 (Linquist et al., 2012). Given the particular importance of rice production, it is crucial to determine whether and how these wetlands can support animal communities, particularly in highly biodiverse tropical regions. However, there are significant gaps in accurately capturing the consequences of rice farming practices for species diversity, especially at the farm scale level.

Considered a measure of taxonomic diversity (TD), species richness (SR) is often used as a tool in conservation efforts (Ahumada et al., 2013). Nevertheless, this estimator may give rise to misinterpretations by presupposing a uniform significance across all species within the ecosystem, which solely considers the presence of species at a site while overlooking abundance, diversity of functional traits, ecosystem services, and phylogenetic history (Webb et al., 2002; Safi et al., 2013). At the same time, an essential component of TD is understanding the change in species composition between sites, referred to as β-diversity (Baselga, 2010), which is influenced by processes such as dispersal limitation and habitat filtering. Importantly, changes in β-diversity can potentially explain how, in response to land-use change, SR might remain constant or even increase at the level of a sampling point yet decline at the level of a study site (Wearn et al., 2016).

Increasingly, the use of trait-based approaches and the incorporation of species' evolutionary history allows to address biodiversity in agroecosystems and gain a better understanding of the spatial and temporal dynamics of species co-occurrence (Moonen and Bàrberi, 2008; Prescott et al., 2016; Sreekar et al., 2021; Jayathilake et al., 2021; Ocampo Ariza et al., 2022). Complementarily, evaluating the structure and abundance of functional groups - clusters based on shared ecophysiological and life-history traits, such as dispersal strategy or a group of species that utilize similar resources - can provide valuable insights into the response to disturbances and land use impacts (Moonen and Bàrberi, 2008). In addition, incorporating measures of phylogenetic diversity can significantly enhance our comprehension of the effects of agricultural land on biodiversity and ecosystem functioning. This approach enables a more comprehensive understanding of the evolutionary impact on biodiversity by considering the relationships between species (Rurangwa et al., 2022). As such, functional diversity (FD) and phylogenetic diversity (PD) offer valuable insights into the ecological roles, functional redundancy, and adaptability of species in ecosystems (Petchey and Gaston, 2007; de Bello et al., 2013). Therefore, integrating these three components—taxonomic diversity, functional diversity, and phylogenetic diversity—becomes a powerful approach to investigating agroecosystem biodiversity in a comprehensive and holistic analysis (Jarzyna and Jetz, 2016).

This study investigated a small mammal community in the tropical wetland agroecosystem dominated by irrigated rice paddies in the Neotropical Pantanal Biome. Small mammals play a crucial role in maintaining ecosystem function in farmlands (Hurst et al., 2014). They consume seeds, non-cultivated plants, and insects (Ness and Morin, 2008; Baraibar et al., 2009), providing essential agricultural pest control (Brown et al., 2007). Moreover, they serve as a food resource for larger mammals and birds (Sieg, 1987) and their habitat specificity, microhabitat use, and short lifespan make them valuable indicators of land use changes (Umetsu and Pardini, 2007).

Our aim was to evaluate the multiple diversity metrics of the small mammal community in a rice-Pantanal agroecosystem of Brazil. More specifically, we aimed to: (i) compare the taxonomic, functional, and phylogenetic diversity of small mammals between rice paddies and the adjacent natural area of forested legal reserve; (ii) examine how abundance-based estimators influence the multiple diversity metrics between the sampling sites when compared to more commonly used incidence-based estimators. We define gamma diversity, also known as regional diversity, as the encompassing diversity of the agroecosystem.

2. Methods

2.1. Study area

Our study area is in the Neotropical Pantanal Biome, Brazil, one of the world's largest wetland ecosystems (Alho and Sabino, 2012). The Upper Paraguay River Basin (UPRB), which encompasses most of the Pantanal, is an area of 361,666 km² within Brazil, with only 10% delimited as protected areas (Jardim et al., 2020). The majority is privately owned, with 18.6% of the land used for agriculture and cattle ranching (MapBiomas, 2021, *available at:* https://mapbiomas.org/). We carried out small mammal sampling in a rice-Pantanal agroecosystem (-20.086050; -56.614764) located in Mato Grosso Sul state **(Fig. 1).** The agroecosystem spans 12,964 ha, with 10,630 ha in the Pantanal domain and around 2,600 ha in the Cerrado domain.

Since 1975, part of the Cerrado areas, approximately 2,100 ha, have been converted to pasture. In 1984, around 3,200 ha were destined for rice paddies, using water from the Miranda River, distributed through 130 km of irrigation and drainage canals (Concone, 2004) **(Fig. 1).** Currently, in some of these areas, crop rotation is carried between rice, corn, and soybeans. There are also about 70 ha of eucalyptus (*Eucalyptus globulus*). The climate in the region is type AW (Köepen classification), tropical hot and humid (Alvares et al., 2013).

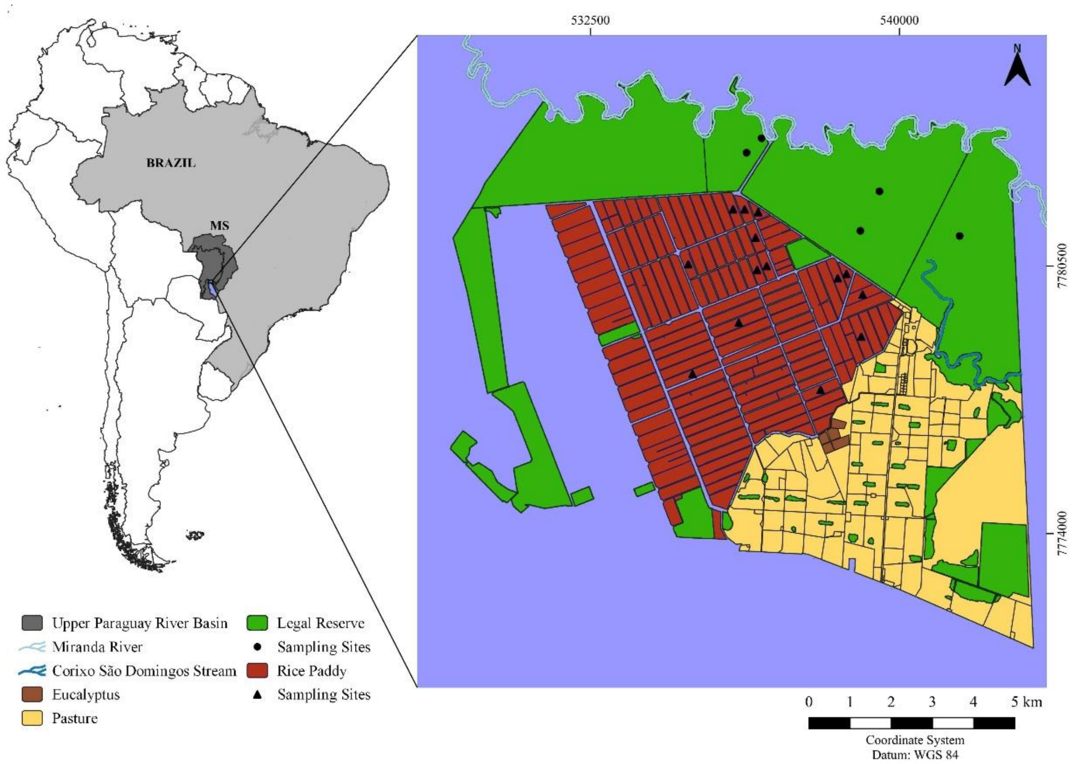

Fig. 1. Map of the study area showing the fourteen sampling transects in the rice paddy (triangles) and five transects inside the Legal Reserve [remnants of natural vegetation] (circles) selected for this study. The colors in the map indicate different land covers: red – rice paddy, light yellow – livestock with pasture, brown – eucalyptus plantation, green: Natural Pantanal Forest under private Legal Reserve.

2.2. Sampling methods

Two sampling habitats were considered: the rice paddy and the legal reserve. We collected the small mammal trapping data during field campaigns conducted throughout the rice growing season from July to November 2019, mostly coinciding with the dry season in the Southern Pantanal (which usually begins in May and peaks in September). An additional data collection campaign was conducted between October and November 2021 to complement the dataset. Notably, our collection period was intentionally chosen to encompass all rice growth stages, ranging from fallow conditions in June-July to harvest in November/December.

We conducted 104 sampling using two types of live traps: Sherman® (25x8x9cm) and Tomahawk® (40x12x12cm). We set up 1,000 meters long transects within each of the five sampling points in the legal reserve, with the traps arranged alternately every 15 meters. We added sub-canopy traps (1.5 meters above the ground) to enhance the capture success of arboreal species (Albanese et al., 2011). In the rice paddy, we sampled 14 points within the study area using 30 traps arranged in two linear transects, each with 15 traps. One transect was positioned inside the agricultural field. At the same time, we placed another along its periphery, on the elevated edges of the rice paddy, which remained dry while the paddy was flooded. Although the tree/shrub vegetation surrounding the rice fields are remnants of natural forests, they are located within the agricultural field and were treated as part of the rice paddy sampling site. Due to the layout of the irrigated rice paddies, the remnants of the natural forest are separated from the crop by water canals (10 to 20 meters wide).

We baited traps with a mixture of banana, peanut candy, oats, sardines, and vanilla essence. Vouchers of adult specimens from both genders have been deposited in the mammal collection of the Federal University of Paraiba (see **Appendix A1. -** for details on small mammals sampling methods and, **Appendix A2.**, voucher list).

Taxonomic nomenclature follows the Taxonomy Committee of the Brazilian Society of Mammalogy (Abreu et al., 2021). Our research adhered to the guidelines of the American Society of Mammalogists (Sikes, 2016) and was authorized under the Biodiversity Authorization and Information System [SISBIO License No. 72681]. Animal handling and euthanasia protocols were authorized by –the Animal Ethics Committee UFPB/CEUA, with the reference number 9192091019.

For appropriate abundance estimation, we calculated sampling sufficiency with individual-based species-accumulation curve (Schilling and Batista, 2008). Additionally, we calculated the following diversity estimators Jackniffe 1, Chao 2, and bootstrap randomization (Gotelli and Chao, 2013), using the *Vegan* package in the R environment (Oksanen, 2022).

2.3. Trait selection for functional diversity

We defined functional traits as morphological, behavioral, or physiological characteristics that influence performance or fitness and respond to abiotic and biotic factors (Petchey and Gaston, 2007). To select the traits for calculating functional diversity indices, we conducted an exploratory analysis using the corrected *Gower* distance, as implemented in the *gawdis v.0.1.3* package (Bello et al., 2021). This method offers improved balance in the contribution of traits (and groups of traits) to overall dissimilarity by providing equitable weights for categorical, continuous, dummy, and fuzzy-coded variables (Bello et al., 2021). We utilized the resulting functional matrix to compute diversity metrics and compared with functional diversity outcomes obtained using the *Gower* distance for dissimilarity matrix calculation, followed by dendrogram generation using the Unweighted Pair Group Method using Arithmetic averages - UPGMA (Podani and Schmera, 2006) **(Fig. S3)**. Ultimately, we opted to employ *Gower's* distance and conducted a Principal Component Analysis (PCA) (Maćkiewicz and Ratajczak, 1993) to explore the contribution of functional traits within our dataset. These methods allowed us to identify groups of functional traits that are highly correlated and play similar roles in the community. Then, we chose only traits that reflect ecological variation that may be important for resource partitioning locally or environmental filtering regionally, focusing on the agricultural system **(Fig. S1.; Table S3.)**. We used a species-based approach (Petchey and Gaston, 2002) and measured four continuous traits in the field (body mass, ear length, hind foot length, and tail length); other traits were extracted from Elton Traits 1.0 (Wilman et al., 2014): diet, daily activity (binary traits), and habitat type (categorical) **(Table 1).**

Table 1. Functional traits used to calculate functional diversity metrics

Functional component	Attribute	Trait values (units)
Size	body mass	Mean (g)
	ear size	Mean (mm)
	hind foot length	Mean (mm)
	tail length	Mean (mm)
Daily activity	crepuscular	0/1
	diurnal	0/1
	nocturnal	0/1
Food type	fruit (pulp)	0/1
	grass	0/1
	invertebrates	0/1
	plant exudates	0/1
	seeds	0/1
	small vertebrates	0/1
Habitat type	arboreal	-
	semiaquatic	-
	terrestrial	-

2.4. Diversity metrics

We chose a multi-diversity approach encompassing Taxonomic, Functional and Phylogenetic diversities **(Table S1.).** We treated the rice paddy and legal reserve as unique sites of interest to determine a diversity value for each assembly. Additionally, we considered the total agroecosystem (rice paddy + legal reserve) to be the gamma diversity.

2.5. Taxomic diversity

We used the Shannon-Weaver and Simpson taxonomic diversity indices using the *Vegan* package (Oksanen, 2022). We also calculated the Pielou index (J') using the Shannon-Wiener diversity values and the logarithm of the species richness of each assemblage. These indices provide a measure of diversity that accounts for changes in richness and abundance, offering a proportional representation of diversity changes. We tested two sample differences with Hutcheson'T test for the Shannon index with the ecolTest package (Hutcheson, 1970; Salinas and Ramirez-Delgado, 2022). Furthermore, we quantified dissimilarity between the legal reserve and rice field with the incidence-based Sorensen (βsor) and abundance-based Bray-Curtis dissimilarity indices in the *Betapart* package (Baselga, 2010). Differences between rice paddy and reserve communities were tested with the PERMANOVA implementation of the *adonis2* function of the *Vegan* package with 1000 permutations.

2.6. Functional diversity

We calculated seven metrics relating to different aspects of functional diversity (Schleuter et al., 2010). The UPGMA approach of calculating the trait space is closely tied to tree-based phylogenetic diversity (Faith, 1992), which provides a consistent framework for comparing different dimensions of biodiversity using tree objects (Mammola et al., 2021). Accordingly, we also computed functional diversity (FD).

To assess divergence between sites and compare with the agroecosystem total, we used Mean Dissimilarity methods, which are based on the notion that functional diversity represents the extent of trait differences between species (de Bello et al., 2013). We used Mean Pairwise Distance (MPD), Mean Nearest Taxon Distance (MNTD), and Rao quadratic entropy (Rao), which considers abundance and enables the use of unified mathematical concepts for taxonomic and phylogenetic diversity (Gotelli and Chao, 2013; Hevia et al., 2016). These methods are also variance-related, and essential in communities with few species, as a single species can significantly affect the variances.

Furthermore, the Rao quadratic entropy (Rao) informs us about the distribution divergence in the functional trait space (Botta-Dukát, 2005). Additionally, we employed methods based on multidimensional spaces. Functional evenness (FEve) measures the regularity of species abundances in functional space, which will be maximized by an even distribution of species and abundances in functional space. All functional diversity metrics were calculated based on incidence and abundance, except for FD, which relies solely on incidence. Additionally, FEve was calculated for abundance (w.abun = TRUE), to ensure it was insensitive to species richness variations, making their interpretation and comparison more straightforward (Mouchet et al., 2010). We carried out all the functional analyses using packages, *Picante* (Kembel et al., 2010) and *dbFD function* in the *FD* package (Laliberté et al., 2022).

2.7. Phylogenetic diversity

A dated phylogenetic tree of the whole species pool **(Fig. 3a)** was generated using ("VertLife Data," 2023) (https://vertlife.org/) which provides a phylogenetic hypothesis for the relationships among taxa by matching the list of species with up-to-date family and genus names, and tip labels of a provided mega tree with branch lengths expressed in million years(Upham et al., 2019).

We used six complementary metrics for phylogenetic diversity (Tucker et al., 2017). We calculated richness using phylogenetic diversity sensu stricto, PD (Faith, 1992). For phylogenetic divergence, the Mean pairwise distance (MPD) and Mean nearest taxon distance (MNTD) were used (Webb, 2000; Webb et al., 2002), with incidence and abundance weighted versions. We also calculated Rao's Quadratic Entropy (Rao, 1982; Botta-Dukát, 2005), representing a phylogenetic diversity index weighted by relative abundance. This index assigns greater weight to the most abundant species in the community (Weiher et al., 2011). We conducted phylogenetic analyses using *Vegan* (Oksanen et al., 2022), *Picante* (Kembel et al., 2010), and *Pez* (Pearse et al., 2015) packages. Differences in abundance-based phylogenetic and functional diversities were tested with Mann-Whitney U test on randomized community matrices of each habitat (rice paddy and reserve) because turnover is high, and the number of species is low. All phylogenetic diversity metrics were calculated based on specie incidence and abundance, except for PD, which relies solely on species richness. All diversity metrics were calculated in R version 4.2.3 (2023-03-15 ucrt).

3. Results

We captured 447 individuals belonging to ten species (seven rodents and three marsupials) with an effort of 12,774 trap nights. The legal reserve had a higher abundance (N=269) than the rice field (N=180) **(Table S2.)**.

Two species were dominant, the Chaco marsh rat (*Holochilus chacarius* Thomas, 1906) (24.16% Relative Abundance, R.A.) in the rice paddy, and the Agile gracile opossum (*Gracilinanus agilis* Burmeister, 1854) (26.17% R.A.) in the legal reserve, collectively representing 50% of the recorded individuals. The other species recorded were the Brazilian guinea pig (*Cavia aperea* Erxleben, 1777), the Mamore arboreal rice rat (*Oecomys mamorae* Thomas, 1906), the savannah mouse hairy-tailed akodont (*Necromys lasiurus* Lund, 1841), the Gray four-eyed opossum (*Philander opossum* Linnaeus, 1758), the Maracaju cerrado mouse (*Cerradomys maracajuensis* Langguth and Bonvicino, 2002), Agricola's gracile opossum (*Cryptonanus agricolai* Gardner, 2007), the elegant rice rat (*Euryoryzomys nitidus*; Thomas, 1884), and the climbing tree rat (*Rhipidomys macrurus* Gervais, 1855).

The species accumulation curve showed stabilization in 10 species with 102 days of sampling **(Fig. S2.)**, indicating that we reached the approximate number of species occurring in the sampled area (Schilling and Batista, 2008). Non-parametric richness estimators converged with this value (Jackniffe 1 = 10.98, Chao = 10.0, and Bootstrap = 10.26).

3.1. Taxonomic, functional, and phylogenetic metrics

Multiple diversity metrics were scaled and summarized in a Cleveland plot (Figure 4). Shannon's index for the agroecosystem was H' = 1.94. Upon separating this area, the highest diversity was observed in the legal reserve (H' = 1.55) compared to the rice paddy (H' = 1.10). Two sample Hutcheson test was significant (H=4.96, p<0.001). The same pattern was observed for the Simpson’s Diversity Index with (D=0.82) for the agroecosystem and more diverse in legal reserve (D= 0.73) than rice paddy (D=0.59) **(Table S2.).**

For the β-diversity (βsor), the dissimilarity between the legal reserve and rice paddy, we obtained a value of β = 0.33 (turnover=0.28, nestedness=0.05), a medium-low dissimilarity value. The PERMANOVA showed a significant difference between the two communities (F=59.54, p<0.001) **(Fig. 2b.)**. However, the relative abundance of species was different between the sampled areas, such as *Holochilus chacarius* (N=103) and *Gracilinanus agilis* (N=117) for the rice paddy and legal reserve, respectively (Fig. 2**a**). Due to the dominance of a species in each area (Pielou's J=0.74 for the legal reserve and J=0.56 for the rice paddy) **(Fig. 2c).** The β-diversity based on abundance using the Bray-Curtis index showed a high dissimilarity between the sites (β=0.95). The PERMANOVA also showed a significant difference between communities (F=27.882, p< 0.001) **(Fig. 2b).**

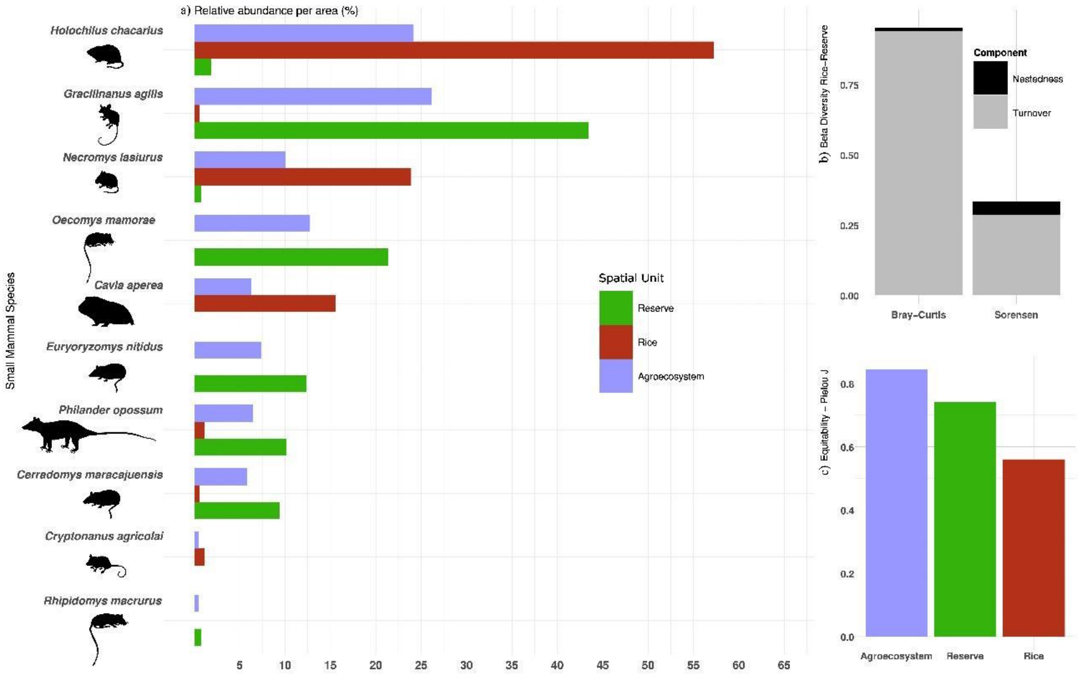

Fig. 2. Relative abundances, Dissimilarity and Dominance of small mammal communities in a rice paddy-Pantanal agroecosystem, in southern Pantanal, Brazil **(a)** Relative abundances in the total agroecosystem, in the rice paddy and in legal forest reserve habitats (%) **(b)** Beta-diversity between rice paddy- forest reserve: Beta Sorensen (β=0.33) / Bray-Curtis index (β=0.95), decomposed in turnover and nestedness; **(c)** Equitability – Pielou J: The Pielou index (J') was calculated using the Shannon-Wiener diversity values and the logarithm of species richness for each sampling unit: agroecosystem (0.85), reserve (0.74), rice (0.56). The most abundant species are dissimilar in the niche space, as shown by the Dendrogram constructed using Gower's Distance and the UPGMA method **(Fig. 3a.; Fig. 3c.).**

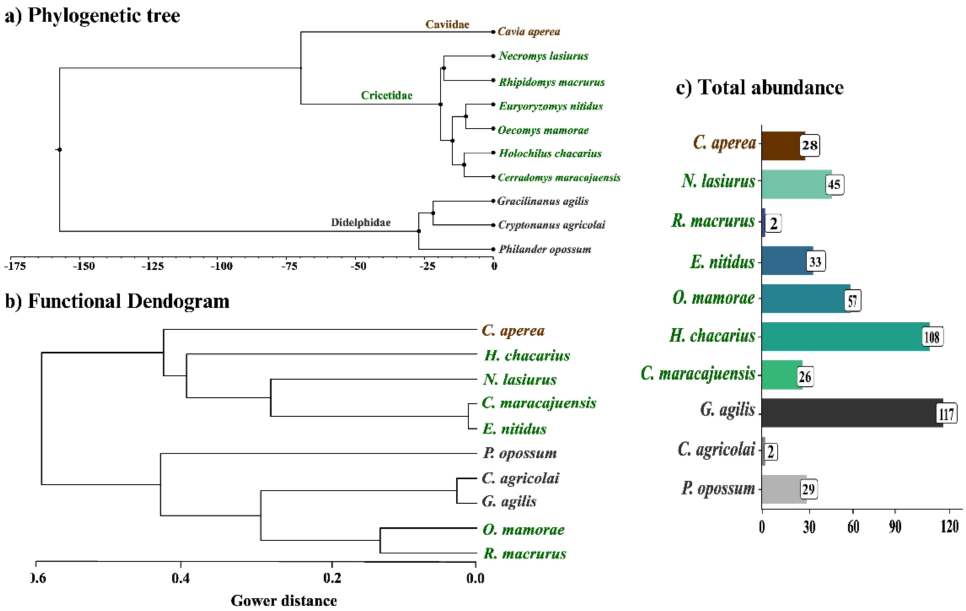

Fig. 3. (a) Phylogenetic tree of the pool of species sampled in a rice paddy-Pantanal agroecosystem, southern Pantanal, Brazil; **(b)** Functional dendrogram by UPGMA of the community of small non-flying

mammals. The dendrogram was constructed using Gower's Distance for the functional characteristics in Table 1; **(c)** Total abundance data of the species trapped in the agroecosystem.

Our findings revealed a functional diversity (FD) value of 1.67 for the agroecosystem. However, this value did not differ significantly between the legal reserve and rice paddies areas. Using the same tree-based phylogenetic diversity approach, the rice paddy assemblage harbored the highest phylogenetic diversity (PD) with a species composition encompassing 444 mya., the legal reserve assembly exhibited a PD of 404 mya. Still for presence-absence data, the rice paddy exhibited the highest mean dissimilarity of functional characteristics (MPD) with a value of 0.81 and the mean nearest taxon distance (MNTD) with 0.44. The legal reserve had values of 0.71 and 0.31, respectively. For the abundance data, the Mann-Whitney test revealed a statistically significant difference in MNTD between rice paddy and legal reserve ($U=295597$; $p < 0.05$), indicating that the diversity of the rice field (0.55) was higher than that of the legal reserve (0.38). Also, there was a significant difference in the diversity of MPD between the sites ($U=53483$; $p < 0.001$), with the rice field (0.38) exhibiting a lower diversity value when compared to the legal reserve (0.51).

Concerning the divergence of phylogenetic diversity, MPD analysis using incidence data demonstrated that the legal reserve assemblage harbored a lower phylogenetic diversity with an average distance between taxa of 153 mya., while the rice paddy harbored, greater phylogenetic diversity with an average distance between taxa of 208 mya. However, the rice paddy exhibited a greater mean distance to the nearest neighbor (MNTD) of 44 mya and the legal reserve of 29 mya. However, the abundance influenced the MPD metric, revealing a statistically significant difference between the legal reserve and the rice paddy ($U=1421605$; $p < 0.001$), with average distances between taxa of 165 mya and 55 mya., respectively. Also, there was a statistically significant difference ($U= 28111$; $p < 0.001$) for MNTD weighted by abundance The rice paddy assembly exhibited the highest mean distance to the nearest neighbor (44 mya.) while the reserve was 38 mya.

Rao's Quadratic Entropy (RAO), the total functional redundancy was estimated to be 0.34, with the rice field displaying a value of 0.27, while the Legal Reserve significantly exhibited a higher value of 0.32 ($U=153$, $p<0.001$). Furthermore, the analysis of functional evenness (FEve) revealed that the feature space within the legal reserve is more evenly distributed in terms of species abundances (0.42) when compared to the rice paddy (0.28), with the Mann-Whitney test showing a significant difference between the two areas (U=120, p=0<0.001) **(Fig. 4).**

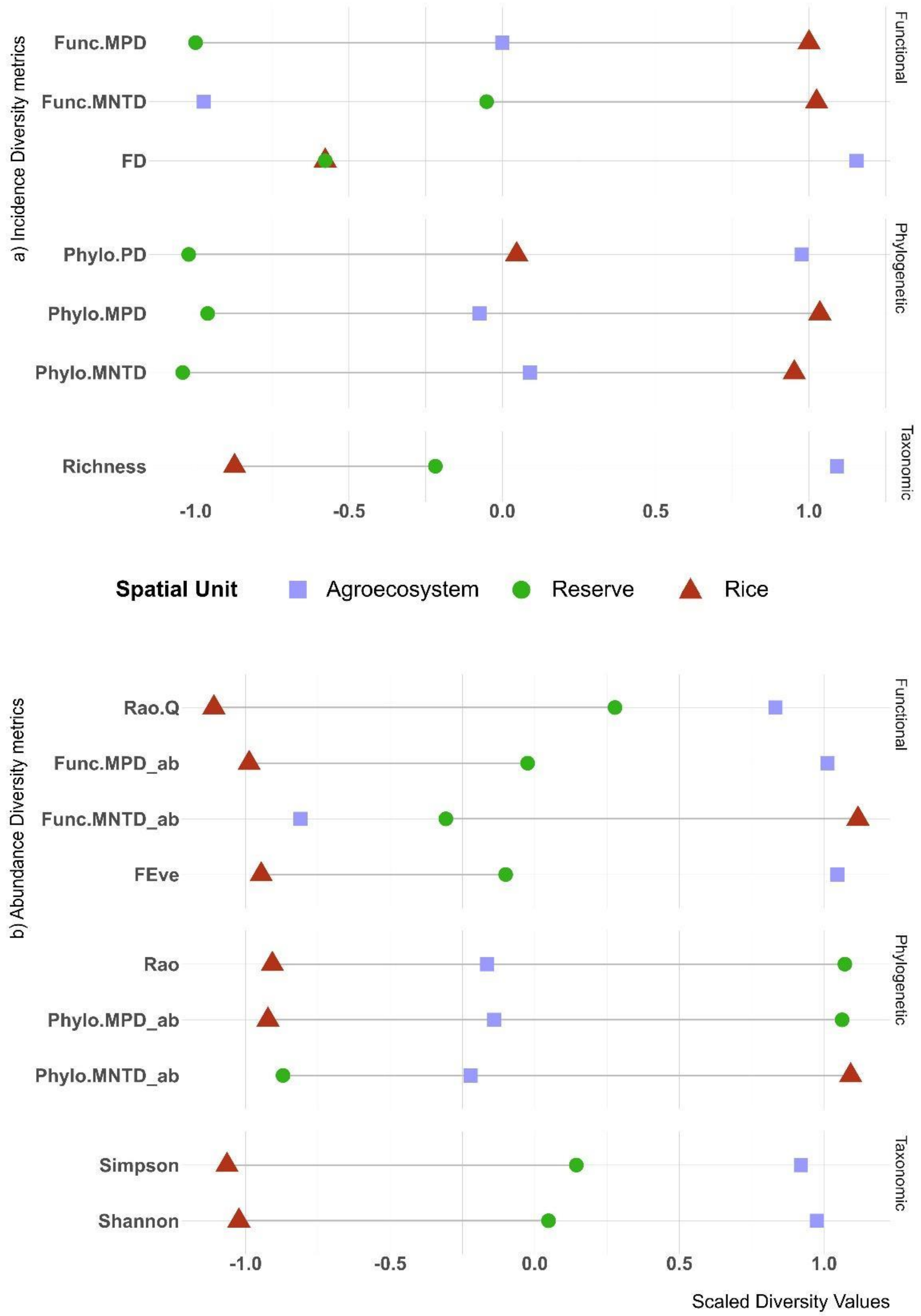

Fig. 4. Multiple Diversity Metrics (Functional, Phylogenetic and Taxonomic) for the Small Mammal Community in a rice paddy-Pantanal agroecosystem, Brazil. **(a)** Diversity metrics with incidence data: Func.MPD, mean pairwise functional distance; Func.MNTD, mean nearest functional taxon distance; FD, functional diversity; Phylo.PD, phylogenetic diversity; Phylo.MPD, mean pairwise phylogenetic distance; Phylo.MNTD, mean nearest phylogenetic taxon distance **(b)** Diversity metrics with abundance data: Rao.Q, functional quadratic entropy; Func.MPD_ab, Func.MNTD_ab, abundance-weighted; FEve, Functional

evenness; Rao, phylogenetic quadratic entropy; Phylo.MPD_ab, Phylo.MNTD_ab, abundance-weighted. The symbols of the figure indicate the spatial units: light purple square, total agroecosystem diversity; green circle, forest legal reserve sampling site and red triangle, rice paddy sampling site.

4. Discussion

4.1. Rice provides new habitats, and the reserve retains resilience

Agronomic practices carried out in rice paddies such as crop growth over a short period of time, inundation and edge management make rice fields historically old, unique and biodiversity rich agroecosystems, especially in Asia (Bambaradeniya and Amarasinghe, 2003). Our results have shown that in the Neotropics, the rice-pantanal agroecosystem sustains a significant diversity of small mammals, hosts 38% of the expected species for the biome (Tomas et al., 2017) and a similar richness when compared to other natural areas in the Pantanal (Alho et al., 1987 [9 spp.], Cáceres et al., 2007 [13 spp.], Andreazzi et al., 2011 [6 spp.]). The species composition in this rice paddy-pantanal agroecosystem does not appear to differ in composition from small mammal communities found in the Upper Paraguay River Basin in natural areas (Cáceres et al., 2007; Hannibal et al., 2017; Gonçalves et al., 2018) and is thus representative of a natural assemblage in an anthropogenic landscape. Species are retained from the Pantanal species pool and the Cerrado biome species pool (*C. agricolai, R. macrurus*) because of the ecotonal characteristics of the study site.

The slight differences in alpha diversity metrics and the low to medium dissimilarity between rice and reserve from the presence-absence matrix (0.33), coupled with non-invasive species exclusive of the rice paddy (3/10), may suggest that in this context this agricultural system induces small perturbations to the adjacent natural system but exerts environmental filtering, as the more abundant species occupy very different niches (Mammola et al., 2021), and adds richness to the agroecosystem as whole. However functional and phylogenetic incidence-based diversity metrics of the rice paddy support this panel of diversity **(Fig. 4).** Higher functional diversity in the reserve arises because of microhabitats of edges with herbaceous plants (Sullivan et al., 2012) that support the presence of open area (*N. lasiurus*) or larger diurnal rodents, exclusive of this habitat (e.g. *C. aperea*) (Westberg, 2011), and of open area marsupials such as *C. agricolai* when trees are present. The semiaquatic oryzomyine rodent *H. chacarius*, dominant in the rice field (57.2% R.A.) **(Fig. 2a)**, is known to thrive in irrigated rice paddies (Hershkovitz, 1955; Yahnke, 2006). We thus concur with a body of literature that appropriate edge presence and management provides habitat for biodiversity (Broughton et al., 2014; Janova and Heroldova, 2016; Benedek and Sîrbu, 2018), by extending it for many small mammals in rice paddy in the Neotropics.

The legal reserve scored higher abundance-based indices, in functional diversity metrics (FEve and Rao) and phylogenetic diversity (Rao and MPD). Due to a loss of functional spaces and increased distances between traits, FEve was lower in the rice paddy, which is indicative of disturbance. This is most likely caused by environmental filtering resulting from the absence of forest cover or by the stronger effects of competitive exclusion resulting from the increased abundance of habitat specialists (Perronne et al., 2017). Meanwhile, the legal reserve assembly possesses a greater genetic and evolutionary diversity. These higher functional and phylogenetic diversities are known to be vital for ecosystem stability in fragmented Neotropical forests (Arroyo-Rodríguez et al., 2012) and maintaining unique genetic resources in sustaining ecosystem function (Hurst et al., 2014), increasing the resilience of the agroecosystem to disturbances (Mouillot et al., 2013). This combination might prove interesting for agroecosystem sustainability in seasonal, fire-disturbed environments like the Pantanal, especially with the increase of climate change-induced extreme events. Moreover, the low beta diversity incidence-based differentiation suggests that some crop-natural ecosystem combinations, with low differentiation, would allow compositionally similar communities to exist but with different functional and phylogenetic diversities to arise when estimated through abundance.

4.2. Impacts of abundance data on multi component diagnostics

The inclusion of abundance gives an enhanced perspective of the diversity of the agroecosystem when compared to presence-absence data as most alpha, functional, phylogenetic and beta diversity metrics become higher in the Legal reserve than in the rice paddy when abundance is considered **(Fig. 4)**. The diversity metrics more affected by abundance are in MPD, both functional and phylogenetic, which are a consequence of the deep phylogenetic relationships of South American small mammal taxa (Webb et al., 2002).

The fact that abundance is more likely to explain the total variation in species-environment relationships than presence-absence data, as demonstrated for birds (A. Cushman and McGarigal, 2004) is not recent in community-level studies. However, our approach comparing incidence and abundance-weighted provides an improved perspective of diversity in this agricultural system, which is seldom used to assess the impact of agroecosystems on terrestrial vertebrate diversity. This statement is especially true for small mammals. Species diversity between sites may be more strongly influenced by fine-scale features of the environment (Serafini et al., 2019). In our context, flooded rice paddies with their edges, and the adjacent natural environment floodplains have structural and functional similarities, as shown by the low beta diversity. However, they are mainly composed of turnover. The similarities between rice paddies and adjacent natural environments have been analyzed worldwide (Elphick, 2000; Natuhara, 2013; Zhou et al. 2016), including the dynamics of abandonment and restoration (Washitani, 2007), making it hard to predict differences between structurally similar habitats. Thus, essencial differences will only be captured in abundance data since common species may exist in both sites (Bello et al., 2007; Thornton et al., 2011).

4.3. Multifunctional health landscapes

In an overview of our work, placing it in the context of the global discourse on agricultural biodiversity, land sparing and land sharing have emerged as critical frameworks for understanding how agricultural landscapes can support ecological conservation alongside food production. The rice-Pantanal agroecosystem characterizes itself as a land-sparing system, with over 20% of its area formally protected under the Brazilian Forest Code. However, the discussion on a land-sharing/-sparing system has gained prominence in the agroecosystem land-sparing, emphasizing the synergistic vision of preserving large areas of land together with surrounding favorable matrices for biodiversity conservation (Kremen, 2015). In the land-sharing context, crop edges act as refuges for many species that are filtered out of the field core by intensive crop management strategies (Yvoz et al., 2021), providing rice fields with habitat quality and structural similarity to the surrounding Pantanal matrix. Thus, although land sparing conserves a high species richness, species populations generally decrease compared to entirely natural habitats (Phalan et al., 2011). Therefore, maintaining protected areas both in the context of land sparing and land sharing also plays a crucial role in increasing species richness, and may be more promising for biodiversity in the long term than using these strategies separately.

Furthermore, our study area promotes wildlife tourism as many other Pantanal farms (de Azevedo and Murray, 2007; Tortato and Izzo, 2017), representing a mixed system with large legally protected areas and crop edges with vegetation that facilitates wildlife observation of endangered mammals, such as *Tapirus terrestris*, the tapir and *Blastocerus dichotomus*, the deer marsh, the giant anteater *Myrmecophaga tridactyla* and carnivores that are commonly spotted in the rice fields, such as *Panthera onca*, *Puma concolor*, *Leopardus pardalis* and *Cerdocyon thous* (de Azevedo and Murray, 2007). Medium and large mammals in the study area showed patterns of intensive use indicative of high habitat permeability, thereby indicating a low contrast in resource availability within the rice-Pantanal system (de Azevedo and Murray, 2007). These results further corroborate our findings for the small mammal community, suggesting that rice cultivation is not entirely detrimental to biodiversity.

Our conclusions must be pondered firstly by the relative temporal limitation of the study. Although we sampled over two years our assessment at the community level scale should be complemented by other surveys since the Pantanal has multi annual drought-fire-flood events, including extreme events, that can have combined impact on community structure over multiple years (Arruda et al., 2022). Spatial limitations of the study are another factor limiting generalizations since 1) local conditions in the Pantanal can have substantial effects over large-scale dynamics (Ivory et al., 2019), 2) our rice-paddy/Pantanal system differs from more traditional planting systems in that it is much more recent than those that occur in more degraded ecosystems that have been cultivated for millennia (Hua et al., 2023), in Asia for example. Sampling limitations due to the flooding regimes of the study area, are a structural bias in the Pantanal, despite our indicative of sampling sufficiency (Andreazzi et al., 2011). Another structural limiting factor for comparison is the small area of distribution of small mammals which leads to the different composition of communities at the ecoregional scale (de la Sancha et al., 2020). Although direct comparison might be difficult, the phylogenetic structure of small mammal Neotropical communities will contain similar phylogenetic elements (sigmodontines, caviomorph and didelphimorph marsupials) as well as functional equivalents.

Researchers should focus future efforts on broadening the comparative estimates of incidence and abundance-based diversity estimators in agroecosystems. Many crops, especially those critical for food security in the Neotropics, such as beans, cassava and sweet potato need more biodiversity studies. Further research should also be conducted regarding our conclusion on the relative friendliness of the rice-paddy matrix. More in-depth studies at the population levels, especially population viability analyses and bioaccumulation data, are needed to verify the long-term quality/effects of the agricultural matrix in maintaining these species. Indeed, an exponential increase in species abundance in rice-paddies can lead to an escalation of ecosystem disservices due to the impact of the availability of prey (Sreekar et al., 2021), through the bioaccumulation and biomagnification of contaminants (Ali and Khan, 2018) in predators. Genome-based demographic estimation of population trends could also complement our study, which needed more time. In summary, we advocate an increased multiscale approach to diversity as presented in this article in order to detect early warning signs of agroecosystem alteration (Kéfi et al., 2014) and encourage research at the local scale level, for important food security crops involving landowners in management policies directly addressing biodiversity conservation.

5. Conclusions

Although poorer in alpha diversity, rice paddies in this agroecosystem in the Pantanal are more diverse functionally and phylogenetically, primarily in incidence-based indices, by providing suitable habitats for open area and semi-aquatic species. The inclusion of abundance data partially alters this conclusion, showing the lack of functional evenness, and functional and phylogenetic redundancy in the rice paddy. Abundance also proves vital for differentiation metrics (beta diversity) uncovering very different small mammal communities because of the dominance that drives species abundance distribution. As a result, abundance-based beta diversity plays a crucial role in assessing agroecosystem impacts, standing out as a critical indicator for understanding how biodiversity varies at different geographical scales, from small plots of land to more significant agricultural landscapes. Abundance-based indices should be used when comparing crops and adjacent natural systems to correctly evaluate each contribution agroecosystem sustainability, especially in seasonal or extreme event prone-environments. Further research at the community level to generalize these conclusions should be carried out within agroecosystems, especially in understudied but strategic crops to food security. At the population levels, estimating population trends in agroecosystems, either evaluating bioaccumulation of contaminants or using population genomics is needed to assess the long-term effects of agricultural matrices on biodiversity after natural vegetation suppression.

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Appendix A. Supporting information

Supplementary Tables

Table S1. Measures of taxonomic, phylogenetic, and functional diversity were used for the analysis.

Taxonomic diversity	Description
Shannon	This species diversity index assigns equal weight to both rare and abundant species and assumes that all species are represented in the sample. In this metric, the higher the value of H' for the area, the greater the estimated diversity of the community (Magurran, 1988).
Simpson	It is a dominance index that reflects the probability of two randomly chosen individuals in the community belonging to the same species. It ranges from 0 to 1, and the higher the value, the greater the probability of the individuals being of the same species, indicating higher dominance and lower diversity (Simpson, 1949).
Eveness	Evenness (j') quantifies the degree of equality among community members in terms of their abundance, as proposed by (Pielou, 1966). Mathematically, it is defined as the ratio of H' (Shannon's diversity) to H_{max}, where H_{max} represents the maximum Shannon's diversity attainable based on the community's richness [H_{max} = 1n (richness)].
β-diversidade (βsor)	This index evaluates the dissimilarity between two compared areas and is divided into two components: nestedness and turnover. The total dissimilarity index ranges from 0 to 1, where values closer to 1 indicate a higher degree of dissimilarity between the paired areas (Baselga, 2010).
β-diversidade (βsor) - Bray curtis index	This index also references total beta diversity but incorporates species abundance (Bray-curtis dissimilarity) (Bray and Curtis, 1957).
Functional diversity	**Description**
Functional diversity (FD)	A tree-based metric, calculated by using a distance matrix of functional traits to create a functional dendrogram of the entire species pool across all samples, and then calculating the total branch lenghts for samples of interest (Petchey and Gaston, 2002).
Mean parwise distance (MPD)	It measures the extent to which the species in a community are closely related in evolutionary history (or traits) or are more distantly related (Weiher et al., 2011); (de Bello et al., 2013).
Mean nearest taxon distance (MNTD)	MNTD is particularly useful when the functional traits of species are strongly influenced by their evolutionary history and can provide insights into the processes that have shaped the functional diversity of a community over time (Webb et al., 2002).
Functional evennes (FEve)	A measure of the regularity of species abundances in funcitonal space, calculated as the shortest minimum sppaning tree that links all species in withinn a community, and wich can be interpreted as the degree off occupation of niches (Villéger et al., 2008).
Rao's quadratic entropy (Rao's Q)	The most common multivariate index of FD is Rao's quadratic entropy (Rao's Q) (Rao, 1982) is a functional trait-based metric that quantifies the diversity of functional traits present in a community. It is a multivariate extension of the classical Simpson Diversity Index (Simpson, 1949) and takes into account the covariance structure between functional traits. Regardless the Simpson Diversity Index, and the RAO-FD, this equation turns out the Functional Redundancy for each community.
Phylogenetic diversity	**Description**

Phylogenetic diversity strictu sensu (PD) or Faith's Index	Faith's Index is described as the sum of the branch lengths connecting all species in an assemblage (Faith, 1992).
Mean parwise distance (MPD)	The average phylogenetic distance between a community. It can be calculated considering incidence data or considering species abundance data. This metric weighs the internal structure of the phylogeny (relationships between species from different families) (Webb et al., 2002). MPD is a measure of divergence (Tucker et al., 2017).
Mean nearest taxon distance (MNTD)	This metric uses the phylogenetic distance matrix to quantify the average of minimum relatedness values between pairs of species (the average value for the nearest neighbor). It can be calculated considering incidence data and species abundance data. MNTD is a metric that weights relationships at the tip of the phylogeny (species of the same genus) (Webb, 2000).
Rao's quadratic entropy (Rao's Q)	Rao's quadratic entropy is a measure of diversity of ecological communities defined by (Rao, 1982) and is based on the proportion of the abundance of species present in a community and some measure of dissimilarity among them. The dissimilarity range from 0 to 1 and is based on a set of specified functional traits or in the phylogenetic dissimilarity.

Table S2. Abundance, sampling effort, and taxonomic diversity of small mammals (rodents and marsupials) captured in the rice-Pantanal agroecosystem. Sampling efforts were calculated per sampling unit: Legal reserve (transects a-e), rice paddy (rice transects 1-14), and agroecosystem (total).

Species	Legal reserve	Rice	Total
Rodentia			
Cavidae: Cavidae			
Cavia aperea	0	28	28
Cricetidae: Sigmodontinae			
Cerradomys maracajuensis	25	1	26
Euryoryzomys nitidus	33	0	33
Holochilus chacarius	5	103	108
Necromys lasiurus	2	43	45
Oecomys mamorae	57	0	57
Rhipidomys macrurus	2	0	2
Didelphimorphia			
Cryptonanus agricolai	0	2	2
Gracilinanus agilis	116	1	117
Philander opossum	27	2	29
Capture success (%)	4	3	3.5
Capture effort	6,026	6,748	12,774
Richness	8	7	10
Shannon	1.94	1.55	1.10
Simpson	0.82	0.73	0.59
Pielou	0.74	0.56	-
Number of individuals	267	180	447

Table S3. List of traits for the small mammals sampled in the study. Size traits were measured in the field using a species-based approach (Petchey and Gaston, 2002), and other traits were extracted from the Elton Traits 1.0.

	Size				**Daily activity**			**Food type**						**Habitat type**		
Scientific name	**Body.mass**	**Ear.size**	**Hind.foot.lenght**	**Tail.lenght**	**Noturnal**	**Crepuscular**	**Diurnal**	**Fruit.pulp.**	**Grass**	**Invertebrates**	**Plant.exudates**	**Seeds**	**Small.vertebrates**	**Arboreal**	**Semia.quatic**	**Terrestrial**
Cavia aperea	416.24	25.43	41.04	0	0	1	0	0	1	0	0	0	0	0	0	1
Cerradomys maracajuensis	65.83	19.67	28.11	150.89	1	1	1	1	1	1	0	1	0	0	0	1
Cryptonanus agricolai	15	17.5	14	153.5	1	1	0	1	0	1	1	0	0	1	0	0
Euryoryzomys nitidus	61.88	20.79	29.38	129.91	1	1	1	1	1	1	0	1	0	0	0	1
Gracilinanus agilis	30.27	22.38	17.17	141.13	1	1	0	1	0	1	1	0	0	1	0	0
Holochilus chacarius	132.48	19.57	38.09	160.79	1	0	0	0	1	1	0	0	0	0	1	0
Necromys lasiurus	40.67	17.27	20.83	79.63	0	0	1	0	1	1	0	0	0	0	0	1
Oecomys mamorae	65.17	20.33	27.88	148.75	1	0	0	1	0	0	0	1	0	1	0	0
Philander opossum	333.04	35.92	36.12	278.5	1	1	1	1	0	1	0	0	1	1	0	0
Rhipidomys macrurus	100	21	27.5	173	1	0	0	1	1	1	0	1	0	1	0	0

Supplementary Figures

Figure S1. Principal component analysis for the functional traits of the small mammal assemblage of the rice-Pantanal agroecosystem, southern Pantanal, Brazil.

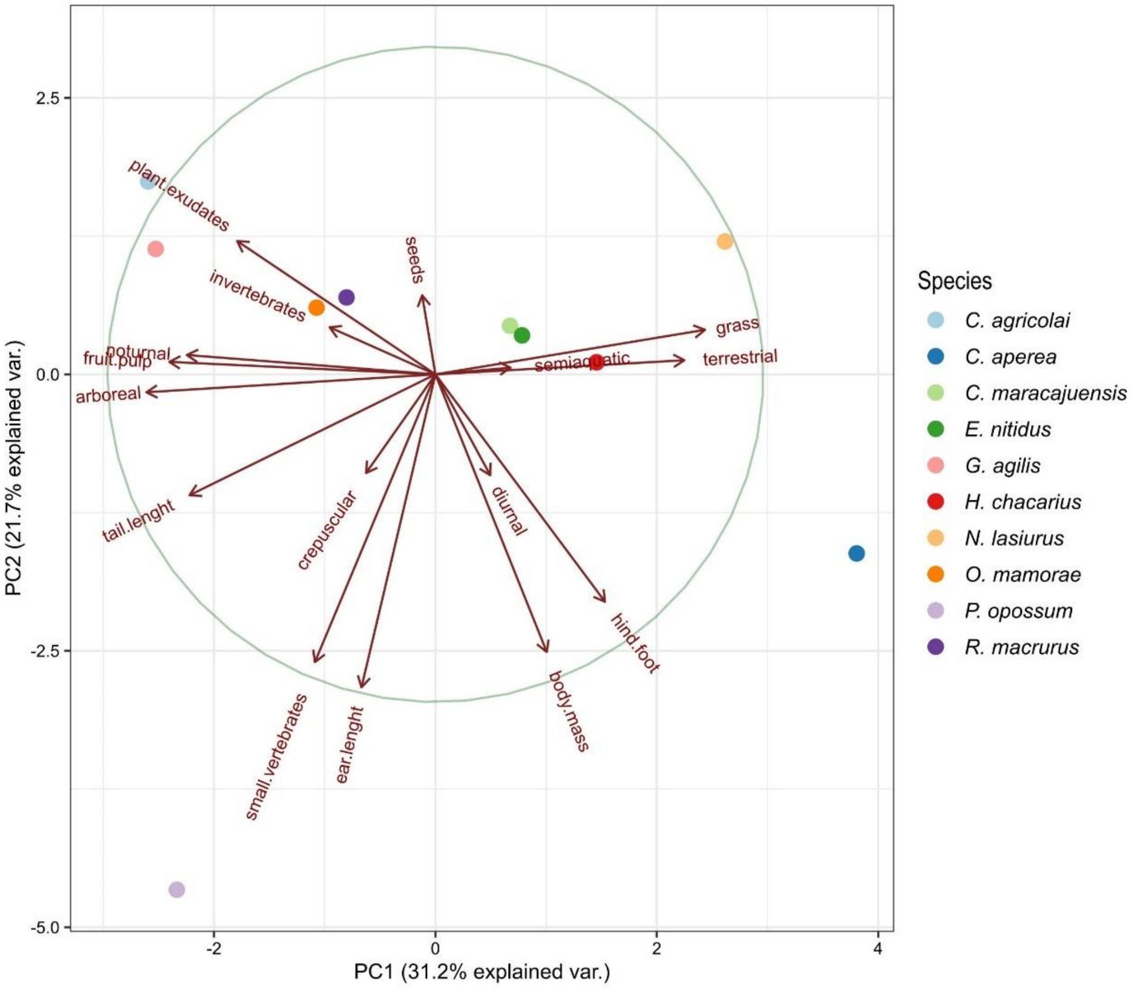


Figure S2. Species accumulation curve in the study area. Black dots represent the observed species richness at each sample size, and the error bars indicate the standard deviation.

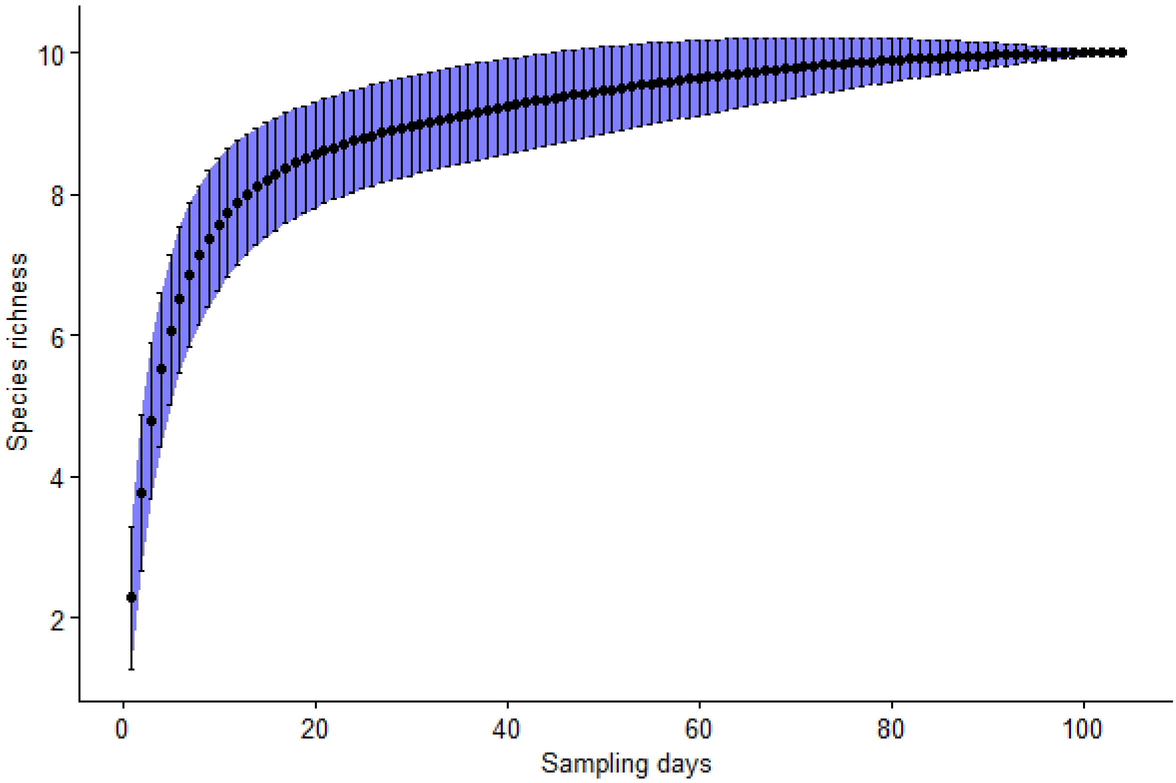
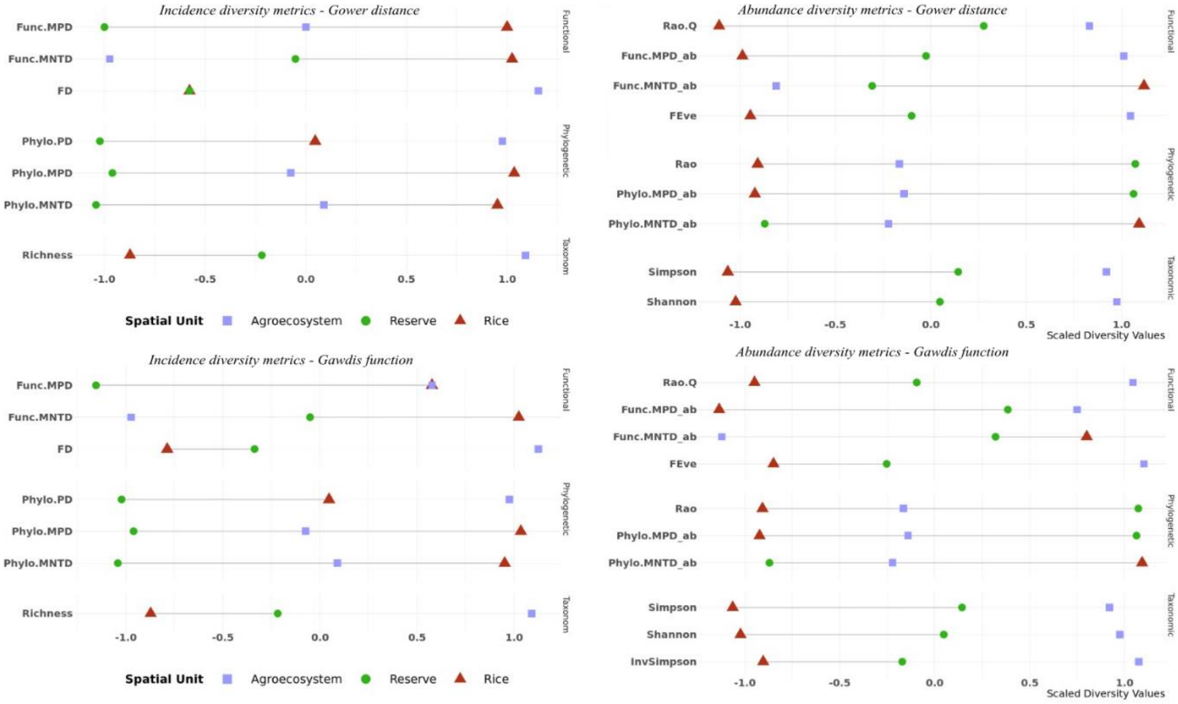


Figure S3. Comparison of functional diversities constructed with Gower's distance and the corrected Gower's distance as implemented in the package 'gawdis'. We kept the original figure because the traits removed were those of habitat specialists. Because our community is not very large in species number, when compared to what would be expected for arthropods or birds, the gawdis suggests a removal of traits that characterize the filtering pattern of the habitat (semiaquatic favouring in rice paddies). In this sense, we interpret the unequal trait contribution as a true pattern not a bias.



Appendix A1. Sampling methods

During the fieldwork, we transported small mammals to a laboratory within the study area (SISBIO License No. 72681; Animal Ethics Committee UFPB/CEUA – 9192091019). The individuals who were not collected were anesthetized using a combination of Ketamine and Acepran (9/1) for rodents and Ketamine and Xylazine (1/1) for marsupials. The administered dose for both anesthetic combinations was 0.1 ml/100g. Each captured individual underwent immediate processing, and recorded body mass, sex, and reproductive condition. To determine their species, we compared ourselves with voucher specimens. We relied on external characteristics, incorporating biometric data (such as body length, tail length, hind foot length, and ear length – all measured in millimeters) and patterns of dorsal and ventral coloration, following the methods outlined in studies by Emmons & Feer (1997); Voss and Jansa (2003); Weksler (2006); Bonvicino et al. (2008); Voss and Jansa (2009).

The vouchers were adult specimens of both genders (N=30). They were euthanized through prior intramuscular anesthesia using ketamine hydrochloride (10 mg/kg) + xylazine hydrochloride (2 mg/kg), followed by deep intravenous anesthesia with sodium thiopental (40 mg/kg). This was followed by the intravenous/intracardiac application of mebezonium iodide + Embutramide + Tetracaine hydrochloride (T61®) (0.3 ml/kg) or intracardiac supplementation with 19.1% potassium chloride (1 ml/kg). The collected animals underwent skin preparation, taxidermy, and skull cleaning with dermestid beetles for later identification based on discrete external and cranial morphological characters. Biological tissue samples (liver and kidney) were collected, preserved in 100% ethanol, and stored alongside voucher specimens in the mammal collection at the Federal University of Paraiba.

Appendix A2. Voucher List

The species collected at the rice-Pantanal agroecosystem (-20.086050; -56.614764), Mato Grosso Sul, Brazil, and deposited in the mammal collection at the Federal University of Paraiba, Brazil were: *Cavia aperea* (UFPB11986♂, UFPB11991♂, UFPB11998♀, UFPB12050♀); *Cerradomys maracajuensis* (UFPB11971♂; UFPB12047♂; UFPB11971♀; UFPB11995♀); *Euryoryzomys nitidus* (UFPB11992♂, UFPB10940♂, UFPB11987♀); *Holochilus chacarius* (UFPB10935♂, UFPB10963♂, UFPB10937♀,); *Necromys lasiurus* (UFPB11970♂, UFPB11964♀); *Oecomys mamorae* (UFPB10961♀, UFPB10962♀, UFPB12145♀, UFPB10962♂, UFPB10966♂);

Rhipidomys macrurus (UFPB10959♂); *Cryptonanus agricolai* (UFPB11393♂); *Gracilinanus agilis* (UFPB10964♂, UFPB10968♂, UFPB10969♂, UFPB11969♀, UFPB11975♀); *Philander opossum* (UFPB10958♀, UFPB11378♀).

Appendix A3. Principal Component Analysis (PCA) based on functional traits.

Principal component analysis showed that more than 52% of the total variations are explained using the first 2 principal components for all traits. The variable "Grass" was found to be the most influential factor for PC1, exhibiting a significantly positive correlation. It was followed by "Habitat type terrestrial" and "Seeds". These characteristics make a greater contribution to PC1 and likely represent traits associated with animals inhabiting terrestrial regions and relying on grasses as their primary food source, such as sigmodontinae rodents. The clustering of *Oecomys mamorae* and *Rhipidomys macrurus* suggests a potential overlap in their ecological niche, as does the grouping of *Cerradomys maracajuensis* and *Euryoryzomys nitidus*. However, the semi-aquatic rodent *Holochilus chacarius*, being the sole representative of its kind, did not exhibit significant overlap, unlike *Necromys lasiurus*. This distinction suggests that these two species may possess distinctive functional characteristics within the agroecosystem. The most influential variable for PC2 is "Ear.length," with a high positive loading, followed by "Small.vertebrates" and "Hind.foot.length." These traits may be related to the size and shape of the ears, as well as a preference for small vertebrates, characteristics that influence the features of the marsupial Philander opossum, positioned in the upper left quadrant. In the upper right quadrant, *Cavia aperea* is distinct from all other species. *C. aperea* is the only folivorous species that lacks a tail, influenced by “hind.foot.length”, “body mass”, and “habitat.type terrestrial” traits. In the lower left quadrant, the marsupials *Gracilinanus agilis* and *Cryptonanus agricolai* are grouped, primarily influenced by “Fruit pulp”, “nocturnal”, and “Plant exudates" traits.

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CAPÍTULO 3

Model Environmental Pollution

Between the hammer and the anvil: exposure to metals through rivers might be higher than through rice-Pantanal agroecosystems in soil and critical tissues of small mammals

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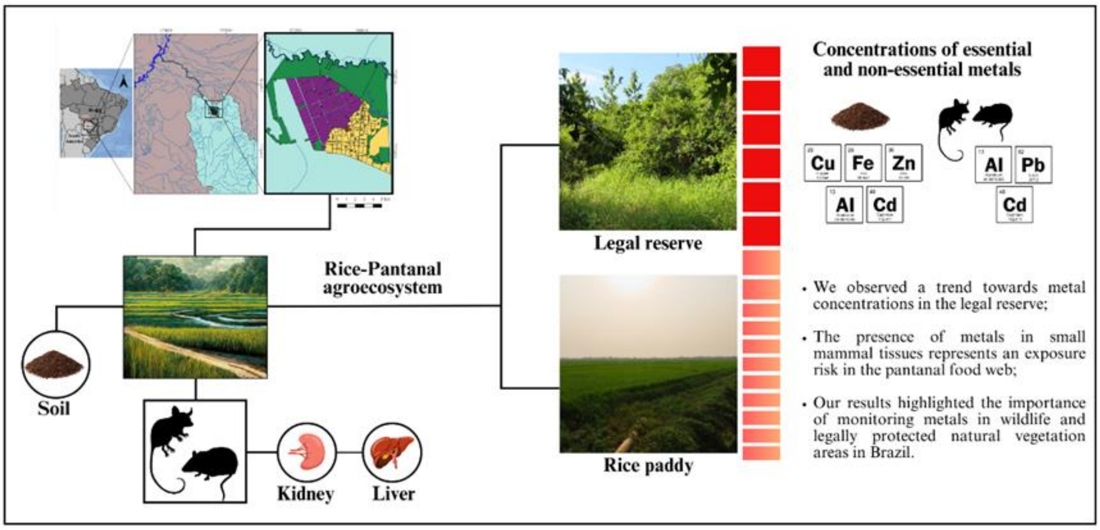
GRAPHICAL ABSTRACT



ABSTRACT

Pantanal harbors a high biodiversity of species and landscapes, providing a wide range of ecosystem services. Yet, the increased use of agrochemicals is a threat to the wetlands. Thus, it is crucial to investigate the exposure of wild species and potential exposure pathways, including soil. Small mammals are potential models for assessing wildlife exposure because they occupy key positions in food webs and accumulate metals in their tissues. Our study focuses on the rice-Pantanal agroecosystem, located in the southern Pantanal. Our main objective was to assess metal contamination (Al, Cd, Cr, Cu, Fe, Pb, and Zn) in two habitats: rice paddy and patches of protected remnant vegetation (legal reserve). This involved quantifying contaminants in soil and critical tissues of small mammals across these habitats. We conducted soil analysis at 14 points across the study area and quantified metals in 41 small mammals, representing 8 species. We found that animals in both habitats had essential and non-essential metals in their tissues. Overall, the highest concentrations of non-essential metals, aluminum (605.86 ± 298.10 mg/kg), and lead (74.09 ± 57.71 mg/kg), were observed in the kidneys of rodents from the legal reserve. Similarly, the highest concentrations of essential metals, copper (28.78 ± 5.05), iron (9,121.46 ± 2,244), and zinc (17.98 ± 3.45) and non-essential metals, aluminum (4,881.48 ± 1,034.69) and cadmium (0.33 ± 0.20), were observed in the soil of the legal reserve. Body condition of the rodents did not differ between habitats, and there was no relationship between the metals bioaccumulation and body condition. The presence of metals in rodent tissues poses a wildlife exposure risk, as these metals can bioaccumulate up the food web. Furthermore, we observed a trend towards higher metal concentrations in the legal reserve, highlighting the need for environmental monitoring policies to protect these legally protected areas of natural vegetation.

Keywords: Pantanal, metals, wildlife, exposure, agriculture, soil

1. Introduction

Wetlands, known as distinctive ecotones between terrestrial and aquatic ecosystems, stand out as one of the planet's most productive environments (Hu et al., 2017). Among these, the Pantanal reigns supreme, encompassing not only the world's largest tropical wetland area but also the grandeur of the world's largest flooded grasslands (Alho and Sabino, 2011). It is located in the center of the Upper Paraguay River basin (hereafter BAP) in South America, with a drainage area of approximately 600,000 km², encompassing Brazil, Bolivia, and Paraguay (Jardim et al., 2020). The Pantanal harbors a high biodiversity of species and landscapes, providing a wide range of ecosystem services and being vital for local populations (Alho and Sabino, 2011; Tomas et al., 2019). Unfortunately, only 10% of the BAP portion is formally protected as conservation units. The majority of the basin's territory is on private lands, where 16.2% of the soil is used for agriculture and livestock, with 99% used for grazing and 1% for agriculture (MapBiomas, 2021).

Recent research has shown that the intensification of human activities and the consequent increase in the use of fertilizers and pesticides can lead to negative impacts on the wetlands and surrounding areas of the plateau, affecting ecosystem functions and the health of the wildlife in the Pantanal (Alho and Sabino, 2011; Schulz et al., 2019; Roque et al., 2021; Viana et al., 2022). Furthermore, studies conducted in the BAP have revealed significant levels of heavy metal contamination across diverse faunal groups, including birds (Marchesi et al., 2015), caimans (Vieira et al., 2011; Quintela et al., 2020), fishes (Riveros et al., 2021; Viana et al., 2022), and mammals (May Júnior et al., 2018; Soresini et al., 2021).

Studies worldwide indicate that current contamination levels in floodplains lead to bioaccumulation in food webs and induce toxicological effects on wildlife (Kooistra et al., 2001; Leuven et al., 2003). Moreover, researchers point out that contaminants represent a silent driver of negative impacts on biodiversity, as they are difficult to measure and still poorly investigated (Sigmund et al., 2023), emphasizing the need to understand the impacts on wild animal populations and integrate bioaccumulation estimates into risk analyses involving wildlife (Fritsch et al., 2022). This understanding relies on determining exposure and dispersal pathways in the environment - soils, sediments, and water - which, in turn, necessitate field data to accurately estimate wildlife exposure (Ahmed et al., 2023; Soliman et al., 2022). In addition, evaluating the amounts of heavy metals in soil and wildlife can reveal information about how elements move through the environment, accumulate, and their potential toxicological impact (García-Sevillano et al., 2014).

After exposure estimation, another challenge is the quantification of contaminants in mammalian tissues. The liver and kidney are main targets for metal quantification as they act as the main detoxification organs in vertebrates and are therefore considered indicators for long-term metal pollution studies (Williams and Iatropoulos, 2002; Dai et al., 2013). The quantification in wild vertebrates requires field data collection of specimens that are increasingly challenging because of growing ethical concerns. Although they are justifiable, they also hamper baseline estimates needed for the development of technical norms to protect wildlife. The development of wildlife indicators is especially needed in the Neotropics where the faunal assemblage is phylogenetically distinct from Neartic or Palearctic counterparts where most model organisms were taken from.

Among Neotropical mammals, small mammals, sigmodontine and caviomorph rodents and didelphid marsupials are potential models for assessing wildlife exposure in contaminated environments because they occupy key positions in food webs (Kaufman et al., 1998). Usually, rodents accumulate high concentrations of metals in their tissues (Damek-Poprawa and Sawicka-Kapusta, 2003; Sumbera, 2003) and have been used as bioindicator species for years (Damek-Poprawa and Sawicka-Kapusta, 2003; Ma et al., 1991; Damek-Poprawa and Sawicka-Kapusta, 2003; Sánchez-Chardi et al., 2007; Beernaert et al., 2007; da Costa et al., 2023). They can also be sampled efficiently and relatively inexpensively using rapid methods (Graipel et al., 2003).

Additionally, our study area, a rice paddy, cattle ranching and ecotourism farm, located in the southern Pantanal, because of its relatively intact status as compared with very degraded biomes such as the Atlantic Forest or the Caatinga, still shows a high density of medium sized mammals, particularly carnivores such as the jaguar (*Panthera onca*), ocelot (*Leopardus pardalis*), crab-eating fox (*Cerdocyon thous*), maned wolf (*Chrysocyon brachyurus*), and raccoon *(Procyon cancrivorus*). The presence of these animals has boosted ecotourism, which is the farm's main income, along with cattle breeding and irrigated rice cultivation. Furthermore, (Concone, 2004)) and (Teribele, 2007) observed that the frequency of sightings of medium and large mammals in rice paddies was higher than in areas of natural vegetation, such as the legal reserve within the farm, which is protected by the Forest Code (Law No. 12,651/2012 - (Brazil, 2012). Although rice is considered one of the three most important crops for food security worldwide (Yuan et al., 2021), its production involves the use of fertilizers and agrochemicals (Satpathy et al., 2014). This agricultural model raises many questions about the exposure of mammals to heavy metals because of farming activities.

Therefore, the main objective of this study was to assess the extent of metal contamination in the tissues (liver and kidney) of small mammals and to evaluate potential exposure pathways through the quantification of contaminants in the soil of a rice-Pantanal agroecosystem in the Southern Pantanal, Brazil. More specifically, our aims were: i) to determine the concentrations of metals (Al, Cd, Cr, Cu, Fe, Pb and Zn) in soil samples from two habitats (legal reserve and rice paddy); ii) to quantify the concentrations of these metals in liver and kidney tissue samples from small mammals in both habitats; iii) to compare the concentrations in the soil and critical tissues of small mammals between habitats to assess the risk of exposure to small mammals in our study area.

2. Material and methods

2.1. Study area

The Brazilian Pantanal covers approximately 140,000 km² of low-lying floodplains in the basin of the upper Paraguay River and its tributaries. The study area (Fazenda San Francisco; municipality of Miranda, state of Mato Grosso do Sul, central Brazil) is located at the south-eastern border of the Pantanal and comprise Miranda River sub-basin (44.740 km2), which is one of the contributing rivers to the Upper Paraguay river basin (Harris et al., 2005). A tropical and wet climate zone characterizes the Pantanal, with an average air temperature of 24ºC and an average annual rainfall of 1,000–1,250 mm. The region has a dry season from April to September and a wet season from October to March (Marengo et al., 2016).

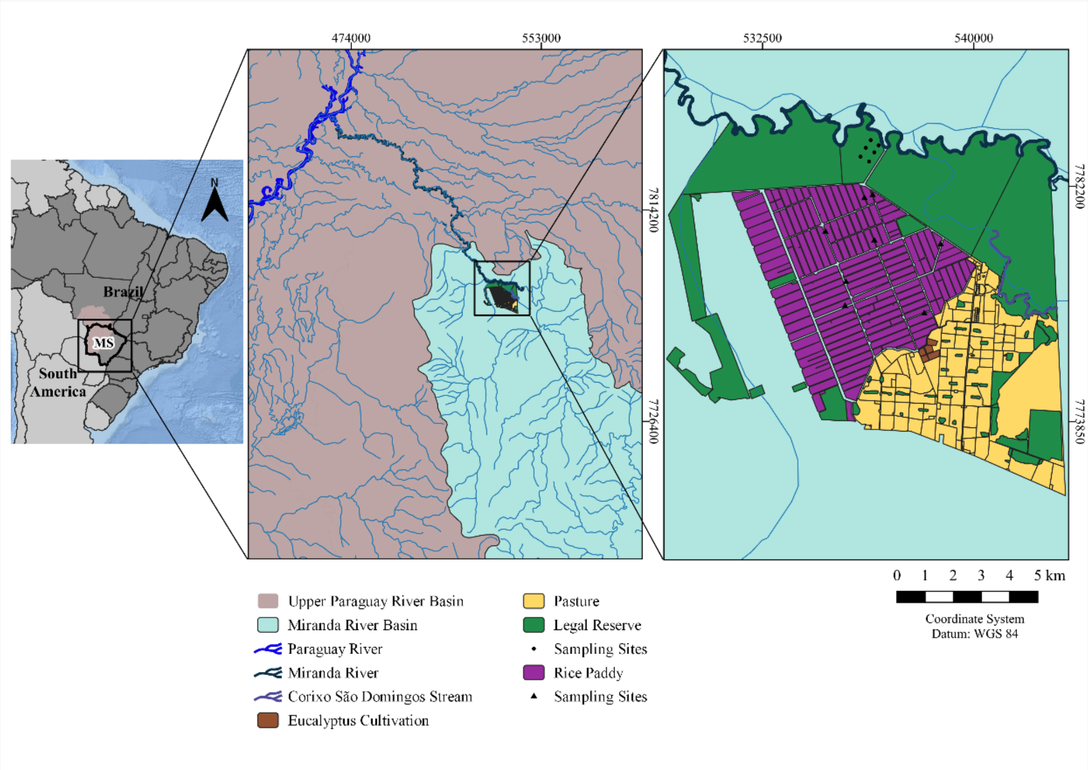


Fig.1. Map of the study area and distribution of small mammal sampling sites, including points for sampling soil (8 in the rice paddy and 6 in the legal reserve), within the rice-Pantanal agroecosystem, Mato Grosso do Sul, Brazil.

2.2. Soil sampling

All soil samples were collected at the end of the field campaign in November 2021. A total of 14 soil sample locations were selected **(Table S1; Fig. 1.)**. Samples were collected from each site at a depth of 0–10 cm from the surface, covering an area of 1 x 1 m, and subsequently air-dried. Subsequently, all samples were transported to the Laboratory of Sanitation in the Department of Hydraulic and Sanitation, University of São Paulo, São Carlos and stored at - 20º C until analysis. To evaluate if the soil samples met the quality standards established for Brazilian soils, we referred to CONAMA Resolution No. 420 (Brasil, 2009). Currently, the Mato Grosso do Sul state does not have its own soil quality standards.

2.3. Sampling of small mammals and tissue

Small mammals were sampled during the rice-growing cycle, which comprised a part of the dry (July–September 2019) and rainy (October–December 2019; October–November 2021).

Sampling was performed using live traps (Sherman® - 25x8x9cm and Tomahawk® - 18x18x39 cm). The traps were placed in linear transects at two sampling sites: rice paddy and Pantanal [legal reserve] **(Fig. 1.)**. In the legal reserve, we set up two parallel transects, each approximately 1,000 meters long. The traps were arranged alternately every 15 meters and, at every 75 meters (5 sampling points) we added sub-canopy traps (1.5 meters above the ground), to enhance the capture success of arboreal species. In the rice field, we sampled 10 points using two linear transects, each equipped with 15 traps, totaling 30 traps at each point. One transect was positioned inside the agricultural field. Another one was placed in the edge of the field, outside the crop. All traps were baited with a mixture of banana, peanut candy, oats, sardines, and vanilla essence.

Animals were identified, sexed, aged (using dental formula for marsupials and pelage color, size, and reproductive stage for rodents), weighed, and had their body length measured. After that, the individuals were anesthetized using a combination of Ketamine and Acepran (9/1) for rodents and Ketamine and Xylazine (1/1) for marsupials. The administered dose for both anesthetic combinations was 0.1 ml/100g. They were euthanized through prior intramuscular anesthesia using ketamine hydrochloride (10 mg/kg) + xylazine hydrochloride (2 mg/kg), followed by deep intravenous anesthesia with sodium thiopental (40 mg/kg). This was followed by the intravenous/intracardiac application of mebezonium iodide + Embutramide + Tetracaine hydrochloride (T61®) (0.3 ml/kg) or intracardiac supplementation with 19.1% potassium chloride (1 ml/kg). Dissections of animals were conducted with stainless steel instruments, and liver and kidney tissues were removed. All samples placed in cryotubes and stored at -20º C until chemical analysis.

Adult specimens of each gender were deposited in the mammal collection at the Federal University of Paraiba. Taxonomic nomenclature follows the Taxonomy Committee of the Brazilian Society of Mammalogy (Abreu et al., 2021). Our research adhered to the guidelines of the American Society of Mammalogists (Sikes, 2016) and was authorized under the Biodiversity Authorization and Information System [SISBIO License No. 72681], as well as the animal-use protocol - Animal Ethics Committee UFPB/CEUA, with the reference number 9192091019.

2.4. Analytical procedures

For analytical procedures, deionized water was used. All acids were purchased from Merck® (analytical grade). All glass materials were cleaned with concentrated nitric acid as described before (Tschopel et al. 1980). The samples for the metals determination were oven-dried at 50°C on glass dishes for 48 hours, homogenized using a pestle and mortar and, each of the weighed samples (about 1.0 g) was taken to a 100 mL beaker to which 10.0 mL of HNO_3 was added and digested near dryness at 80°C on a hot plate; 1.0 ml of

H_2O_2 was added to aid in the total digestion of organic matter. For the biological matrices (liver and kidney), the samples remained in the oven for 72 hours and the same procedure was performed until complete digestion of the sample.

The digested samples were cooled at room temperature, filtered by using filter papers and collected in a 100 ml beaker. The filter papers were washed with ca. 20 mL of water and the contents of the beaker were transferred to 100 ml volumetric flasks for soil and 50 ml volumetric flasks for biological matrices. Blanks and standards were run with each batch of samples. The detection of metals followed the SM 3111B Method and was made using an atomic absorption spectrophotometer (VarianTM, model 240 FS) (APHA, 1999). The detection limits were Al - 0.001 mg L^{-1}, Cr - 0.003 mg L^{-1}, Cd - 0.0006 mg L^{-1}, Pb - 0.001 mg L^{-1}, Cu - 0.003 mg L^{-1}, Fe - 0.005 and Zn - 0.002 mg L^{-1}.

2.5. Statistical analysis

The Shapiro-Wilk test was used to assess the normality of the data, as well Levene test was employed to test for the homogeneity of variance. We employed the *t-test* (α - 0.05) to compare the average concentration of metals in the soil and tissues (liver and kidney) of small mammals between the samples from the legal reserve and the rice paddy. We used the non-parametric Wilcoxon-Mann-Whitney when the data did not meet the assumptions of normality. We employed the analysis of Covariance (ANCOVA) to compare the body condition (BCI) between the habitats (legal reserve and rice paddy). Additionally, we used an ANCOVA design to compare metal concentrations in critical tissues between the legal reserve and the rice paddy with the heavy metal concentrations in soil samples as covariate. Sexes and age of mammals were not used as variables because of the lack of sample size in these subgroups.

Since our objectives were to understand and track the exposure of small mammals in the rice-Pantanal agroecosystem, we chose to analyze our results separately for each taxonomic order, because Didelphimorphia marsupials and Rodentia have different diets (da Costa et al., 2023), and deep phylogenetic divergence (166-123 mya) (Álvarez-Carretero et al., 2022). Comparison of the same species between legal-reserve and rice paddy was not possible because of the strong habitat filtering of the agroecosystem. As a consequence the dissimilarity between the small mammals assemblages between these two habitats is 95% (Gomes de Sá et al. 2024) hampering species specific value comparison. Didelphimorphia tissues were not compared between the sampling sites because they were abundant in the legal reserve and rare in the rice paddy where trees were suppressed, and most species are arboreal. Therefore, the comparison between sites was restricted to rodents, which had a tissue sample size greater than 5 at both sampling sites. Furthermore, in the legal reserve, all the rodents belong to the Sigmodontinae family (tribe Oryzomyini) and are similar both functionally and phylogenetically at the scale of our comparisons. Consequently, they share similar physiological traits, which simplifies discussions regarding exposure pathways. The body condition residual index (BCI) was estimated as a regression of body length and body weight (e.g. Peig and Green, 2010). Individuals with positive BCI values were considered to have higher body condition, while negative values were considered to have lower body condition. At the same time, our sample size allowed us to discuss the metals concentration and the BCI of the didelphid marsupial *Gracilinanus agilis* in the legal reserve. The final concentrations were expressed as the mean ± standard deviation (SD).

3. Results

3.1. Metal concentration in soil samples

All essential and non-essential metals were detected in soil samples. Three metals were not detected in all samples from the rice paddy site. Samples showed concentrations below the detection limit, Cr (40% of samples detected), Cd (50%) and Pb (40%) **(Table 1).**

Table 1. Concentrations of essential (Cu, Cr, Fe, and Zn) and non-essential metals (Al, Cd, Pb) (mg/kg) in the soil for the Legal Forest reserve and Rice paddy. in the South Pantanal agroecosystem, Mato Grosso do Sul state, Brazil.

Site	Metal	Mean ± SD	Range (min-max)	(%) samples detected	Soil Quality Standards (Brazil, 2009)*
Legal reserve (N=6)	Cu	28.78 ± 5.05	23.63-37.19	100%	60
	Cr	1.59 ± 0.52	0.73-2.32	100%	75
	Fe	9,121.46 ± 2,244	5,786.67-12,346.32	100%	-
	Zn	17.98 ± 3.45	12.72-21.32	100%	300
	Al	4,881.48 ± 1,034.69	3,513.37-5,861.62	100%	-
	Cd	0.33 ± 0.20	0.05-0.59	100%	1.3
	Pb	1.88 ± 1.38	0.95-3.97	100%	72
Rice paddy (N=10)	Cu	8.50 ± 2.42	4.18-11.67	100%	60
	Cr	2.68 ± 2.96	0.14-6.01	40%	75
	Fe	5,592.89 ± 3,337.43	2,529.80-13,543.43	100%	-
	Zn	9.10 ± 5.72	4.69-24.37	100%	300
	Al	2,083 ± 356.16	1,584.53-2,651.75	100%	-
	Cd	0.20 ± 0.11	0.10-0.33	50%	1.3

Pb	3.10 ± 2.21	0.96-6.20	40%	72

*CONAMA Resolution 420 (Brazil, 2009) soil quality standards

Significant differences in metal concentration between the reserve and rice-paddy were found for essential Copper (Cu, p<0.01), Iron (Fe, p=0.015) and Zinc (Zn, p<0.01), as well as for non-essential metals Aluminum (Al, p<0.01) and Cadmium (Cd, p= 0.017) **(Table 1; Fig.2.)**. Concentrations were significantly higher in the legal reserve than in the rice paddy. Chromium and lead concentration were higher in the rice paddy than in the legal reserve although not statistically significant (Cr, p=0.069; Pb=0.257).

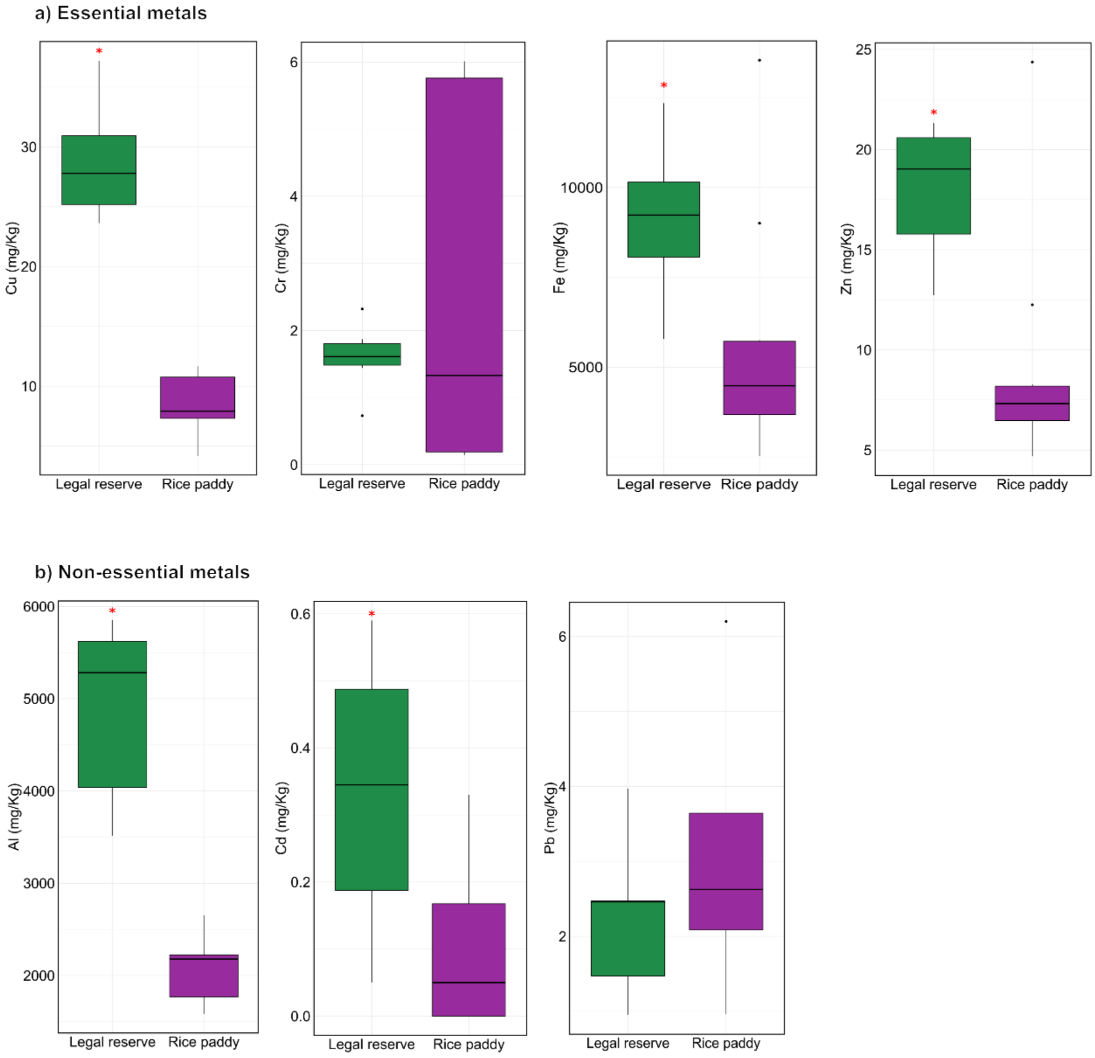


Fig. 2. Concentrations of metals in soil samples from rice-Pantanal agroecosystem, Brazil: forest legal reserve (green) and rice paddy (purple). (a) Essential metals - Cooper (Cu), Chrome (Cr), Iron (Fe) and Zinc (Zn); b) Non-essential metals - Aluminum (Al), Cadmium (Cd) and Lead (Pb) (mg/kg). *= Indicates a significant difference in Cu (p<0.01), Fe (p=0.015), Zn (p<0.01), Al (p<0.01) and Cd (p=0.017), between sites. Outliers are represented by individual data points beyond the box plot whiskers.

3.2. Small mammals and exposure to metals

We sampled 42 individuals of 8 small mammal species in the rice-Pantanal agroecosystem for the purpose of heavy metal quantification. Additional information of the faunal assemblage can be found in Gomes de Sá et al. (2024). Within the rice paddy, 20 individuals were captured, one marsupial (*Philander opossum*) and three rodent species (*Cavia aperea*, *Holochilus chacarius* and *Necromys lasiurus*). In the legal reserve, we sampled 22 individuals, one marsupial (*Gracilinanus agilis*) and three rodent species (*Cerradomys maracajuensis*, *Euryoryzomys nitidus* and *Oecomys mamorae*)**.** We did not detect Chromium in any of the tissue sampled. The metal concentration for each tissue and site are shown in **Table 2**.

Overall metal concentrations in G. agilis in the legal reserve and body condition index (BCI)

For *G. agilis* (N=8), in the legal reserve, aluminum was not detected in any tissue sample. The metal concentration pattern was Fe>Zn>Pb>Cd>Cu in both tissues, except for Cu which was not detected in the kidney samples **(Table S2.)**. The marsupial *G. agilis* showed an average body weight (BW) of 33.8±9.16 (mean ± standard deviation) and an average body length (BL) of 102 mm ± 4.56. Also, *G. agilis* exhibited both positive and negative values of the body condition residual index (BCI). Most individuals (n=5) had negative body condition values. However, we did not find a significant relationship between BCI and the concentration of non-essential metals - Cd (p=0.337) and Pb (p=0.424).

Table 2. Metal concentrations (mg/kg) in critical tissues of marsupials (Didelphimorphia) and rodents (Rodentia) for the two sampling sites (Legal reserve and Rice paddy) in the rice-pantanal agroecosystem, Mato Grosso do Sul, Brazil. Values are represented as x ± SD, where x = average and SD = standard deviation, N = number of animals, ND = undetected values of concentration of heavy metals.

Didelphimorphia

		Metal	**Mean ± SD**	**Range (min-max)**	**samples detected (%)**
Legal reserve	Liver (N= 8)	Cu	19.90	19.90	12.5%
		Cr	ND	-	-
		Fe	1,051.67 ± 444.09	464.5-1,944.56	100%
		Zn	171.09 ± 41.48	94.7-220.5	100%
		Al	ND	-	-
		Cd	18.35 ± 4.69	3.5-27	75%
		Pb	42.03 ± 7.80	34.4-50	37.50%
	Kidney (N=8)	Cu	ND	-	-
		Cr	ND	-	-
		Fe	759.30 ± 862.33	860-2,747.7	50%
		Zn	294.21 ± 152.19	115.3-507.7	100%
		Al	ND	-	-
		Cd	118.12 ± 28.67	86.3-152.5	75%
		Pb	142.38 ± 104.94	19.53-275	50%
Rice paddy	Liver (N=2)	Cu	75.5 ± 83.01	16.8-134.2	100%
		Cr	ND	-	-
		Fe	528.9 ± 158.95	416.5-641.3	100%
		Zn	198.1 ± 101.11	126.6-269.6	100%
		Al	92.40	92.4	50%
		Cd	0.5	0.5	50%
		Pb	27.2	27.2	50%
	Kidney (N=2)	Cu	41.05 ± 14.21	31-51.1	100%
		Cr	ND	-	-
		Fe	321 ± 55.86	281.5-360.5	100%
		Zn	145.45 ± 3.88	142.7-148.2	100%
		Al	89.50	89.5	50%
		Cd	ND	-	-

		Pb	5.30	5.30	50%
Rodentia					
		Metal	**Mean ± SD**		
Legal reserve	Liver (N=14)	Cu	14.01 ± 1.86	11.42-15.52	28.57%
		Cr	ND	-	-
		Fe	609.31 ± 293.88	195.07-1,424.44	100%
		Zn	252.60 ±168.29	108.3-578.8	100%
		Al	300.94 ± 109.47	116.1-391	28.57%
		Cd	15.05 ± 11.71	4.9-42.4	100%
		Pb	49.6 ± 19.39	26.8 - 76.9	42.86%
	Kidney (N=14)	Cu	5.50	5.5	7.14%
		Cr	ND	-	-
		Fe	329.17 ± 174,51	3.4-569.1	100%
		Zn	283.57 ± 213.35	70.8-746.94	100%
		Al	605.86 ± 298.10	289.5-1,092.1	35.7%
		Cd	56.86 ± 49.37	3.4-146.94	100%
		Pb	74.09 ± 57.71	4.9-171.1	78.57%
Rice paddy	Liver (N= 18)	Cu	38.91 ± 35.36	5.77-125.1	72.2%
		Cr	ND	-	-
		Fe	1,005.25 ± 723.82	87.1-2,788.46	100%
		Zn	279.15 ± 137.83	47.1-624.71	100%
		Al	4.20	4.2	5.56%
		Cd	10.98 ± 10.14	1.3-39.82	100%
		Pb	18.9	18.9	5.56%
	Kidney (N= 18)	Cu	100.22 ± 164.57	2.4-345.7	22.2%
		Cr	ND	-	-
		Fe	851.31 ± 1,116.89	42.86-4,361.41	100%
		Zn	407.69 ± 402.44	37.2-1,680.17	100%
		Al	18.30	18.3	5.56%
		Cd	58.56 ± 48.34	5.3-150	100%
		Pb	59.20 ± 53.84	1.96 -157.66	33.3%

Metal concentrations in rodents between sites and body condition index (BCI)

Since rodents were captured at both sampling sites, with 18 individuals in the rice paddy and 14 in the legal reserve, our results were comparable between the sampling sites. All rodent species exhibited internal concentrations of both essential and non-essential metals **(Tab. S2).** Overall, the accumulation pattern of sigmodontine rodents in the legal reserve (*C. maracajuensis*, *E. nitidus*, and *O. mamorae*) was Fe>Al>Zn>Pb>Cd>Cu, except for *E. nitidus*, which lacked aluminum in their tissues. In contrast, the accumulation pattern for the rice paddy rodents (*C. aperea*, *N. lasiurus* and *H. chacarius*) was slightly different: Fe>Zn>Pb>Cd>Cu>Al. Once again, aluminum was not detected in *H. chacarius*.

The rodents differed in body weight and body length between the two sites. Within the legal reserve, rodents had an average body weight (BW) of 59.2±12.7 (mean ± standard deviation) and an average body length (BL) of 121.7 mm ±7.60. In contrast, rodents from the rice paddy displayed a higher body weight of 131.2±95.3 and body length of 175.2 mm ±69.7. Indeed, it was expected that the rodents from the rice paddy would show a greater variation in body length and weights, as they belong to distinct groups: *C. aperea* (Caviomorph) and, *H. chacarius* and *N. lasiurus* (sigmodontines). Additionally, the body condition residual index (BCI) showed positive and negative values in both sites. Positive values of BCI measuring 0.072±0.080 in the legal reserve and 0.201±0.126 in the rice paddy. The high body conditions prevailed in the legal reserve (71.4%), while in the rice paddy, less than half of the individuals (44.4%) exhibited high body condition index (BCI). However, the habitats (legal reserve and rice paddy) do not significantly affect the BCI of rodents ($p=0.757$).

Rodents from both legal reserve and rice paddy sites showed concentrations of all the metals evaluated (essential and non-essential metals) in the liver and kidney tissues. The rice paddy exhibited the highest concentrations of copper (Cu), zinc (Zn) and iron (Fe) in both liver and kidney tissues. The differences in liver tissue concentrations of metals between the habitats were statistically significant for copper ($p<0.01$) and iron ($p<0.05$), but not significant for zinc ($p=0.357$) **(Fig. 3a.)**. In the kidney, the concentrations were not significantly different between sites - Cu ($p=0.241$), Zn ($p=0.357$) and Fe ($p=0.135$) **(Fig. 3b.)**.

The legal reserve exhibited the highest concentrations of lead (Pb) and aluminum (Al) in both tissues. In addition, we emphasize that lead was only detected in one liver sample in the rice paddy (18.9 mg/kg), as well as aluminum (4.20 mg/Kg) **(Fig. 3a.)**. The concentrations in the liver from the legal reserve were as follows: Pb (n=6) - 49.6 ± 19.39 mg/kg and Al (n=4) - 300.94 ± 109.47 mg/kg. In the kidney, the concentrations were as follows: Pb (n=11) - 74.09 ± 57.71 mg/kg and Al (n=5) - 605.86 ± 298.10 mg/kg. The concentration values found in both tissue samples from the legal reserve were statistically significant for lead: liver ($p < 0.01$) and kidney ($p < 0.01$), and for aluminum: liver ($p < 0.05$) and kidney ($p < 0.05$). Lastly, cadmium exhibited higher mean concentrations in the liver of species from the legal reserve (n=14) - 15.05 ± 11.71 mg/kg. On the other hand, the highest concentrations in the kidney were found in the rice paddy (n=18) - 58.56 ± 48.34 mg/kg **(Fig. 3b.).** There were no statistically significant differences between the sites for the Cd in tissues: liver (p=0.189) and kidney (p=0.866).

Finally, there were no significant differences in the overall concentrations of tissues of essential metals (Cu, p=0.390; Fe, p=0.194 and Zn, p=0.192) concerning the BCI for rodents. Likewise, we did not find significant differences for non-essential metals (Al, p=0.442; Cd, p=0.096 and Pb, p=0.621). In addition, for the metals that showed significant differences in any of the tissue concentrations between sites (Aluminum, Copper, Iron and Lead), there were no significant differences in the concentrations due to the interaction between BCI and the habitats (legal reserve and rice paddy), namely Al (p=0.175), Cu (p=0.901), Fe (p=0.73) and Pb (p=0.348).

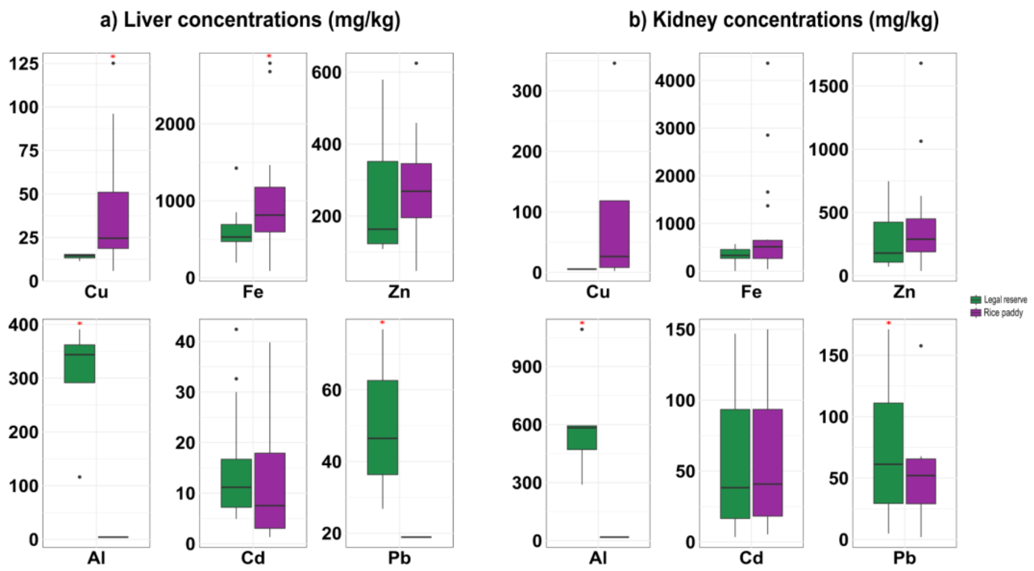


Fig. 3. Concentration of metals in critical tissues of rodents from the legal reserve (green) and rice paddy (purple) in the rice-Pantanal agroecosystem, Mato Grosso do Sul, Brazil. *= indicates a significant difference in essential metals, outliers are presented by individual data points in the boxplots. **a)** Concentrations in the liver tissues: Cu, $p<0.01$; Fe, p=0.03; Al, p=0.02; Pb, $p<0.01$, Wilcoxon test. The

number of outliers in each group: legal reserve – Fe= 1, Al= 1 and Cd= 2; rice paddy – Cu= 1, Fe= 2, Zn= 1. **b)** Al, p=0.02; Pb, p= 0.01, Wilcoxon test). Outliers are presented by individual data points in the boxplots. The number of outliers in each group: legal reserve – Al= 1; rice paddy – Cu= 1, Fe= 4, Zn= 2 and Pb= 1.

Relationship between bioaccumulation in rodent tissues and soil data for non-essential metals

The analysis of covariance (ANCOVA) showed that the type of habitat (legal reserve or rice paddy) has a significant effect on the concentrations of aluminum in the liver ($p = 0.01$) and kidney ($p = 0.014$) while controlling for soil concentration as a possible exposure source. However, the concentration of aluminum in soil has not been shown to have a significant effect on the tissues - liver ($p = 0.962$) and kidney ($p = 0.516$). The interaction term between habitat and the concentration of Al in the soil was not significant for concentrations in the liver ($p = 0.981$) and kidney ($p = 0.783$). These results suggest that factors other than the aluminum concentration in the soil likely influence the aluminum concentration in rodent tissues. Likewise, the ANCOVA revealed a significant difference in lead concentrations between habitats for the liver ($p < 0.01$) and the kidney ($p = 0.041$), while controlling for the lead concentrations in the soil. However, the concentration of lead in soil has not been shown to have a significant effect on the tissues - liver ($p = 0.425$) and kidney ($p = 0.845$). In addition, the interaction between lead concentration in the soil and habitat did not have a significant effect on lead concentrations in the liver ($p = 0.56413$) and kidney ($p = 0.272$). Our results suggest that the relationship between the concentration of aluminum and lead in the soil and in rodent tissues may be more complex than soil mediated exposure. Finally, we decided not to carry out additional tests for cadmium based on the absence of a significant difference in the Wilcoxon test between the groups (legal reserve and rice paddy) for concentrations in the tissues and soil.

4. Discussion

In Brazil, studies of metal levels in small mammals are very scarce (e.g. (Machado et al., 2020; da Costa et al., 2023). Our study is the first to evaluate exposure to essential and non-essential metals in critical tissues of small mammals and soil data from the world's largest neotropical wetland, the Pantanal, thus providing essential data to assess metal exposure in non-flying Neotropical small mammals. The presence of all the metals assessed in the soil (Cu, Cr, Fe, Zn, Al, Cd and Pb) at both sampling sites (legal reserve and rice paddy) is a strong indicator of contamination in the study area. Ferreira et al. (2019) also reported contamination by Cr, Fe, Zn, Al, Cd, and Pb in sediment samples from the Miranda River, which constitutes our study area in the Pantanal Sul-Mato-Grossense. This finding is consistent with our results regarding soil contamination in the southern Pantanal region. Moreover, our initial hypothesis was that the concentrations of metals in the soil and in the rodents' tissues would be higher in the rice paddy due to the use of fertilizers and pesticides in crops. Yet, our results revealed that concentrations of non-essential metals in rodent tissues (Pb and Al) and soil for both essential and non-essential metals (Cu, Fe, Zn, Al, Cd) from the legal reserve were significantly higher than concentrations of these metals in the rice paddy. The highest concentration of these metals in the legal reserve deserves further discussion due to the complexity of the dynamics of metal contamination in the Pantanal.

The impacts of pesticides and erosion are spreading as agriculture and livestock farming in the highlands of the Upper Paraguay Basin (UPRB) increase, endangering ecosystem functions as well as the health of humans and animals (Hunt et al., 2016; Albuquerque et al., 2016). Indeed, since large-scale soybean and corn production intensified in the UPRB in 2015, the use of pesticides has increased (Roque et al., 2021). In the highland drainage areas surrounding the Pantanal, which correspond to the Upper Paraguay River Basin (UPRB), pesticides like cypermethrin, endosulfan, 2,4-D, atrazine, L-cyhalothrin, permethrin, and glyphosate are currently widely applied (Pignati et al., 2017). As well the Miranda River Basin (hereafter MRB) is located in a region of intense agricultural activity, especially monocultures such as irrigated rice, cotton, sugarcane, corn and soybeans (de Carvalho Dores, 2016; de Magalhães Neto and Evangelista, 2022). These activities result in an increase in the production of pollutant loads of diffuse origin along the MRB, since heavy metals can be transported from one environment compartment to another (Moreschi et al., 2015). Our results do not rule out our initial hypothesis but suggest that rice paddies may not be the only or main source of contamination in the study area. Furthermore, high concentrations of Fe and Al can be related to the geochemical composition of the Pantanal soil (Schiavo et al., 2012). We also highlight that since the 1980s, the high concentration of Fe has been associated with ore reserves in the Pantanal region, which raises several concerns about metal exposure and risk to the maintenance of local biodiversity (Del Lama et al., 2011; Riveros et al., 2021; Viana et al., 2022). Moreover, our unprecedented data showed diffuse iron contamination in rodent tissue but with higher levels of Fe in the rice paddy. Overall, iron exhibited the highest concentrations in the liver of rodents from the rice paddy (1,005.25 ± 723.82 mg/kg), highlighting the high levels of Fe and consequent exposure to fauna in the Pantanal biome and contribution of the agricultural activities in local scale farm for that. These findings are very important and should be evaluated with caution, as prolonged exposure to Fe can pose serious risks to the health of animals since mammals do not possess any physiological pathway for iron excretion (Papanikolaou and Pantopoulos, 2005).

Furthermore, higher concentrations of Cu were detected in the liver of rodents inhabiting the rice paddy. In addition, although we found no significant differences for Zn between the habitats, the rodents from the rice paddy tended to exhibit slightly higher concentrations in their tissues than the rodents from the legal reserve. The intensive application of agrochemicals and fertilizers in rice paddies emerges as a plausible primary source of contamination for rodents in this habitat. Studies have demonstrated that rice paddies can accumulate elevated levels of Zn (Satpathy et al., 2014), along with Cu, given that copper is commonly utilized in fertilizers as a micronutrient (Xiaorong et al., 2007). Nevertheless, Cu and Zn can be found in significant amounts in the liver for a variety of reasons, including their involvement in the body's detoxification processes (Okati and Rezaee, 2013). The average values found for zinc in the liver indicate critical exposure values, as they exceeded the threshold levels of 274 mg/kg for the kidney and 465 mg/kg for the liver in mammals, indicating that zinc can become toxic, leading to various detrimental effects on the organism (Eisler, 1993).

On the other hand, concentrations of non-essential metals Al and Pb found in the rodent tissues from the legal reserve, suggests that animals in the rice-Pantanal agroecosystem may be exposed to different sources between habitats. Small mammals tend to have a low immigration rate and are limited to a small area or home range, which can give an accurate picture of local contamination (Ostfeld, 1990; Prevedello et al., 2008; Al Sayegh Petkovšek et al., 2014). Furthermore, the sigmodontinae rodents in the legal reserve seem to be exclusive to this habitat, as they do not enter the rice paddy due to the environmental filter coming from the agricultural area, as highlighted by (Gomes de Sá et al., 2024), reinforcing our results regarding the diffuse sources of pollution for rodents in the legal reserve and for rodents in the rice paddy. At the same time, the body condition of small mammals does not seem to have been affected by either the habitat or the accumulation of metals, as no significant differences were observed in any of these variables.

The concentration of non-essential metals (Al and Pb) in the tissues was shown to be influenced by the habitat in which the rodents live, showing highest concentrations in the legal reserve, but the concentration in the soil was not related to this influence. Additionally, we found no statistical differences in tissue cadmium concentrations between the habitats, with rodents from both the legal reserve and the rice paddy exhibiting high concentrations of Cd in their tissues. Factors unrelated to direct soil exposure are likely influencing the concentration of non-essential metals in the rodents' tissues. In mammals, the main route of exposure to contaminants is through diet (Ma et al., 1991) and it is expected that non-essential metals are transferred through the food chain more than essential metals (Pascoe et al., 1996; Rogival et al., 2006). Neotropical sigmodontine rodents have a frugivorous/omnivorous diet, consisting mainly of plants, invertebrates, fruits, as well as parts of plants such as seeds and flowers (Prado and Chiquito, 2019). Plants growing in contaminated soils can accumulate various non-essential metals, and water can also serve as an exposure route for plants (Wijnhoven et al., 2007). Aluminum can accumulate in plants at levels of up to 200 mg/kg through water (Kabata-Pendias, 2010). Through their diet, aluminum can accumulate in the tissues of vertebrates, including the central nervous system, where it acts as a neurotoxin (Walton, 2007). Although detected in less than half of the rodents in the legal reserve, the concentrations of aluminum in the liver (300.94 ± 109.47 mg/kg) and kidney (605.86 ± 298.10 mg/kg) can cause severe chronic effects on the population viability of small mammals, since aluminum concentrations (200 mg/l) are correlated with lower testosterone levels, affecting the testes and epididymal weights of adult rodents (Abu-Taweel et al., 2011; Miska-Schramm et al., 2017).

Moreover, exposure to Pb and Cd can have serious and harmful toxic effects on both animal physiology and human health, even at low concentrations (Damek-Poprawa and Sawicka-Kapusta, 2003). These effects include impacts on excretory, nervous, and hematopoietic functions, bone fractures, and negative effects on the reproductive system of mammals. Additionally, both Pb and Cd are potentially carcinogenic (Sánchez-Chardi and Nadal, 2007; Sánchez-Chardi et al., 2007; European Food Safe Authority, 2012). The bioaccumulation of Cd in all rodent tissues raises concerns about widespread contamination at community level. Furthermore, high levels of Pb and Cd were found in the tissues of the arboreal marsupial *Gracilinanus agilis* from the legal reserve. Interestingly, Al is not detected in any individuals of *G. agilis*, shown for this animal, levels of bioaccumulation tends to be different than rodents, probably due their insectivorous diet (Paglia et al., 2012; Veltman et al., 2007). Small insectivorous mammals tend to have higher metal concentrations because insects accumulate high amounts of metals in their bodies (Pascoe et al., 1996; Wijnhoven et al., 2007). This is particularly true for Pb, which is stored and immobilized in the insect's exoskeleton upon ingestion (Roberts and Johnson, 1978).

The concentrations of lead in rodent tissues were found to be high, particularly in the kidneys, exceeding the effect concentrations observed in kidneys of small mammals, which range from 25 to 1506 mg/kg dry weight (Ma, 1989); (Ma et al., 1991; Stansley and Roscoe, 1996). Additionally, the toxicological risks associated with lead concentration can be even higher, as Pb accumulates more in bone structures than in the liver and kidneys of small mammals (Stansley and Roscoe, 1996); (Torres and Johnson, 2001). At the same time, the concentrations in the kidneys of *G. agilis* exceed the critical concentration of 105 - 210 mg/kg dry weight observed for threshold doses that lead to subclinical symptoms in the kidneys of small mammals (Ma, 1987; Cooke, 2011). Although we have not assessed the individual health of small mammals. These findings indicate some animals may be very close to intoxication, particularly those in the legal reserve due to widespread contamination.

About exposure to heavy metals in the rice-Pantanal agroecosystem, the study area appears to have diffuse pollution with different sources of exposure for the soil, rodents, and the arboreal marsupial *G. agilis*. However, the presence of non-essential metals in small mammals may result from either temporary or permanent exposure. The presence of metals in both soil and animals represents a route of exposure for fauna, with the potential for perpetuation in the trophic chain. This poses a risk not only for the animals themselves but also for predators, as it is highly likely that the metals will magnify throughout the food webs.

In the future, it would be interesting to investigate the magnitude of the effects of metal contamination at the population level and how the flooding and dynamics of the

Miranda River impact on soil contamination and the consequent exposure of small mammals to contaminants. Interestingly, our concerns are raised mainly in the legal reserve, because of the environmental filter caused by the rice paddies, small mammals may be forced to live in conditions that are not ideal in terms of exposure in the areas of remaining vegetation setting an ecological trap for the species that live in these legally protected but contaminated riverine areas (Hale and Swearer 2016). Finally, we reiterate that the determination of soil quality values in Mato Grosso do Sul is a fundamental step towards clarifying sources of exposure and monitoring widespread contamination in the area encompassing the Upper Paraguay river basin, specifically in the Southern Pantanal.

5. Conclusions

Both the concentrations of metals in the soil and in the critical tissues of small mammals indicate potential contamination by non-essential metals (Al, Pb, and Cd) as well as essential metals reaching toxic levels in both habitats (legal reserve and rice paddy). We observed a trend towards higher metal concentrations in the legal reserve, highlighting the need for environmental monitoring policies to protect these legally protected areas of natural vegetation. This study represents the first assessment of metal contamination in the soil and critical tissues of small mammals in the Brazilian Pantanal, providing crucial data indicating the exposure to contaminants for the fauna. These findings can serve as a foundation for further research in Brazil and South America, where studies on this subject remain limited.

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Supplementary material

Between the hammer and the anvil: exposure to metals through rivers might be higher than through rice-Pantanal agroecosystems in soil and critical tissues of small mammals

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Table S1. Sampling sites of soil, showing the land use types and geographic coordinates.

Legend	Point	Land use	Coordinates	
			Lat	Long
S1	Legal reserve	Riparian vegetation	-20,0439	-56,6494
S2	Legal reserve	Riparian vegetation	-20,0463	-56,6506
S3	Legal reserve	Riparian vegetation	-20,0472	-56,6514
S4	Legal reserve	Riparian vegetation	-20,0440	-56,6498
S5	Legal reserve	Riparian vegetation	-20,0463	-56,6514
S6	Legal reserve	Riparian vegetation	-20,0470	-56,6529
S7	Barreiral Q-08	Rice	-20,0975	-56,6342
S8	Setor A Q-08	Rice	-20,0753	-56,6287
S9	Setor B Q-08	Rice	-20,0875	-56,6608
S10	Setor F Q-01	Rice	-20,0742	-56,6512
S11	Setor F Q-02	Rice	-20,0952	-56,6611
S12	Setor M Q-05	Rice	-20,0606	-56,6546
	Setor M Q-05	Rice (edge)	-20,0606	-56,6546
S13	Setor M Q-08	Rice	-20,0714	-56,6678
	Setor M Q-08	Rice (edge)	-20,0714	-56,6678
S14	Triangulo Q-07	Rice	-20,0596	-56,6518

Tab. S2. Number of individuals (N) and average metal concentrations (mg/kg) in the small mammal species from the two sampling sites (Legal reserve and Rice paddy) in the rice-pantanal agroecosystem, Mato Grosso do Sul, Brazil. ND = Not Detected values.

Site	**Species**	**N**	**Concentration of metals (mg/kg)**							**Tissues**
			Cu	**Cr**	**Fe**	**Zn**	**Al**	**Cd**	**Pb**	
Legal reserve	*Gracilinanus agilis*	8	19.9	ND	1,051.67±444.09	171.09±41.48	ND	18.35±4.69	42.03±7.8	Liver
			ND	ND	759.30±862.33	294.21152.19	ND	118.12±28.67	142.38±104.94	Kidney
	Cerradomys maracajuensis	4	11.42	ND	464.76±196.01	313.88±199.8	343.8	24.95±15.06	67.7	Liver
			ND	ND	428.51±132.09	285.92±155.96	470.6	58.85±40	68.46±81.02	Kidney
	Euryoryzomys nitidus	4	14.88±0.83	ND	952.27±321.61	366.76±170.11	ND	11.93±5.41	ND	Liver
			ND	ND	183.36±35.03	538.91±155.37	ND	114.36±35.03	53.9±10.35	Kidney
	Oecomys mamorae	6	ND	ND	477.05±87.36	135.65±49.59	290.22±123.34	10.53±9.61	45.98±19.28	Liver
			5.5	ND	360.15±79.15	111.78±41.17	639.67±332.96	17.21±10.37	83.65±61	Kidney
Rice paddy	*Philander opossum*	2	75.50±83	ND	528.9±158.95	198.10±101.11	92.4	0.5	27.2	Liver
			41.05±14.21	ND	321±55.86	145.45±3.88	89.5	ND	5.3	Kidney
	Cavia aperea	6	81.8±35.33	ND	572.4±282.46	250.45±119.12	4.2	2.66±1.33	ND	Liver
			119.42±195.9	ND	720.87±469.31	342.45±356.57	ND	16.64±5.28	1.96	Kidney

	Holochilus chacarius	6	20.83±10.84 42.64	ND ND	860.66±439.23 1,193.77±1615.01	308.65±189.65 498.33±612.61	ND ND	12.83±8.42 53.15±37.25	18.9 45	Liver Kidney
	Necromys lasiurus	6	19.07±8.32 ND	ND ND	1,582.69±926.60 639.28±1,094.40	278.34±111.37 382.28±180.24	ND 18.3	17.46±11.81 105.89±41.22	ND 77.07±56.98	Liver Kidney

CONCLUSÃO GERAL

Os resultados desta tese demostram que um terço dos mamíferos terrestres brasileiros (319) ocorrem em agroecossistemas e 205 no ambiente dentro do cultivo (in crop) no Brasil. Esse número deve aumentar devido a subamostragem nos biomas Caatinga e Pampa e em todos os tipos de cultivos exceto culturas perenes. Modelos de exposição utilizando uma única espécie são insuficientes uma vez que os espaços funcionais de mamíferos em diferentes tipos de agroecossistemas incluem três a dois grupos funcionais distintos. Baseado na probabilidade de ocorrência dos grupos funcionais nos diferentes tipos de agroecossistemas Espécies Genéricas Modelo podem ser utilizadas para sistemas de pastagem, plantações de árvores e cultivos anuais com as características funcionais terrestre, crepuscular, de tamanho médio a grande e dieta de vertebrados. Para agrofloresta e culturas perenes os traços encontrados sugerem Espécies Modelo Genéricas arborícolas e com dietas frugívora e nectarívora. Para diagnosticar efeitos em escala de paisagem, o estudo de multidiversidade (taxonômica, funcional e filogenética) no sistema modelo de avaliação arroz-Pantanal mostrou que as estimativas de diversidade baseadas em incidência indicaram um arroz mais diverso enquanto estimativa baseadas em abundância mostram uma reserva legal mais diversa. O estudo sugere que para avaliar impactos de agroecossistemas em comunidades de pequenos mamíferos a estimativa das abundâncias é essencial. Os contaminantes de metais dosados em tecidos críticos (fígado e rim) nos pequenos mamíferos indicam a presença dos metais pesados não essenciais Alumínio e Chumbo em maiores concentrações na reserva legal do que no arrozal. As análises indicam que a presença destes metais em tecidos críticos não advém da exposição pelo solo, mas provavelmente pelo regime de cheia da reserva legal. Os resultados permitem orientar o setor público regulatório e a academia na avaliação de impactos da agricultura sobre a biodiversidade de mamíferos e do risco ambiental de contaminantes (agrotóxicos e metais) para organismos não-alvo. A avaliação do sistema modelo arroz-pantanal, fornece um protocolo inédito para acessar os impactos de agroecossistemas na comunidade de pequenos mamíferos, utilizando múltiplas métricas de diversidade juntamente com a quantificação de contaminantes na fauna. Este protocolo pode ser aplicado em outros sistemas agrícolas, a fim de ampliar o conhecimento sobre a ocorrência e a saúde de mamíferos em áreas agrícolas, além de direcionar futuras pesquisas de conservação e auxiliar na criação de legislações para o monitoramento e fiscalização de contaminantes no meio ambiente.

ANEXO 1

MamInAgro - Um banco de dados para
registros da ocorrência de mamíferos silvestres em agroecossistemas
https://maminagro.azurewebsites.net/swagger/index.html
https://lume.ufrgs.br/handle/10183/272994

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE INFORMÁTICA
CURSO DE ENGENHARIA DE COMPUTAÇÃO

LAURIEN SANTIN

MamInAgro - Um banco de dados para registro de observações de espécies da fauna brasileira

Monografia apresentada como requisito parcial para a obtenção do grau de Bacharel em Engenharia da Computação

Orientador: Prof. Dr. Renata de Matos Galante

Porto Alegre
2024

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RESUMO

Em tempos de mudanças climáticas cada vez mais acentuadas, a preservação de ecossistemas e desenvolvimento de propostas de utilização sustentável dos recursos naturais se mostram altamente necessários. Para isso, é importante que se tenha um monitoramento constante e consistente da biodiversidade. Nesse contexto, queremos desenvolver um sistema para reunir informações de observações de espécies mamíferas em paisagens agrícolas brasileiras, chamado MamInAgro. O sistema será composto de um banco de dados, modelado para permitir o armazenamento do maior número possível de informações comumente divulgadas em estudos da área; um backend e uma interface, com algumas opções de processos de inserção de dados, para popular o banco e mais tarde servir de base para construir visualizações que ajudem na análise dos dados e permitir seu uso em diferentes estudos sobre observação de espécies e suas paisagens relacionadas.

Palavras-chave: Banco de dados. ocorrências de espécies. agroecossistema. aplicação.

MamInAgro - A database for brazilian fauna species recording

ABSTRACT

In times of ever-growing climate change, ecosystem preservation measures and the development of sustainable ways of resource consumption are highly necessary. For that, it's important to have constant and consistent biodiversity monitoring. In this context, we are developing a system to group information on mammal species observations inside Brazilian agricultural landscapes, called MamInAgro. The system will be composed of a database, modeled in order to allow storage of as much information disclosed in studies from the area as possible; a backend and a user interface, with a few different options to save data, so we can, at a later point, develop views to aid data analysis and allow its use for different studies on the sighted species and the related landscapes.

Keywords: Database, Species occurrences, agroecosystem, application.

LISTA DE ABREVIATURAS E SIGLAS

API	*Application Programming Interface*
DNA	*Deoxyribonucleic acid*
GBIF	*Global Biodiversity Information Facility*
IBAMA	Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis
LA	*Living Atlas*
ORM	*Object Relational Mapper*
RNA	*Ribonucleic acid*
SGBD	Sistema de Gerenciamento de Banco de Dados
SiBBr	Sistema de Informação sobre a Biodiversidade Brasileira
TCC	Trabalho de Conclusão de Curso

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SUMÁRIO

1 INTRODUÇÃO

Vivemos um período crítico para a conservação da biodiversidade do nosso planeta. Mudanças climáticas, aumento de áreas de produção agropecuária, introdução de espécies exóticas, aumento da paisagem urbana, construções relacionadas à infraestrutura de cidades; fatores para a redução, ou desaparecimento de espécies não faltam. Fala-se muito em medidas para desacelerar o declínio na biodiversidade, ou em salvar determinada espécie, mas é importante saber, antes de tudo, qual a situação da nossa variedade biológica.

Nesse cenário, a agricultura é considerada uma das maiores responsáveis pela redução da diversidade dentro das paisagens (ZINGG et al., 2019), porém não são completamente hostis, e podem proporcionar condições favoráveis para a vida selvagem (TSCHARNTKE et al., 2012). Assim, um balanço deve ser feito entre atender à crescente demanda por alimentos e manter a conservação da biodiversidade (TSCHARNTKE et al., 2012).

Um fator potencialmente prejudicial para esse balanço, é o alto consumo de agrotóxicos no Brasil. O seu acúmulo e depósito no meio ambiente expõe outros organismos, além de seus alvos, a resíduos e efeitos de seus subprodutos (AKTAR; SENGUPTA; CHOWDHURY, 2009). Devido ao processo de bioacumulação de pesticidas organoclorados e de bio-ampliação, no ciclo predador-presa, os mamíferos estão entre os potencialmente em risco (JIN et al., 2010), em especial porque algumas espécies de roedores são favorecidas pelo ambiente de culturas de grãos. Além disso, a presença desses roedores atrai predadores, tornando esses ambientes relevantes para estudos de efeitos da deposição de agrotóxicos no ambiente. (IMD, 2022)

Já existem fontes de $datasets$, tanto a nível de Brasil, quanto mundialmente, que organizam dados de ocorrências de espécies. Porém, o objetivo deste trabalho é desenvolver a ferramenta que além de agregar ocorrências, adiciona caracterização dos ambientes onde os animais estão, especialmente paisagens agrícolas. Uma aplicação voltada para esse tipo de estudo, com possibilidade de ampliação do escopo dos dados, incluindo a relação de produtos utilizados nas paisagens e outros dados relevantes, não disponíveis em agregadores mais gerais. Experimentos foram conduzidos com usuários, para avaliar a usabilidade da interface, com resultados majoritariamente positivos e algumas sugestões de melhorias.,

Este trabalho está dividido em seis capítulos, além da Introdução. No capítulo

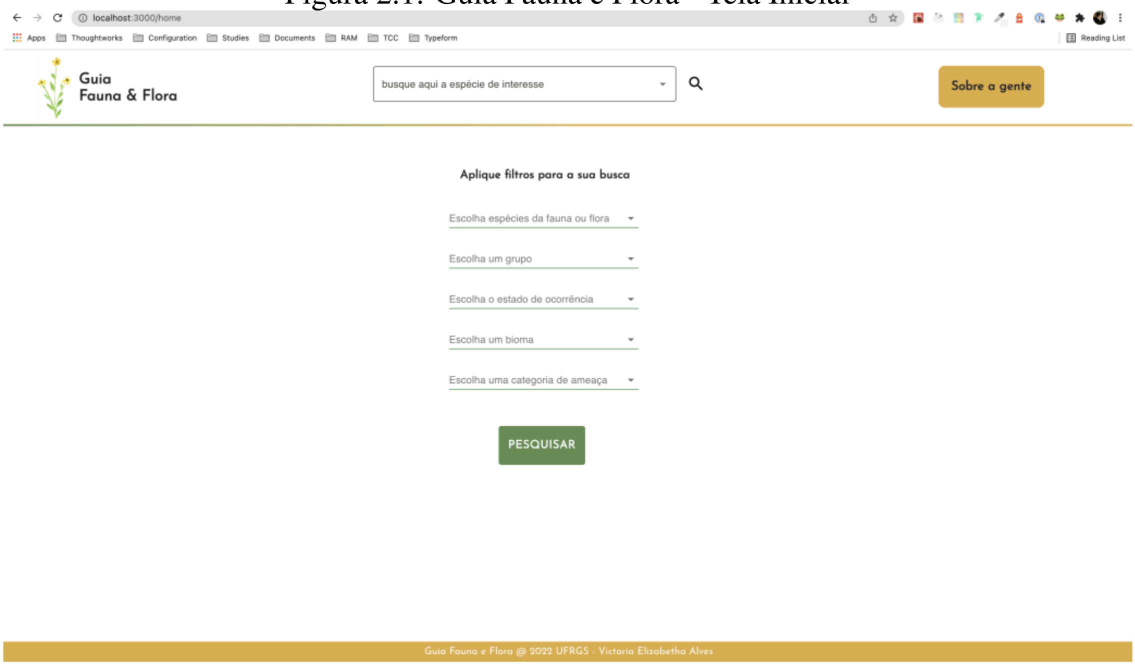
2, outras ferramentas de registros de espécies são apresentadas e comparadas: o Guia Fauna e Flora, o SiBBr (Sistema de Informação sobre a Biodiversidade Brasileira), o GBIF (*Global Biodiversity Information Facility*), o $eBird$ e o $iNaturalist$. No capítulo 3, caracteriza-se o cenário agrícola brasileiro, as consequências para a biodiversidade e os desafios enfrentados na preservação da biodiversidade biológica, seguido da descrição da forma como os requisitos foram levantados, até tomarem forma na aplicação $MamInAgro$. A aplicação é apresentada no capítulo 4, com sua arquitetura e tecnologias utilizadas. Avaliações realizadas por meio de um formulário são discutidas no capítulo 5, juntamente de próximos passos, para aprofundamento do trabalho e melhoria da ferramenta. O capítulo final (6) faz uma rápida retomada do que foi apresentado ao longo do trabalho, e sumariza as suas contribuições.

2 TRABALHOS RELACIONADOS

Esse capítulo apresenta três ferramentas com funcionalidades similares ao que estamos propondo. O primeiro deles é o trabalho de um TCC, focado na divulgação de dados de espécies ameaçadas de extinção. Depois, é apresentado um nodo brasileiro do GBIF, responsável por agregar as informações de *datasets* de ocorrências de espécies brasileiras. Por fim, o capítulo fala do próprio GBIF, um agregador internacional de dados da biodiversidade mundial.

2.1 Guia Fauna e Flora

Figura 2.1: Guia Fauna e Flora - Tela Inicial



Fonte: (ALVES, 2022)

A plataforma Guia Fauna e Flora (ALVES, 2022) foi proposta com o objetivo de disponibilizar uma diversidade de consultas e visualizações das espécies da fauna e flora brasileira, ameaçadas de extinção. Suas funcionalidades foram pensadas para facilitar a análise dos locais, dimensão e possíveis agentes promotores de ameaça para as espécies. A plataforma propriamente dita, não está disponível para ser usada, mas sua definição pode ser encontrada em (ALVES, 2022).

Os dados disponíveis na plataforma foram retirados do Portal Brasileiro dos Dados Abertos, sendo inseridos no banco da plataforma para consulta do usuário final.

Logo na tela inicial, a plataforma apresenta opções de busca, incluindo uma barrinha na parte superior, e filtros mais específicos no centro da tela, como mostrado na Figura 2.1.

A barra de pesquisa mostra sugestões de espécies ao ser clicada, e essas sugestões mudam dinamicamente, de acordo com o que for sendo digitado.

Clicando em espécie listada, a plataforma exibe uma tela com algumas informações:

- Se a espécie é Fauna ou Flora
- Grupo
- Família
- Categoria de ameaça
- Bioma em que a espécie é encontrada
- Principais causas de ameaça
- Estados de ocorrência
- Se a espécie é exclusiva do Brasil

Caso um filtro seja adicionado, a aplicação é redirecionada para uma tela com resultados mais detalhados da pesquisa: a lista de resultados e alguns gráficos, servindo tanto para pesquisa de uma espécie, quanto para busca de informações agregadas, relacionadas aos filtros escolhidos. Na Figura 2.2, foi selecionado o filtro "Fauna"e estado de ocorrência "RS".

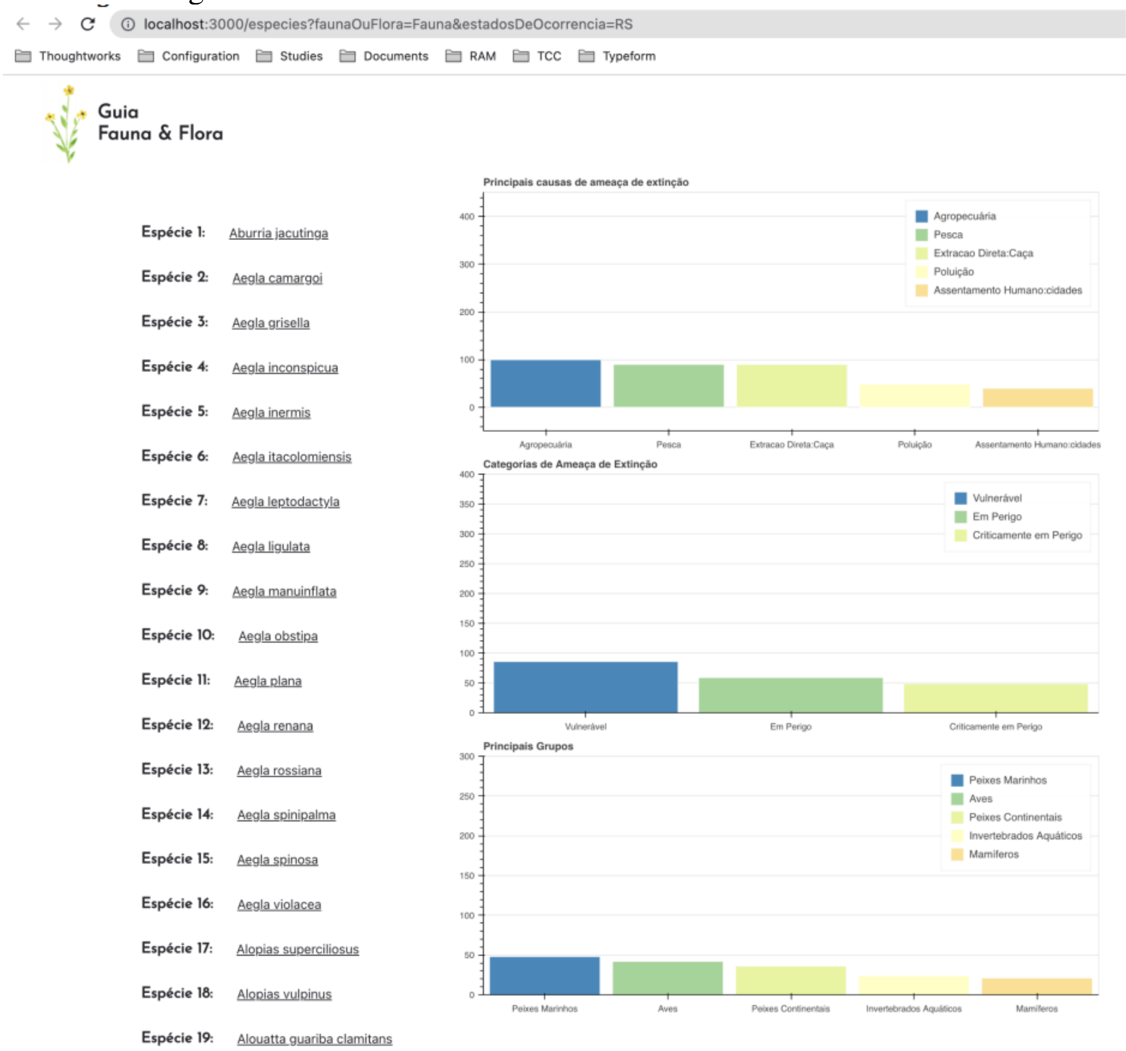
Por fim, a ferramenta possui uma página, acessível pelo botão no canto superior direito da tela, com informações do projeto, como a motivação por trás dele e seu objetivo.

2.2 Global Biodiversity Information Facility

Origem do padrão adotado pelo *Living Atlas* (LA) e o Sistema de Informação sobre a Biodiversidade Brasileira (SiBBr), o GBIF (GBIF, 2023) disponibiliza uma gama de ferramentas para compartilhar informação sobre quando e onde espécies foram encontradas. A rede também sugere um formato padronizado e boas práticas para a disponibilização das informações.

A sua tela inicial (Figura 2.3) mostra contadores que redirecionam para páginas de listagem e pesquisa de ocorrências, *datasets*, instituições e artigos. No menu *Get data*,

Figura 2.2: Guia Fauna e Flora - Resultado da busca com filtros



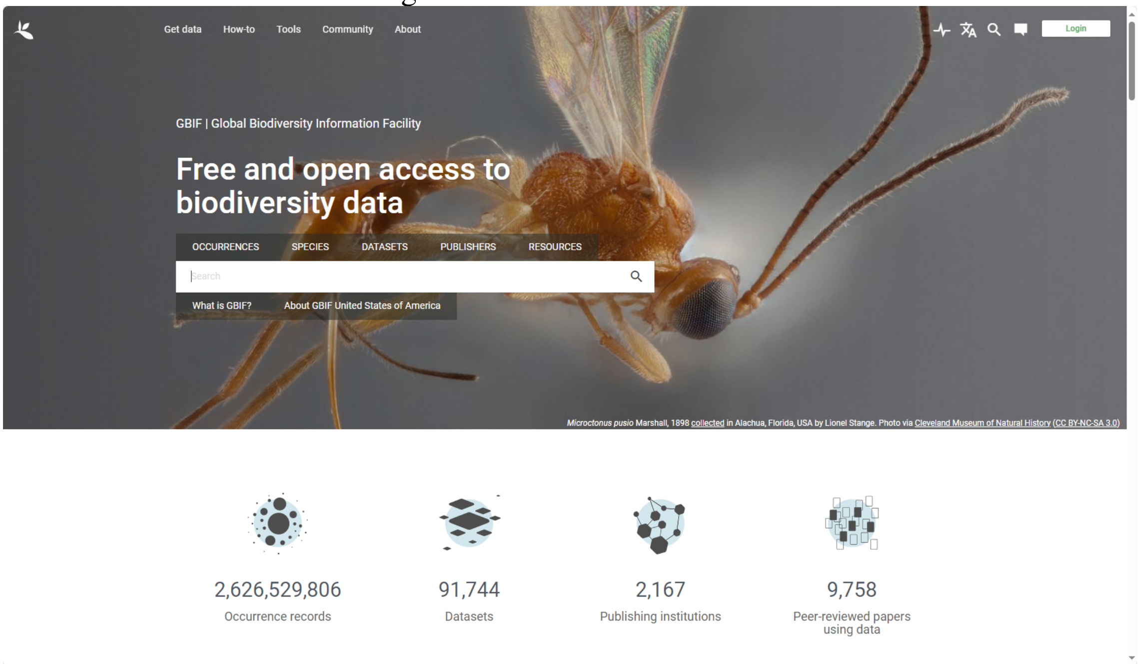
Fonte: (ALVES, 2022)

localizado na parte superior, também aparecem opções para buscar espécies, *snapshots* de ocorrências e orientações para uso da API do GBIF.

A ocorrência no GBIF (Figura 2.4) apresenta os dados em uma tabela, em que cada linha contém o nome do campo, o valor interpretado, o valor original do *dataset*, e observações sobre o dado.

O GBIF armazena também dados específicos do *dataset*. Acessando a página de um *dataset*, pode-se ver a descrição de seu conteúdo, métricas, atividades e um *link* para *download* dos dados. Dentre as informações disponíveis, estão escopo temporal e geográfico, metodologia, contatos e citação. Para métricas, temos a distribuição taxonômica das ocorrências, contagem de problemas encontrados nos dados e distribuição temporal das ocorrências.

Figura 2.3: GBIF - Tela inicial



Fonte: https://www.gbif.org/

2.3 Sistema de Informação sobre a Biodiversidade Brasileira

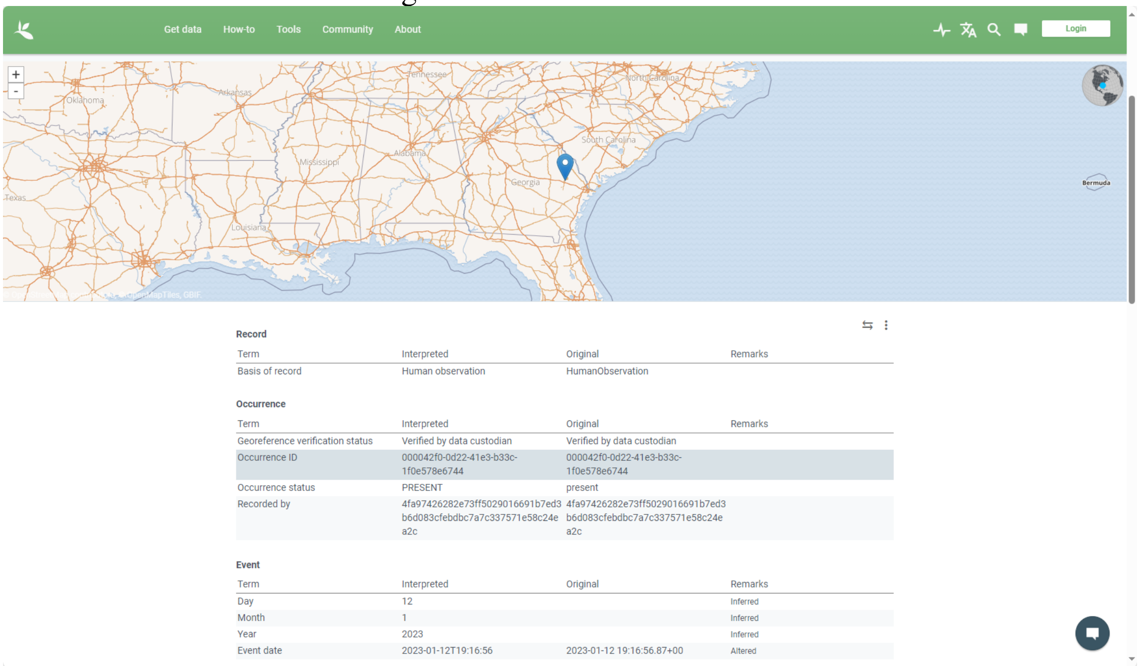
O SiBBr atua como nó brasileiro do GBIF e foi desenvolvido em cima da plataforma do LA, código aberto desenvolvido pelo governo Australiano. Similar ao GBIF, o SiBBr oferece visualizações para listas de espécies registradas no Brasil, ocorrências, e agrupamentos dos dados por instituição e coleções. É responsável pela organização, indexação, armazenamento e disponibilização de dados e informações sobre a biodiversidade e os ecossistemas brasileiros (GOVERNO FEDERAL DO BRASIL, 2023).

Na página inicial, é possível clicar nos contadores de Instituições, Coleções, Conjuntos de Dados e Ocorrências para ser levado para listagens dessas informações, com opções de busca e filtros. Na listagem de ocorrência, temos filtros de:

- Nome científico
- Forma de vida
- Ano de ocorrência
- Tipo de registro
- Coleção
- Recurso de dados

Através dos menus, localizados na parte superior, o portal também disponibiliza

Figura 2.4: GBIF - Ocorrência



Fonte: https://www.gbif.org/occurrence/4089794301

outros tipos de visualização:

- Por área (Figura 2.8): a partir de um endereço, ou da localização do usuário, a aplicação mostra uma lista de ocorrências na região. É possível determinar o raio desejado para a busca, e como resultado exibe uma lista com contagens das ocorrências agregadas por grupo, a lista de cada espécie discriminada e um mapa com as localizações das ocorrências marcadas - que responde, caso o usuário selecione uma espécie da lista.
- Por Região: similarmente, essa tela mostra um mapa, e opções para explorar por regiões do Brasil, biomas, estados, municípios e alguns tipos de unidade de conservação. Uma vez selecionada a região desejada, a tela é similar à exploração por área, tendo o mapa com localizações, a lista de espécies e o agrupamento por grupos.
- Por Ocorrência: nessa opção, é possível fazer pesquisas mais avançadas; o site tem a opção de busca simples por texto, busca avançada, permitindo preencher várias espécies numa única busca, nome científico, grupo de espécies, instituição ou coleção, entre outras opções de filtros. Também possui uma aba para buscas por ID de eventos, com algumas opções de identificações. Por fim, a última aba é busca espacial, com um mapa e ferramentas de desenho, para determinar uma área.

Figura 2.5: SiBBr - Tela inicial

Fonte: https://sibbr.gov.br/

A partir das listagens, pode-se acessar os perfis das espécies, que possuem *links* para recursos relacionados (como página no GBIF), galeria de fotos, informações de nomenclatura, taxonomia, além de listas de ocorrências, menções em literatura, sequenciamento de DNA/RNA e parceiros de dados.

Para uma determinada espécie, cada registro de ocorrência segue o padrão determinado pelo GBIF, com múltiplas informações sobre localização, método de ocorrência, informações da qualidade do dado, entre outras informações.

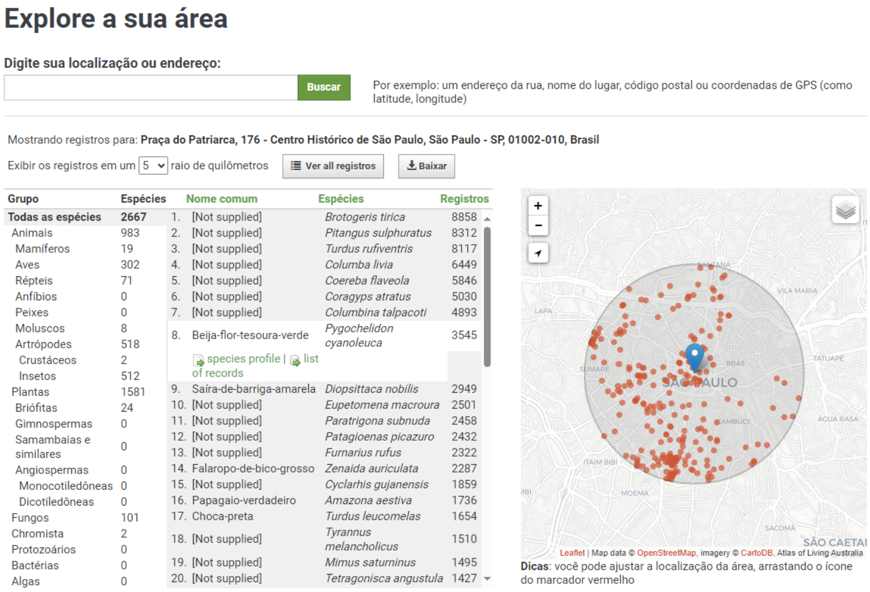
A aplicação também permite que usuários façam inserção de dados. Instituições podem publicar conjuntos de dados, e qualquer um pode registrar ocorrências através de um aplicativo vinculado.

2.4 Outros

Outras duas plataformas, com objetivos similares são o *eBird*(CORNELL LAB OF ORNITHOLOGY, 2024) e o *iNaturalist*(INATURALIST, 2008). Voltadas para o público geral, elas permitem que qualquer pessoa se cadastre e registre suas observações de espécies.

No caso do *eBird*, focado em pássaros, possui um formulário com três passos para submeter avistamentos. Primeiro, a localização dos avistamentos é selecionada, via

Figura 2.6: SiBBr - Exploração por área



Fonte: https://ala-hub.sibbr.gov.br/ala-hub/explore/your-area/#-23.5475|-46.6361|12|ALL_SPECIES

coordenadas geográficas, nome do local, ou posicionamento de pinos em um mapa; então, data, comentários e o tipo de avistamento são requisitados. Dependendo do tipo de avistamento (isto é, se a procura por pássaros era o seu objetivo), dados de tempo, distância percorrida e número de pessoas com você são pedidos também. Por fim, o *site* mostra uma lista de pássaros, separados em algumas categorias, para que o usuário possa marcar todos os avistados e/ou ouvidos.

A plataforma mostra algumas informações de cada espécie, como nome científico, grau de ameaça, fotos e áudios do canto dos pássaros.

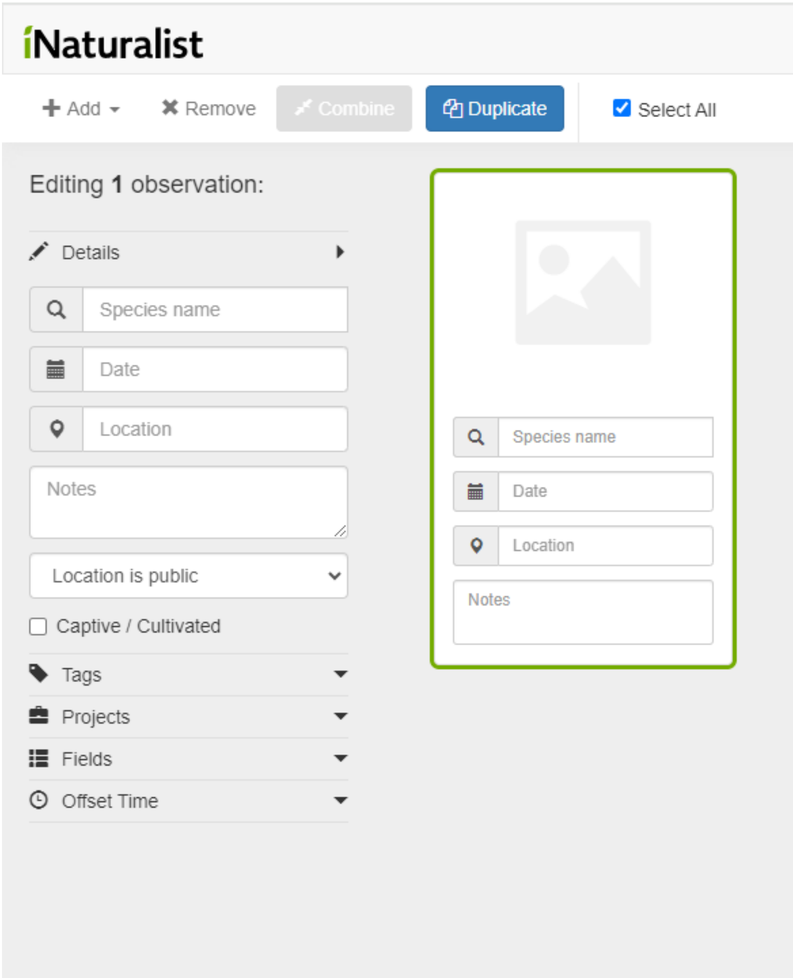
Figura 2.7: eBird - Envio de avistamentos

Fonte: https://ebird.org/submit/map

No $iNaturalist$, o foco maior é em fotografias tiradas do que foi avistado. É possível inserir dados sem imagens, mas o número de informações pedidas é menor: localização, também escolhida através de um mapa, com coordenadas e um raio de acurácia; nome da espécie avistada, data e hora da observação. Também é possível marcar uma observação como duplicata.

A aplicação também mostra dados das espécies para a informação do usuário, como a página da Wikipedia(WIKIPEDIA FOUNDATION INC., 2024) da espécie, classificação de ameaça e dados taxonômicos.

Figura 2.8: iNaturalist - Upload de observação



Fonte: https://www.inaturalist.org/observations/upload

2.5 Análise Comparativa

Dos cinco exemplos, o mais diferente é o Guia Fauna e Flora, que tem um objetivo ligeiramente diferente dos demais. É perceptível que os objetivos e funcionalidades do GBIF e SiBBr são praticamente iguais, uma vez que um é de certa forma uma especialização do outro. O *eBird* e o *iNaturalist* são similares na parte de registro de avistamentos, mas têm um foco maior no público geral, permitindo que qualquer pessoa crie um cadastro e envie suas observações.

Quanto às funcionalidades, o SiBBr cobre praticamente tudo o que é necessário para o armazenamento e consulta de ocorrências de espécies brasileiras de uma forma genérica. Apesar de permitir o registro de ocorrências, e portanto, ter alguns dados relacionados às localizações dos animais, não possui caracterizações mais profundas do local, como distinguir entre uma ocorrência em área de floresta de um avistamento de um animal em uma plantação.

Tabela 2.1: Comparação entre os trabalhos relacionados

Funcionalidade	Guia Fauna e Flora	SiBBr	GBIF	eBird	iNaturalist
Permite inserção de dados		x	x	x	x
Registros de ocorrência das espécies		x	x	x	x
Métricas sobre resultados de buscas	x	x	x	x	
Busca com filtros	x	x	x	x	x
API para consultas			x		
Busca por área	x	x	x	x	x
Métricas de tendências			x	x	x
Descrição dos *datasets*		x	x		

Tendo por base o padrão existente, definido pelo GBIF e as funcionalidades disponibilizadas pelas plataformas existentes, este trabalho propõe uma melhoria na caracterização da localização que a ocorrência aconteceu, pensando na utilização por futuros estudos do efeito de agrotóxicos em mamíferos dentro de ecossistemas agrícolas. As seguintes funcionalidades são essenciais:

- Inserção de *datasets*;
- Consultas de dados inseridos;
- Descrição da metodologia usada para compilação do *dataset*;
- Descrição da paisagem onde se deu a ocorrência

3 REQUISITOS DA APLICAÇÃO

Este capítulo descreve o processo usado para elaborar a aplicação $MamInAgro$.

3.1 Domínio da Aplicação

Dentro do quadro de crescimento populacional mundial, apesar do declínio no aumento, já são mais de 8 bilhões de pessoas (BRASIL, 2022). Alimentadas principalmente pelo agronegócio, este ainda se faz fundamental para a segurança alimentar da população. Após a modernização das técnicas agrícolas, denominada Revolução Verde, na década de 1970, o cultivo agro econômico cresceu bastante no Brasil e a nível mundial, adquirindo estruturas monocultoras e dependentes de agrotóxicos (RICO; CAVICHIOLI, 2018). Como resultado, se torna um grande fator na redução da diversidade de paisagens ecológicas.

Outro fator potencialmente prejudicial é o uso de agrotóxicos. O Brasil, maior consumidor de agrotóxicos do mundo (IMD, 2022), em anos recentes, mais que dobrou a quantidade de diferentes agrotóxicos aprovados para uso (RODRIGUES; LOPES; SILVA, 2022). Não apenas podem contaminar através do ar, chuvas, penetração no solo e águas subterrâneas (DUTRA RODRIGO MARCIEL MENDONçA OLIVEIRA DE SOUZA, 2017), como sofre o processo de bio-ampliação no ciclo predador-presa, expondo diversos organismos além dos intencionados a seus efeitos.

Apesar disso, algumas espécies ainda se beneficiam do ambiente proporcionado pela paisagem agrícola, e para entender os efeitos dos agrotóxicos em seus organismos, o primeiro passo é monitorar as populações de animais que habitam agroecossistemas. Que mamíferos vivem nas paisagens agrícolas, e como se utilizam delas? Onde as ocorrências são mais comuns? Dentro de culturas, ou fora? Na borda? Quais as características das culturas mais aproveitadas pelas espécies? Essas são algumas perguntas que a aplicação desenvolvida por esse trabalho se propõe a começar a responder.

3.2 Levantamento de Requisitos

O processo de construção do modelo foi realizado com base em um documento, escrito após a elaboração de um relatório técnico para o Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA)(IMD, 2022), que gerou perguntas que poderiam ser respondidas por consultas, além de uma proposta inicial de tabelas para armazenar as informações, com definições do significado dos campos necessários em cada uma. A lista inicial foi analisada e aprimorada até chegarmos na estrutura atual, com a proposta validada através de reuniões.

As perguntas que os dados devem ser capazes de responder são:

- Quais espécies têm registros de presença em paisagens agrícolas no território brasileiro?
- Como podemos categorizar as espécies como de interesse para avaliação de risco baseado na frequência de ocorrência em agroecossistemas?
- Qual a frequência de ocorrência em agroecossistema das espécies listadas na literatura?
- Como categorizar as espécies como de interesse para avaliação de risco baseado no grau de endemismo e grau de risco de extinção segundo ICMBio 2018?
- Quais as culturas utilizadas por mamíferos?
- Em que estágio fenológico de desenvolvimento da cultura os mamíferos usam e usam mais as culturas?
- Quais espécies de mamíferos utilizam quais culturas e em que frequência?
- Dos mamíferos que usam agroecossistemas, qual a frequência de uso dos diferentes habitats (cultivo/borda/área silvestre adjacente)?
- Qual a frequência de ocorrência de mamíferos em agroecossistemas segundo grupos taxonômicos?

O conjunto inicial de tabelas, sugerido no documento, foi:

- Bibliografia - Com dados de referenciamento de estudos responsáveis pela coleta de dados;
- Taxonomia - Árvore de taxonomia, inicialmente proposta como tabela única;
- Metodologia de Coleta - Inicialmente pensada para incluir diversos tipos de dados sobre a coleta de informações, como tipo de registro, descrição do método, desenho amostral da localização escolhida pelo estudo, esforço amostral, período de coleta;
- Georreferenciamento - Localização da ocorrência, com coordenadas, município, UF, bioma;
- Ocorrência do Registro da Espécie - Data e hora, tipo de espaço ($incrop$, $offcrop$,

borda), ambiente de captura, tamanho do ambiente, descrição de paisagem;

- Paisagem - Descrição da área amostrada pelo estudo, com coordenadas geográficas, tipo de área, tamanho, descrição e estatísticas da paisagem (como número de fragmentos, tamanho de bordas);
- Abundâncias
- Cultivos - Caracterização das áreas de plantação, com espécie do cultivo, variedade, fenologia, área plantada, tempo desde o plantio;
- Agroecossistema - Classificação do ecossistema, para agrupamento posterior em consultas (ex: Agrofloresta, Plantação de Árvores, Pastagem para Agropecuária, etc.);
- Risco de Extinção - Dados de ameaça da espécie, e o órgão responsável pela divulgação da classificação;

Além dessas, tabelas para Dados Biológicos (do indivíduo da ocorrência), Agrotóxicos, Amostras biológicas e Efeito foram consideradas, mas deixadas para uma versão posterior da aplicação.

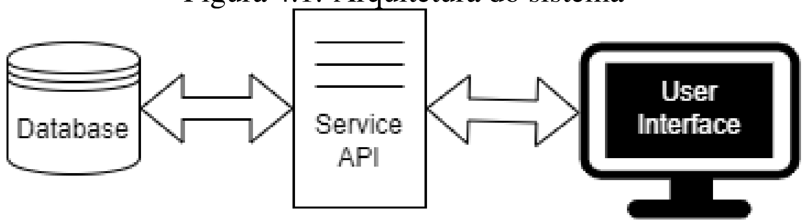
4 APLICAÇÃO

Este capítulo apresenta a implementação escolhida para o banco de dados *MamInAgro*. O objetivo principal é reunir informações de avistamentos de espécies animais dentro de agroecossistemas. Dessa forma, o banco precisa ter uma estrutura capaz de acomodar as mais diversas maneiras como essas informações são disponibilizadas através de estudos da área.

Uma estrutura de dados foi definida para o banco, e uma API com operações básicas de leitura, escrita e deleção foi implementada para facilitar o acesso, e futuramente servir uma plataforma *web*, para realização de consultas sobre os dados.

4.1 Arquitetura

Figura 4.1: Arquitetura do sistema



O foco principal do projeto está no modelo do banco de dados, e como comportar as informações com diferentes níveis detalhamentos. Para isso, optamos por uma arquitetura simples, focada inicialmente no banco de dados em si, e um serviço responsável pelas consultas e manipulações nos dados.

Para o banco, PostgreSQL (THE POSTGRESQL GLOBAL DEVELOPMENT GROUP, 2023) foi escolhido por ser um SGBD relacional, facilitando a normalização do banco e a construção de $queries$; gratuito, *open source* e bastante completo em suas funcionalidades.

Para o serviço, foi escolhido o $framework$ .Net (MICROSOFT, 2023b), principalmente pela familiaridade da autora com a tecnologia. Além disso, é uma tecnologia *open source*, com suporte às linguagens C#, F# e Visual Basic, é multiplataforma e recebe contínuas atualizações, com melhorias de segurança e novas $features$ para facilitar o desenvolvimento.

Por fim, a interface para o usuário final será desenvolvida em ReactJS (META OPEN SOURCE, 2023), biblioteca para Javascript desenvolvida pela Meta, empresa do

Facebook, e proporciona uma série de funcionalidades para facilitar a criação de interfaces para o usuário, junto da linguagem Typescript (MICROSOFT, 2023c), um *superset* do Javascript, para adição de tipagem de dados, o que também ajuda na velocidade de desenvolvimento e *debug*, em caso de erros.

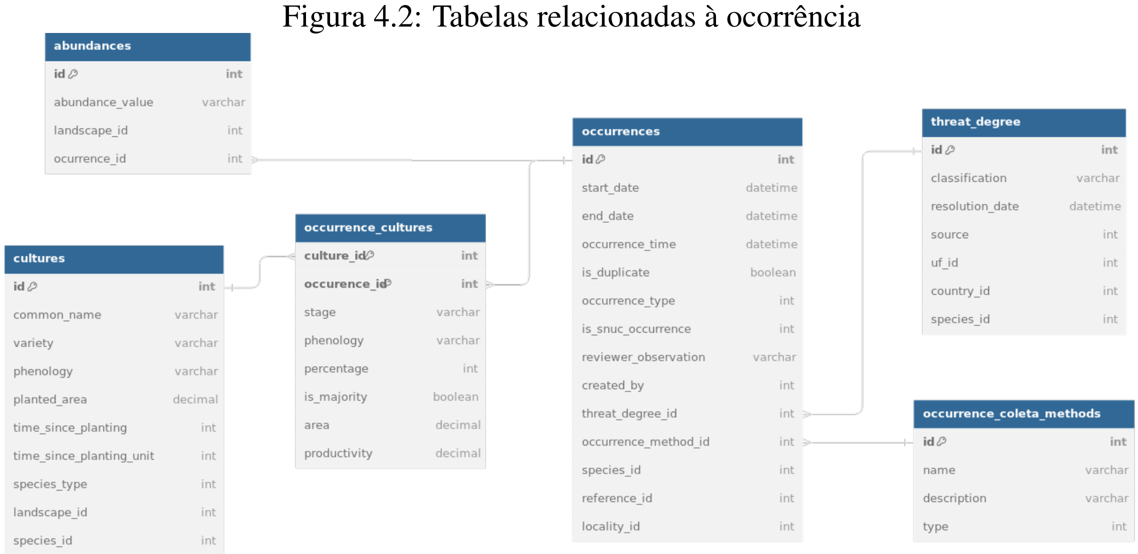
4.2 Banco de Dados

A modelagem do banco de dados resultou em 32 tabelas, contando tabelas de relacionamento. A ferramenta (HOLISTICS, 2023) foi utilizada para geração do diagrama entidade relacionamento, para fins de documentação (Figura A.1). Todas as tabelas (com exceção das relacionamento) possuem um identificador (ID) inteiro, autoincremental.

Para melhor entendimento do modelo criado, dividiremos as tabelas como sendo relacionadas a quatro tópicos: ocorrência (tabelas com seus campos e ligações mostradas na Figura 4.2), localidades (tabelas mostradas na Figura 4.3), estudo (tabelas mostradas na Figura 4.4) e taxonomia (tabelas mostradas na Figura 4.5). Uma visão geral, com todas as tabelas pode ser vista na Figura A.1, no Anexo A, ao final deste documento.

4.2.1 Ocorrência

Figura 4.2: Tabelas relacionadas à ocorrência



A principal entidade do sistema é o que chamamos de *Occurrence*. Refere-se à ocorrência de uma espécie, em um determinado tempo e localização. Sua tabela possui uma data de início, data de fim e hora exata da ocorrência. Os três campos são opcionais, já alguns estudos não divulgam esses dados. Além disso, possui um booleano para identificar ocorrências duplicadas, isto é, quando um animal já registrado é observado novamente, e um booleano para identificar avistamentos dentro de uma unidade de conservação. Possui um campo para observações adicionadas pelo usuário que revisar a inserção do dado.

Por fim, possui relacionamentos com algumas tabelas:

- Users: indica o usuário que inseriu a ocorrência no sistema
- Threat Degrees: relaciona a ocorrência ao grau de risco da espécie naquele momento
- Occurrence Methods: identifica o método utilizado para avistar o espécime
- Species: a espécie avistada
- References: o trabalho que publicou o dado da ocorrência
- Localities: A localidade onde aconteceu o avistamento

Além disso, o banco possui outras tabelas, para informações adicionais relacionadas à ocorrência:

- Abundances: A abundância é a descrição de quantos espécimes foram avistados em uma determinada ocorrência, e essa quantidade pode não ser exata, ou numérica, dependendo do método de avistamento. Portanto, possui um campo de texto para o valor da abundância, e relacionamentos com a ocorrência e a paisagem.
- Cultures: Cultures é outra tabela importante. Uma vez que o objetivo é criar uma base de ocorrências de espécies em agroecossistemas, se faz necessária a qualificação das características agrícolas da paisagem. Aqui, guardamos o nome comum da plantação relacionada à paisagem, a variedade e fenologia da cultura. A entidade também possui um campo para a área plantada, tempo desde o plantio e outro campo para a unidade de tempo. Tudo isso é opcional, pois não podemos garantir que o estudo terá essas informações. Além disso, possui um campo para o tipo de espécie de cultura, enumerado em *Pasture*, *Exotic Pasture*, *Plantation* e *Other*. Isso serve para identificar plantações descritas de forma genérica (como por exemplo, apenas "*Pasture*")

  Por fim, tem relacionamento com a paisagem onde a cultura está localizada, e a espécie da planta da cultura, se for especificada no estudo.
- Occurrence Cultures: Tabela de relacionamento entre Ocorrência e Cultura. Possui

as referências para as respectivas tabelas, além de outros dados relacionados ao estado da cultura no momento da ocorrência. São eles: estágio da cultura, fenologia, porcentagem (uma ocorrência pode acontecer em uma área com diversas culturas), área da cultura, produtividade e um booleano para indicar se essa cultura ocupa a maioria do espaço onde aconteceu a ocorrência.

- Threat Degrees: Indica o grau de ameaça de uma espécie em um determinado momento. Para isso, possui um campo de texto para classificação de risco, data da resolução que determinou o grau de ameaça e fonte da informação, enumerada em "Iucn"(NATURE; RESOURCES, 2023), "Mma"(BRASIL, 2023), "Uf"(para resoluções estaduais) e "National"(para resoluções nacionais).
  A entidade é relacionada com:
  - Espécie
  - Uf: para resoluções estaduais, a Unidade Federativa que publicou a informação
  - Country: para resoluções nacionais, o país que publicou a informação
- Occurrence Coleta Methods: Descreve os possíveis métodos de coleta de ocorrências. Guarda o nome, descrição e tipo de método de coleta de uma ocorrência. O tipo é enumerado e pode ser direto ou indireto. Exemplos de métodos diretos são armadilhas de queda, redes de neblina; exemplos de métodos indiretos são fezes, pegadas, vocalização.

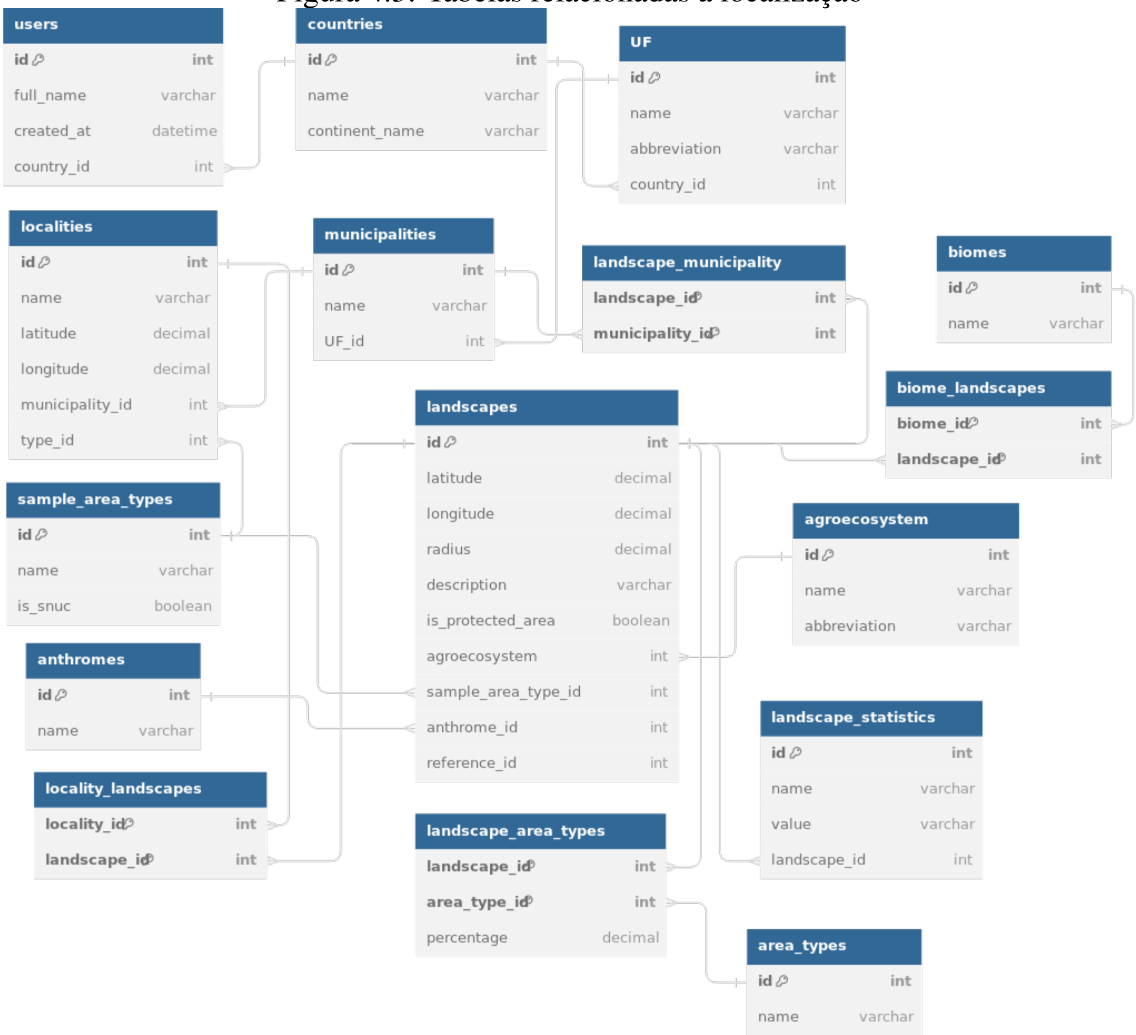
4.2.2 Localidade

A segunda principal entidade do sistema é a paisagem, criada para descrever as características de uma região. Possui coordenadas para latitude e longitude, além de um campo para o raio ao redor do ponto geográfico, que delimita o lugar e um booleano para marcar se é uma região protegida.

Seus relacionamentos são:

- Agroecosystem: o agroecossistema em que a paisagem está inserida
- Sample Area Types: o tipo de área de amostragem
- Anthromes: o antroma da região
- References: o estudo relacionado à descrição da paisagem

Figura 4.3: Tabelas relacionadas à localização



Também referente a localizações, temos outras entidades:

- Agroecosystem: Definição de um agroecossistema, ou um ecossistema em um ambiente agrícola. Possui nome e abreviatura.
- Anthromes: Definição de um antroma, isto é um "padrão ecológico global, formado pela interação direta de humanos com ecossistemas"(LAB, 2023). Possui apenas seu nome.
- Area Types: Caracterização do tipo de área de uma paisagem. Possui apenas um nome. Exemplos de tipos são: área agrícola, silvestre, área protegida.
- Landscape Area Types: Tabela de relacionamento entre Area Types e Landscape. Além de possuir as relações, possui um campo para determinar a porcentagem daquele tipo de área dentro da paisagem.
- Biomes: Assim como antromas, queremos saber qual o bioma relacionado a uma paisagem. Aqui, também guardamos apenas seu nome.

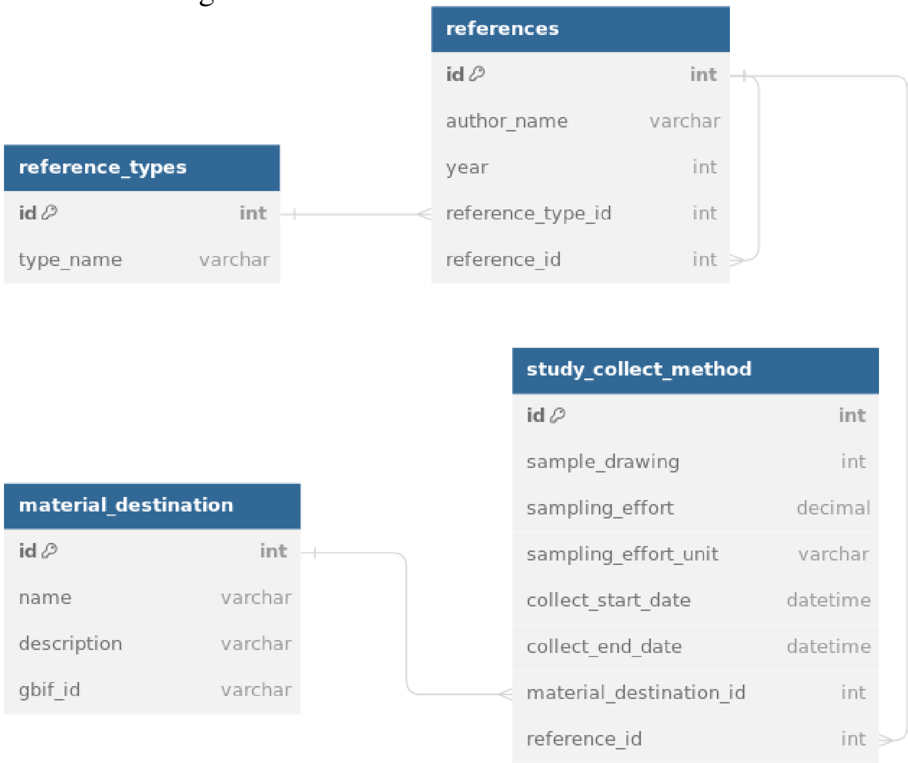
- Countries: Tabela com nome do país e do continente em que se encontra. Não achamos necessária a criação de uma tabela para normalizar o nome dos continentes, portanto não é um relacionamento.
- Biome Landscapes: entidade relacionamento entre *Biomes* e *Landscapes*.
- Ufs: Tabela de estados/Unidades Federativas. Possui nome, sigla e relação com o país a que pertencem.
- Municipalities: Tabela de municípios. Armazena apenas o nome e uma ligação com o estado (ou Unidade Federativa) onde se encontra.
- Landscape Municipalities: entidade relacionamento entre *Landscapes* e *Municipalities*.
- Landscape Statistics: Entidade para descrever estatísticas conhecidas da paisagem. Possui um campo texto para nome da estatística e outro, também texto, para valor, além da relação com a paisagem que está sendo descrita. Alguns exemplos de estatísticas são Número de Fragmentos, Tamanho de Bordas.
- Localities: Localidade é uma coordenada geográfica. Sem raio, como a paisagem, é usada para marcar um ponto exato no mapa. Possui um nome, latitude e longitude e uma relação com Sample Area Type.
- Landscape Localities: Entidade relacionamento entre *Landscapes* e *Localities*.
- Sample Area Types: Definição de tipos de áreas amostradas nos estudos. Possui nome e um booleano para identificar áreas Snuc. Exemplos de tipos de amostras são Cultura, Área Protegida.
- Users: Apesar de não termos autenticação ainda, guarda as informações de um usuário. Nome completo e uma referência para o país que estão representando no sistema.

4.2.3 Referência

Para identificação da origem de dados e histórico de inserções, o sistema guarda informações do estudo de onde vieram os registros das ocorrências. A entidade *Reference* guarda o nome do autor, ano do estudo, tipo de referência e chave para o BibTex, além de uma relação com a própria entidade Referência, já que o estudo pode referenciar outro estudo já inserido no banco.

Para suporte da descrição de referência, temos as seguintes tabelas:

Figura 4.4: Tabelas relacionadas à referência



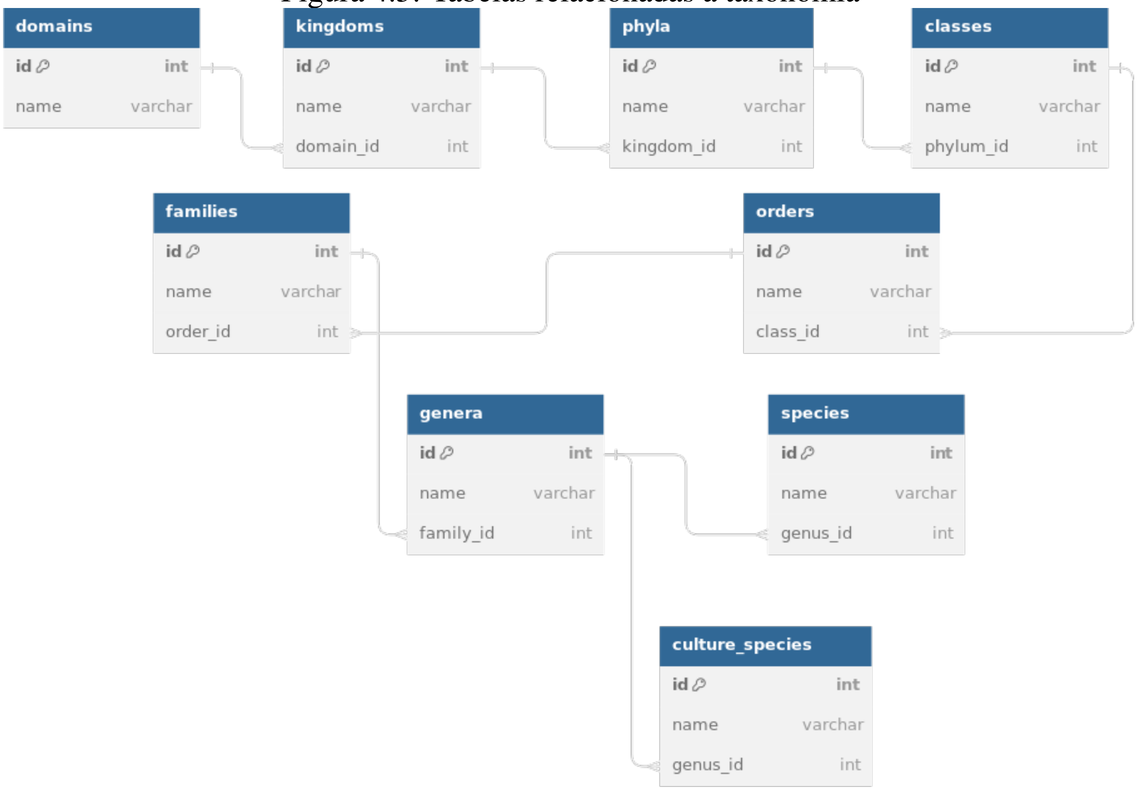
- Reference Types: Tabela para unificar a definição de tipos de referência. Possui apenas o nome o tipo (artigo, monografia, etc.).
- Material Destinations: Entidade para descrever o destino do material coletado, como "Solto/mantido em natureza", ou "Depositado em coleção/museu". Contém nome, descrição e uma identificação Gbif (GBIF, 2023).
- Study Collect Methods: Definição da metodologia de coleta do estudo. Armazena o tipo de desenho amostrado, enumerado em *Grid*, *Transect* e *Random*. Também possui campos para o esforço amostral (número) e unidade de esforço amostral (texto); datas de início e fim da coleta, e referências para o destino do material e o estudo (referência) propriamente dito.

4.2.4 Taxonomia

Por fim, temos tabelas para classificar as espécies, tanto animais quanto plantas em sua taxonomia completa.

- Domains: Estando no "topo", o Domínio possui apenas um nome.
- Kingdoms: Reinos possui nome, e relação com o Domínio do qual faz parte.

Figura 4.5: Tabelas relacionadas a taxonomia



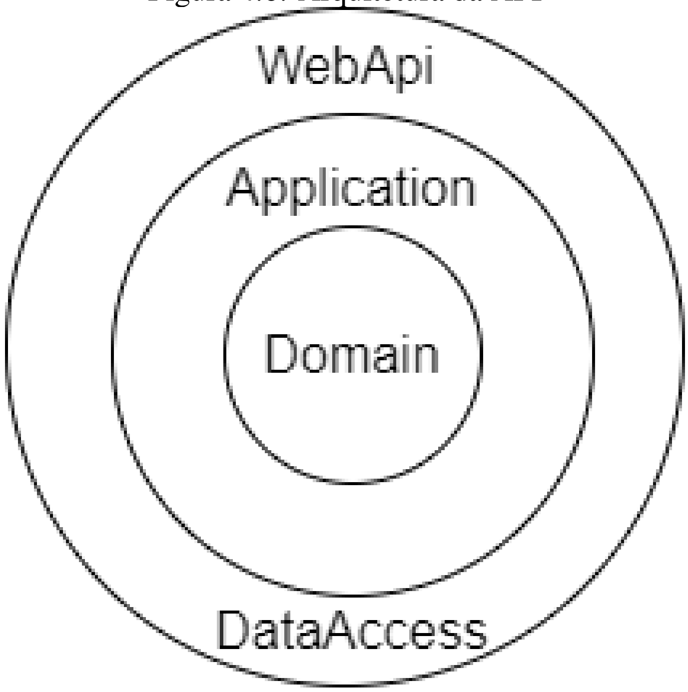
- Phyla: Filos possui nome e relação com o seu Reino.
- Classes: Possui nome e relação com o Filo ao qual a Classe pertence.
- Orders: Ordem possui nome e relação com a Classe a qual pertence.
- Families: Famílias possuem um nome e relação com a Ordem á qual pertence.
- Genera: Gêneros possui nome e relação com a sua Família.
- Culture Species: Espécie da cultura, criada para facilitar a divisão entre espécies animais (objetos principais de estudo) das plantações. Possui apenas o nome e uma relação com seu Gênero.
- Species: Tabela para dados de espécies animais. Possui nome e referência para o gênero da espécie.

4.3 API de Serviço

O primeiro passo, depois de definido o modelo de dados, foi implementar um serviço que fizesse o processamento dos dados para inserção e consultas no banco. A abordagem para manutenção do banco de dados escolhida foi a chamada *code-first*, onde usa-se um ORM, como o *Entity Framework Core* (MICROSOFT, 2023a), para mapear as entidades de forma que as tabelas e relações sejam todas criadas automaticamente. Esse processo facilita a manutenção de um banco através de diferentes ambientes e compartilhamento entre os desenvolvedores.

Além disso, o serviço foi implementado seguindo o padrão de *Clean Architecture* (MARTIN, 2017), de modo que o domínio da aplicação, isto é, o modelo das entidades, não depende das tecnologias escolhidas (com exceção da linguagem de programação), permitindo que quaisquer mudanças necessárias sejam implementadas sem muito impacto.

Figura 4.6: Arquitetura da API



O projeto foi dividido em quatro camadas, chamadas *WebApi*, *DataAccess*, *Application* e *Domain*, como representado na Figura 4.6.

Domain é a camada central, onde está o modelo dos dados e definição de enumerações usadas pelo serviço.

Application é onde estão implementados os serviços, responsáveis por processar os dados antes de salvar, ou devolver para o usuário. Também contém os modelos de dados como apresentados para o usuário, permitindo transformações para diferentes visualizações, ou esconder dados sensíveis quando necessário.

DataAccess é a camada responsável pelo acesso e configuração do banco de dados. Desde o mapeamento dos campos para as entidades definidas no *Domain*, *Migrations* para criar/atualizar o banco de dados, até repositórios para realização de operações de leitura e escrita.

Por fim, *WebApi* é a camada de apresentação, que contém os *Controllers*, definindo os *endpoints* (interfaces para recebimento de chamadas para o serviço). Também é a camada de inicialização da aplicação.

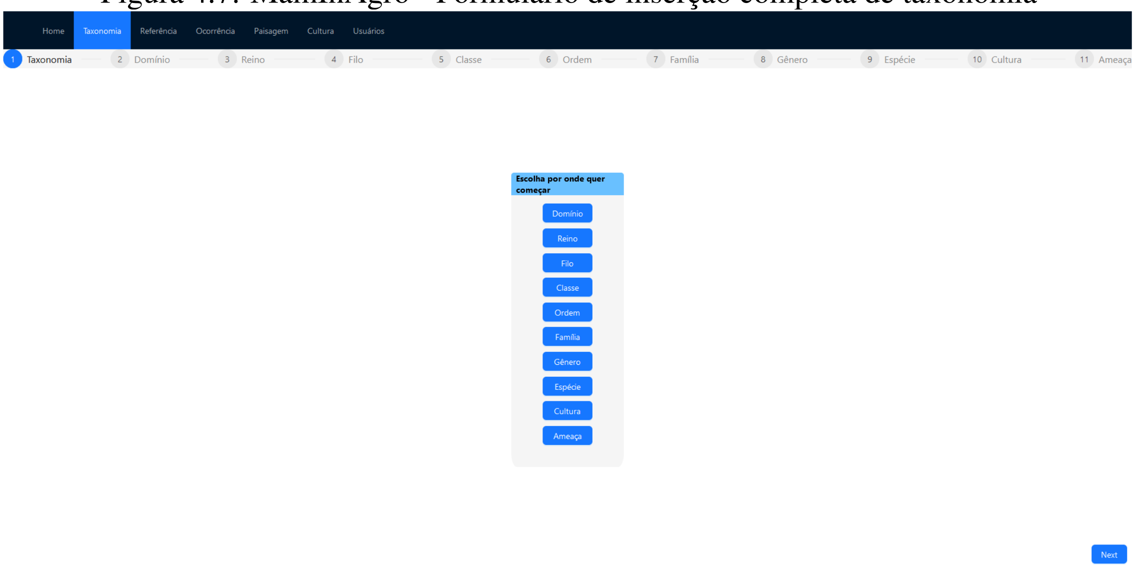
4.4 Interface de Usuário

Para realizar a população das tabelas do banco, foram construídas 34 telas, com diferentes abordagens na construção de formulários. Divididas em seis menus, as telas foram agrupadas sob:

- Taxonomia
- Referência
- Ocorrência
- Paisagem
- Cultura
- Usuários

4.4.1 Taxonomia

Figura 4.7: MamInAgro - Formulário de inserção completa de taxonomia

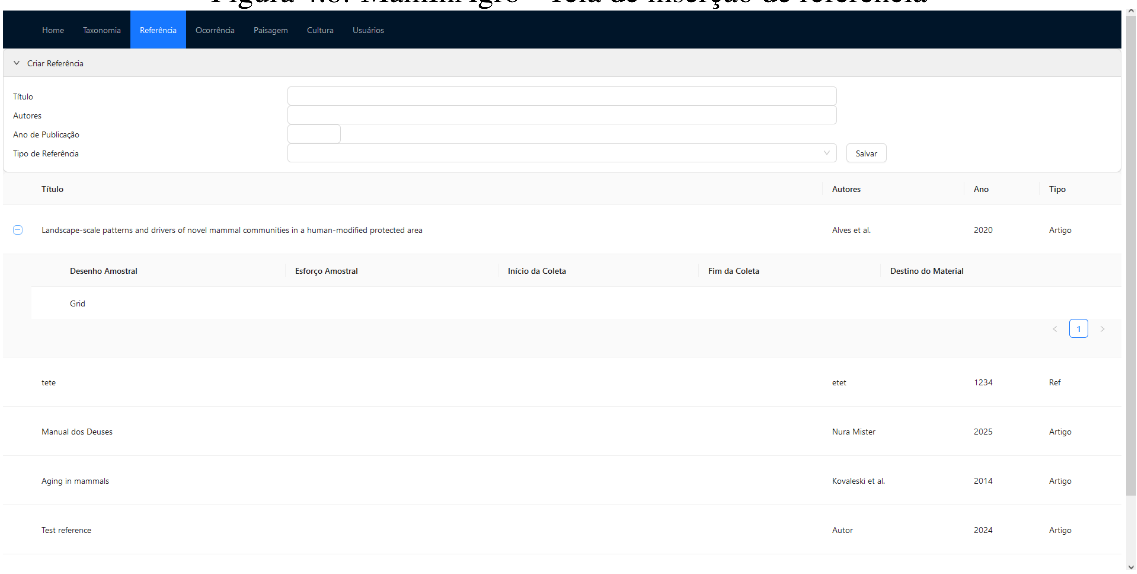


A seção de Taxonomia possui dez telas, sendo nove delas correspondentes às tabelas de taxonomia. A décima, chamada "Taxonomia Completa"(Figura 4.7), é um formulário com diversos passos, para que o usuário possa seguir de um nível taxonômico para outro com poucos cliques, além de permitir inserção de dados sobre risco de extinção da espécie inserida (ou de qualquer outra), ao final do processo.

Nessa seção, as telas não possuem listagem de dados já inseridos, apenas o próprio formulário, no centro da tela. O formulário de múltiplos passos começa com uma seleção, caso o usuário saiba que algumas entidades já existem na base, permitindo que comece em qualquer nível de taxonomia. Ao passar para o seguinte, o dado inserido aparecerá como opção para seleção, no campo de relacionamento entre tabelas.

4.4.2 Referência

Figura 4.8: MamInAgro - Tela de inserção de referência



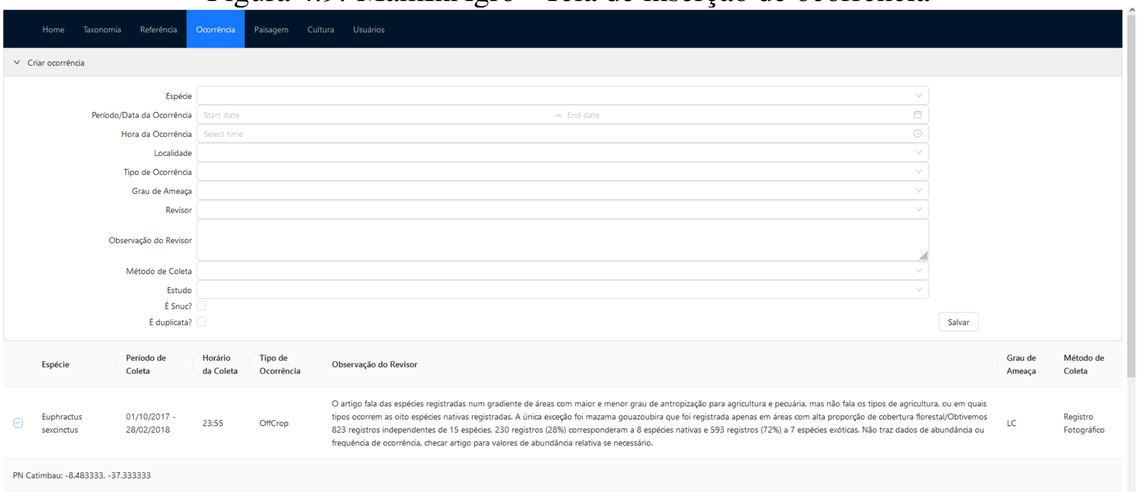
A seção de Referência possui cinco telas, da mesma forma que a anterior, sendo quatro delas correspondentes a tabelas do banco, e uma contendo um formulário com múltiplos passos, para inserção de toda a informação relacionada à Referência: a referência em si, tipos de referência, métodos de coleta do estudo e destinos de materiais pós coleta.

Aqui, as telas são compostas por um formulário no topo da página, que pode ser ocultado, e uma tabela abaixo, exibindo os dados já inseridos. No caso da referência, que pode possuir múltiplos métodos de coleta relacionados, cada linha da tabela pode ser expandida para exibi-los (Figura 4.8). O mesmo acontece na tabela de métodos de coleta, onde expandindo a linha mostra o nome da referência relacionada.

O formulário com múltiplos passos também é um pouco diferente: o primeiro passo possui dois formulários (colapsáveis, assim como nas páginas individuais), para inserção de parâmetros (Tipos de Referência e Destinos de Material), e os próximos passos permitem inserir uma referência (porém sem a tabela embaixo) e seus métodos de coleta.

4.4.3 Ocorrência

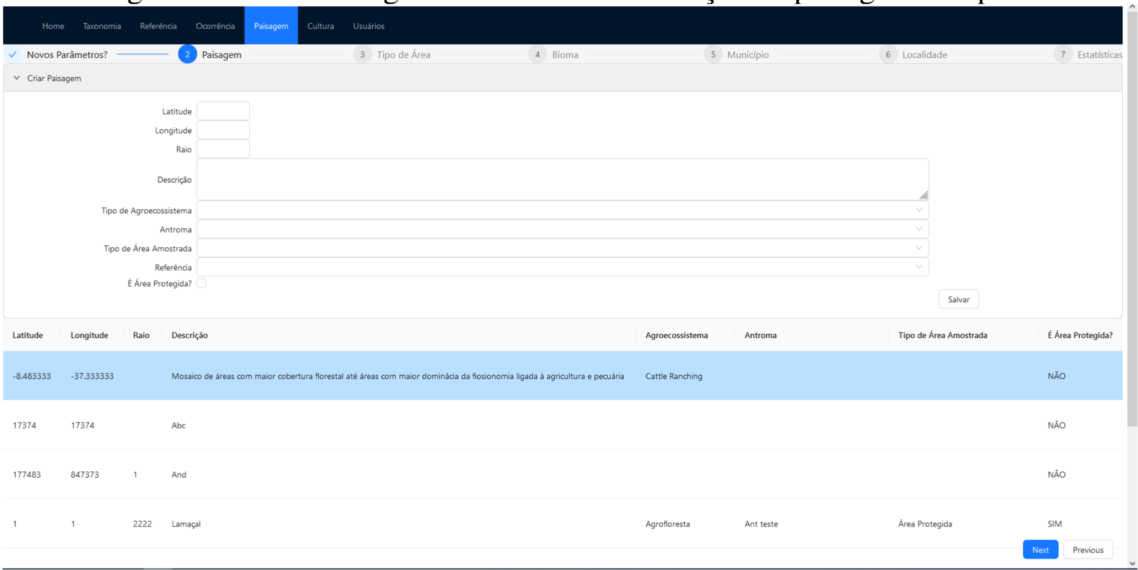
Figura 4.9: MamInAgro - Tela de inserção de ocorrência



Esta seção possui quatro telas e todas são correspondentes a uma tabela do banco de dados: Ocorrência, Graus de Ameaça, Métodos de Coleta da Ocorrência e Abundância. Apesar de possuírem relação entre si, não formavam um conjunto coeso o bastante para agregar em um único formulário. Dessa forma, Graus de Ameaça aparece no formulário da taxonomia, pois tem relação próxima com a espécie; Métodos de Coleta aparece no formulário de Cultura Completa, pois é um parâmetro da ocorrência e Ocorrência também aparece no formulário Cultura Completa.

Da mesma forma que as telas da seção anterior, essas também possuem tabelas para exibir os dados já inseridos abaixo dos formulários. Apenas a tabela de ocorrências possui linhas expansíveis, com a informação de localidade (Figura 4.9).

Figura 4.10: MamInAgro - Formulário de inserção de paisagem completa



4.4.4 Paisagem

A parte de Paisagem é a maior da aplicação, possuindo onze páginas. Dez correspondendo a tabelas únicas e uma com um formulário maior, contendo inserções que não aparecem sozinhas. Diferente das outras seções, contudo, nem todas as páginas individuais aparecem no formulário completo.

São as páginas individuais:

- Paisagem
- País
- UF
- Município
- Localidades
- Antroma
- Bioma
- Tipo de Agroecossistema
- Tipo de Área
- Tipo de Área Amostrada

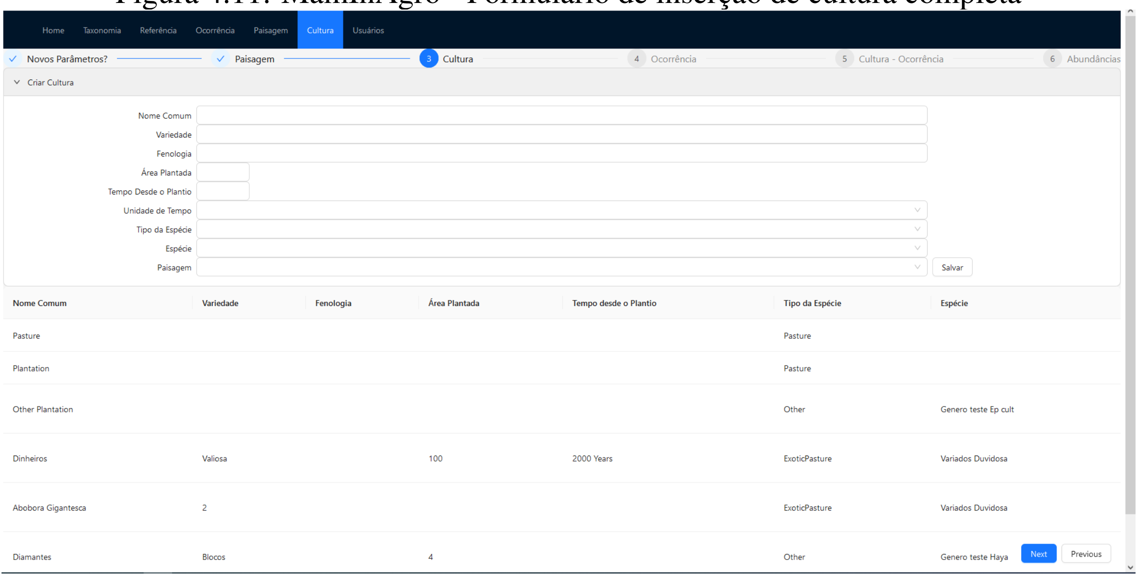
No formulário completo, da mesma forma que o anterior, esse começa com um passo para inserção de parâmetros necessários para os passos seguintes. Então permite a inserção de paisagem, ou seleção de uma pré-existente (clicando em uma linha da tabela de paisagens, como mostrado na Figura 4.10). Se o usuário optar pela inserção, a paisagem criada será referenciada pelos passos seguintes, da mesma forma que se uma linha da tabela for clicada. Nos passos seguintes, é possível salvar o relacionamento da paisagem inserida ou selecionada com diversas outras entidades:

- Tipo de Área
- Bioma
- Município
- Localidade
- Estatísticas da paisagem

Para todas as entidades seguintes à paisagem, é possível inserir várias relações para uma única paisagem, e no caso da localidade, é possível inserir uma nova, para ser relacionada, dentro do próprio passo. A localidade é salva, e depois relacionada à paisagem em um botão azul, no canto inferior esquerdo da tela. No caso das estatísticas, cada uma é relacionada a uma única paisagem, não sendo considerada um parâmetro. Por isso, a tabela abaixo do formulário pode exibir todas as estatísticas já criadas, ou apenas as relacionadas a uma paisagem, caso já tenha sido selecionada.

4.4.5 Cultura

Figura 4.11: MamInAgro - Formulário de inserção de cultura completa



Dentro de cultura temos apenas três telas: Cultura, Ocorrência-Cultura e Cultura

Completa.

A cultura é uma entidade cujos campos são fáceis de entender sozinhos, em sua maior parte, com exceção do campo de paisagem. Sendo uma entidade complexa, sem uma propriedade que facilite sua distinção das demais, fica difícil para o usuário escolher uma paisagem dentre uma lista. Da mesma forma, para a Ocorrência-Cultura, é difícil distinguir entre ocorrências, para selecionar uma dentre as várias já inseridas. Pensando nisso, o formulário de Cultura Completa começa com a já implementada inserção de parâmetros básicos, e segue com a inserção de Paisagem. Da mesma forma que na seção anterior, é possível escolher uma paisagem já inserida, a partir da tabela, ou criar uma na hora, para ser referenciada no formulário seguinte, Cultura. A cultura também pode ser criada ali (Figura 4.11), ou escolhida da lista, seguida da ocorrência. Tanto a cultura como a ocorrência aparecerão pré-selecionadas na penúltima do formulário: Ocorrência-Cultura, restando apenas os campos descrevendo as características da cultura no momento específico da ocorrência. Por fim, o formulário é encerrado com um passo para inserção de abundâncias, que podem se referir tanto a uma ocorrência (vários indivíduos de uma única vez), ou a uma paisagem (tantos indivíduos habitam determinada região).

4.4.6 Usuários

Por fim, a última seção possui apenas uma página, para inserir os dados dos revisores, responsáveis por criar os dados no sistema e adicionar suas observações, em especial dentro das ocorrências. Nesse momento, o sistema não possui autenticação, portanto não é necessário $login$ para realizar as operações dentro da plataforma, mas futuramente, quando essa funcionalidade for implementada, essa tabela vai guardar os dados dos usuários cadastrados.

5 AVALIAÇÃO DA USABILIDADE

Este capítulo dedica-se a apresentar os testes realizados por usuários na plataforma. Uma vez que o público-alvo da aplicação são pesquisadores da área, que já demonstraram interesse, auxiliando no desenvolvimento do modelo de dados utilizado, os testes foram focados na usabilidade dos formulários de inserção. Um questionário online foi utilizado como ferramenta para medir o nível de dificuldade dos usuários durante o uso.

A avaliação foi dividida em duas etapas: uma série de perguntas demográficas, a fim de caracterizar os usuários que participaram dos testes, seguidos de uma série de instruções sobre operações a serem realizadas, e escalas de dificuldade encontradas em cada uma das operações. Os voluntários também marcaram uma opção, confirmando seu consentimento na participação do experimento. O formulário completo, com todas as perguntas pode ser visto no Anexo B.

5.1 Resultados

O experimento obteve dez respostas. Metade dos respondentes se identificaram como mulheres, metade como homens (Figura 5.1). Sua faixa etária está entre 22 e 41 anos (Figura 5.2), e 80% são da área da computação, 10% da biotecnologia, e 10% têm formação em história e radiologia (Figura 5.4). Três participantes estão cursando ensino superior, cinco têm ensino superior concluído e três concluíram pós-graduação (Figura 5.3).

Na segunda etapa, os voluntários foram instruídos a explorar um menu da ferramenta por vez. Iniciando no menu taxonomia, o formulário pedia para pular a opção "Taxonomia Completa", e navegar primeiro pelos outros itens, tentando fazer algumas inserções e depois retornar à primeira opção do menu, e repetir as operações realizadas. Como mostrado na Figura 5.5, todos conseguiram realizar as operações de inserção, tanto nos formulários separados, como no formulário em conjunto. Apenas um participante reportou não conseguir relacionar duas entidades.

Seguindo para o menu referência, as instruções se repetiram, pedindo que os usuários acessassem a tela de "Estudo"apenas no final. Dessa vez, ninguém reportou não conseguir realizar as operações, e todos acharam relativamente fácil utilizar os formulários (Figura 5.6).

Figura 5.1: Gênero dos participantes

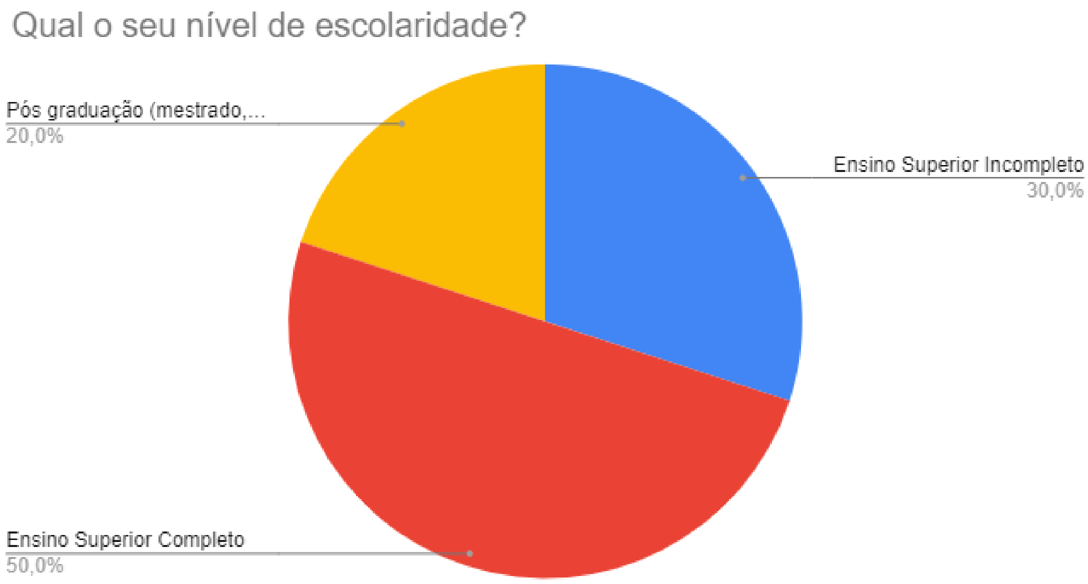
Fonte: Google Forms

No menu ocorrência, as respostas não foram tão positivas: em todas as operações, pelo menos uma pessoa não conseguiu, ou achou difícil usar os formulários. O resultado, mostrado na Figura 5.7 era esperado, pois é a seção mais abstrata da aplicação, mas demonstra bem que melhorias na interface precisam ser feitas.

O resultado se repetiu na parte de paisagens (Figura 5.8). O surpreendente foi a resposta "Não consegui"na pergunta sobre inserção de parâmetros, levantando a questão de qual foi a dificuldade encontrada: algum *bug*, ou falta de entendimento sobre as nomenclaturas? As outras operações foram completadas com sucesso por todos, mas alguns acharam difícil também.

Por fim, no menu cultura, as pessoas voltaram a achar as operações fáceis, como mostrado na Figura 5.9 (com uma exceção, no formulário de múltiplos passos, que teve um participante que não conseguiu realizar a operação). Também surpreendente, pois eram formulários complexos também, corroborando a hipótese de que o formulário com múltiplos passos facilitou o processo.

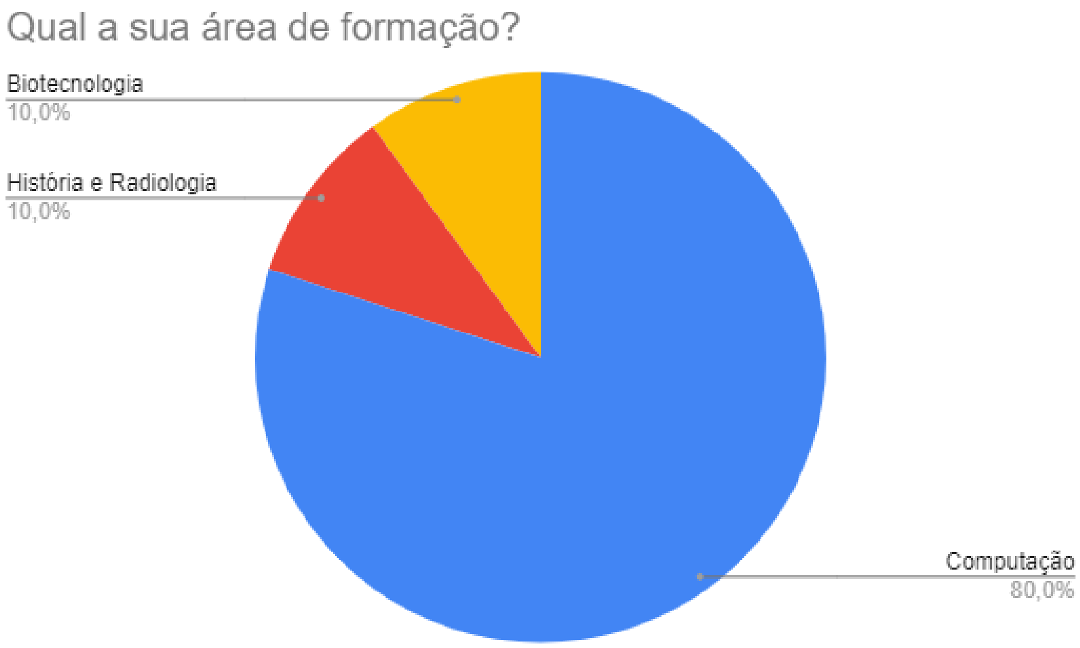
Figura 5.3: Escolaridade dos participantes



Fonte: Google Forms

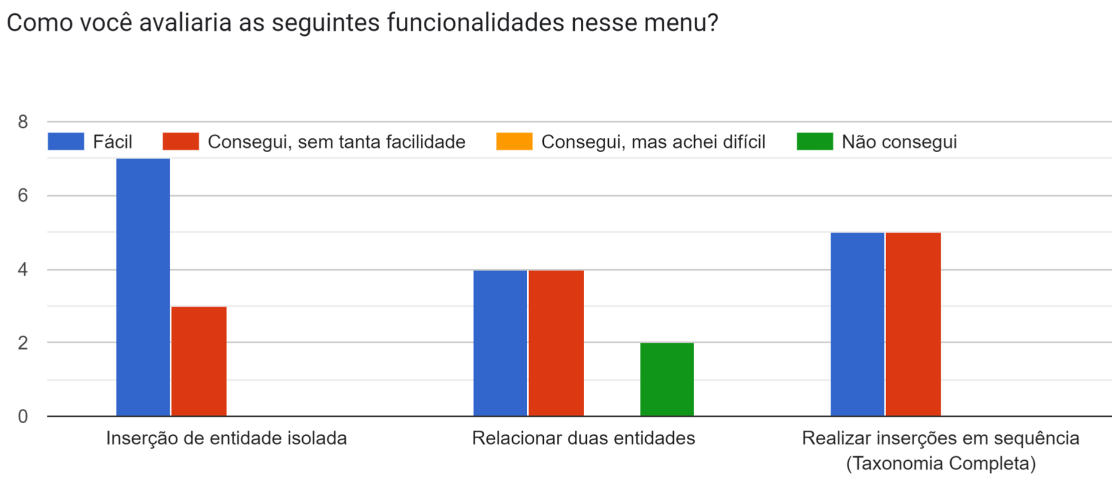
das espécies avistadas e até mesmo a evolução da saúde dos indivíduos coletados e identificados. Há bastante território para cobrir.

Figura 5.4: Áreas de formação



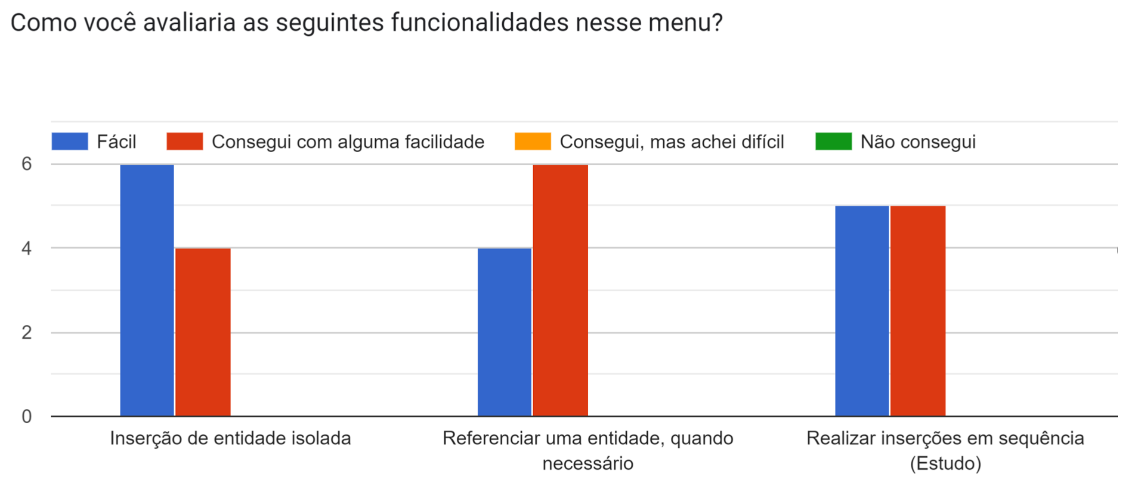
Fonte: Google Forms

Figura 5.5: Respostas do menu taxonomia



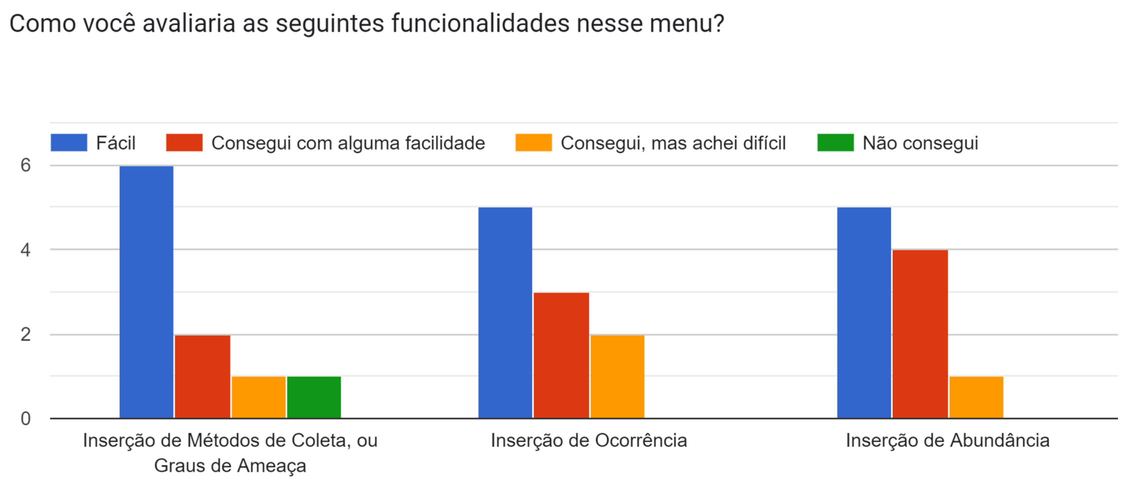
Fonte: Google Forms

Figura 5.6: Respostas do menu referência



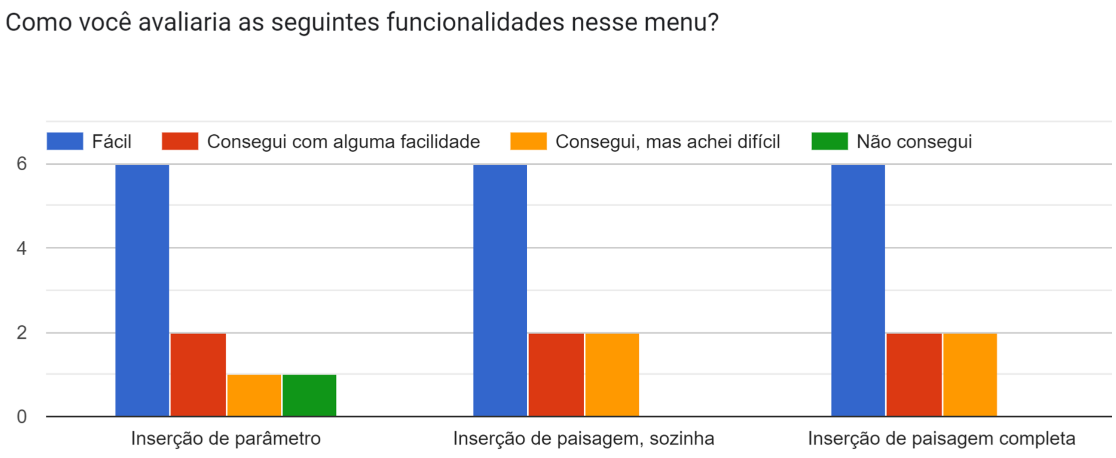
Fonte: Google Forms

Figura 5.7: Respostas do menu ocorrência



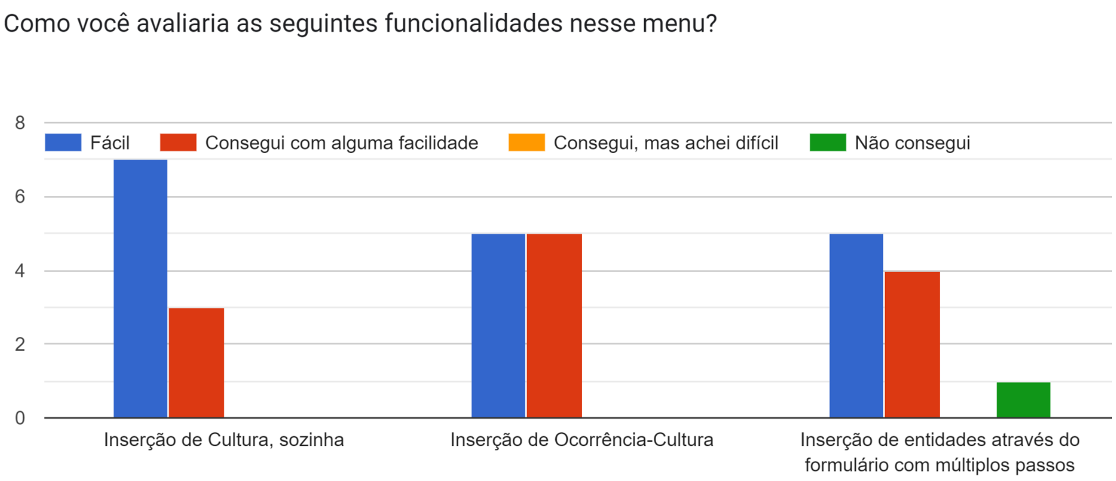
Fonte: Google Forms

Figura 5.8: Respostas do menu paisagens



Fonte: Google Forms

Figura 5.9: Respostas do menu cultura



Fonte: Google Forms

6 CONCLUSÃO

O campo de estudo da conservação da biodiversidade tem muitos desafios a enfrentar, e a coleta e organização de informações sobre o estado da fauna brasileira é um aspecto importante e complexo. Conversando com alguns voluntários, o interesse por variantes do sistema proposto é claro.

Na tentativa de auxiliar no estudo do estado da diversidade de mamíferos brasileiros, especificamente dentro de agroecossistemas, desenvolvemos um sistema, utilizando postgreSQL no banco de dados, um serviço em C#, utilizando o framework .net e ORM EF Core e uma interface web em typescript em conjunto com a biblioteca React. Cobrimos apenas um pequeno pedaço das funcionalidades necessárias para o sistema, mas já foi um bom começo.

Importante frisar que a avaliação da usabilidade precisa ser expandida, especialmente entre pesquisadores da área, que terão mais autoridade para dizer que o sistema está funcionando como deveria, ou não.

Por fim, podemos sumarizar as contribuições desse trabalho em um exemplo de utilização de ferramentas modernas para criação de um $backend$, um $frontend$ e um modelo de dados para buscar informações sobre a conservação ambiental do nosso país tão rico em sua biodiversidade. Trabalhemos para mantê-la rica.

No futuro, depois do modelo ser propriamente validado por pesquisadores da área, consultas customizadas podem ser implementadas, para permitir análises mais profundas sobre os dados. As melhorias sugeridas pelos voluntários desse trabalho também podem entrar como novos requisitos e novas tabelas podem ser adicionadas, para aumentar ainda mais a capacidade da ferramenta de caracterizar as paisagens e os animais envolvidos nas ocorrências.

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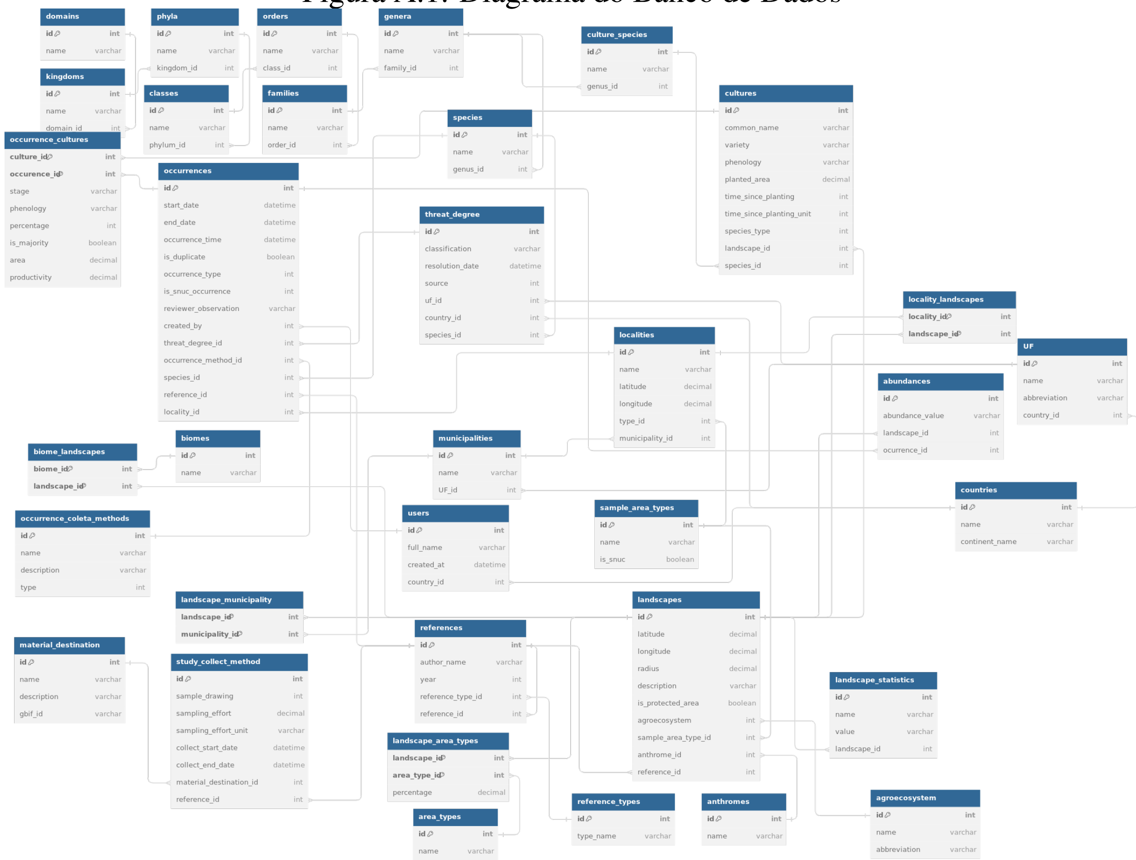
APÊNDICE A — DIAGRAMA COMPLETO DO BANCO DE DADOS

Figura A.1: Diagrama do Banco de Dados



APÊNDICE B — FORMULÁRIO DE AVALIAÇÃO POR USUÁRIOS

Avaliação da Plataforma MamInAgro

Este experimento objetiva avaliar a experiência de usuários na plataforma MamInAgro. Por favor,
leia atentamente este documento e esclareça todas as suas dúvidas antes de concordar em
participar.
Procedimentos:
Inicialmente, os usuários respondem um questionário para coletar informações de caracterização. Depois, o usuário será submetido a uma pequena lista pré-definida de atividades a
serem realizadas na plataforma, enquanto respondem perguntas relacionadas às atividades. O tempo
total do experimento será de aproximadamente 30 minutos. Os voluntários podem a qualquer
momento e por qualquer motivo interromper os testes.
Os dados obtidos ao longo do experimento serão utilizados apenas neste estudo e de forma
totalmente anônima.

* Indica uma pergunta obrigatória

1. Caso você esteja de acordo com este termo, marque a opção abaixo. *

Marcar apenas uma oval.

◯ Aceito participar deste experimento. Declaro que fui devidamente informado sobre os objetivos da pesquisa, os procedimentos envolvidos nos testes aos quais vou me submeter e foi-me garantido o sigilo de minhas informações e o direito de retirar minha participação a qualquer momento.

Questões Demográficas

Esse pedaço do questionário possui como objetivo caracterizar o usuário, para que mais tarde possa-se identificar características que deem direções para a melhoria da plataforma.

2. Qual a sua idade?

3. Qual a sua área de formação?

____

4. Qual o seu nível de escolaridade?

Marcar apenas uma oval.

- ( ) Ensino Médio Incompleto
- ( ) Ensino Médio Completo
- ( ) Ensino Superior Incompleto
- ( ) Ensino Superior Completo
- ( ) Pós graduação (mestrado, doutorado) Incompleto
- ( ) Pós graduação (mestrado, doutorado) Completo

5. Qual sua identidade de gênero?

Marcar apenas uma oval.

- ( ) Mulher
- ( ) Homem
- ( ) Gênero não-binário

6. Qual seu nível de experiência com internet?

Marcar apenas uma oval.

	1	2	3	4	5	
Tota	( )	( )	( )	( )	( )	Muito experiente

Atividades na plataforma - Taxonomia

Entrando na plataforma, em:
https://ashy-moss-09f60e010.4.azurestaticapps.net

Agora vamos percorrer a plataforma, executando algumas atividades, para avaliar sua experiência de usuário.
Primeiro, vamos percorrer o menu "Taxonomia".
Pulando a opção "Taxonomia Completa", navegue pelas outras opções do menu.
Observe os formulários, tente salvar dados em algum (ou alguns) deles.
Depois, volte para "Taxonomia Completa", e repita as operações.

7. Como você avaliaria as seguintes funcionalidades nesse menu? *

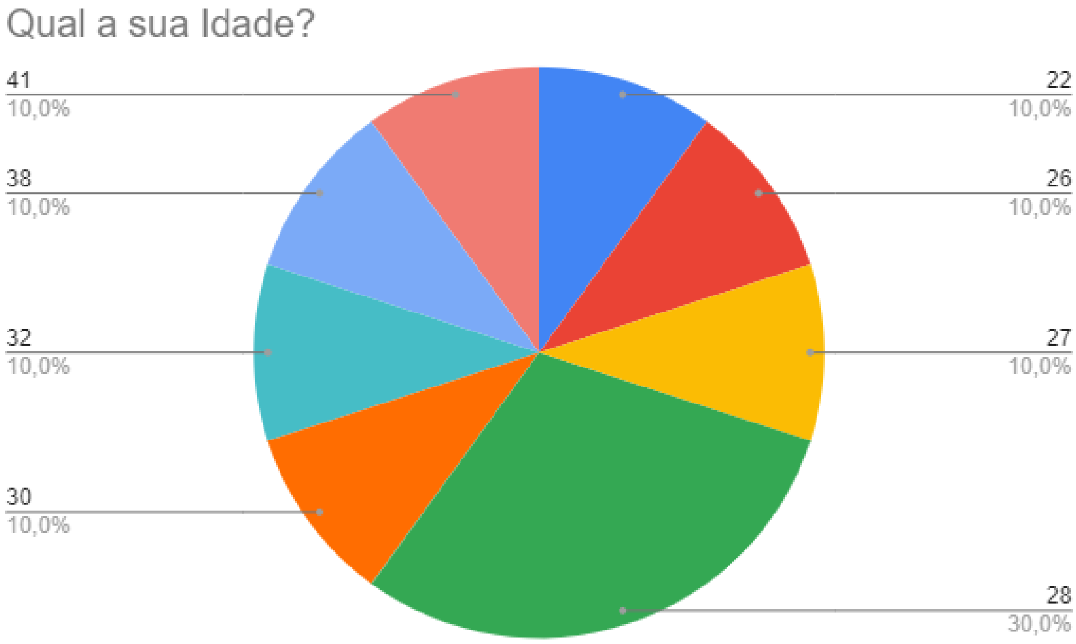
Marcar apenas uma oval por linha.

	Fácil	Consegui, sem tanta facilidade	Consegui, mas achei difícil	Não consegui
Inserção de entidade isolada	◯	◯	◯	◯
Relacionar duas entidades	◯	◯	◯	◯
Realizar inserções em sequência (Taxonomia Completa)	◯	◯	◯	◯

Atividades na plataforma - Referência

Agora, vamos para o segundo menu: Referência.
Da mesma forma, comece pulando a primeira opção (Estudo). Navegue pelas telas, e tente inserir algum (ou alguns) dado(s).
Depois, volte para a opção Estudo, e repita as operações.

Figura 5.2: Faixa etária dos participantes



Fonte: Google Forms

5.2 Considerações e Próximos Passos

Os resultados negativos eram esperados, devido à complexidade do sistema, às mínimas instruções e à grande maioria ser da área da computação, e não ter conhecimento sobre algumas das terminologias utilizadas. Apesar disso, a maioria ainda achou o sistema de fácil utilização, mostrando que com algumas melhorias simples, a utilização pode ser facilmente acessível.

Dentre os comentários no campo de sugestões, ao final da pesquisa, alguns apontaram a falta de responsividade da plataforma em dispositivos móveis, e outros pediram clarificação sobre as nomenclaturas e um guia no primeiro acesso.

Fora isso, a plataforma ainda tem muito escopo a ser desenvolvido. O trabalho não incluiu a exploração de visualizações dos dados, filtros, ou maneiras de permitir ao usuário customizar consultas. A adição de mapas, tanto para entrada de informações nos formulários, quanto para visualização das localizações inseridas também é algo pensado para o futuro. Além disso, poderíamos importar dados diretamente do SiBBr, ou do próprio GBIF, mas um mapeamento dos nomes dos campos seria necessário.

O próprio banco de dados também pode ser expandido, permitindo ampliar a caracterização das culturas, incluindo informações de agrotóxicos, características biológicas

8. Como você avaliaria as seguintes funcionalidades nesse menu? *

Marcar apenas uma oval por linha.

	Fácil	Consegui com alguma facilidade	Consegui, mas achei difícil	Não consegui
Inserção de entidade isolada	⬭	⬭	⬭	⬭
Referenciar uma entidade, quando necessário	⬭	⬭	⬭	⬭
Realizar inserções em sequência (Estudo)	⬭	⬭	⬭	⬭

Atividades na plataforma - Ocorrência

Prosseguindo para a próxima seção, a dificuldade aumenta um pouco.
Para a inserção de uma ocorrência, é necessário inserir uma Localidade primeiro (sob o menu Paisagem) e um Usuário (no menu Usuário).
Esse menu também não possui uma opção para reunir todas as entidades em uma só.

9. Como você avaliaria as seguintes funcionalidades nesse menu? *

Marcar apenas uma oval por linha.

	Fácil	Consegui com alguma facilidade	Consegui, mas achei difícil	Não consegui
Inserção de Métodos de Coleta, ou Graus de Ameaça	⬭	⬭	⬭	⬭
Inserção de Ocorrência	⬭	⬭	⬭	⬭
Inserção de Abundância	⬭	⬭	⬭	⬭

Atividades na plataforma - Paisagem

Essa é a maior seção da plataforma.
Explore os menus de parâmetros, e depois tente inserir uma Paisagem.
Então, repita a operação pela opção "Paisagem Completa", seguido de informações adicionais da Paisagem.
Note que no passo "Paisagem", em "Paisagem Completa", se você desejar reutilizar uma paisagem que já existe, basta clicar na sua linha, na tabela de Paisagens.

10. Como você avaliaria as seguintes funcionalidades nesse menu? *

Marcar apenas uma oval por linha.

	Fácil	Consegui com alguma facilidade	Consegui, mas achei difícil	Não consegui
Inserção de parâmetro	◯	◯	◯	◯
Inserção de paisagem, sozinha	◯	◯	◯	◯
Inserção de paisagem completa	◯	◯	◯	◯

Atividades na plataforma - Cultura

Para a última parte da plataforma, temos a parte de Cultura, e retomada da Ocorrência.
Comece inserindo uma Cultura, seguido de uma Ocorrência-Cultura.
Então prossiga para "Cultura Completa", para inserção de várias possíveis entidades.
Da mesma forma que em "Paisagem Completa", é possível selecionar Paisagens, Ocorrências e Culturas pré-existentes nas suas respectivas tabelas.

11. Como você avaliaria as seguintes funcionalidades nesse menu? *

Marcar apenas uma oval por linha.

	Fácil	Consegui com alguma facilidade	Consegui, mas achei difícil	Não consegui
Inserção de Cultura, sozinha	◯	◯	◯	◯
Inserção de Ocorrência-Cultura	◯	◯	◯	◯
Inserção de entidades através do formulário com múltiplos passos	◯	◯	◯	◯

Comentários

12. Se desejar, por favor explique as dificuldades que encontrou no processo, ou deixe sugestões para melhoria

ANEXO 2

SALT-ASSISTED SOLID-LIQUID MINIATURIZED EXTRACTION FOR THE DETERMINATION OF PESTICIDES IN LIVER AND KIDNEY TISSUES OF WILDLIFE RODENTS FROM THE SOUTH AMERICAN WETLAND PANTANAL BY UHPLC-MS/MS AND GC-MS/MS (*In prep.)*

SALT-ASSISTED SOLID-LIQUID MINIATURIZED EXTRACTION FOR THE DETERMINATION OF PESTICIDES IN LIVER AND KIDNEY TISSUES OF WILDLIFE RODENTS FROM THE SOUTH AMERICAN PANTANAL WETLAND BY UHPLC-MS/MS AND GC-MS/MS

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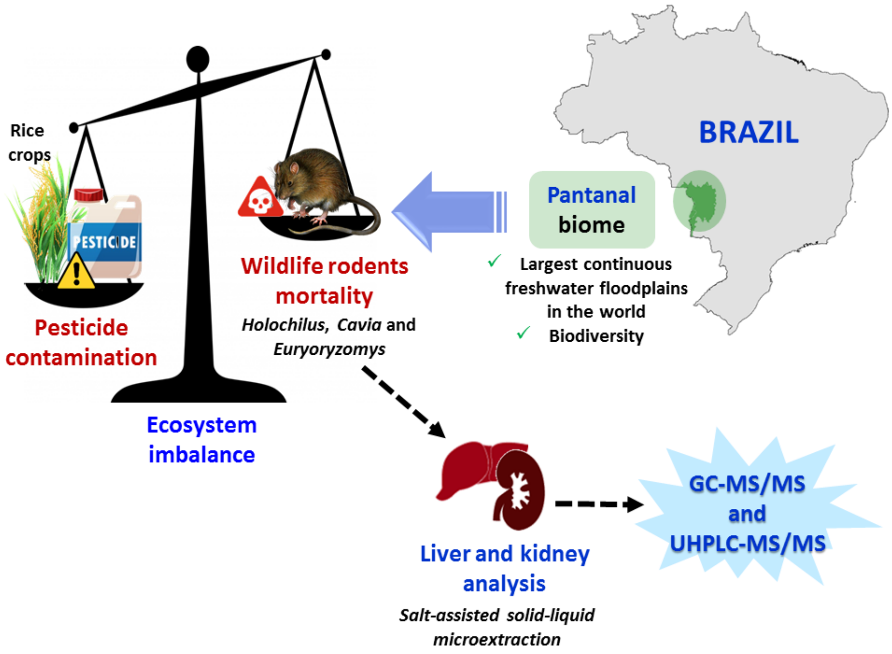
GRAPHICAL ABSTRACT



ABSTRACT

A methodology using UHPLC-MS/MS and GC-M/MS was developed to determine 138 pesticide residues in liver and kidney tissues of wildlife rodents of the genus *Holochilus*, *Cavia* and *Euryoryzomys* from the South American Pantanal wetland. The sample preparation employed ice-cold acetonitrile for extraction followed by a simple salt-assisted solid-liquid microextraction (SALLE) with NaCl aqueous solution. The proposed methods were validated according to the European Commission guideline SANTE/12682/2021. Linearity presented $r^2 \geq 0.990$ for all analytes in both chromatographic techniques, and recoveries ranged from 70 to 120% with RSD ≤20%. For 136 analytes the limit of quantitation (LOQ) was 10 μg kg^{-1}, that corresponds to limit of detection (LOD) of 3 μg kg^{-1}. Only acetochlor and cycloate, analyzed by GC-MS/MS, presented LOD and LOQ of 12 and 40 μg kg^{-1}, respectively. Seven pesticides were detected in a range of 1.6 (<LOQ) to 142 μg kg^{-1}in a total of eighty samples from forty individuals. The results demonstrated that the method developed provides reliable, simple, and rapid determination of a wide range of pesticides in wildlife tissues, being useful for routine analyses of environmental monitoring.

Keywords: microextraction, pesticide residues, mammals in crop, rice paddy

Introduction

The latest UN projections suggest that the world's population could grow to 8.5 billion in 2030 and 9.7 billion in 2050, before peaking at around 10.4 billion people during the 2080s (UN DESA, 2022). As a consequence of wanting to ensure crop yields for a growing population, there has been an exponential increase in the use of pesticides (Geissen et al., 2021). More than 500 active ingredients are used in the formulation of synthetic products, belonging to more than 100 chemical classes and different modes of action (Bernhardt et al., 2017). Since 2008, Brazil has led the world ranking of pesticide consumption (Albuquerque et al., 2016), with a market valued at USD $10 billion per year (Bombardi, 2019).

The role of pesticides as drivers in the loss of agricultural biodiversity has been debated worldwide: recently, a review pointed to chemical pollution, including pesticides, as the second most important factor for the worldwide decline in insect populations (Sánchez-Bayo and Wyckhuys, 2019), with effects on the population reduction of pollinators and on natural enemies of crop pests (Sánchez-Bayo, 2021), adverse effects on the immune system of bees (Di Prisco et al., 2013), delays in metamorphosis and population declines of anurans (Freitas et al., 2017; Kenko, 2022), bioaccumulation in mammals (Oliveira et al., 2021; Fritsch et al., 2022), and population reduction in birds (Biswas et al., 2022). These effects are worrying as key and abundant species anchor trophic interactions and are responsible for vital ecosystem services in their communities.

The knowledge gaps of the toxic effects of pesticides, the degree of contamination, biomagnification of compounds, entry routes of molecules into ecological processes, and their impacts on ecosystem services make risk mitigation challenging (Bruhl and Zaller, 2019). A key step to risk assessment is to characterize the exposure of non-target species in their natural habitats in a multiscale approach, assessing the spatiotemporal patterns of contamination pathways. However, basic information about the exposure and monitoring of wildlife on contamination by pesticides is lacking (Fritsch et al., 2022).

The environmental risk analysis (ERA) of pesticides, evaluates the physicochemical properties of the active substance and their toxicity through acute and chronic tests mostly on laboratory rats and mice (US EPA, 1993; EFSA, 2009). In the United States of America and in the European Union wildlife species are part of ERA either because they are endangered (US EPA) or because they are focal species (abundant, easily captured, high metabolism, diverse diet, foraging and size) in post-registration surveys (EFSA, 2019). However, Brazil lacks pesticide risk assessment for animals, except for bees

(Cham et al., 2017), despite its high biodiversity and large agricultural sector. Brazil is also in need of adequate monitoring of pesticide residues in freshwater (Albuquerque et al., 2016) despite having a large freshwater system. As a whole risk to ecosystem services at large scales can seldom be evaluated (but see Roque et al. 2019). The Pantanal biome, one of the most important wetlands on the planet (Jardim et al., 2020), extends over 179,300 km² in Brazil (78%), Bolivia (18%) and Paraguay (4%). Despite harboring an important biodiversity, including several endangered species, only 5,71% are under protected areas and 93% under private properties (Tomás et al., 2018). Within this context our study site is a farm that combines wildlife sighting tourism, irrigated rice cultivation, livestock farming and tree plantation. Ecotourism represents the majority of the net gains, driven mainly by high frequency of sightings of birds and mammals in the agricultural area compared to areas of natural vegetation was observed (Concone and Giordano, 2013), with species such as the ocelot *Leopardus pardalis* and also endangered mammals such as the jaguar *Panthera onca*, the giant anteater *Myrmecophaga tridactyla* and the marsh deer *Blastocerus dichotomus* (IUCN, 2022). Furthermore, the most common rodents in rice fields are, in decreasing abundance, *Holochilus chararius* the marsh rat, *Cavia aperea* the cavy and *Necromys lasiurus* the field rat (Gomes de Sá et al., 2024). Rice paddies are favorable for the survival and abundance of these species, which attracts carnivores, which drives tourism.

These rice paddies, with an abundant biodiversity, are regularly treated with the use of pesticides (EFSA, 2009; Parsons et al. 2010) and thus a relevant environment for pesticide monitoring initiatives. Additionally rice planting in the study area is carried out strictly during the dry season, between April and September (Harris et al., 2005). In the rainy season, plains are flooded (Alho and Sabino, 2011), and pesticides residues can spread over long distances throughout the Upper Paraguay Basin (BAP) due to runoff and leaching processes (Roque et al., 2021). Thus, pesticide quantification in our study site has important consequence at the population levels because of the abundance of wildlife, at the species levels, including endangered species, at the community level because of intense trophic interactions at the site and because of ecosystem consequences through runoff and leaching.

Within this multi-level approach, exposure and synergy of compounds are of utmost importance since most are unknown in wildlife. The use of multi-residue analyses constitutes an important tool to better understand the accumulation processes of complex mixtures (Scholz et al., 2022), which may be caused by simultaneous exposure to various compounds, or by successive and cumulative exposures (Fritsch et al., 2022). For multi-residue determination of pesticides in general and in wildlife animal the use of the techniques of ultra-high performance liquid chromatography and gas chromatography both coupled with tandem mass spectrometry, UHPLC-MS/MS and GC-MS/MS respectively, are the most suitable and allow analysis with high selectivity and sensitivity (Jesús et al., 2018; Schanzer et al., 2021).

Finally, since small mammals are short-lived and abundant in agricultural fields worldwide (Bilenca et al., 2007; Braga et al., 2015; Phukon and Borah, 2019), and occupy key positions in trophic networks as the diet of several species of carnivores (Kaufman et al., 1998), they should be sensitive to the exposure to pollutants (Marcheselli et al., 2010). We established a multi-residue method for the determination of pesticides in liver and kidney tissues of wildlife rodents using salt-assisted solid-liquid miniaturized extraction and analysis by UHPLC-MS/MS and GC-MS/MS, following the application in these small mammals captured in the Brazilian Pantanal.

Materials and methods

Chemicals, reagents, materials and equipments

All analytical standards were obtained from LGC Standards (London, United Kingdom) and Sigma-Aldrich (USA). Atrazine-d5 was used as surrogate standard (SS) and triphenyl phosphate as internal standard (IS). In the case of SS, a solution containing 20 µg L^{-1} in acetonitrile (MeCN) was prepared and stored at -5 °C. Individual stock standard solutions (1000 mg L^{-1}) of each compound were prepared in appropriate solvent HPLC grade (acetonitrile, methanol and toluene, purchased from Merck, Germany) considering the purity of solid standard, and stored at -5 °C. From the stock solutions, individual solutions at 10 mg L^{-1} were prepared and kept at -5 °C. The mixture solutions (10 mg L^{-1}) were prepared in acetonitrile and also stored at -5 °C.

Eppendorf microtubes (2 mL) and 0.22 µm nylon syringe filters were purchased from Axygen Scientific (EUA) and Agilent Technologies (EUA), respectively. Ultra Purified water was obtained with a Milli-Q Direct UV3 system from Millipore (France). Ammonium formate, and sodium chloride were purchased from Sigma-Aldrich (St. Louis, USA). Formic acid 98.5% was acquired from Sigma-Aldrich (Darmstadt, Germany).

The equipment used in our work included a Vortex mixer model QL-901 (Microtécnica, Brazil), analytical balance AUW-220D from Shimadzu (Japan), refrigerated centrifuge model SL703 (SoLab Científica, Brazil) and automatic micropipettes (Brand, Germany).

Instrumentation

GC-MS/MS analysis

The analyses were performed with an Agilent Intuvo 9000 gas chromatograph (Santa Clara, CA, USA) equipped with an Agilent 7693 autosampler and coupled with an Agilent 7010B triple quadrupole mass spectrometer with a high efficiency source (HES). The separation was performed through a HP-5 MS UI planar column (30 m x 0.25 mm x 0.25 µm) coupled with a guard-chip as pre-column (150 ºC). The injector temperature was 280 ºC, the injection volume was 1 µL in splitless mode using an ultra-inert inlet liner with glass wool. High purity helium (99.999%) was used as carrier and quench gas, and nitrogen as collision gas. The carrier gas was used under constant flow rate at 1.2 mL min^{-1}. The oven temperature was programmed as follows: 60 °C for 1 min; 40 °C min^{-1} to 170 °C, and then 10 °C min^{-1} to 310 °C and hold for 3 min. The total run time was 20.75 min. Transfer line temperature was 300 °C. The electron-ionization ion source was operated at 300 °C for source temperature and 150 °C for quadrupole temperature. The dynamic multiple reaction monitoring mode was used for data acquisition. For the quantification and identification of the pesticides, two transitions were used, where the highest intensity was chosen to quantify and the 2nd most intense was used to confirm the compounds.

UHPLC-MS/MS analysis

Samples were analyzed using a Waters Acquity UPLC system with Xevo TQ-XS mass spectrometer equipped with electrospray ionization source (Milford, USA), nitrogen generator (Peak Scientific, Inchinnan, Scotland) and argon gas 6.0 (White Martins, Rio de Janeiro, Brazil) as collision gas for the MS/MS system. An Acquity UPLC™ HSS T3 (100 x 2.1 mm, 1.8 µm) column and mobile phase consisted of (A) ultrapure water and (B) methanol:acetonitrile (1:1, v/v), both with 0.1% (v/v) formic acid and 5 mmol L^{-1} ammonium formate at flow rate 0.500 mL min^{-1} were employed. The ionization source by electrospray operated in positive and negative mode (ESI+ and ESI-) and mass analyzer in selected reaction monitoring (SRM) mode with two transitions for each pesticide, where the highest intense transition was chosen for quantification and the 2nd most intense for identification of the pesticides.

Samples

During September and November 2021, forty free-ranging rodents were collected from one rice paddy system in the south Pantanal (-20.086050; - 56.614764). The animals were collected from two sites: *Cavia aperea* and *Holochilus chacarius* in the rice fields, and *Euryoryzomys nitidus* in the legal reserve (a protected area in the agroecosystem with remnants of natural vegetation).

Rodents were captured using live traps baited with a mixture of banana, oats, sardines, and vanilla essence. The specimens were then transported to the laboratory located in the field, where the animals were sedated using a combination of ketamine and acepromazine (0.1 mL/100 g) and then sacrificed by cardiac puncture. All procedures involving the handling of animals were conducted by certified personnel and treated following legal and ethical procedures (ICMBio/MMA nº 72681/ CEUA nº 9192091019). The liver and kidneys were immediately removed, weighed, and frozen at -20°C prior to chemical analyses.

Capybara liver and kidney were used as blank matrices for sample preparation and method validation experiments. The capybara was chosen because it belongs to the same order (*Rodentia*) as the study rodents (*Cavia aperea*, *Euryoryzomys nitidus* and *Holochilus chacarius*). Before being used in subsequent experiments, all blank matrices were tested for the analytes of interest. The pesticides were not found in both blank sample matrices.

Sample Preparation

The sample preparation consisted in a simple salt-assisted solid-liquid microextraction method. 0.100 (± 0.010) g of liver or kidney samples were weighed into a microtube and spiked with the mixture solutions. Then, 400 µL of ice-cold MeCN (< -10 °C) solution with SS (20 µg L^{-1}) were added, and the samples were shaken immediately using a vortex for 1 minute. Afterwards, 75 µL of 5 mol L^{-1} NaCl aqueous solution were added to the tubes, followed by vortexing (1 min) and centrifugation (10000 rpm, -10 °C for 10 min). A portion of the organic layer was diluted 1:1 (v/v) in ultrapure water and filtered in nylon syringe filters (0.22 µm) prior injection into the UHPLC-MS/MS system. Another portion of the organic layer was collected, filtered in nylon syringe filters (0.22 µm) and injected into the GC-MS/MS system.

Results and discussion

Evaluation of method conditions

Evaluation of extraction procedure

All studied analytes evaluated during the experiments are listed in Table S1. Initially, three extraction methods were evaluated in capybara liver, since this matrix becomes more complex than kidney (e.g., fatty substances and proteins), and also to achieve the best method of extraction of pesticides. The number of pesticides to be determined by UHPLC is greater than in GC (> 100 compounds) and, therefore, the initial tests were injected only in this system.

A miniaturized modified QuEChERS and MSPD methods were compared with the salt-assisted solid-liquid microextraction. All experiments were performed using 0.100 g of liver, 400 µL of ice-cold MeCN and 400 µL 5 mol L^{-1} of NaCl aqueous solution. For pesticides recovery, all experiments were spiked at 10 µg kg^{-1} (n= 4). The salt-assisted solid-liquid microextraction was conducted by extracting 0.100 g of liver with iced MeCN in microtubes after spiking with the studied pesticides. Then, the saturated saline solution was added to promote partitioning of aqueous and organic phases. For the modified QuEChERS, a d-SPE step was performed after the partitioning using the saturated NaCl solution, using 20 mg of $MgSO_4$, 10 mg C18 and 10 mg of PSA. The MSPD method was performed using 100 mg of C18 with 0.100 g of liver and two ceramic homogenizers, addition of ice-cold MeCN and the NaCl aqueous solution. All the microtubes were submitted for centrifugation at 10000 rpm for 10 minutes at -10 °C and the supernatants of the three experiments were diluted 2-fold in ultrapure water before UHPLC-MS/MS analysis.

The preliminary results indicated that miniaturized QuEChERS provided inadequate average recovery value (134%) for almost all pesticides.

Choice of extraction solvent and salts

Protein precipitation is a simple sample preparation and significantly reduces the matrix effect. Previous studies have shown that low-temperature organic solvents can contribute to protein precipitation in human and animal livers (Kim et al., 2020; Kadar et al., 2019; Bussy et al., 2018), and then we proposed to use ice-cold MeCN instead of MeCN at room temperature. MeCN is the most commonly organic solvent used in multiresidue pesticide methods and animal tissue sample preparation, allowing the extraction of pesticides in a wide range of polarities and reducing the solubility of matrix coextractives (proteins, lipids, etc) at the same time (Moloney et al., 2018). In this study, the addition of formic acid (1% v/v) to ice-cold MeCN was used to improve the extraction efficiency of herbicide pesticides such as 2,4-D, imazapyr and imazethapyr. The experiments with acid were conducted using or not the drying agent magnesium sulfate ($MgSO_4$). The use of acid resulted in higher overall pesticide recoveries (120-150%) whether or not $MgSO_4$ is used, indicating the presence of proteins bound to pesticides (Hajeb et al., 2022). Formic acid is used to precipitate proteins as well as to aid in the release of compounds of interest from the sample (Park et al., 2021). However, the use of high acid concentration may induce more presence of proteins and affect the detection of pesticides. For this reason, it was preferred not to use formic acid and $MgSO_4$ in subsequent experiments.

Effect of NaCl aqueous solution

Sodium chloride (NaCl) is an effective and widely used salt to induce phase separation of MeCN from aqueous phase. High concentrations of salt reduce the solubility of analyte in the aqueous phase and increase its partition into the organic phase (Torbati et al., 2019; Farajzadeh et al., 2014). The concentration and volume of NaCl aqueous solution has a significant impact on protein precipitation and transfer of polar analytes (e.g. acephate, carbendazim, methamidophos) during extraction performance of the SALLE, respectively. According to Abolghasemi et al. (2020) better extraction will be achieved by saturating aqueous solutions with NaCl. In our study, we employed 5 mol L^{-1} of NaCl aqueous solution and investigated the range of 75 to 400 µL in order to induce the separation between organic and aqueous phases. The volume was selected visually (Figure S1). At higher volumes, more volume of organic phase than aqueous phase was observed, indicating the existence of dissolved water in the organic phases. The experiment conducted using 50 µL was unable to promote the phase separation. At 75 µL, it was able to notice a clear extract after phase separation and collect aliquots for both chromatographic systems. Then, this volume was chosen for the validation process.

Method validation

Under optimal conditions, the proposed method was validated according to SANTE/11312/2021 (European Commission, 2021). Parameters such as matrix effect (ME), linearity, accuracy, precision, in terms of repeatability and intermediate precision, limit of quantification (LOQ) and limit of detection (LOD) were determined. The selectivity was ensured by the use of a selected reaction monitoring (SRM) working method and by the specific retention time of each analyte. No interfering peaks from other compounds were found in the range of the retention times of the selected pesticides. All of the following validation data are summarized in Table 1.

Chromatographic analyses coupled to mass spectrometry are susceptible to matrix effects due to ionization suppression or enhancement of the analyte/matrix combination in the ionization source. These effects could affect the reliability of results (Raposo; Barceló, 2021).

The ME of the proposed method was evaluated by comparison of the slopes of the calibration curves prepared with or without the matrix, using the following equation:

$ME (\%) = (X_2 - X_1)/X_1 \times 100$

where X_2 corresponds to the slope of the curves prepared in the blank matrix extract (capybara liver) and X1 corresponds to the slopes of the curves prepared in solvent (acetonitrile).

According to the SANTE/11312/2021 guideline (European Commission, 2021), ME is significant when higher than ±20%. Positive values denote an enhanced ion signal and negative values represent an ion signal suppression (Raposo; Barceló, 2021). Results shown in Table 1 indicate that all of the compounds investigated by UHPLC-MS/MS presented negative matrix effects, except for triadimefon which exhibited a positive value of 4.5%. An ion suppression higher than -20% was measured for thirteen pesticides, while the other compounds presented values within the range of acceptable. In contrast, high values of positive ME were obtained for most of the analytes determined by GC-MS/MS, showing great enhancement of their signals. Therefore, the matrix-matched calibration procedure was applied for the validation experiments and sample analysis by both techniques.

Matrix-matched calibration curves were prepared by spiking a mixture of the studied analytes in blank capybara liver and kidney extracts (n= 3). Seven calibration standards ranging from 4 to 400 μg kg^{-1}, corresponding to 0.5 to 50 μg L^{-1}, were plotted for the UHPLC-MS/MS analysis, and six calibration standards ranging from 20 to 800 μg kg^{-1} (5 to 200 μg L^{-1}) for the GC-MS/MS analysis. Determination coefficients (r^2) were >0.990 for all analytes in both chromatographic techniques indicating good linearity.

Accuracy and precision were evaluated considering the recovery results from blank capybara liver and kidney samples spike at 5, 10 and 20 μg kg^{-1} for UHPLC-MS/MS and 10, 20 and 40 μg kg^{-1} for GC-MS/MS (n= 7).

Considering the validation results presented in Table 1, 28 pesticides were validated using GC-MS/MS with the selected conditions achieving practical method LOQ of 10 μg kg^{-1}, that corresponds to a method LOD of 3 μg kg^{-1}, for most of the pesticides with exception of acetochlor and cycloate that LOD and LOQ were 12 and 40 μg kg^{-1}, respectively. These levels are very adequate for monitoring programs. Using UHPLC-MS/MS, 110 compounds were validated achieving practical method LOQ of 10 μg kg^{-1} and method LOD of 3 μg kg^{-1} for all pesticides.

Insert Table 1

Application of the proposed method in wildlife rodents

The established method was applied to the analysis of 80 samples of liver and kidney from wildlife rodents. The results are presented in Table 2. Six pesticides (acephate, methamidophos, metconazole, tebuconazole, thiacloprid and tricyclazole) were detected in 10 samples of *Holochilus chacarius,* while clethodim and thiacloprid were detected in 4 samples of *Cavia aperea*. Figure 1 presents a comparison of the UHPLC-MS/MS signals for the pesticide tricyclazole for a blank sample, the method LOQ, samples of liver L1 and L6.

Insert Table 2

Insert Figure 1

Conclusions

A multi-residue method was developed and validated for the determination of 138 pesticides in kidney and liver samples from wildlife rodents. The sample preparation method utilizes protein precipitation combined with salt assisted solid-liquid microextraction prior to analysis by GC-MS/MS and UHPLC-MS/MS. From 80 samples of kidney and liver analyzed, seven pesticides were detected in a range of 5 to 142 µg kg-1. The method was able to analyze a large number of pesticides even in very small amounts of samples (100 mg). In addition, this method is easy, rapid, and simple to perform, being useful for routine analyses.

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Table 1. Validation results for the proposed: matrix effect (ME), method LOQ, accuracy (recovery), and precision, in terms of repeatability and intermediate precision

	Analyte UHPLC-MS/MS	ME (%)	LOQ µg kg^{-1}	Matrix = Rodent kidney						Matrix = Rodent liver					
				Repeatability (n= 7)			Intermediate precision (n=7)			Repeatability (n =7)			Intermediate precision (n=7)		
				5	10	20	5	10	20	5	10	20	5	10	20
				Rec (RSD), %			Rec (RSD), %			Rec (RSD), %			Rec (RSD), %		
1	Acephate	-11.2	5	103 (9)	96 (8)	87 (14)	84 (9)	74 (6)	103 (12)	92 (4)	92 (4)	80 (13)	93 (9)	82 (8)	85 (3)
2	Acetamiprid	-25.8	5	96 (18)	109 (12)	104 (14)	91 (10)	86 (7)	100 (10)	81 (9)	80 (18)	98 (15)	94 (8)	82 (3)	93 (6)
3	Ametryn	-18.3	5	82 (10)	113 (7)	110 (15)	89 (12)	85 (5)	102 (6)	88 (17)	83 (18)	79 (14)	96 (9)	87 (6)	79 (3)
4	Atrazine	-15.0	5	110 (13)	106 (8)	105 (15)	110 (9)	108 (7)	114 (11)	106 (13)	99 (16)	83 (15)	118 (9)	97 (7)	115 (5)
5	Azaconazole	-15.3	5	99 (8)	119 (6)	112 (15)	116 (11)	102 (4)	112 (12)	112 (11)	101 (13)	92 (13)	111 (9)	110 (9)	115 (3)
6	Azoxystrobin	-10.7	5	99 (11)	117 (9)	105 (17)	101 (14)	93 (7)	110 (13)	115 (17)	102 (13)	95 (13)	119 (10)	104 (10)	112 (3)
7	Benalaxil	-12.4	5	98 (9)	104 (7)	110 (19)	90 (14)	82 (6)	95 (7)	109 (18)	98 (15)	74 (11)	103 (6)	89 (5)	77 (2)
8	Bitertanol	-2.0	5	114 (15)	113 (6)	112 (18)	87 (18)	85 (3)	103 (17)	99 (18)	93 (15)	90 (14)	71 (19)	89 (9)	106 (4)
9	Buprofezin	-17.6	5	104 (9)	101 (9)	96 (17)	71 (19)	87 (8)	92 (9)	74 (18)	72 (19)	70 (17)	99 (10)	95 (10)	103 (5)
10	Carbaryl	-11.2	5	85 (11)	95 (8)	101 (14)	116 (10)	108 (8)	110 (9)	71 (10)	89 (13)	81 (16)	117 (12)	111 (11)	109 (7)
11	Carbendazim	-10.5	5	89 (11)	97 (6)	108 (13)	85 (6)	87 (3)	106 (10)	72 (8)	81 (9)	87 (12)	99 (5)	98 (7)	111 (1)
12	Carbofuran-3-OH	-10.3	5	108 (9)	113 (7)	103 (16)	101 (10)	95 (5)	110 (11)	112 (9)	99 (7)	93 (11)	116 (10)	107 (12)	113 (4)
13	Carboxin	-17.4	5	113 (9)	118 (7)	103 (15)	108 (9)	107 (6)	115 (9)	114 (13)	94 (14)	71 (18)	74 (9)	112 (13)	109 (5)
14	Chlorantraniliprole	-10.0	5	103 (7)	115 (11)	101 (15)	81 (20)	85 (7)	92 (5)	118 (15)	105 (16)	86 (14)	101 (14)	95 (10)	101 (8)
15	Cletodim	-13.5	5	117 (12)	115 (10)	104 (16)	116 (17)	101 (8)	90 (6)	116 (14)	92 (9)	80 (14)	105 (12)	109 (11)	103 (4)
16	Clomazone	-11.3	5	116 (12)	108 (8)	99 (15)	111 (8)	111 (6)	112 (10)	119 (15)	100 (18)	87 (15)	112 (7)	110 (9)	108 (4)
17	Clothianidin	-10.7	5	113 (11)	106 (4)	119 (14)	115 (7)	111 (10)	101 (9)	114 (10)	102 (14)	119(13)	114 (7)	105 (5)	110 (6)
18	Cyazofamid	-16.4	5	109 (8)	106 (7)	112 (17)	105 (15)	104 (5)	111 (13)	105 (16)	103 (15)	91 (16)	116 (7)	112 (9)	114 (5)
19	Cyproconazole	-16.1	5	112 (13)	108 (9)	98 (17)	115 (17)	103 (9)	117 (14)	94 (13)	111 (12)	99 (16)	110 (6)	115 (9)	116 (2)
20	Demeton-S-methyl-sulfone	-24.5	5	112 (10)	108 (7)	109 (14)	116 (10)	108 (5)	113 (10)	109 (6)	115 (9)	101(11)	106 (6)	115 (12)	113 (5)
21	Diazinon	-8.7	5	108 (9)	105 (9)	105 (19)	110 (14)	108 (7)	112 (14)	107 (18)	101 (17)	88 (15)	108 (6)	119 (10)	114 (4)

22	Diclosulam	-12.3	5	110 (9)	105 (8)	113 (16)	91 (17)	93 (7)	95 (8)	104 (17)	105 (12)	93 (16)	109 (9)	107 (7)	112 (2)
23	Dicrotophos	-11.5	5	110 (11)	99 (8)	96 (13)	73 (13)	76 (6)	112 (12)	99 (9)	95 (5)	89 (9)	90 (10)	89 (10)	97 (3)
24	Diflubenzuron	-15.8	5	118 (11)	108 (10)	107 (17)	89 (15)	91 (9)	105 (15)	81 (19)	92 (13)	85 (16)	111 (15)	103 (9)	89 (5)
25	Dimethoate	-10.2	5	118 (11)	108 (7)	106 (15)	116 (7)	103 (6)	116 (8)	102 (9)	99 (6)	96 (11)	107 (7)	114 (11)	104 (3)
26	Dimethomorph	-12.1	5	119 (11)	114 (6)	116 (15)	116 (13)	99 (6)	109 (13)	118 (14)	113 (14)	102(12)	111 (8)	111 (10)	111 (2)
27	Dimoxystrobin	-11.7	5	110 (11)	120 (7)	114 (17)	106 (15)	100 (7)	113 (12)	104 (17)	96 (16)	92 (14)	103 (8)	109 (9)	116 (3)
28	Diniconazole	-15.3	5	116 (8)	111 (8)	114 (16)	110 (17)	99 (5)	105 (15)	103 (14)	102 (8)	89 (16)	107 (4)	103 (9)	98 (4)
29	Diuron	-19.4	5	95 (11)	102 (6)	94 (13)	103 (8)	105 (4)	108 (11)	78 (9)	101 (12)	91 (12)	111 (6)	113 (7)	111 (5)
30	Epoxiconazole	-12.7	5	118 (12)	117 (7)	107 (17)	109 (12)	102 (5)	113 (13)	115 (17)	99 (13)	90 (15)	114 (7)	112 (8)	115 (3)
31	Ethephon	-16.3	5	118 (9)	115 (10)	100 (16)	110 (15)	97 (7)	110 (14)	117 (19)	99 (17)	88 (15)	109 (7)	110 (7)	111 (3)
32	Ethiofencarb	-12.9	5	106 (11)	114 (8)	108 (15)	103 (10)	92 (7)	108 (10)	100 (11)	94 (13)	90 (15)	105 (13)	102 (12)	106 (5)
33	Ethoxysulfuron	-20.2	5	119 (9)	113 (11)	114 (18)	98 (15)	90 (7)	100 (16)	118 (17)	114 (10)	85 (20)	118 (14)	97 (8)	102 (2)
34	Fenamidone	-15.6	5	101 (12)	103 (7)	110 (16)	111 (14)	98 (7)	110 (13)	97 (14)	99 (13)	90 (16)	115 (15)	107 (11)	110 (3)
35	Fenamiphos	-13.5	5	109 (11)	104 (8)	110 (16)	104 (14)	90 (7)	101 (14)	101 (19)	82 (8)	75 (13)	75 (16)	80 (14)	77 (5)
36	Fenazaquin	-28.4	5	106 (8)	108 (10)	101 (15)	72 (20)	81 (9)	102 (17)	71 (20)	72 (16)	71 (17)	87 (10)	84 (11)	89 (7)
37	Fenbuconazole	-16.5	5	103 (12)	106 (5)	112 (17)	106 (13)	96 (7)	111 (13)	120 (17)	99 (14)	93 (14)	105 (5)	108 (10)	115 (4)
38	Fenoxycarb	-21.2	5	114 (11)	103 (9)	110 (15)	94 (15)	95 (6)	104 (14)	108 (17)	95 (15)	93 (17)	113 (10)	104 (10)	109 (6)
39	Fipronil	-6.3	5	114 (14)	104 (8)	107 (17)	87 (14)	88 (8)	112 (7)	90 (17)	92 (14)	96 (14)	118 (10)	102 (10)	108 (5)
40	Flupicolid	-17.6	5	119 (10)	114 (6)	115 (17)	106 (16)	107 (7)	113 (12)	115 (13)	114 (20)	95 (14)	101 (5)	115 (10)	115 (4)
41	Fluquinconazole	-12.1	5	120 (14)	105 (9)	110 (15)	110 (13)	101 (11)	118 (12)	115 (20)	98 (16)	88 (11)	104 (5)	107 (9)	115 (6)
42	Flusilazole	-17.5	5	107 (12)	105 (7)	111 (17)	116 (13)	109 (6)	117 (14)	100 (14)	100 (15)	93 (18)	110 (7)	105 (7)	100 (4)
43	Flutolanil	-14.6	5	110 (15)	115 (9)	103 (18)	119 (14)	104 (7)	113 (12)	105 (16)	93 (16)	87 (13)	114 (8)	112 (10)	115 (5)
44	Flutriafol	-14.1	5	119 (12)	112 (7)	116 (15)	107 (13)	107 (6)	114 (12)	117 (12)	110 (16)	96 (12)	119 (8)	115 (10)	117 (4)
45	Hexythiazox	-24.6	5	117 (5)	107 (11)	106 (17)	95 (18)	88 (8)	102 (19)	107 (12)	96 (18)	83 (18)	104 (10)	94 (9)	99 (5)
46	Imazalil	-10.5	5	107 (9)	119 (8)	108 (15)	117 (16)	106 (6)	116 (14)	103 (20)	77 (11)	70 (20)	102 (11)	104 (11)	103 (6)
47	Imidacloprid	-29.9	5	105 (9)	103 (5)	107 (16)	103 (7)	94 (7)	103 (13)	108 (8)	113 (8)	101(12)	116 (5)	111 (11)	112 (4)
48	Indoxacarb	-16.0	5	111 (9)	104 (8)	109 (15)	112 (12)	95 (10)	96 (7)	89 (14)	105 (17)	98 (15)	110 (10)	110 (9)	111 (5)

49	Iprovalicarb	-7.3	5	106 (10)	116 (9)	110 (17)	97 (14)	94 (6)	109 (11)	101 (16)	96 (13)	91 (14)	114 (8)	104 (9)	114 (3)
50	Mephosfolan	-14.8	5	110 (13)	118 (7)	111 (15)	106 (7)	97 (6)	112 (9)	111 (12)	103 (10)	96 (9)	118 (8)	109 (9)	116 (3)
51	Mepronil	-12.7	5	109 (12)	116 (8)	105 (17)	105 (13)	98 (6)	111 (13)	102 (17)	93 (15)	88 (14)	102 (10)	108 (12)	113 (4)
52	Metalaxyl	-10.5	5	113 (12)	115 (7)	106 (16)	114 (9)	101 (5)	115 (10)	111 (12)	100 (11)	92 (14)	104 (7)	111 (9)	99 (4)
53	Metconazole	-13.4	5	119 (10)	108 (8)	105 (17)	99 (16)	94 (8)	106 (14)	86 (18)	83 (16)	78 (18)	114 (12)	101 (9)	105 (5)
54	Methamidophos	-0.6	5	90 (14)	80 (6)	78 (14)	101 (7)	82 (6)	88 (8)	77 (6)	72 (6)	71 (10)	100 (12)	84 (9)	83 (5)
55	Methidathion	-9.2	5	106 (11)	93 (8)	82 (11)	109 (5)	116 (7)	114 (12)	97 (11)	89 (19)	90 (12)	116 (8)	105 (11)	104 (6)
56	Methiocarb	-15.2	5	107 (9)	106 (9)	112 (15)	109 (14)	100 (8)	108 (12)	116 (14)	101 (19)	91 (16)	102 (11)	110 (12)	111 (5)
57	Methiocarb sulfone	-14.9	5	103 (12)	109 (8)	97 (15)	103 (11)	99 (6)	108 (9)	109 (9)	96 (10)	90 (16)	111 (8)	113 (12)	117 (6)
58	Methomyl	-7.2	5	93 (9)	112 (10)	118 (12)	114 (6)	96 (7)	106 (6)	119 (17)	106 (16)	115(16)	101 (6)	100 (10)	116 (5)
59	Methoxyfenozide	-10.1	5	113 (10)	107 (10)	117 (19)	112 (14)	109 (9)	117 (12)	111 (15)	100 (16)	79 (14)	110 (8)	103 (9)	115 (5)
60	Metolachlor	-19.8	5	108 (10)	109 (7)	113 (17)	109 (12)	101 (7)	111 (13)	105 (15)	101 (15)	93 (16)	102 (9)	108 (10)	111 (3)
61	Metsulfuron methyl	-8.8	5	114 (8)	101 (8)	89 (15)	93 (13)	82 (8)	102 (9)	114 (11)	90 (7)	79 (15)	110 (9)	93 (9)	96 (4)
62	Mevinphos	-8.9	5	117 (11)	104 (8)	98 (16)	103 (10)	92 (6)	105 (8)	88 (11)	91 (7)	82 (11)	102 (16)	97 (10)	91 (5)
63	Monocrotophos	-4.8	5	114 (11)	100 (7)	98 (14)	83 (9)	82 (4)	103 (9)	105 (9)	95 (5)	91 (12)	100 (6)	97 (9)	110 (3)
64	Monolinuron	-14.7	5	113 (12)	106 (12)	104 (15)	118 (10)	111 (6)	111 (12)	118 (13)	97 (9)	92 (14)	109 (5)	100 (12)	118 (10)
65	Myclobutanil	-10.1	5	120 (12)	108 (8)	101 (17)	93 (14)	83 (5)	111 (13)	93 (16)	87 (14)	70 (11)	105 (10)	91 (7)	78 (3)
66	Oxadixyl	-18.4	5	106 (9)	105 (7)	111 (15)	117 (9)	107 (5)	117 (10)	95 (10)	108 (9)	97 (10)	107 (4)	115 (9)	102 (5)
67	Penconazole	-15.0	5	111 (13)	108 (9)	114 (16)	93 (14)	93 (7)	109 (12)	104 (17)	101 (17)	87 (15)	104 (10)	99 (9)	106 (5)
68	Pencycuron	-12.8	5	114 (9)	118 (8)	109 (17)	95 (18)	91 (6)	91 (7)	104 (18)	93 (17)	86 (16)	114 (11)	100 (9)	103 (3)
69	Penoxsulam	-11.5	5	118 (8)	110 (8)	115 (18)	105 (16)	93 (8)	92 (8)	120 (12)	119 (4)	107(15)	107 (7)	105 (10)	108 (3)
70	Picoxystrobin	-12.6	5	97 (10)	99 (8)	113 (15)	82 (9)	88 (7)	112 (12)	97 (14)	96 (14)	95 (16)	96 (7)	98 (7)	112 (4)
71	Phosalone	-18.4	5	104 (10)	112 (9)	102 (17)	89 (15)	91 (5)	100 (14)	102 (16)	92 (11)	87 (19)	108 (10)	100 (10)	105 (5)
72	Phosmet	-16.4	5	100 (11)	105 (8)	116 (16)	116 (11)	111 (5)	98 (3)	117 (13)	106 (13)	97 (13)	108 (7)	117 (9)	117 (4)
73	Piperonyl butoxide	-14.5	5	109 (7)	112 (8)	114 (16)	72 (10)	81 (4)	102 (13)	84 (9)	83 (16)	88 (13)	92 (4)	92 (6)	104 (3)
74	Pirimicarb	-11.6	5	109 (11)	101 (7)	95 (15)	104 (11)	93 (7)	112 (11)	92 (11)	87 (11)	82 (12)	117 (12)	105 (10)	114 (4)

75	Pirimiphos-ethyl	-22.3	5	116 (10)	111 (10)	103 (17)	82 (19)	82 (8)	98 (16)	106 (19)	91 (19)	83 (15)	98 (9)	94 (9)	102 (5)
76	Pirimiphos methyl	-9.4	5	120 (7)	117 (10)	106 (18)	107 (14)	101 (7)	101 (10)	113 (18)	95 (19)	85 (13)	102 (8)	111 (10)	114 (6)
77	Procymidone	-10.7	5	105 (10)	111 (7)	110 (16)	91 (11)	92 (5)	112 (12)	83 (12)	88 (14)	92 (16)	104 (7)	102 (8)	115 (4)
78	Prometryn	-13.3	5	109 (11)	118 (8)	114 (15)	98 (15)	100 (6)	119 (14)	91 (18)	84 (20)	78 (16)	107 (10)	102 (10)	110 (4)
79	Propargite	-16.1	5	116 (8)	109 (12)	97 (17)	97 (19)	87 (9)	101 (16)	78 (17)	86 (18)	81 (17)	111 (11)	99 (10)	101 (3)
80	Propiconazole	-13.2	5	105 (10)	105 (8)	112 (16)	111 (14)	100 (7)	111 (14)	112 (18)	97 (17)	87 (15)	107 (7)	108 (9)	109 (4)
81	Propoxur	-7.4	5	103 (10)	104 (8)	108 (15)	115 (7)	107 (6)	117 (9)	107 (10)	106 (11)	95 (15)	105 (5)	119 (9)	104 (5)
82	Propyzamide	-13.7	5	117 (13)	115 (9)	104 (17)	118 (11)	101 (7)	114 (12)	107 (14)	94 (17)	85 (14)	112 (9)	112 (12)	111 (3)
83	Pyraclostrobin	-13.4	5	109 (8)	113 (8)	103 (17)	113 (17)	101 (8)	112 (14)	101 (18)	90 (16)	83 (15)	111 (8)	111 (9)	112 (4)
84	Pyrazophos	-9.7	5	116 (9)	106 (7)	111 (17)	99 (18)	89 (5)	101 (15)	112 (17)	107 (13)	98 (16)	119 (9)	100 (10)	103 (4)
85	Pyridaben	-19.9	5	98 (9)	90 (11)	82 (15)	71 (20)	82 (7)	104 (13)	86 (18)	75 (18)	70 (18)	107 (12)	95 (12)	95 (6)
86	Pyridaphenthion	-11.3	5	115 (11)	107 (6)	110 (17)	113 (11)	107 (6)	115 (12)	113 (14)	106 (14)	92 (11)	108 (6)	119 (9)	118 (3)
87	Pyrimethanil	-10.7	5	93 (11)	96 (8)	101 (16)	114 (13)	112 (8)	96 (10)	76 (12)	85 (18)	75 (17)	105 (8)	113 (14)	112 (6)
88	Quinalphos	-19.5	5	98 (9)	105 (12)	112 (16)	120 (13)	106 (7)	107 (11)	91 (14)	100 (17)	92 (16)	114 (9)	111 (9)	111 (4)
89	Quinoxyfen	-15.9	5	106 (5)	117 (13)	98 (18)	114 (18)	103 (9)	96 (9)	111 (20)	80 (11)	71 (18)	111 (9)	108 (14)	102 (7)
90	Rotenone	-14.8	5	101 (8)	102 (8)	110 (16)	101 (11)	94 (7)	106 (13)	114 (16)	100 (14)	96 (15)	112 (12)	107 (10)	110 (2)
91	Saflufenacil	-15.2	5	114 (7)	105 (7)	103 (16)	92 (15)	95 (6)	118 (9)	105 (10)	110 (8)	99 (14)	111 (13)	103 (11)	113 (6)
92	Simazine	-15.0	5	117 (9)	107 (8)	108 (14)	113 (7)	104 (10)	113 (12)	115 (8)	103 (13)	88 (15)	117 (7)	113 (11)	119 (6)
93	Spinosad A	-17.1	5	111 (9)	99 (7)	110 (17)	106 (19)	102 (5)	106 (7)	98 (18)	78 (10)	79 (16)	119 (12)	111 (9)	112 (4)
94	Spinosad D	-9.4	5	105 (8)	103 (7)	108 (17)	105 (18)	105 (8)	115 (16)	95 (17)	89 (19)	80 (17)	118 (12)	113 (9)	106 (5)
95	Tebuconazole	-6.3	5	113 (6)	106 (9)	107 (16)	105 (12)	101 (7)	110 (14)	108 (18)	101 (15)	89 (16)	98 (6)	108 (9)	108 (4)
96	Tebufenozide	-15.8	5	103 (8)	112 (9)	118 (18)	106 (16)	103 (7)	114 (14)	119 (18)	109 (15)	86 (14)	104 (6)	99 (7)	115 (6)
97	Tebufenpyrad	-17.7	5	106 (8)	103 (9)	110 (16)	98 (17)	90 (6)	101 (15)	87 (16)	101 (16)	95 (17)	103 (10)	102 (9)	101 (5)
98	Terbuthylazine	-10.5	5	115 (8)	102 (6)	110 (10)	90 (3)	113 (3)	112 (10)	87 (8)	97 (19)	103(11)	87 (5)	111 (7)	107 (4)
99	Tetraconazole	-35.8	5	117 (10)	117 (8)	106 (17)	97 (13)	94 (5)	111 (13)	110 (11)	115 (10)	99 (14)	114 (14)	111 (13)	111 (4)
100	Thiabendazole	-21.4	5	120 (5)	108 (7)	107 (15)	118 (10)	103 (7)	104 (11)	108 (8)	104 (12)	86 (19)	104 (10)	113 (17)	104 (3)

101	Thiacloprid	-16.2	5	111 (10)	105 (7)	101 (13)	112 (9)	106 (6)	114 (10)	111 (8)	107 (9)	91 (11)	105 (8)	118 (9)	116 (4)
102	Thiobencarb	-24.7	5	106 (9)	107 (9)	109 (19)	107 (9)	114 (7)	113 (15)	119 (16)	102 (17)	89 (14)	118 (11)	107 (7)	113 (5)
103	Thiophanate methyl	-9.9	5	114 (15)	105 (5)	104 (15)	106 (11)	105 (5)	110 (10)	95 (13)	101 (8)	86 (10)	109 (9)	98 (8)	105 (3)
104	Triadimefon	4.5	5	115 (11)	107 (8)	109 (15)	113 (13)	107 (5)	114 (14)	111 (9)	105 (14)	91 (15)	109 (7)	115 (9)	115 (3)
105	Tricyclazole	-11.1	5	95 (17)	96 (13)	106 (12)	119 (11)	103 (18)	112 (9)	84 (19)	75 (18)	115 (9)	105 (19)	109 (10)	99 (6)
106	Trifloxysulfuron	-16.3	5	117 (7)	109 (5)	112 (16)	115 (16)	106 (6)	110 (14)	117 (11)	113 (10)	100(13)	111 (5)	117 (8)	114 (3)
107	Trifloxystrobin	-22.6	5	104 (7)	110 (9)	97 (16)	107 (17)	97 (6)	111 (14)	104 (17)	92 (16)	84 (14)	106 (7)	109 (8)	110 (4)
108	Triflumuron	-35.6	5	106 (10)	106 (7)	111 (9)	95 (12)	88 (6)	106 (15)	72 (18)	94 (16)	87 (18)	104 (12)	97 (8)	108 (5)
109	Vamidothion	-16.7	5	119 (5)	106 (8)	100 (17)	111 (10)	101 (6)	104 (12)	108 (6)	105 (9)	88 (13)	114 (7)	110 (14)	106 (4)
110	Zoxamide	-16.7	5	105 (10)	116 (7)	106 (18)	96 (17)	89 (5)	102 (15)	99 (16)	94 (14)	90 (14)	115 (11)	98 (7)	102 (2)

	Analyte GC-MS/MS	**ME (%)**	**LOQ µg kg^{-1}**	**Matrix = Rodent kidney**						**Matrix = Rodent liver**					
				Repeatability (n=7)			**Intermediate precision (n=7)**			**Repeatability (n =7)**			**Intermediate precision (n=7)**		
				10	**20**	**40**	**10**	**20**	**40**	**10**	**20**	**40**	**10**	**20**	**40**
				Rec (RSD), %			**Rec (RSD), %**			**Rec (RSD), %**			**Rec (RSD), %**		
1	Acetochlor	134	40			120 (7)			93 (6)			111(11)			98 (5)
2	Aldrin	22	10	101 (10)	96 (17)	98 (6)	77 (13)	114 (12)	82 (8)	72 (10)	74 (17)	82 (9)	81 (13)	84 (6)	74 (3)
3	Benfluralin	57	10	114 (14)	119 (10)	107 (8)	107 (8)	116 (17)	85 (5)	98 (9)	93 (13)	98 (9)	118 (10)	104 (4)	85 (4)
4	BHC-alpha	48	10	108 (7)	107 (8)	98 (7)	87 (9)	110 (15)	81 (6)	88 (9)	80 (14)	82 (18)	95 (10)	93 (3)	82 (4)
5	BHC-beta	41	10	107 (7)	111 (8)	103 (6)	89 (8)	118 (15)	90 (8)	85 (8)	84 (13)	91 (9)	101 (10)	101 (3)	90 (4)
6	BHC-gamma	-3	10	107 (6)	99 (16)	93 (7)	87 (8)	115 (11)	80 (6)	83 (9)	70 (15)	85 (7)	93 (8)	90 (2)	80 (5)
7	Bromophos-ethyl	329	10	92 (7)	88 (15)	89 (5)	79 (9)	118 (14)	83 (8)	71 (9)	72 (14)	70 (20)	88 (11)	86 (3)	78 (2)
8	Bupirimate	295	10	105 (9)	105 (15)	107 (6)	96 (6)	118 (15)	102 (8)	74 (16)	76 (9)	72 (7)	83 (18)	79 (6)	70 (4)
9	Cadusafos	167	10	118 (10)	121 (9)	112 (10)	86 (10)	115 (15)	87 (5)	97 (17)	94 (16)	109 (9)	120 (6)	115 (5)	93 (8)
10	Chlorobenzilate	318	10	95 (7)	100 (8)	97 (5)	87 (7)	115 (14)	94 (8)	71 (9)	71 (9)	113 (2)	91 (12)	119 (3)	108 (5)
11	Cycloate	63	40			116 (7)			92 (6)			109(12)			71 (5)
12	DCPA	74	10	119 (9)	118 (9)	112 (6)	89 (8)	120 (18)	90 (7)	93 (7)	91 (14)	96 (6)	99 (12)	95 (4)	86 (4)

13	DDD-o,p'	84	10	96 (6)	99 (9)	97 (5)	83 (8)	119 (19)	90 (8)	70 (9)	78 (10)	74 (17)	90 (14)	88 (5)	80 (2)
14	DDD-p,p'	179	10	103 (7)	96 (15)	96 (5)	77 (8)	110 (12)	78 (7)	80 (14)	77 (16)	86 (6)	92 (14)	87 (5)	80 (4)
15	DDE-p,p'	72	10	96 (6)	95 (16)	95 (5)	76 (8)	109 (18)	88 (9)	71 (9)	72 (15)	70 (3)	83 (16)	75 (8)	71 (6)
16	Dichloran	259	10	120 (3)	109 (20)	115 (6)	98 (10)	119 (13)	93 (6)	104 (13)	101 (16)	113 (9)	112 (11)	111 (5)	95 (4)
17	Diphenylamine	396	10	112 (11)	120 (7)	117 (9)	95 (8)	120 (14)	90 (5)	99 (13)	96 (11)	105(20)	109 (10)	105 (5)	82 (9)
18	Endosulfan ether	16	10	111 (6)	116 (11)	108 (6)	88 (8)	117 (16)	91 (6)	87 (8)	88 (17)	91 (9)	101 (10)	98 (3)	86 (5)
19	Etrimfos	157	10	114 (11)	120 (8)	116 (8)	95 (8)	117 (15)	88 (6)	93 (15)	98 (16)	110(10)	113 (11)	109 (5)	94 (5)
20	Fosthiazate	3144	10	119 (15)	119 (18)	119 (9)	91 (12)	118 (12)	86 (5)	102 (17)	109 (10)	98 (20)	106 (17)	101 (5)	88 (6)
21	Heptachlor	63	10	115 (8)	112 (11)	103 (5)	85 (8)	114 (18)	86 (7)	86 (10)	84 (16)	86 (9)	93 (11)	89 (5)	80 (4)
22	Hexachlorobenzene	20	10	95 (10)	93 (12)	89 (6)	75 (12)	91 (17)	77 (6)	71 (8)	78 (13)	80 (10)	85 (12)	82 (6)	71 (6)
23	Pebulate	52	10	108 (10)	105 (18)	107 (5)	89 (6)	105 (14)	91 (6)	87 (17)	83 (18)	103 (8)	100 (7)	99 (3)	92 (4)
24	Sulfotep	106	10	118 (14)	121 (8)	115 (8)	92 (9)	118 (14)	90 (5)	108 (12)	102 (16)	108 (9)	108 (9)	108 (5)	94 (4)
25	Tefluthrin	51	10	119 (15)	117 (8)	110 (8)	93 (9)	118 (19)	87 (7)	111 (10)	104 (13)	103(11)	110 (9)	104 (4)	90 (4)
26	Transfluthrin	71	10	117 (12)	107 (14)	101 (10)	80 (8)	109 (15)	76 (9)	115 (11)	105 (17)	103(10)	108 (8)	99 (6)	85 (6)
27	Triallate	59	10	116 (11)	116 (11)	107 (7)	90 (8)	115 (18)	88 (7)	94 (10)	88 (18)	97 (9)	100 (7)	103 (16)	87 (4)
28	Trifluralin	56	10	113 (13)	120 (9)	109 (8)	96 (10)	117 (17)	85 (6)	95 (8)	94 (13)	100 (9)	110 (11)	101 (3)	85 (4)

Table 2. Results for 11 positive samples obtained from application of the validated method

	Compounds ($\mu g\ kg^{-1}$)						
Samples	Acephate	Clethodim	Methamidophos	Metconazole	Tebuconazole	Thiacloprid	Tricyclazole
Kidney							
K1	12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
K2	142	n.d.	<LOQ	n.d.	n.d.	5	n.d.
K3	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
K4	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.
K5	n.d.	n.d.	n.d.	<LOQ	n.d.	n.d.	n.d.
K6	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.
Liver							
L1	25	n.d.	n.d.	n.d.	10	n.d.	5
L2	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.
L3	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.
L4	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.
L5	n.d.	n.d.	n.d.	n.d.	10	n.d.	5
L6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	20
L7	n.d.	n.d.	n.d.	n.d.	8	n.d.	n.d.

n.d.= not detected; K4 and L2 to L4 were samples of *Cavia aperea*.

Table S1. Compounds determined by UHPLC-MS/MS and GC-MS/MS, with their respective retention time (t_R), electrospray ionization (ESI) mode, cone voltage (CV), precursor and product ions, and collision energies (CE).

	Analyte (UHPLC-MS/MS)	t_R (min)	ESI	CV (V)	Quantification		Identification	
					1st transition	CE (eV)	2nd transition	CE (eV)
1	Acephate	2.10	+	20	183.9 → 142.8	10	183.9 → 94.6	25
2	Acetamiprid	3.91	+	30	223.0 → 126.0	20	223.0 → 56.1	15
3	Ametryn	6.29	+	25	228.1 → 186.1	20	228.1 → 68.1	35
4	Atrazine	5.93	+	48	216.1 → 174.1	16	216.1 → 96.0	22
5	Azaconazole	6.31	+	34	300.0 → 159.1	28	300.0 → 231.1	18
6	Azoxystrobin	7.84	+	15	404.1 → 372.0	16	404.1 → 328.9	30
7	Benalaxil	9.69	+	25	326.1 → 148.0	20	326.1 → 91.0	30
8	Bitertanol	9.43	+	30	338.1 → 70.1	8	338.1 → 98.9	16
9	Buprofezin	11.42	+	20	306.1 → 201.0	12	306.1 → 115.9	16
10	Carbaryl	5.53	+	30	202.1 → 145.1	10	202.1 → 117.1	24
11	Carbendazim	2.94	+	24	192.1 → 160.1	18	192.1 → 132.1	30
12	Carbofuran-3-OH	3.73	+	34	238.0 → 181.0	10	238.0 → 163.0	16
13	Carboxin	5.64	+	5	236.0 → 143.0	15	236.0 → 87.0	25
14	Chlorantraniliprole	7.16	+	15	481.6 → 283.9	23	481.6 → 451.1	25
15	Cletodim	10.91	+	25	360.0 → 164.0	20	360.0 → 268.1	10
16	Clomazone	6.82	+	23	240.0 → 125.0	18	240.0 → 89.0	46
17	Clothianidin	3.56	+	25	250.0 → 132.0	15	250.0 → 169.0	10
18	Cyazofamid	9.11	+	25	325.0 → 107.9	15	325.0 → 261.0	10
19	Cyproconazole	7.76	+	5	292.2 → 70.2	20	292.2 → 125.1	30
20	Demeton-S-methyl-sulfone	3.15	+	32	263.0 → 169.1	17	263.0 → 121.0	17
21	Diazinon	9.82	+	20	305.1 → 169.0	22	305.1 → 96.9	35
22	Diclosulam	6.41	+	46	406.1 → 161.0	30	406.1 → 378.0	15
23	Dicrotophos	3.30	+	30	238.0 → 112.0	10	238.0 → 193.0	10
24	Diflubenzuron	8.57	+	34	311.1 → 158.1	12	311.1 → 141.1	28
25	Dimethoate	3.82	+	20	230.0 → 198.8	10	230.0 → 124.8	22
26	Dimethomorph	7.77	+	30	388.1 → 165.0	30	388.1 → 300.9	20
27	Dimoxystrobin	9.06	+	20	327.1 → 205.2	10	327.1 → 116.1	21
28	Diniconazole	9.55	+	10	326.1 → 70.2	25	326.1 → 159.0	30
29	Diuron	6.14	+	25	233.0 → 72.0	18	233.0 → 159.9	25
30	Epoxiconazole	8.42	+	15	330.0 → 121.0	22	330.0 → 101.0	50
31	Ethephon	10.76	+	25	409.0 → 186.0	16	409.0 → 145.0	40
32	Ethiofencarb	5.81	+	10	226.1 → 107.0	15	226.1 → 164.0	10
33	Ethoxysulfuron	7.96	+	25	398.9 → 261.0	16	398.9 → 218.0	24
34	Fenamidone	7.78	+	5	312.1 → 92.0	25	312.1 → 236.1	14
35	Fenamiphos	8.59	+	27	304.1 → 217.1	24	304.1 → 202.1	36
36	Fenazaquin	12.25	+	5	307.2 → 161.0	15	307.2 → 57.2	20
37	Fenbuconaloze	8.83	+	15	337.0 → 70.1	20	337.0 → 125.0	30
38	Fenoxycarb	8.84	+	10	302.1 → 88.0	20	302.1 → 116.1	11
39	Fipronil	9.33	-	34	434.9 → 329.9	16	434.9 → 249.9	28
40	Flupicolid	8.11	+	40	383.0 → 172.9	20	383.0 → 365.0	15
41	Fluquinconazole	8.24	+	25	376.0 → 348.8	20	376.0 → 206.9	25
42	Flusilazole	8.77	+	5	316.0 → 165.0	25	316.0 → 247.0	20
43	Flutolanil	8.32	+	25	324.1 → 262.1	20	324.1 → 65.0	35

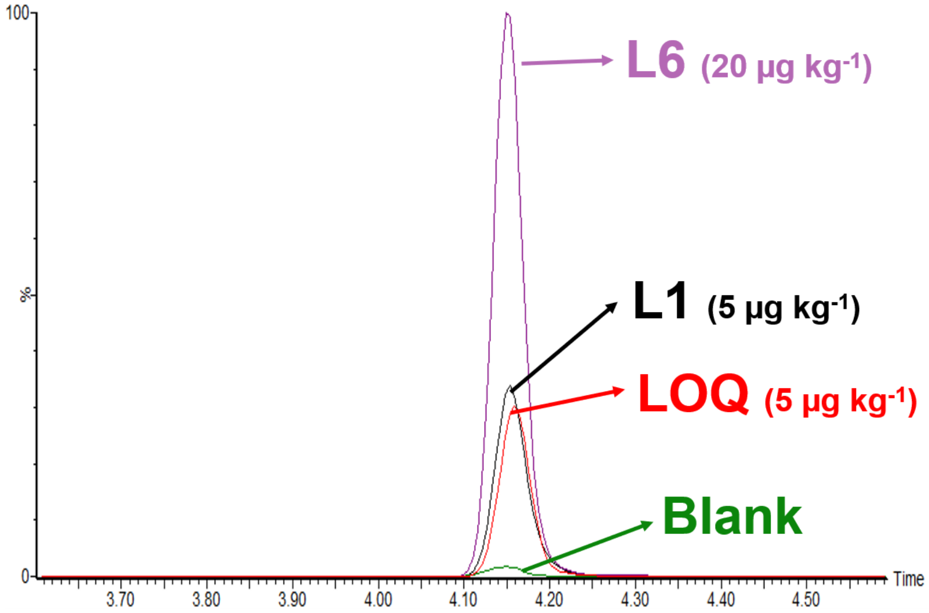
44	Flutriafol	6.00	+	25	302.1 → 70.1	16	302.1 → 122.9	30
45	Hexythiazox	11.72	+	10	353.0 → 168.1	25	353.0→ 228.1	15
46	Imazalil	5.79	+	31	297.1 → 159.0	22	297.1 → 69.0	18
47	Imidacloprid	3.65	+	25	256.1 → 209.0	12	256.1 → 174.0	20
48	Indoxacarb	10.71	+	30	528.1 → 202.9	40	528.1 → 217.9	25
49	Iprovalicarb	8.18	+	20	321.1 → 119.1	20	321.1 → 203.1	10
50	Mephosfolan	5.00	+	34	270.1 → 140.0	24	270.1 → 75.1	22
51	Mepronil	8.20	+	15	270.1 → 119.0	25	270.1 → 91.0	35
52	Metalaxyl	6.46	+	10	280.1 → 220.1	15	280.1 → 192.1	20
53	Metconazole	9.43	+	5	320.1 → 70.0	25	320.1 → 125.0	30
54	Methamidophos	1.78	+	30	141.9 → 93.9	12	141.9 → 124.8	14
55	Methidathion	6.95	+	30	303.0 → 144.8	10	303.0 → 84.9	20
56	Methiocarb	7.18	+	25	226.0 → 169.0	10	226.0 → 121.0	20
57	Methiocarb sulfone	4,09	+	31	258.1 → 107.1	38	258.1 → 122.1	19
58	Methomyl	2.98	+	15	162.9 → 88.0	10	162.9 → 105.9	10
59	Methoxyfenozide	8.39	+	15	369.2 → 149.1	15	369.2 → 313.2	10
60	Metolachlor	8.83	+	17	284.1 → 176.1	25	284.1 → 252.1	15
61	Metsulfuron methyl	5.26	+	28	382.0 → 167.0	16	302.0 → 198.9	22
62	Mevinphos	3.72	+	15	225.1 → 127.1	15	225.1 → 193.1	10
63	Monocrotophos	3.03	+	15	224.1 → 127.1	16	224.1 → 98.1	12
64	Monolinuron	5.74	+	15	215,0 → 126.0	15	215.0 → 99.0	30
65	Myclobutanil	8.04	+	25	289.1 → 70.2	15	289.1 → 125.1	30
66	Oxadixyl	4.83	+	20	279.1 → 219.0	12	279.1 → 132.3	25
67	Penconazole	9.00	+	15	284.0 → 70.1	15	284.0 → 159.0	25
68	Pencycuron	10.26	+	30	329.1 → 124.9	30	329.1 → 281.0	16
69	Penoxsulam	6.31	+	42	484.2 → 195.1	30	484.2 → 164.1	40
70	Phosalone	10.20	+	12	367.9 → 181.9	14	367.9 → 110.9	42
71	Phosmet	7.32	+	28	318.0 → 160.0	22	318.0 → 77.0	46
72	Picoxystrobin	9.42	+	10	368.0 → 145.1	25	368.0 → 205.1	10
73	Piperonyl butoxide	11.41	+	20	356.3 → 176.9	10	356.3 → 119.0	35
74	Pirimicarb	4.75	+	25	239.1 → 72.4	18	239.1 → 182.1	15
75	Pirimiphos methyl	10.18	+	42	306.3 → 164.1	22	306.3 → 108.1	30
76	Pirimiphos-ethyl	11.63	+	42	334.1 → 198.1	23	334.1 → 182.1	25
77	Procymidone	9.42	+	10	368.0 → 145.0	22	368.0 → 205.0	10
78	Prometryn	7.49	+	25	242.0 → 158.0	25	242.0 → 200.1	20
79	Propargite	12.18	+	10	368.3 → 231.2	15	368.3 → 175.2	15
80	Propiconazole	9.39	+	15	342.1 → 158.9	25	342.1 → 69.1	20
81	Propoxur	5.20	+	15	210.1 → 110.9	12	210.1 → 92.9	25
82	Propyzamide	7.80	+	31	256.1 → 190.0	16	256.1 → 173.0	23
83	Pyraclostrobin	10.04	+	25	388.1 → 163.0	25	388.1 → 193.9	12
84	Pyrazophos	10.01	+	33	374.0 → 222.1	22	374.0 → 194.0	32
85	Pyridaben	12.80	+	5	365.1 → 147.1	24	365.1 → 309.1	12
86	Pyridaphenthion	8.24	+	31	341.0 → 189.0	22	341.0 → 92.0	34
87	Pyrimethanil	6.62	+	25	200.0 → 107.0	24	200.0 → 82.0	24
88	Quinalphos	9.20	+	15	299.0 → 162.9	24	299.0 → 96.9	30
89	Quinoxyfen	11.16	+	15	308.0 → 197.0	30	308.0 → 161.9	35
90	Rotenone	8.99	+	10	395.0 → 213.1	24	395.0 → 192.1	20
91	Saflufenacil	7.69	+	26	501.1 → 198.0	38	501.1 → 349.0	20
92	Simazine	4.91	+	40	202.0 → 124.0	16	202.0 → 96.0	22
93	Spinosad A	10.00	+	35	732.6 → 142.0	30	732.6 → 98.1	35

94	Spinosad D	10.57	+	40	746.5 → 142.0	31	746.5 → 98,1	35
95	Tebuconazole	8.97	+	30	308.2 → 70.1	24	308.2 → 124.9	40
96	Tebufenozide	9.26	+	10	353.2 → 133.1	10	353.2 → 105.1	20
97	Tebufenpyrad	11.08	+	15	334.0 → 145.0	25	334.0 → 117.0	25
98	Tetraconazole	8.45	+	15	372.0 → 159.0	25	372.0 → 70.1	20
99	Tetradifona	8.05	+	56	294.0 → 197.2	21	294.0 → 225.0	19
100	Thiabendazole	3.21	+	45	202.0 → 174.9	25	202.0 → 130.9	30
101	Thiacloprid	4.27	+	35	253.0 → 125.8	20	253.0 → 90.0	40
102	Thiobencarb	9.94	+	25	258.1 → 125.1	15	258.1 → 89.1	35
103	Thiophanate methyl	5.15	+	25	343.0 → 151.0	20	343.0 → 93.0	35
104	Triadimenol	7.71	+	30	296.1 → 70.0	10	296.1 → 98.9	15
105	Tricyclazole	4.14	+	10	190.0 → 163.0	20	190.0 → 136.0	25
106	Trifloxystrobin	10.76	+	25	409.2 → 185.9	14	409.2 → 145.0	40
107	Trifloxysulfuron	6.23	+	30	438.0 → 182.0	20	438.0 → 139.0	45
108	Triflumuron	9.71	+	5	359.0 → 156.1	20	259.0 → 139.1	30
109	Vamidothion	3.66	+	25	288.0 → 146.0	10	288.0 → 188.0	25
110	Zoxamide	9.69	+	15	336.0 → 187.1	20	336.0 → 159.0	36
	Atrazine-d5 (SS)	5.89	+	45	221.1 → 179.3	15	221.1 → 101.0	18
	Triphenyl phosphate (IS)	9.80	+	40	327.2 → 152.1	37	327.2 → 215.2	28

			Quantification		**Identification**			
	Analyte (GC-MS/MS)	**t_R (min)**	**1st transition**	**CE (V)**	**2nd transition**	**CE (eV)**	**3rd transition**	**CE (eV)**
1	Acetochlor	8.60	174.0 → 146.1	10	146 → 131.1	10	222.9 → 132.2	20
2	Aldrin	9.39	254.9 → 220.0	20	262.9 → 192.9	35	-	-
3	Benfluralin	6.95	292.0 → 264.0	5	292.0 → 206.0	10	-	-
4	BHC-alpha	7.27	216.9 → 181.0	5	180.9 → 145	15	218.9 → 183.0	5
5	BHC-beta	7.64	216.9 → 181.1	5	181.0 → 145.0	15	218.9 → 183.1	5
6	BHC-gamma	7.74	181.0 → 145.0	15	216.9 → 181.0	5	218.9 → 183.1	5
7	Bromophos-ethyl	10.43	358.7 → 302.8	15	302.8 → 284.7	15	-	-
8	Bupirimate	11.19	272.9 → 193.1	5	272.9 → 108.0	15	-	-
9	Cadusafos	7.08	158.8 → 97.0	15	157.9 → 96.9	15	-	-
10	Chlorobenzilate	11.54	139.1 → 111.0	10	139.1 → 75.1	30	251.1 → 139.1	15
11	Chlorthal-dimethyl	9.51	331.8 → 300.9	10	298.9 → 221.0	25	300.9 → 223.0	25
12	Cycloate	6.74	154.1 → 83.1	5	154.1 → 72.1	5	-	-
13	DDD-o,p'	11.16	235.0 → 165.1	25	235.0 → 200.1	10	199.1 → 164.1	20
14	DDD-p,p'	11.72	237.0 → 165.1	25	237.0 → 200.1	15	165.1 → 115.0	35
15	DDE-p,p'	11.00	246.1 → 176.2	30	315.8 → 246	15	317.8 → 246	15
16	Dichloran	7.46	206.1 → 176.0	10	124.1 → 73.0	10	160.1 → 124.1	10
17	Diphenylamine	6.70	169.0 → 168.2	15	167.0 → 166.2	20	168 → 167.2	15
18	Endosulfan ether	8.38	240.9 → 205.9	20	169.9 → 99.0	30	271.8 → 236.8	15
19	Etrimfos	8.11	292.1 → 181.1	5	181.1 → 56.1	25	-	-
20	Fosthiazate	9.76	195.0 → 103.0	5	195.0 → 60.0	20	199.0 → 102.0	5
21	Heptachlor	8.83	271.7 → 236.9	15	273.7 → 236.9	15	273.7 → 238.9	15
22	Hexachlorobenzene	7.40	283.8 → 248.8	15	283.8 → 213.9	30	281.8 → 211.9	30
23	Pebulate	5.64	128.0 → 57.1	5	128.0 → 72.0	0	161.0 → 128.0	5
24	Sulfotep	7.04	201.8 → 145.9	10	237.8 → 145.9	10	321.8 → 201.9	10
25	Tefluthrin	7.99	177.1 → 127.1	15	177.1 → 87.0	30	197.0 → 141.1	10

26	Transfluthrin	8.64	163.1 → 91.1	10	163.1 → 143.1	20	165.1 → 91.1	10
27	Triallate	8.12	142.9 → 83.0	15	268.0 → 184.1	20	270.0 → 186.1	20
28	Trifluralin	6.92	306.1 → 264.0	5	264.0 → 160.1	5	264.0 → 206.0	5
	Atrazine-d5 (SS)	7.14	205.0 → 127.0	10	205.0 → 105.0	15	-	-
	Triphenyl phosphate (IS)	13.03	325.1 → 77.1	40	325.1 → 169.1	20	-	-

Figure 1. Comparison of the UHPLC-MS/MS signals for the pesticide tricyclazole



ANEXO 3

Health of *Holochilus chacarius* (Rodentia: Cricetidae)
in rice agroecosystem in a neotropical wetland assessed
by histopathology

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RESEARCH

Health of *Holochilus chacarius* (Rodentia: Cricetidae) in rice agroecosystem in a neotropical wetland assessed by histopathology

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Abstract Small mammals have a short lifetime and are strictly associated with their environment. This work aimed to use histopathology to assess the health of *Holochilus chacarius* in a rice agroecosystem in the Pantanal of Mato Grosso do Sul. During necropsy, fragments of the lung, kidney, skin, liver, and reproductive system of 33 animals were collected and submitted to histological processing. Tissue damages were evaluated as mild, moderate, and severe and arranged in a matrix for further statistical analysis. Furthermore, we used generalized linear models to verify the influence of tissue changes on the body condition, obtained by a regression between body mass and length. In the lungs, we found an intense inflammatory infiltrate associated with anthracosis that had a negative influence on the body's condition. Also, we observed degenerative and inflammatory changes in the liver, kidneys, skin, and reproductive system that ranged from mild to moderate. The histopathological lesions observed in this study may be associated with environmental alterations of anthropic origin such as the exposure to soot from wildfires and heavy metals, evidenced by lesions in the lung, kidney, and liver. The present study provided a histopathological matrix as a new approach that allows to classify and quantify the tissue alterations. Tissue changes when associated with body condition demonstrated to be an effective tool to assess the health of small free-living mammals, showing that these animals can be used as bioindicators of environmental condition.

Keywords Small mammals · Histopathology · Health · Bioindicator

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Introduction

Studies that evaluate the health of wildlife that inhabit anthropized environments are vital for monitoring ecosystems from a One Health perspective (Deem et al., 2001). Indeed, the use of a health approach related to a given wildlife species at tissue, cellular, and molecular levels enables to assess the presence of toxic substances, heavy metals, or gases from burning in a particular environment (Acevedo-Whitehouse & Duffus, 2009; Destoumieux-Garzón et al., 2018; Prestes & Vincenci, 2019).

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Wildlife health may be monitored at both population (reproductive indexes, demographic parameters, and genetic variables) and individual levels (body condition, clinical signs, pathology, toxicology, hematology, and serum biochemistry analysis) (Blazer et al., 2018; Leotta et al., 2012; Leroy et al., 2017; Maceda-Veiga et al., 2015). Histopathological analysis has been used as an effective method for detecting harmful biological effects such as cell death and degenerative and inflammatory processes caused by environmental contamination (Ben Ameur et al., 2012; Liebel et al., 2013; Sonne et al., 2007). In this context, ecopathology, a branch of pathology that intends to understand how environmental risk factors are related to tissue damage (Faye et al., 1999), supports the interpretation about the interactions between the macro and microenvironment.

The Pantanal is the largest wetland known with vast biological diversity and different phytophysiognomies including semi-deciduous forest, savannah-like formations, and lowland sandy flood plains (Alho et al., 2011). During the last decades, this biome has been altered by agriculture and the replacement of native pastures by exotic grasses for livestock (Li et al., 2020; Libonati et al., 2020). Moreover, the prolonged drought and forest fires reported from 2019 to 2021 throughout the entire biome caused a negative impact on the ecosystem resulting in the death of wildlife, starvation, and respiratory syndromes by the presence of carbon monoxide and nitrogen dioxide (Berlinck et al., 2022; Garcia et al., 2021).

Because small mammals have short lifetime, they respond quickly to natural and human disturbances with strong year-to-year variation at population levels, thus being considered bioindicators of the environmental condition (Pearce & Venier, 2005). Among these mammals, the sigmodontine rodent *Holochilus chacarius*, a semiaquatic herbivorous marsh rat associated with wetland in the Pantanal (Brandão & Nascimento, 2015; Do Prado et al., 2021; Gonçalves et al., 2015), tolerates human disturbance, including burned areas and irrigated crops, with high densities in rice plantations (Alleva et al., 2006; Martino & Aguilera, 1989; Semedo et al., 2022; Zanardi et al., 2020). In the present work, we aimed to investigate the influence of tissue changes on the health of a small rodent *H. chacarius* collected in an agroecosystem at the South Pantanal.

Material and methods

Biological samples from 21 males and 12 females of *H. chacarius* were collected in the rice agroecosystem situated at the southern Pantanal Biome (−20.086050; −56.614764), during October and November 2021. The studied area encompasses 3200 ha of irrigated rice with water supply pumped from the Miranda River, adjacent to 3600 ha of a forest reserve. This agroecosystem uses agrochemicals as herbicides (GAMIT® clomazone and naphtha and glyphosate), fungicides, and insecticides to prevent hemipterans. A previous study detected two insecticides (acephate and thiacloprid) and two fungicides (tebuconazole and tricyclazole) in liver and kidney samples from small mammals in this area (Cordeiro-Estrela, P. C. E. A, personal communication). Furthermore, the surrounding areas were burned by the fires that occurred in 2021 (Leal Filho et al., 2021), strongly affecting the region.

We recorded information about sex, reproductive status, body weight (g), and head-body length (mm) of sampled animals. The residual of ordinary linear regression between body weight and head-body length was used to define a body condition (BC). The procedures were performed in accordance with the licenses CEUA-PB no. 9192091019, CEUA UCDB 006/2022, and SISBIO 73472. The capture of wild animals was authorized by the Capture License ICMBio/MMA no. 72681.

Histopathological examination

In general, several organs can be affected by environmental contamination. We chose, for the histopathological study, the liver and kidneys due to their damage in the process of detoxification and waste excretion, respectively (Sonne et al., 2007). Also, we investigated lungs (Mutluoğlu et al., 2012; Nguyen, 2023) and skin (Scarff, 1991) due to the direct contact with the contaminated environment by heavy metals, pesticides sprayed on the rice plantation, and fires. The reproductive system was investigated to evaluate changes related to environmental effects on gametogenesis (Sonne et al., 2014). Paired organs, such as kidneys and lungs, were randomly chosen because environmental contamination results in the systemic pathway. Fragments of all these organs were fixed with 10% buffered formalin and processed for routine histological procedures. In order to evaluate tissue damage and inflammation pattern, the slides were stained with hematoxylin and eosin (HE). Furthermore, for renal protein and ferric pigments visualization, we also used special staining techniques such as Periodic Acid-Schiff (PAS) and Prussian blue, respectively. Additionally, Congo red and Masson's trichrome were used to detect renal amyloidosis and fibrosis, respectively. The histological slides were analyzed using the light composite microscope Carl Zeiss Microscopy GmbH (MOC) model Axio Scope A1. Tissue images were captured with the aid of Zen software and photographed by an Axiocam 503 color camera attached to the MOC.

Each tissue was individually analyzed, and the pathological findings were classified as (i) regressive, (ii) circulatory, and (iii) inflammatory and repair changes (Binkowski et al., 2013). The intensity of lesions was classified as absent (0), mild (focal-1), moderate (multifocal to coalescing-2), or severe (extensive-3) and tabulated forming a matrix adapted from Leopold (1971) for further analysis, thus obtaining a quantitative score for each animal.

Effects of pathologies on body condition

Generalized linear models (GLM) were created to investigate the effects of lesions in the lung, liver, skin, kidney, and reproductive system on the body condition (BC) of *H. chacarius*. In addition, a complete model was created using all variables (all lesions) and a null model. In this study, BC was used as a health assessment parameter (Santos et al., 2018, 2022). All candidate models were compared in a model selection approach based on the Akaike information criterion corrected for small samples (AICc) (Akaike, 1974); considering all models with ΔAICc $\leq$ 2 plausible, analyses were performed using the "AICcmodavg" package version 2.3–1 (Mazerolle, 2020) in R 3.5.0 (Team R Development Nucleo, 2018).

Results

Histopathological changes

Fragments of the liver, lung, skin, kidney, and genitals (n=5) of all individuals (n=33) were collected, totalizing 165 fragments analyzed (Table 1). The lesions varied in terms of intensity from mild to moderate except lung which had intense changes. Inflammation, degeneration, and congestion were found in the liver, lung, kidneys, and reproductive system. In the skin, we observed only inflammation that ranged from mild to moderate. We draw attention to the exogenous particles found in the lung and liver. Ferric pigment, amyloids, and fibrosis were not observed. The pathological changes recorded for each tissue, along with the different intensities observed, are presented in Table 2. Combining all the changes and scores, an individual could reach a maximum score of 78. Overall, the results showed that the maximum value obtained was 28 (n=2) (Table 2).

In the lungs, we observed atelectasis, congestion, emphysema, and pigments with greater intensity (intense-3) (Table 2). Furthermore, we detected that 28 out of 33 animals (84.8%) had pneumoconiosis, expressed by the presence of exogenous anthracosis-like particles (Fig. 1 A and B, Table 1). Areas of moderate to severe obstructive atelectasis observed in 81.8% of the individuals were characterized by mixed inflammatory infiltration and red blood cells in the alveolar sacs, together with the thickness of the alveolar septa (Fig. 1A). Around these areas, compensatory pulmonary emphysema was observed (Fig. 1B). In the lungs of three animals, microfilariae were found inside blood vessels and bronchi (Fig. 1C).

Renal tubules showed protein deposit (75.7%), confirmed by Periodic Acid-Schiff (PAS) staining (Fig. 2A, Table 1). The kidneys showed regressive changes, classified as mild to moderate, such as degeneration of renal tubule epithelial cells in 57.5% of the sampled animals (Fig. 2B, Table 1). Glomerular atrophy was also observed in 69.7% and glomerular hypertrophy in 72.7% of the evaluated individuals (Fig. 2C, Table 1), resulting in a decrease of the Bowman's space. Furthermore, we observed multifocal areas of hemorrhage and congestion in the renal parenchyma.

In the liver, circulatory disorders, mainly congestion (Fig. 3A), were classified as mild to moderate. Hydropic degeneration of hepatocytes was observed in nine individuals (27.3%), manifested by intense intracytoplasmic vacuolization (Fig. 3B, Table 1). Five animals (15%) presented portal spaces with the proliferation of bile duct epithelial cells (Fig. 3C, Table 1); among them, two were severely

Table 1 Histopathological levels of lesions in different tissues of *Holochilus chacarius* (*n*=33) collected in Pantanal region, during 2021. Results are expressed by the number of individuals and the percentual of occurrence in parenthesis

Tissue	Lesion	Intensity			
		Mild	Moderate	Severe	Absent
Liver	Congestion	12 (36.4%)	11 (33.3%)	2 (6.7%)	8 (24.2%)
	Fibrosis	5 (15.1%)	0 (0%)	0 (0%)	28 (84.8%)
	Inflammation	9 (27.3%)	3 (9.1%)	0 (0%)	21 (63.6%)
	Degeneration	5 (15.1%)	4 (12.1%)	1 (3%)	23 (69.7%)
	Exogenous particles	8 (24.2%)	4 (12.1%)	0 (0%)	21 (63.6%)
Skin	Inflammation	13 (39.4%)	3 (9.1%)	0 (0%)	17 (51.5%)
Lung	Congestion	8 (24.2%)	10 (30.3%)	5 (15.1%)	10 (30.3%)
	Hemorrhage	4 (12.1%)	2 (6.7%)	1 (3%)	26 (78.8%)
	Atelectasis	16 (48.5%)	8 (24.24%)	3 (9.1%)	6 (18.2%)
	Inflammation	6 (18.2%)	20 (60.6%)	3 (9.1%)	4 (12.1%)
	Degeneration	2 (6.7%)	0 (0%)	0 (0%)	31 (93.9%)
	Emphysema	6 (18.2%)	11 (33.3%)	11 (33.3%)	5 (15.1%)
	Exogenous particles	15 (45.4%)	12 (36.4%)	1 (3%)	5 (15.1%)
Reproductive	Congestion	6 (18.2%)	3 (9.1%)	1 (3.03%)	23 (69.7%)
	Inflammation	3 (9.1%)	0 (0%)	0 (0%)	30 (90.9%)
	Quantity of sperm	6 (18.2%)	10 (30.3%)	0 (0%)	17 (51.5%)
	Degeneration	12 (36.4%)	6 (18.2%)	0 (0%)	15 (45.4%)
	Necrosis	0 (0%)	0 (0%)	0 (0%)	33 (100%)
	Pigments	1 (3.0%)	1 (3.0%)	0 (0%)	31 (94%)
Kidney	Congestion	5 (15.1%)	10 (30.3%)	4 (12.1%)	14 (42.4%)
	Hemorrhage	8 (24%)	1 (3%)	0 (0%)	24 (73%)
	Inflammation	6 (18.2%)	4 (12.1%)	0 (0%)	23 (69.7%)
	Glomerular atrophy	10 (30.3%)	12 (36.3%)	10 (30.3%)	1 (3%)
	Degeneration	12 (36.4%)	5 (15.1%)	2 (6.7%)	14 (42.4%)
	Glomerular hypertrophy	4 (12.1%)	18 (54.5%)	2 (6.7%)	9 (27.3%)
	Protein accumulation	17 (51.5%)	8 (24.2%)	0 (0%)	8 (24.2%)

parasitized by the helminth of the genus *Capillaria*, evidenced by parasite migration and egg deposition in the hepatic parenchyma surrounded by inflammatory cells (Fig. 4A and B). We detected the presence of blackened pigments inside and outside the blood vessels, similar to those found in the lung (Fig. 3A). No significant changes were noticed in the skin. Few individuals had nonspecific inflammation of mild to moderate intensity (Table 2). In this particular tissue, 48.4% (16/33) of the areas of inflammation were focal with few polymorphonuclear cells in connective tissue (Table 1).

The male reproductive system was evaluated from the observation of the testis and epididymis. Both tissues showed foci of degeneration of the seminiferous tubules and epididymal ducts in 18 animals (54%), ranging from mild to moderate, associated with the presence of multinucleated giant cells observed in only two animals (Table 1). The absence of sperm in these two structures, even in animals within the reproductive period, was considered a regressive change (Fig. 5A), which was observed in 15 animals (45.5%). In the reproductive system of females, few tissue changes were found, such as the presence of the lipofuscin pigment in one individual (Fig. 5B, Table 1).

Body condition

GLM analysis showed that the BC of *H. chacarius* was negatively influenced by lung lesions (ΔAICc: 0; AICcWt: 0.70; 95% CI = −3.67 to −0.44, *p*-value = 0.01). Individuals with greater intensity of pulmonary lesions had lower BC (Table 3).

Table 2 Histopathological matrix showing different lesion intensities classified as (0) absent, (1) mild, (2) moderate, and (3) intense of *Holochilus chacarius* sampled in a rice agroecosystem in the Pantanal, Mato Grosso do Sul, between October and November 2021

ID	Liver						Skin		Lung								Reproductive							Kidney								OVERALL
	Circulatory Alteration	Inflammatory and Repair Alteration		Regressive Alteration			Inflammatory and Repair Alteration		Circulatory Alteration		Inflammatory and Repair Alteration		Regressive Alteration				Circulatory Alteration	Inflammatory and Repair Alteration	Regressive Alteration					Circulatory Alteration		Inflammatory and Repair Alteration	Regressive Alteration					
	Congestion	Fibrosis	Inflammation	Degeneration	Exogenous Particles	Total	Inflammation	Total	Congestion	Hemorrhage	Atelectasis	Inflammation	Degeneration	Emphysema	Exogenous Particles	Total	Congestion	Inflammation	Quantity of Sperm	Degeneration	Necrosis	Pigments	Total	Congestion	Hemorrhage	Inflammation	Glomerular Atrophy	Degeneration	Glomerular Hypertrophy	Protein Accumulation	Total	
EGS 37	2	1	0	0	0	3	1	1	3	0	1	2	0	2	2	10	1	0	1	1	0	0	3	3	0	1	2	2	2	1	11	28
EGS 76	1	0	1	0	0	2	1	1	3	1	1	2	0	2	1	10	0	0	2	2	0	0	4	2	0	0	2	3	2	2	11	28
EGS 38	1	0	0	0	0	1	0	0	3	0	3	3	0	3	2	14	2	0	1	0	0	0	3	0	2	0	2	0	3	1	8	26
EGS 64	2	0	0	2	2	6	0	0	1	0	3	3	0	3	2	12	0	0	0	1	0	0	1	2	0	0	2	1	0	2	7	26
EGS 30	2	1	1	0	0	4	0	0	2	0	2	2	0	2	2	10	2	0	2	0	0	0	4	3	0	0	1	0	2	1	7	25
EGS 51	2	1	1	0	2	6	0	0	1	0	2	1	0	0	2	6	0	0	2	2	0	0	4	1	1	0	2	1	2	1	8	24
EGS 31	0	0	0	0	0	0	0	0	3	2	2	2	0	3	1	13	3	0	0	0	0	2	5	2	0	0	1	0	0	2	5	23
EGS 33	1	0	0	0	1	2	0	0	1	0	2	2	0	2	3	10	1	0	2	2	0	0	5	2	0	0	0	1	2	1	6	23
EGS 72	0	0	1	0	1	2	1	1	0	1	1	2	0	0	1	5	1	0	1	2	0	0	4	2	0	2	2	3	2	0	11	23
EGS 39	1	0	0	0	0	1	1	1	3	0	3	0	0	3	0	9	1	0	2	0	0	0	3	2	0	0	0	2	2	2	8	22
EGS 71	0	1	1	0	0	2	2	2	2	0	1	2	0	1	1	7	0	0	0	2	0	0	2	2	0	1	1	2	2	1	9	22
EGS 98	1	0	1	1	0	3	0	0	1	3	0	1	0	1	1	7	0	0	0	1	0	0	1	1	1	0	3	1	2	2	10	21
EGS 27	2	0	0	0	1	3	0	0	2	0	1	1	0	1	2	7	0	0	2	1	0	0	3	2	0	0	1	1	2	1	7	20
EGS 34	2	0	2	0	0	4	1	1	0	0	1	2	0	2	1	6	0	0	0	0	0	1	1	2	0	1	2	0	2	1	8	20
EGS 50	1	0	0	3	2	6	2	2	0	0	1	2	0	1	2	6	0	0	0	1	0	0	1	0	1	0	2	0	0	2	5	20
EGS 61	0	0	2	2	0	4	1	1	2	1	1	2	0	3	1	10	0	0	0	1	0	0	1	0	0	0	1	1	2	0	4	20
EGS 22	3	1	0	0	0	4	0	0	2	0	2	3	0	3	2	12	1	1	0	0	0	0	2	0	0	0	1	0	0	0	1	19
EGS 32	1	0	0	2	0	3	0	0	1	0	1	2	0	2	0	6	0	1	2	0	0	0	3	3	0	0	1	1	2	0	7	19
EGS 36	1	0	0	0	0	1	0	0	0	0	2	2	0	3	2	9	0	0	0	1	0	0	1	2	1	0	1	2	2	0	8	19
EGS 41	2	0	0	0	0	2	0	0	0	2	2	0	1	0	1	6	0	0	1	1	0	0	2	3	1	0	2	0	2	1	9	19
EGS 57	2	0	0	0	0	2	1	1	0	0	1	2	0	1	2	6	0	0	2	1	0	0	3	1	0	2	0	1	0	2	6	18
EGS 59	1	0	0	1	1	3	1	1	2	0	1	2	0	2	2	9	0	0	0	0	0	0	0	1	0	0	2	1	1	0	5	18
EGS 60	3	0	1	0	2	6	1	1	1	0	1	1	0	2	0	5	0	0	0	0	0	0	0	0	1	1	2	1	0	1	6	18
EGS 23	1	0	0	0	1	2	1	1	0	0	0	2	0	2	2	6	2	0	2	0	0	0	4	0	0	0	0	0	2	1	3	16
EGS 40	0	0	1	1	0	2	1	1	0	0	0	2	0	3	1	6	0	0	2	1	0	0	3	0	0	1	1	0	1	1	4	16
EGS 58	1	0	0	0	1	2	1	1	2	0	1	2	1	2	1	9	0	0	1	0	0	0	1	0	0	0	0	0	1	2	3	16
EGS 62	0	0	0	1	0	1	0	0	2	1	0	2	0	2	1	8	0	0	1	1	0	0	2	1	0	0	0	1	2	1	5	16
EGS 63	2	0	2	0	0	4	0	0	2	0	1	2	0	0	1	6	1	0	0	0	0	0	1	0	1	2	0	1	0	1	5	16
EGS 25	2	0	1	0	1	4	0	0	1	0	1	2	0	3	1	8	0	0	0	0	0	0	0	0	0	0	0	0	2	1	3	15
EGS 48	0	0	0	2	0	2	0	0	2	0	2	1	0	3	0	8	0	0	0	0	0	0	0	0	0	0	2	0	3	0	5	15
EGS 42	1	0	0	0	0	1	1	1	1	0	0	0	0	0	1	2	0	1	0	2	0	0	3	0	0	2	0	0	1	1	4	11
EGS 49	2	0	0	0	1	3	2	2	0	0	0	1	0	1	0	2	0	0	0	1	0	0	1	0	0	0	1	0	0	1	2	10
EGS 65	0	0	0	1	0	1	0	0	0	0	1	0	0	3	1	5	0	0	0	0	0	0	0	0	1	1	0	2	0	0	4	10

Discussion

In the present study, we highlight that the main histopathological alterations observed were lung and liver pigments, emphysema, and atelectasis, as well as degeneration in the lung, liver, kidney, and testis. In addition, the observed anthracosis-like found in the liver and lungs could be a consequence of intense fires in the study area (Garcia et al., 2021; Leal Filho et al., 2021). Indeed, it has been reported that histopathological changes are related to environmental impacts caused by the use of pesticides in agriculture and/or the presence of heavy metals and pollutants resulting from fires (Lushchak et al., 2018; Sánchez-Chardi et al., 2008).

We found that the intense lung lesions negatively influenced the BC of the analyzed animals, probably as a consequence of the anthracosis observed in 28 of the 33 animals due to the inhalation of soot and carbon monoxide from the fires in the Pantanal region of Mato Grosso do Sul (Leal Filho et al., 2021; Li et al., 2020). It has been found that the toxicity

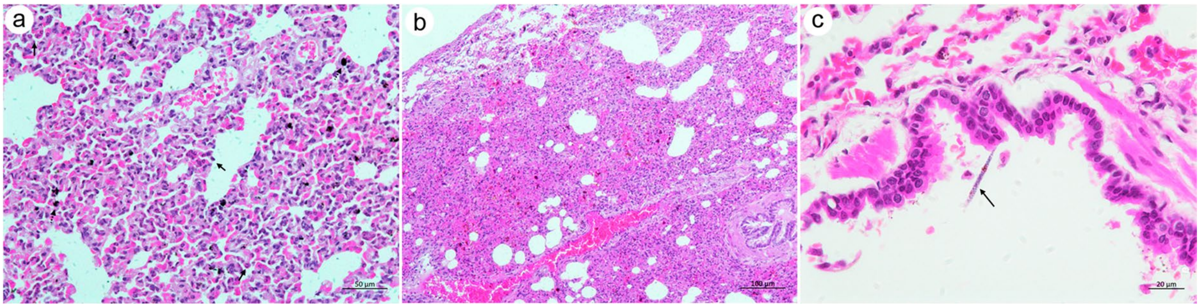

Fig. 1 Lung of *Holochilus chacarius*. **a** Dark-colored particles (full arrow), inflammatory cells, and red blood cells in the alveolar space. **b** Atelectasis and emphysema. **c** Microfilaria within the lumen of a bronchus (arrow)

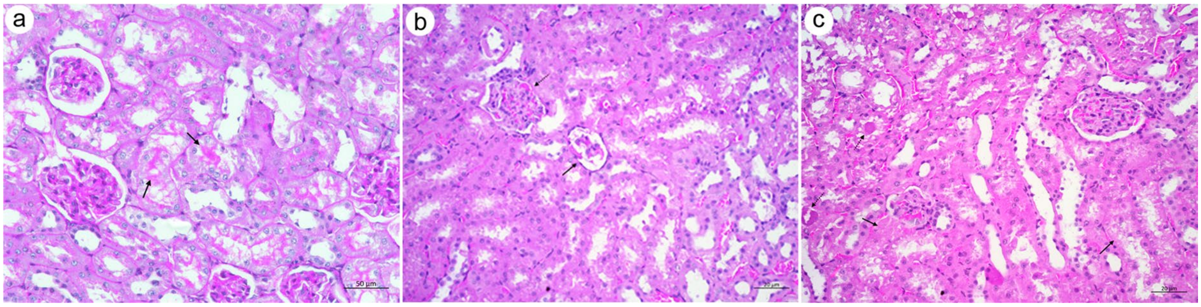

Fig. 2 Kidney of *Holochilus chacarius*. **a** Protein deposit in tubules (arrows) detected by PAS staining. **b** Atrophy of renal glomerulus (full arrow) and hypertrophied glomerulus, with the absence of Bowman's space (dotted arrow). **c** Vacuolization of renal tubule epithelial cells and presence of protein (dotted arrow)

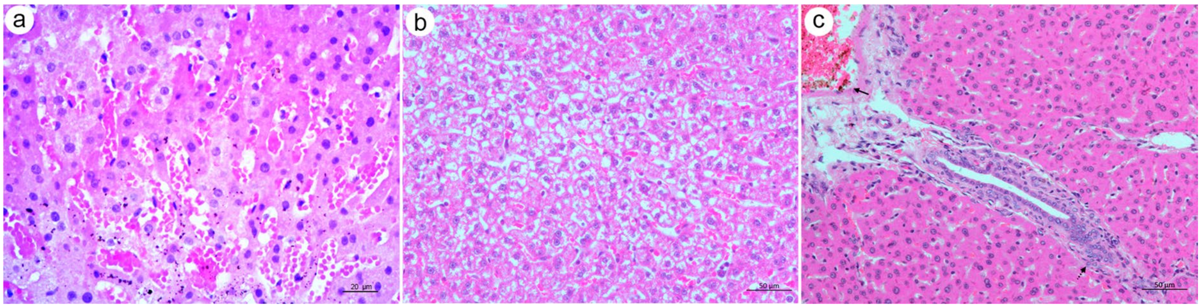

Fig. 3 Liver of *Holochilus chacarius*. **a** Congestion and presence of blackened pigments inside and outside the blood vessels. **b** Liver showing hydropic degeneration of hepatocytes. **c** Portal spaces with proliferation of bile duct epithelial cells (dotted arrow) and dark-colored pigments (full arrow)

caused by carbon monoxide and heavy metals in the lungs, especially when inhaled, can cause a decrease in BC (Mutluoğlu et al., 2012; Pandey & Madhuri, 2014). Some reports have shown that emphysema, a common finding in our study, is directly linked to a reduction in the BC of animals (Rich et al., 2020; Tsegaye et al., 2016) since progressive lung diseases negatively interfere with food consumption (Fehrenbach, 2006). In fact, the presence of pulmonary emphysema and its consequent dyspnea activates the sympathetic

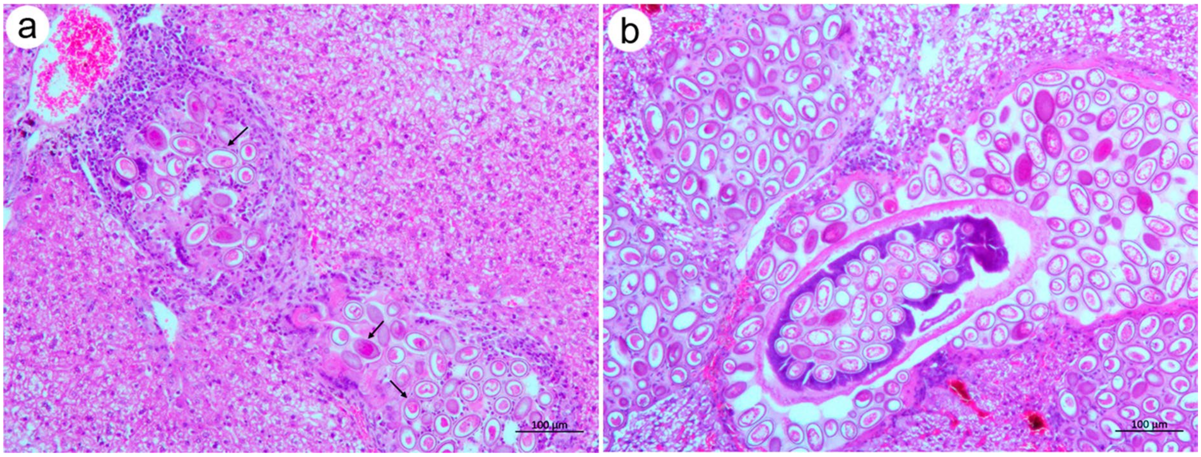

Fig. 4 Liver of *Holochilus chacarius* showing a nematode of the genus *Capillaria*. **a** Mixed inflammatory reaction around eggs (arrows) in the parenchyma. **b** Eggs and cross section of a parasite

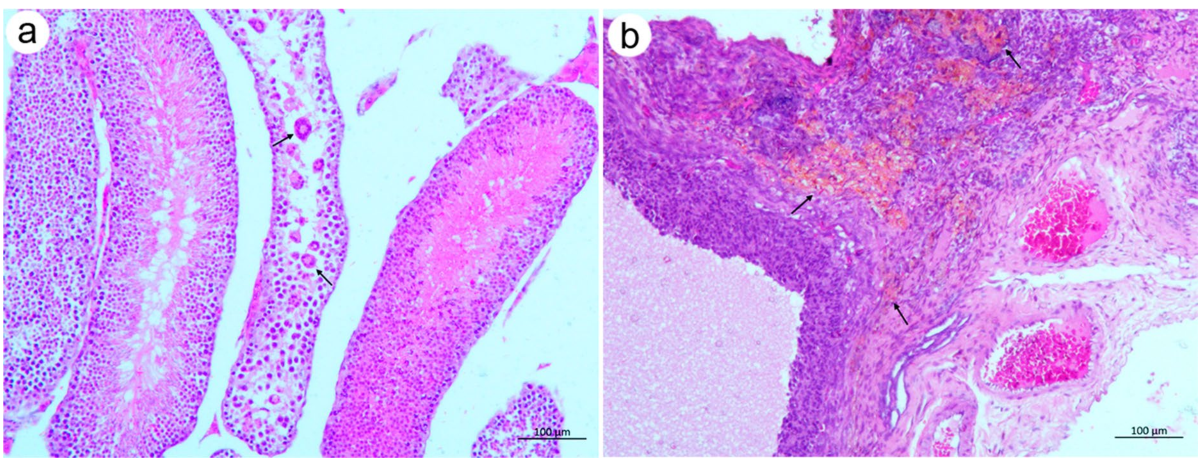

Fig. 5 Reproductive system of *Holochilus chacarius*. **a** Seminiferous tubules of a male, showing degeneration with disorganized germ cells and multinucleated giant cells (arrows). Note tubules without lumen and the absence of sperm. **b** Ovary of a female in the reproductive period, showing lipofuscin pigment

Table 3 Models used to explore the variables that influenced the body condition of *Holochilus chacarius* captured in the Southern Pantanal

Models	K	AICc	ΔAICc	AICcWt	Cum.Wt
Model 2 (Model 2 (body condition~lung injuries)	3	276.79	0.00	0.70	0.70
Null model (body condition~1)	2	280.40	3.61	0.12	0.82
Model 5 (body condition~liver injuries)	3	281.99	5.20	0.05	0.87
Model 4 (body condition~kidney injuries)	3	282.14	5.35	0.05	0.92
Model 3 (body condition~skin injuries)	3	282.74	5.95	0.04	0.95
Model 1 (body condition~reproductive injuries)	3	282.80	6.01	0.03	0.99
Model 6 (body condition~all injuries)	7	285.25	8.46	0.01	1.00

K, number of parameters; *AICc*, Akaike information criterion; *ΔAICc*, delta akaike information criterion; *AICcWt*, Akaike's weight; *Cum.Wt*, accumulated Akaike weight.

nervous system by increasing the release of catecholamines, which causes increased energy consumption because of hypermetabolism (Hofford et al., 1990). It is important to point out that the loss of physical condition in free-living animals can be an association of many causes such as parasitosis or nutritional and metabolic diseases (Aguirre et al., 1999).

The observed tissue lesions of regressive, inflammatory, and circulatory origin in the kidneys may be related to the action of heavy metals, which have different pathophysiological pathways: (i) oxidative stress, which increases the resistance of the vascular system and increases blood pressure; (ii) increase of free radicals in the body, causing inflammation; and (iii) enzymatic alterations and apoptosis that culminate in degenerative and necrotic alterations (Sabath & Robles-Osorio, 2012).

Most anthropic activities carried out in the Pantanal, such as agriculture, result in the runoff and leaching of metals and pesticides into the plain, potentially threatening wildlife (de Oliveira Roque et al., 2021). Previous studies from Pantanal have reported mercury levels in wood stork *Mycteria americana* (Del Lama et al., 2011), caimans *Caiman yacare* (Vieira et al., 2011), and jaguars *Panthera onca* (May Junior et al., 2018).

The presence of hyaline casts and the accumulation of proteins in the renal tubules from the sampled *H. chacarius* were also evidenced in free-living bears during the exposure to heavy metals such as mercury (Sonne et al., 2007). Furthermore, many evidence as observed in our study, such as hyaline cylinders, hyperemia, tubular cell swelling, inflammatory infiltrate, abnormalities in the glomeruli, and tubular lesions, have been associated with cadmium and lead, which have a remarkable nephrotoxicity (Yuan et al., 2014). Our results also showed glomerular dilation, tubular degeneration, and atrophy, most likely a consequence of the pesticides in the environment, as reported by Sonne et al. (2008) in arctic foxes. Moreover, neonicotinoid insecticides also used in the studied area (Cordeiro-Estrela., P.C.E.A., personal communication) have been experimentally demonstrated to be associated with histopathological lesions in rats (Vivek & Jain, 2020).

The hydropic degeneration evidenced in the liver of 27.3% of the evaluated animals could be a consequence of the exposure to toxins, bacteria, virus, or free radical action (Myers et al., 2012). However, biotoxins and heavy metals such as cadmium, copper, lead, and iron have been associated with hepatocyte vacuolization in both experimental (Kotak et al., 1993, 1996) and natural conditions (Shahsavari et al., 2019). The presence of hydropic degeneration in the liver can also be associated with environmental pollutants (Stehr et al., 1998) since toxic substances are able to produce oxidative stress and cell death (Ekinci-Akdemi et al., 2020). Moreover, the capillariasis observed in two individuals could be associated with hepatocyte vacuolization, alongside significant tissue compromise due to the presence of the parasite within the parenchyma (Resendes et al., 2009; Teixeira et al., 2018). The discrete inflammatory infiltrate detected in the skin of 15 animals was considered non-specific, since free-living animals are exposed to a variety of traumatic and infectious stimuli which can end up altering the dermis microbiota (Gimblet et al., 2017; Hubalek, 2000; Scarff, 1991).

In the last decade, there has been a growing demand for the use of wildlife species as bioindicators of environmental condition (Alleva et al., 2006; Chiarelli & Roccheri, 2014; Sonne et al., 2014; Zanardi et al., 2020). Our results showed that the histopathological study constitutes a remarkable tool to monitor the environmental health by using small mammals as bioindicators, in accordance with Gerber et al. (2017). Furthermore, since small mammals have a significant importance in the trophic network acting as prey for birds, serpents, and carnivores, toxic agents could bioaccumulate in these predatory animals (Sánchez-Chardi et al., 2008). We emphasize that, as our histopathological matrix showed that only two sampled animals had a score of 28, out of a maximum score of 78, the tissue lesions observed in the *H. chacarius* would not be interfering with the general health status of the studied animals. It has been reported that human encroachment, such as fires and environmental contamination by pesticides, may have a negative health effect on the wildlife (Alleva et al., 2006). Indeed, the Pantanal region was strongly affected by the fires that occurred in 2021 (Leal Filho et al., 2021), which could be related to the severe lung lesions observed in 27 out of 33 sampled animals.

In the One Health approach, the sanitary condition of the environment may have a strong influence on wildlife, domestic animals, and human health (Baptista et al., 2021; Camargo et al., 2010; Quadros et al., 2016; Zanardi et al., 2020). Since ecopathology demands to associate tissue changes with natural environmental conditions (Faye & Lancelot, 2006; Lau, 2010), our study showed that some observations in the light of histopathology could be associated with the human presence.

Conclusion

Our work demonstrates that the use of the histopathological matrix associated with models and body condition index proved to be an effective tool in health analysis. In fact, although we observed lesions in the liver, kidneys, skin, and reproductive system, our results strongly showed that only lung lesions influenced body condition. Tissue changes found in the study animals could be attributed to the presence of anthropogenic activities, such as fires, indicating free-living *H. chacarius* as a bioindicator in the studied area.

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Author contribution All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Amanda Costa Rodrigues, Caio Lucca Cação Tognini Ozório, Erica Fernanda Gomes de Sá, Filipe Martins Santos, Gisele Braziliano de Andrade, Heitor Miraglia Herrera, Nayara Yoshie Sano, and Pedro Cordeiro-Estrela. The first draft of the manuscript was written by Amanda Costa Rodrigues, Filipe Martins Santos, Gisele Braziliano de Andrade, and Heitor Miraglia Herrera, and all authors commented on previous versions of the manuscript. Julia Gindri Bragato Pistori revised the submitted manuscript. All authors read and approved the final manuscript.

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Data availability No datasets were generated or analyzed during the current study.

Declarations

Ethics approval The research had an acceptance of the Ethics Committee in the Use of Animals of the Dom Bosco Catholic University (no. 006/2022), of the Federal University of Paraíba (no. 9192091019), and of the System of Authorization and Information in Biodiversity (no. 73472). The license to capture wild animals was authorized by the Chico Mendes Institute for Biodiversity Conservation (no. 72651). The authors have no relevant financial or non-financial interests to disclose.

Competing interests The authors declare no competing interests.

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