UNIVERSIDADE ESTADUAL PAULISTA
“JÚLIO DE MESQUITA FILHO”
INSTITUTO DE BIOCIÊNCIAS – RIO CLARO

PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA, EVOLUÇÃO E BIODIVERSIDADE

INTERAÇÃO ENTRE COMPORTAMENTO E FISIOLOGIA EM MORCEGOS FRUGÍVOROS

PEDRO HENRIQUE MIGUEL

Rio Claro – SP
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PEDRO HENRIQUE MIGUEL

Tese apresentada ao Instituto de Biociências do Câmpus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Doutor em Ecologia, Evolução e Biodiversidade.

Orientador: Dr. Ariovaldo Pereira Da Cruz-Neto

Coorientador: Dr. Augusto Florisvaldo Batisteli

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RESUMO

A perda e fragmentação de habitat, resultantes de mudanças no uso e cobertura da terra por ações humanas, estão entre as principais causas da perda de biodiversidade em todo o mundo. Os morcegos frugívoros, sendo importantes dispersores de sementes, podem ser particularmente afetados por essas mudanças ambientais, inclusive quanto a seu comportamento, fisiologia e sua relação com patógenos. A personalidade reflete a estabilidade nas respostas comportamentais individuais ao longo do tempo (ou seja, o padrão de repetibilidade), e diferentes traços de personalidade podem estar associados, formando síndromes comportamentais. No entanto, poucos estudos analisaram a existência de traços de personalidade e síndromes comportamentais em morcegos frugívoros, o impacto da perda de habitat e a infecção viral. Neste contexto, esta tese aborda três assuntos principais, estruturada em três capítulos: (1) a existência de personalidade e síndromes comportamentais em morcegos frugívoros, (2) os efeitos da perda de habitat no comportamento e fisiologia desses morcegos, e (3) a influência da degradação do habitat e do comportamento na ocorrência de vírus em morcegos. O primeiro capítulo destacou a presença de padrão de alta repetibilidade individual em comportamentos de docilidade, atividade e ousadia em *Artibeus lituratus* e *Carollia perspicillata*, mas com síndromes comportamentais diferentes para cada espécie. Em *C. perspicillata*, os três comportamentos estavam correlacionados, enquanto em *A. lituratus*, apenas docilidade e ousadia estavam correlacionadas. Esses resultados sugerem variações nas pressões seletivas específicas de cada espécie. O segundo capítulo investigou os efeitos da perda de habitat no comportamento de docilidade e na fisiologia dos morcegos, utilizando a região Cantareira-Mantiqueira como área de estudo. Os resultados mostraram que a cobertura florestal influencia significativamente os níveis de estresse fisiológico, indicados pelo proxy da análise de células brancas feitos através da razão neutrófilo/linfócito, e a condição corporal dos morcegos. Maior cobertura florestal foi associada a menores níveis de estresse fisiológico, indicados pela razão neutrófilo/linfócito. Também houve uma relação positiva entre a razão neutrófilo/linfócito e a docilidade. No entanto não houve uma relação direta significativa entre a cobertura florestal e o comportamento de docilidade. Isso sugere que a influência do ambiente sobre o comportamento pode ser mediada por efeitos fisiológicos, como o estresse. O terceiro capítulo analisou a influência da perda de habitat e da docilidade na ocorrência de vírus da família Coronaviridae em morcegos na Mata Atlântica, com amostras coletadas da saliva e de material retal para detecção viral. Foi observado que a ocorrência de vírus aumenta com a porcentagem de cobertura florestal e com a docilidade dos morcegos. Áreas com maior cobertura florestal podem suportar populações mais densas de morcegos, facilitando a transmissão viral, enquanto morcegos mais dóceis são mais propensos a infecções devido ao aumento das interações sociais. Esses achados ressaltam a importância de integrar análises comportamentais, ecológicas e imunológicas nas estratégias de conservação e monitoramento de zoonoses, contribuindo para a compreensão dos impactos das mudanças ambientais na biodiversidade e na saúde dos ecossistemas. A compreensão detalhada dos comportamentos individuais e suas correlações com o ambiente e a fisiologia é essencial para entender melhor as respostas dos morcegos às mudanças ambientais e os potenciais riscos de zoonoses.

Palavras-chave: Mata Atlântica, Ocorrência viral, Perda de habitat, Personalidade, Quirópteros

ABSTRACT

Habitat loss and fragmentation, resulting from changes in land use and cover due to human activities, are among the main causes of biodiversity loss worldwide. Frugivorous bats, being important seed dispersers, can be particularly affected by these environmental changes, impacting their behavior, physiology, and their relationship with pathogens. Personality reflects the stability of individual behavioral responses over time (i.e., the pattern of repeatability), and different personality traits can be associated, forming behavioral syndromes. However, few studies have examined the existence of personality traits and behavioral syndromes in frugivorous bats, the impact of habitat loss, and viral infection. In this context, this thesis addresses three main topics, structured into three chapters: (1) the existence of personality and behavioral syndromes in frugivorous bats, (2) the effects of habitat loss on the behavior and physiology of these bats, and (3) the influence of habitat degradation and behavior on the occurrence of viruses in bats. The first chapter highlighted the presence of a pattern of high individual repeatability in docility, activity, and boldness behaviors in *Artibeus lituratus* and *Carollia perspicillata*, but with different behavioral syndromes for each species. In *C. perspicillata*, the three behaviors were correlated, whereas in *A. lituratus*, only docility and boldness were correlated. These results suggest variations in species-specific selective pressures. The second chapter investigated the effects of habitat loss on docility behavior and bat physiology, using the Cantareira-Mantiqueira region as the study area. The results showed that forest cover significantly influences stress levels, indicated by the proxy of white cell analysis through the neutrophil/lymphocyte ratio, and the bats' body condition. Greater forest cover was associated with lower physiological stress levels, indicated by the neutrophil/lymphocyte ratio. There was also a positive relationship between the neutrophil/lymphocyte ratio and docility. However, there was no direct significant relationship between forest cover and docility behavior. This suggests that the influence of the environment on behavior may be mediated by physiological effects, such as stress. The third chapter analyzed the influence of habitat loss and docility on the occurrence of viruses from the Coronaviridae family in bats in the Atlantic Forest, with samples collected from saliva and rectal swabs for viral detection. It was observed that the occurrence of viruses increases with the percentage of forest cover and with the bats' docility. Areas with greater forest cover may support denser bat populations, facilitating viral transmission, while more docile bats are more prone to infections due to increased social interactions. These findings emphasize the importance of integrating behavioral, ecological, and immunological analyses into conservation strategies and zoonosis monitoring, contributing to the understanding of the impacts of environmental changes on biodiversity and ecosystem health. A detailed understanding of individual behaviors and their correlations with the environment and physiology is essential for better understanding the responses of bats to environmental changes and the potential risks of zoonoses.

Keywords: Atlantic Forest, Chiroptera, Habitat loss, Personality, Viral occurrence

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INTRODUÇÃO GERAL

Através da seleção natural, os animais desenvolveram diversas adaptações fenotípicas aos seus ambientes que podem afetar diretamente a sua aptidão, sendo importantes para a compreensão da evolução e da seleção dos ambientes em que esses animais podem ser encontrados (ENDLER, 1977; HOLTMANN et al., 2017; KAWECK; EBERT, 2004). Essas adaptações fenotípicas podem incluir características morfológicas, fisiológicas e comportamentais, todas essenciais para o desempenho das espécies em diferentes contextos ambientais. Por exemplo, Smith e Skulason (1996) sugerem que diversos táxons podem desempenhar papéis importantes na divergência e especiação populacional, podendo envolver vários tipos de traços, incluindo traços morfológicos e comportamentais, e aqueles relacionados à história de vida. Assim é importante analisar o comportamento como o resultado de características morfológicas e/ou fisiológicas, de modo que as condições atuais dessas características governam ou restringem os comportamentos das espécies (BENCA et al., 2009; SIH et al., 2010).

É fundamental ressaltar que a consistência comportamental é acompanhada por uma plasticidade comportamental limitada, de forma que os traços de personalidade podem restringir a capacidade de um indivíduo de lidar com certas pressões do ambiente, como a abundância de predadores. Isso pode levar a uma distribuição não aleatória de fenótipos comportamentais nos diferentes ambientes, gerando uma covariância fenótipo-ambiente (COTE et al., 2010; DEWITT et al., 1998; SIH et al., 2004). Essa covariância pode ser explicada através do conceito em que os indivíduos se estabelecem em um habitat que melhor se adapta à sua própria personalidade, para reduzir o estresse e evitar ajustes dispendiosos de comportamentos (CARRETE; TELLA, 2010; EDELAAR et al., 2008; HOLTMANN et al., 2017). Entre os traços de personalidade mais comumente estudados estão ousadia, agressividade e exploração (GOSLING et al., 2001; RÉALE et al. 2007; WOLF; WEISSING 2012).

As síndromes comportamentais são compostas por conjuntos correlacionados de traços de personalidade e podem incluir traços relacionados ao comportamento e ecologia dos animais (SIH; BELL, 2008). Neste contexto, a síndrome é definida, considerando a variação interindividual, quando indivíduos de uma mesma população apresentam correlação consistente entre dois ou mais diferentes tipos de personalidade. Por exemplo, traços como ousadia e exploração podem estar correlacionados de maneira estável, manifestando-se de forma consistente em diferentes contextos e situações. Isso implica que, em vez de focar exclusivamente em um comportamento, podemos considerar como esses comportamentos correlacionados interagem entre si para formar padrões (SIH et al., 2004a SIH; BELL, 2008).

A plasticidade comportamental pode explicar a classificação espacial de fenótipos em diferentes ambientes, mostrando que a expressão de traços comportamentais é flexível (BEAMAN et al., 2016; TUOMAINEN; CANDOLIN, 2011; SOL et al., 2013). Quando as condições ambientais são estáveis ao longo do tempo, o ajuste comportamental a esses ambientes também pode gerar covariância entre os tipos de comportamento e o ambiente (CARRETE; TELLA, 2010; EDELAAR et al., 2008).

Esforços comportamentais e fisiológicos para dominar a situação do meio podem ser determinantes e importantes na fisiologia animal (KOOLHAAS et al. 1999). Além disso, os indivíduos tendem a mostrar diferentes respostas fisiológicas a diferentes situações (KOOLHAAS et al. 1999). A variação individual em comportamento agonístico pode ser considerada um exemplo de variação geral no enfrentamento de desafios ambientais, onde indivíduos altamente agressivos adotam um estilo proativo e indivíduos submissos adotam um estilo mais passivo ou reativo (KOOLHAAS et al., 2007).

Em mamíferos, assim como em outros animais, é sugerido que as diferenças individuais nas respostas ao estresse refletem a variação na personalidade (KORTE et al. 2005), levando a uma repetibilidade individual das respostas ao longo do tempo. Espera-se que os animais passivos tenham respostas mais altas do eixo hipotálamo-hipófise-adrenal (HPA) aos estressores (ELLIS et al. 2006). Por exemplo, em aves, a exploração rápida, de alto risco e alta capacidade de resposta a um novo objeto parece estar associada a níveis elevados de corticosterona (MARTINS et al. 2007; RICHARD et al. 2008). Essa repetibilidade tende a ser especialmente pronunciada quando os indivíduos se alimentam em áreas de baixa competição; em áreas de alta competição essas diferenças individuais tendem a menos consistentes (ELLIS et al. 2006).

Cinco gradientes de traços comportamentais são frequentemente utilizados para descrever a personalidade animal: ousadia, atividade, exploração, agressividade e docilidade (RÉALE et al., 2008). Existem diversos estudos que analisam personalidade utilizando essas cinco métricas em insetos (MING et al., 2018; SCHUETT et al., 2018), anfíbios (KELLEHER; SILLA; BYRNE, 2018), répteis (WARD-FEAR et al., 2018), aves (CORNELIUS et al., 2017; METTKE-HOFMANN et al., 2005; WILLIAMS et al., 2012) e mamíferos (BRECK et al., 2019; KOWALSKI et al., 2019; MYERS; YOUNG, 2018).

Os morcegos são essenciais para os ecossistemas devido aos seus diversos papéis ecológicos, como polinização, controle de insetos e dispersão de sementes, sendo os frugívoros particularmente importantes para a regeneração de florestas e a manutenção da biodiversidade vegetal (HOWE; MIRITI, 2004; MEDELLÍN; GAONA, 1999). O estudo do comportamento desses animais é importante para entender como as mudanças ambientais, como a perda e fragmentação de habitat, afetam suas atividades e interações sociais (FAHRIG, 2003; MIGUEL et al., 2019). A degradação ambiental pode não só alterar os padrões de comportamento e fisiológicos dos morcegos, mas também pode influenciar a transmissão de patógenos, incluindo vírus da família Coronaviridae, cuja persistência e disseminação estão frequentemente ligadas à modificação dos habitats naturais (TAYLOR et al., 2001; GIBB et al., 2020)

A perda de habitat é um dos principais fatores das mudanças na paisagem, podendo levar à formação de fragmentos florestais isolados (BROOKS et al., 2002; FAHRIG, 2003). Esse processo é uma ameaça significativa para a biodiversidade global (BETTS et al., 2014; FRANKLIN et al., 2002; LINDENMAYER; FISCHER, 2007) e reduz a diversidade de espécies locais (BENDER et al., 1998; ORTEGA-ÁLVAREZ; MACGREGOR-FORS, 2009). A perda de habitat também pode afetar a dinâmica de transmissão de patógenos, incluindo a ocorrência de vírus, uma vez que a degradação ambiental pode alterar as interações entre espécies e aumentar o risco de disseminação viral (ALROY, 2017; MULLU, 2018).

Os vírus da família Coronaviridae são notáveis por sua capacidade de persistir em reservatórios naturais (TAYLOR et al., 2001; GIBB et al., 2020). Nesse contexto, os morcegos são reconhecidos como reservatórios significativos para esses vírus e as mudanças no habitat e comportamento desses animais podem influenciar a ocorrência e a transmissão de vírus (TANG et al., 2008; WOO et al., 2012; MUYLAERT et al., 2022).

Tendo em vista todas essas questões, nesta tese, abordamos três assuntos principais: (1) a existência de traços de personalidade e síndromes comportamentais em duas espécies de morcegos frugívoros, (2) os efeitos da perda de habitat no comportamento e fisiologia de morcegos frugívoros; e (3) a influência da perda de habitat na ocorrência de vírus da família Coronaviridae em morcegos.

Dessa forma, esta tese estrutura-se em três capítulos:

Capítulo 1: *Personality and behavioral syndromes in two species of fruit bats (Chiroptera: Phyllostomidae)*

Neste estudo, investigamos a presença de personalidade e síndromes comportamentais em morcegos frugívoros, focando em machos das espécies *Artibeus lituratus* e *Carollia perspicillata*. Analisar os padrões de repetibilidade ao longo do tempo é a principal maneira de aferir personalidades. Através de testes repetidos após 48 horas, analisamos a repetibilidade individual em relação aos comportamentos de atividade, docilidade e ousadia. Nossos resultados indicaram um padrão de alta repetibilidade individual para ambos os morcegos, mas diferenças nas síndromes comportamentais entre as espécies. Enquanto *C. perspicillata* apresentou correlações entre os três comportamentos, com indivíduos menos dóceis sendo também mais ousados e ativos, *A. lituratus* mostrou uma correlação positiva apenas entre docilidade e ousadia, sem relação com a atividade. Esses achados sugerem que *C. perspicillata* pode ter uma variação comportamental mais restrita, potencialmente influenciada por pressões seletivas específicas da espécie. Embora os resultados se limitem a machos, a identificação de síndromes comportamentais nesses morcegos frugívoros destaca a importância da variação individual nas funções ecológicas que eles desempenham, especialmente no contexto da dispersão de sementes.

Capítulo 2: *Investigating the effects of habitat loss in the Atlantic Forest on the behavior and physiology of three species of Neotropical fruit bats*

Analisamos como a perda de habitat afeta o comportamento e a fisiologia de morcegos frugívoros na região da Cantareira-Mantiqueira, um exemplo paisagens localmente fragmentada na Mata Atlântica. O estudo foi realizado em gradientes de cobertura florestal que variaram entre 11% a 91%, e incluiu 326 morcegos das espécies *A. lituratus* (n = 63), *C. perspicillata* (n = 67) e *Sturnira lilium* (n = 96). Os morcegos foram capturados utilizando redes de neblina, onde avaliamos o comportamento de docilidade por meio do teste de saco de manuseio, além da condição corporal pelo índice de massa escalado. Também investigamos a razão neutrófilo-linfócito (razão N/L) como indicador de estresse fisiológico. A análise por modelagem de equações estruturais revelou que a cobertura florestal tem um impacto significativo nos níveis de estresse fisiológico dos morcegos, com maiores coberturas associadas a menores níveis de estresse, sugerindo melhores condições nutricionais em habitats com maior cobertura florestal. Embora não tenha sido encontrada uma relação direta significativa entre a cobertura florestal e a docilidade, observou-se uma correlação positiva entre comportamento e a razão N/L, indicando que a perda de habitat pode afetar indiretamente o comportamento através do aumento do estresse fisiológico.

Capítulo 3: *Habitat Loss and Behavior Influence the Occurrence of Coronaviruses in Bats from a Neotropical Biodiversity Hotspot*

Investigamos a influência da perda de habitat e da docilidade sobre a ocorrência de coronavírus em morcegos na Mata Atlântica, um hotspot de biodiversidade global caracterizado por altos índices de fragmentação. Foram capturados 326 morcegos, dos quais 15% testaram positivo para coronavírus. A análise utilizou um modelo logístico binomial, considerando como preditores a docilidade dos morcegos e a porcentagem de cobertura florestal em um raio de 2 km. Os resultados indicaram que a ocorrência viral aumenta com a maior cobertura florestal e com a docilidade dos morcegos, sugerindo que áreas com maior cobertura podem suportar populações mais densas, facilitando a transmissão viral. Além disso, morcegos mais dóceis, possivelmente devido a interações sociais mais frequentes, mostraram maior probabilidade de infecção por coronavírus. Este conjunto de dados destaca a importância de considerar fatores comportamentais e ecológicos ao avaliar a dinâmica de doenças em ambientes fragmentados.

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CAPÍTULO 1: Personality and behavioral syndromes in two species of fruit bats (Chiroptera: Phyllostomidae)

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Personality and behavioral syndromes in two species of fruit bats (Chiroptera: Phyllostomidae)

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Highlights

- We assessed the personality and behavioral syndromes in two Neotropical fruit bats
- Aggressiveness, activity and boldness showed individual repeatability for both
- The three behaviors were correlated in *Carollia perspicillata*
- For *Artibeus lituratus*, only aggressiveness and boldness were correlated
- Our results highlight the effect of individual variation on their ecological roles

Abstract

Personality indicates consistency in individual behavioral responses across time and different personality traits may be correlated in behavioral syndromes. Investigating such dimensions of the behavior in frugivorous animals is crucial given the potential link between individual variation and their ecological significance as seed dispersers. However, few studies have explored the existence of personality traits and behavioral syndromes in fruit bats. In this context, this study aims to test whether the Neotropical fruit bats *Artibeus lituratus* and *Carollia perspicillata* present (1) individual repeatability (i.e. personality) related to the behaviors: activity, aggressiveness and boldness and (2) correlation between these personality axes, constituting behavioral syndromes. All tests were repeated after 48-h to analyze repeatability. We found high individual repeatability of aggressiveness, activity and boldness for both species, but different behavioral syndromes for each of them. For *C. perspicillata*, the three behaviors were correlated to each other, with the most aggressive individuals being bolder and more active. For *A. lituratus*, aggressiveness and boldness were also positively correlated, but activity did not correlate with the other two behaviors. Considering these different syndromes, our results suggest that *C. perspicillata* may demonstrate a potentially reduced variation in behavioral profiles compared to *A. lituratus*, likely influenced by species-specific selective pressures. Although our results are restricted to males the existence of behavioral syndromes in these frugivorous bats contribute to understanding the importance of individual variation on the ecological functions they provide.

Keywords: Activity, Docility, *Artibeus lituratus*, Boldness, *Carollia perspicillata*, Individual variation.

1 INTRODUCTION

The evolution of animal behavior benefits from the wide variation of strategies between individuals because this flexibility confers greater adaptability to populations facing environmental fluctuations. Despite the well-known behavioral flexibility of individuals depending on the environmental stimuli, interindividual variation and its potential consequences for ecological and evolutive processes has gained increased attention in wildlife species. In this sense, animal personality can be defined as the consistency of a given individual behavioral response that remains stable over time (BELL et al., 2009; DINGEMANSE; WRIGHT, 2020; RÉALE et al., 2007; SIH et al., 2004), . Different dimensions of animal behavior can be studied in isolation and still be referred to as a continuum of a given personality axis when showing repeatability at the individual level (CAREAU; GARLAND, 2012; SIH; BELL, 2008). Because behavioral decisions affect fitness via processes such as resource acquisition and predator avoidance, animal personality traits are potentially under evolutive pressures through natural selection and have important ecological consequences (DINGEMANSE et al., 2004; SIH et al., 2004; ROBERTS; SHERRATT, 2002). For instance, bolder and more active individuals play a crucial role in dispersing and establishing new populations (DOHM, 2002; HAYES; JENKINS, 1997), increasing genetic diversity and promoting the long-term persistence of populations at regional scale (DINGEMANSE et al., 2002; MINDERMAN et al., 2009).

Moreover, animal personality is linked with wildlife conservation, influencing how species respond to environmental changes (CAREAU; GARLAND, 2012; DINGEMANSE et al., 2004; SIH et al., 2004; VILLEGAS-RÍOS et al., 2018; COCKREM, 2022). For instance, studies by Careau and Garland (2012), Dingemanse et al. (2004), Sih et al. (2004), Villegas-Ríos et al. (2018), and Cockrem (2022) emphasize how specific personality traits can grant greater adaptability and resilience amidst environmental fluctuations. The boldness of individuals can enable them to explore new habitats or exploit fresh resources, thereby enhancing their survival prospects during periods of ecological degradation (CAREAU; GARLAND, 2012). Similarly, individuals may employ proactive coping strategies, such as seeking alternative food sources or adjusting behavior to changing conditions, potentially bolstering their fitness in evolving environments (DINGEMANSE; DE GOEDE., 2004). Additionally, certain personality types may foster social cooperation and coordination, enabling individuals to adeptly navigate resource competition or collaborate in collective defense against environmental stressors (SIH et al., 2004).

When two or more personality traits are correlated within a population, they constitute a behavioral syndrome (SIH et al., 2004; SIH; BELL, 2008). Although some specific personality traits or behavioral syndromes are better suited to certain contexts than others, fluctuations in environmental conditions should promote the coexistence of a range of personality types within populations (SIH et al., 2012; NICOLAUS et al., 2016). Understanding these individual differences is important for predicting their potential impact on individual fitness and behavior selection (STAMPS; GROOTHUIS, 2010).

A classical axis of animal personality is the boldness/shyness gradient, which reflects the propensity of an individual face a risky situation, usually measured by their reaction to the proximity of predators or humans (CHAPMAN et al., 2011; STAMPS, 2007; WILSON et al., 1994). Boldness is therefore associated with the capacity to use riskier habitat patches such as those with higher predation risk, because bolder individuals cope better with these challenges (COTE et al., 2010; DINGEMANSE et al., 2003; FRASER et al., 2001; STAMPS, 2007). Moreover, research has demonstrated that boldness can be regarded as a consistent personality trait and can influence animal behavior across various contexts, such as foraging, social interaction, and response to environmental stimulus (COCKREM, 2022; DAMMHAHN; ALMELING, 2012; FRASER et al., 2001)

The capacity to move between habitat patches is linked to another aspect of animal personality, the activity gradient, primarily associated with individual propensity for locomotion and movement in the environment (DINGEMANSE et al., 2002; REALE et al., 2007; VERBEEK et al., 1994). This concept reflects in free living individuals the same tendency found in laboratory experiments, where spatial movement is prominently related to the activity gradient (see COTE et al. 2010 for a review). Furthermore, compelling evidence indicates correlations between activity levels and various ecological factors, including individual dispersal distance (DINGEMANSE et al., 2003; FRASER et al., 2001; HOSET et al., 2010), seasonal dispersal patterns (CHAPMAN et al., 2011; VAN OVERVELD et al., 2014), and home range sizes (MINDERMAN et al., 2009; VAN OVERVELD et al., 2011). Hence, activity level represents an informative personality trait for investigating individual and population-level processes in the fields of behavioral and movement ecology (COTE et al., 2010; SIH et al., 2012).

A third personality axis, the docility gradient, refers to an animal's tendency to remain calm and submissive in situations of restriction or containment but may also extend to interactions with conspecifics, predators, and environmental stimuli. These responses is often mediated by glucocorticoid hormones and usually assessed through tests involving the animal's immobility in response to specific stimuli, such as the handling bag test (BIRO; STAMPS, 2008; BOON et al., 2007; CAREAU et al., 2015; MARTIN; REALE 2008; TCHABOVSKY et al., 2024). It is commonly used as a measure of self-preservation, which has evolved due to the intricate trade-off between longevity and reproductive success (RÉALE et al., 2007; VAN OERS; SINN, 2013; ZWOLAK; SIH, 2020). Despite the relevance of each of these personality traits per se, the interplay between them and their impact on individual behavior can provide valuable insights into the complex dynamics of personality in animals.

Despite the relevance of each of these personality traits per se, the interplay between them and their impact on individual behavior can provide valuable insights into the complex dynamics of personality in animals, elucidating the interplay between personality traits and their consequences for individual behavior in animals (SHAPIRO et al., 2021; SIH; BELL, 2008). For instance, while more aggressive individuals may excel in intraspecific conflicts for scarce food resources, their heightened aggression might lead to increased energy expenditure, including in the care of offspring. This allocation of resources towards offspring care, though beneficial in the short term, could potentially result in a trade-off leading to shorter lifespans due to the strain on the individual's health and longevity (NICOLAUS et al., 2016; SHAPIRO et al., 2021; WEBBER; WILLIS, 2020).

The investigation of personality syndromes encompassing these three traits is of paramount importance for conservation (RÉALE et al., 2007; RÉALE et al., 2009; MACKINLAY; SHAW 2023). Investigating the potential syndromes involving the docility, boldness, and activity gradients holds the potential to yield profound insights into the mechanisms by which personality shapes different behavioral profiles within populations (CAREAU; GARLAND 2012; RÉALE et al., 2007; SIH et al., 2012). For instance, a syndrome in which increased aggression coincides with a greater willingness to take risks in resource acquisition results in distinct behavioral patterns that may affect the survival of individuals (RÉALE et al., 2007; RÉALE et al., 2009; ZWOLAK; SIH, 2020). Additionally, some studies report reduced activity levels as a more cautious approach when exploring the environment and moving in fragmented habitats, as in the passerine *Pyriglena leucoptera*, which suggests adaptations to avoid predation risks in response to vegetation structure (BÍZ et al., 2017; CORNELIUS et al., 2017). Syndromes like these have the potential to provide invaluable insights into the correlated manifestation of personality traits and their subsequent consequences (CAREAU; GARLAND 2012; DINGEMANSE et al., 2004; RÉALE et al., 2007). However, despite the importance of studying personality and syndromes, it's worth noting that some groups, as is the case of bats, remain underrepresented in these studies.

Bats serve as valuable models for the study of animal personality and behavioral syndromes for exploring the role of individual variation to conservation and ecosystem functioning, as their individual behavioral traits can impact the persistence of populations and several ecological processes, including resource use, predation dynamics, pollination and seed dispersal (MUYLAERT et al. 2017; SIMMONS; CONWAY, 2003). Understanding how personality influences fruit-eating animals such as bats is crucial, as it can significantly impact seed dispersal, an ecological process vital for maintaining ecosystems (BOBROWIEC; GRIBEL, 2010; FLEMING; HEITHAUS, 1981; MEDINA et al., 2007). This influence could affect decisions related to resource consumption in specific locations, such as more degraded regions (AZEVEDO; YOUNG, 2021; ZWOLAK; SIH, 2020). Personality traits and behavioral syndromes in bats can shape various aspects of frugivory, potentially leading to trade-offs in the quality of seed dispersal, such as transporting seeds to locations with a higher probability of germination, among individuals with different behavioral tendencies (DINGEMANSE et al., 2004; SIH et al., 2012; ZWOLAK; SIH, 2020).

Bats, representing around 20% of all mammals on the planet, exhibit significant ecological and morphological diversity (SIMMONS; CONWAY, 2003). Among the 288 species of Neotropical bats, approximately 83 consume fruits and disperse seeds from around 460 plant species (GORRESEN; WILLIG, 2004; MICKLEBURGH et al., 2002; SIMMONS, 2005). *Artibeus lituratus* and *Carollia perspicillata* (Phyllostomidae) are important seed dispersers abundant in preserved areas, small forest patches, and disturbed rural and urban environments (MUYLAERT et al., 2017; BALLESTEROS; RACERO-CASARRUBIA 2012, JARA-SERVÍN et al. 2017, NUNES et al. 2017). It is important to highlight that the mentioned environmental disturbance encompasses a variety of sources, including urbanization, agriculture, deforestation, and other human activities. These disturbances are increasing primarily due to anthropogenic land use (EWERS; DIDHAM, 2006; FAHRIG, 2003; MUYLAERT et al., 2017). The species mentioned, found in urban, rural, and disturbed environments, face significant challenges due to these disturbances, which can influence their ecology and behavior (MUYLAERT et al., 2017; NUNES et al. 2017; ZWOLAK; SIH, 2020). However, it's worth noting that the few personality studies in bats are most focused on insectivorous species from temperate regions as models (BOYER et al., 2020; MENZIES et al., 2013; SHAPIRO et al., 2021; WEBBER; WILLIS, 2020; BOYER et al., 2020). In general, studies conducted with insectivorous bats have demonstrated repeatability in behaviors such as activity, boldness, aggressiveness, and sociability (MENZIES et al., 2013; SHAPIRO et al., 2021; WEBBER; WILLIS, 2020). Additionally, these studies have identified behavioral syndromes, such as correlations between activity and sociability, and aggressiveness and activity (MENZIES et al., 2013; WEBBER; WILLIS, 2020). However, research on frugivorous bats is scarce, with few studies available (although see HARTEN et al., 2021). Inhabiting the mentioned environmental disturbance gradient, *A. lituratus* and *C. perspicillata* are expected to exhibit a marked variation in personality traits that enhances that behavioral flexibility at population level. Exploring the personality of frugivorous animals, especially those capable of thriving in degraded environments, can yield valuable insights for bat conservation and forest restoration efforts.

In this context, this study aimed to test whether the frugivore bats *A. lituratus* and *C. perspicillata* present individual repeatability related to the behaviors: activity, docility and boldness, indicating they are personality traits. We choose these traits because activity can be associated with the search for resources (DINGEMANSE et al., 2002; REALE et al., 2007), docility can influence competition strategies (RÉALE et al., 2007; VAN OERS; SINN, 2013; ZWOLAK; SIH, 2020), and boldness can affect the exploration of new environments and risk-taking (COTE et al., 2010; DINGEMANSE et al., 2003), which are some important evolutionary characteristics. Moreover, we tested whether these species present correlation between these personality axes, constituting behavioral syndromes and whether they differ regarding the constitution of such syndromes

We present three hypotheses regarding the personality traits and behavioral syndromes of *A. lituratus* and *C. perspicillata*. First, we hypothesize that docility, activity, and boldness are personality traits for these bat species, given the influence of genetic, environmental, and individual factors on the formation and expression of these behavioral traits (BOYER et al., 2020; MENZIES et al., 2013; KUO et al., 2023; WEBBER; WILLIS, 2020). Therefore, we could expect that differences in hormonal responses, lifetime experiences, and social interactions can shape divergent behavioral tendencies among individuals (MENZIES et al., 2013; WEBBER; WILLIS, 2020).

Our second hypothesis is that there are behavioral syndromes for these two species, composed by the correlation between boldness and activity because individuals that are bolder tend to exhibit more active behaviors (ADAMS et al., 2009; BERNARD; FENTON 2002; BIANCONI et al., 2006;). Regarding this hypothesis, we predict a negative correlation between activity and docility. This expectation stems from the premise that highly active individuals may encounter more threats and competitors for resources and mates, conflicting situations that potentially reduce the benefits of passive behaviors (BIRO; STAMPS, 2008; NICOLAUS et al., 2016; WOLF; WEISSING, 2012). We also predict that individuals displaying higher levels of boldness will also exhibit increased activity (BIRO; STAMPS, 2008; NICOLAUS et al., 2016; WOLF; WEISSING, 2012). We also expect to find a negative relationship between boldness and docility in the study species, where bolder bats would be less likely to exhibit passive behaviors (RÉALE et al., 2007; STAMPS, 2007; VAN OERS; SINN, 2013). Last, we hypothesize that study species would differ regarding the constitution of behavioral syndromes as they have distinct life history traits, especially regarding their foraging habitats, movement ecology and home range size (BIRO; STAMPS, 2008; VAN OERS; SINN, 2013).

2 MATERIAL AND METHODS

For bat collection, we used 6 mist nets (dimensions 12 × 3 m; mesh 16 × 16 mm), installed in trails of approximately 2 m wide beneath forest fragments, opened at at 6:00 PM and closed at 11:00 PM (TREVELIN et al., 2017). Bats were captured from December 2021 to March 2022 at two sites of São Paulo State, southeastern Brazil: the Edmundo Navarro de Andrade State Forest, Rio Claro municipality (22°24'56"S; 47°31'17"W) and the Federal University of São Carlos, São Carlos municipality (21°59'05"S; 47°52'50"W). The sites are c.a. 60 km apart and both areas are located in Atlantic Forest regions with nearby urbanization and exhibit similar characteristics with the predominance of seasonal semideciduous forest. These areas share not only the same vegetation formation but also are subject to similar influences from the surrounding urban environment.

We captured 27 adult males of each species. Although we acknowledge the importance of using both sexes in personality studies, the decision to use only male bats was a strategy to minimize the potential variation that could arise from differences between the sexes and related to different reproductive stages of females. After captured, each individual was placed in a cloth bag (25 cm high × 20 cm long) for the first measurement of docility (see below). All animals captured on each collection night were kept individually in cloth bags until the end of the collection period (11:00 PM), when they were transported to the Laboratory of Animal Physiology at the Institute of Biosciences of the Universidade

Estadual Paulista “Júlio de Mesquita Filho” at Rio Claro. In the lab, they were housed in individual cages (40 × 40 × 40 cm) and kept in a climatic chamber at 26 ºC and photoperiod 12/12 h for 2 days before the other behavioral tests. Individuals were fed daily with a piece of papaya (Carica papaya) and received water ad libitum and bats were kept in the lab for a total of 7 days.

Bats were tested in a random order and the behavioral tests were run consecutively starting with the docility test, then the activity test and boldness test at last. Each test was conducted twice with each individual in an interval of 48 h between replicates to allow estimating individual repeatability of behaviors. With exception of the first docility test, which was performed in the field right after the bats were taken off from the nets, all the other tests were carried out in the lab.

2.1 Docility test

The handling bag test was performed as a measure of docility (CAREAU et al., 2015; MARTIN; REALE, 2008). This test consists of quantifying movement time, i.e., the time in seconds the animal struggles from the moment the animal is placed in the bag within a period of 180 seconds. Individuals who moved for a longer time were considered less docile (CAREAU et al., 2015; MARTIN; REALE, 2008). This test was performed right after capture in the field and repeated in the lab after 48 h before beginning the other experiments.

2.2 Activity test

To measure activity level of the individuals, we adapted the open field test (MARTIN; RÉALE, 2008; MINDERMAN et al., 2009). For this, we built an arena measuring 230 × 110 × 110 cm (length × height × width), with the sides, top and bottom covered with PVC screen (mesh 5 × 7 mm) and transparent plastic on the front side, so that the animals' activities could be recorded using a Sony Handycam HDR-CX405 camcorder positioned 2.5 m from this side. This arena was built in a closed room free of weathering and external interference, with temperature varying between 22ºC to 26ºC.

We individually placed each bat in this arena after 6:00 pm, when these animals begin their activities in the wild, and filmed them for 30 minutes. We watched the videos to assess activity, defined as the sum of the duration in seconds of flights inside the arena within that interval. We assume animals that moved longer as more active (BREHM et al., 2019; METTKE-HOFMAN et al., 2014).

2.3 Boldness test

The boldness test was performed right after the activity test in another closed room, free from weathering and external interference. For this test, the bats were replaced in the same individual cages they were kept since brought to the lab (40 × 40 × 40 cm) and after a short interval (i.e. 3 min) were fed in a round plastic bowl (5 cm in diameter) with papaya, the same food they received since captured. After introduce the bowl in the cage, the same researcher stand up 30 cm distant from the cage, which was positioned 1.5 m above ground, and recorded the latency for the animal start eating. We assumed that individuals who took less time to start eating were bolder. Five *A. lituratus* and three *C. perspicillata* individuals did not approach the bowl in one of the replicates of the boldness tests within a 5 min interval and were discarded from the boldness repeatability and behavioral syndromes analysis. After the first and second replicate of the boldness test, bats were weighed to the nearest 1 g and then returned to the climate-controlled room.

2.4 Statistical analysis

In order to assess the existence of personalities, we performed repeatability tests using the 'rptGaussian' function in the 'rptR' package (BREHM et al., 2019; STOFFEL et al., 2017). This function uses linear mixed-effects modeling to provide a repeatability estimate that ranges from 0 to 1 and its confidence interval is calculated using parametric bootstrapping and Bayesian method. The method tests whether the behavioral responses are consistent at the individual level, that is, whether the repeatability estimate differs from zero, through a likelihood ratio test (STOFFEL et al., 2017). We first calculated the adjusted repeatability estimates by including in all models potential confounding effects, namely body mass, site (Rio Claro and São Carlos) and trial (first vs. second test) as independent variables. In parallel, we also calculated non-adjusted repeatability estimates, which do not take into account any potential effect of the independent variables. We set individual identity as a random factor, and defined 1000 iterations for parametric bootstrapping for all these models. To assess the effect of those independent variables in behavioral responses, we rebuild the same linear mixed-effects models using the packages 'lme4' (BATES et al., 2007) and 'lmerTest' (KUZNETSOVA et al., 2015). To enhance the comprehension of the biological meaning of the boldness and docility measurements, latency to feed during boldness tests and time struggling in the handling bag tests were multiplied by -1. Therefore, a latency to feed of zero seconds is the highest boldness value and zero seconds of struggling time is the highest docility value when addressing its relationship with docility and activity when analyzing the behavioral syndromes (see below).

To test whether the behaviors studied were correlated in behavioral syndromes, we first estimated patterns of covariance between pairs of the behavioral measurements by means of mixed-effects multivariate models following the Markov chain Monte Carlo (MCMC) procedure using the “MCMCglmm” package (HADFIELD 2010). We built one model for each bat species where the response variables were docility, boldness and activity, all standardized to mean = 0 and standard deviation = 1, setting informative priors (expected variance V = diag (3); degree of belief nu = 1) and 1 500 000 iterations. After a burn in of 500 000 iterations thinned by 100 iterations, density plots were visually checked to ensure proper model mixing and convergence. The covariances obtained indicated the phenotypic variation for each of the behaviors, which can be partitioned into two components. The within-individual variation denotes adjustments in their responses between replicates of a given test and indicates essentially behavioral plasticity at individual level; on the other side, among-individual variation reflects the differences between individuals for a given behavioral test (DOCHTERMANN; DINGEMANSE 2013). Therefore, behavioral syndromes were assumed when there was a significant correlation between the among-individual variance for any pair of behavioral traits (i.e. when their 95% confidence interval did not overlap zero, MARTINIG et al. 2022) and reported as β being the median of 10 000 estimates of correlation coefficients produced by the MCMC procedure. All analyzes were conducted in the R software (R CORE TEAM, 2022). Adjusted repeatability estimates, denoted as R_{adj}, are followed by their confidence intervals as CI [minimum – maximum] and the respective non-adjusted repeatability estimate ($R_{non-adj}$), and other values are presented as mean ± standard deviation unless otherwise noted.

2.5 Ethical Note

This study received ethical approval from the Coordination of the Animal Use Ethics Committee (CEUA) and adhered to the ASAB/ABS guidelines. Following the completion of the study, all captured bats were released at their original capture location.

3 RESULTS

Docility exhibited significant repeatability in both species. For *C. perspicillata*, the adjusted repeatability coefficient (R_{adj}) was 0.640 (CI = 0.378–0.839; $R_{non-adj} = 0.569$; n = 24; $p < 0.001$), while for *A. lituratus* it was 0.747 (CI = 0.521–0.901; $R_{non-adj} = 0.704$; n = 22; $p < 0.001$). Activity also showed repeatability for *C. perspicillata* (R = 0.516; CI = 0.188–0.783; $R_{non-adj} = 0.458$; n = 24; p = 0.014) and *A. lituratus* (R = 0.494; CI = 0.155–0.789; $R_{non-adj} = 0.453$; n = 22; p = 0.013). Boldness exhibited high repeatability for both species; in *C. perspicillata*, the repeatability coefficient was 0.915 (CI = 0.836–0.968; $R_{non-adj} = 0.865$; n=24; $p < 0.001$), while for *A. lituratus*, it was 0.813 (CI = 0.613–0.928; $R_{non-adj}$

= 0.813; n = 22; $p < 0.001$). None of the behaviors studied was affected by body mass, study sites or trials for both species (Table 2).

Positive correlations were found between boldness and docility for *C. perspicillata* (β = -0.720; Table 3; Fig. 1A; Fig. 2) and for *A. lituratus* (β = -0.512; Table 3; Fig. 1B; Fig. 2). Additionally, activity was positively correlated with the boldness for *C. perspicillata* (β = 0.798; Table 3; Fig. 1C; Fig. 2), but not for *A. lituratus* (Table 3; Fig. 1D; Fig. 2). Similarly, the activity was negatively correlated with docility for *C. perspicillata* (β = -0.791; Table 3; Fig. 1E; Fig. 2), but not for *A. lituratus* (Fig. 1; Fig. 2).

Table 1. Variation of docility, boldness, and activity in *Artibeus lituratus* and *Carollia perspicillata* between two replicates of the tests, all measured in seconds as time struggling during handling-bag tests (zero seconds of struggling time is the highest docility value), latency to feed near an observer (zero seconds is the highest boldness value) and time moving inside a flight tent, respectively.

	Artibeus lituratus			*Carollia perspicillata*		
	Mean	Sd	Range	Mean	Sd	Range
Docility						
First trial	-55.3	33.3	-131, -110	-37.6	29.2	-92, -66
Second trial	-52.6	31.4	-140, -15	-38.1	28.7	-102, -5
Boldness						
First trial	-92.7	73.6	-263, -10	-95.2	65.3	-225, -20
Second trial	-99.2	85.4	-280, -10	-93.6	74.9	-255, -20
Activity						
First trial	66.8	73.4	2, 318	48.4	29.8	6, 112
Second trial	57.4	54.4	3, 180	37.0	22.9	6, 82

Table 2. Results of linear mixed effects models assessing the effect of body mass, site (Rio Claro and São Carlos) and trial (first and second test) on individual responses of two Neotropical frugivorous bats (*Carollia perspicillata* and *Artibeus lituratus*) during behavioral tests of docility, boldness and activity. SE: Standard error; *: significant p-values at $\alpha = 0.05$.

	Carollia perspicillata			*Artibeus lituratus*		
	Estimate (SE)	t value	p	Estimate (SE)	t value	p
Docility						
Intercept	46.513 (62.509)	0.744	0.465	-19.349 (68.553)	-0.282	0.781
Body mass	-0.670 (3.405)	-0.197	0.846	0.994 (0.941)	1.057	0.305
Site	24.991 (13.686)	1.826	0.082	9.205 (23.232)	0.396	0.697
Trial	1.667 (4.913)	0.339	0.738	-1.524 (5.131)	-0.297	0.770
Activity						
Intercept	62.046 (54.53)	1.138	0.268	258.284 (132.757)	1.946	0.067
Body mass	-0.966 (2.969)	-0.325	0.748	-2.231 (1.821)	-1.226	0.236
Site	20.107 (11.935)	1.685	0.107	-33.662 (44.948)	-0.749	0.464
Trial	-9.458 (5.163)	-1.832	0.080	-8.667 (15.18)	-0.571	0.574
Boldness						
Intercept	-31.442 (165.584)	-0.190	0.851	217.201 (175.037)	1.241	0.231
Body mass	6.794 (9.025)	0.753	0.460	-0.876 (2.403)	-0.365	0.720
Site	-38.930 (36.277)	-1.073	0.295	-64.594 (59.331)	-1.089	0.291
Trial	8.625 (5.853)	1.474	0.154	12.524 (11.05)	1.133	0.270

Table 3. Among-individual correlation coefficients (medians and their 95% credible interval) of personality traits in two Neotropical fruitbats. Significant correlations, in bold, were assumed when the 95% credible interval does not overlap zero.

	Carollia perspicillata	*Artibeus lituratus*
Docility vs. Boldness	**-0.720 (-0.922, -0.426)**	**-0.512 (-0.854, -0.054)**
Docility vs. Activity	**-0.791 (-0.943, -0.545)**	-0.383 (-0.813, 0.183)
Boldness vs. Activity	**0.798 (0.563, 0.946)**	0.239 (-0.316, 0.751)

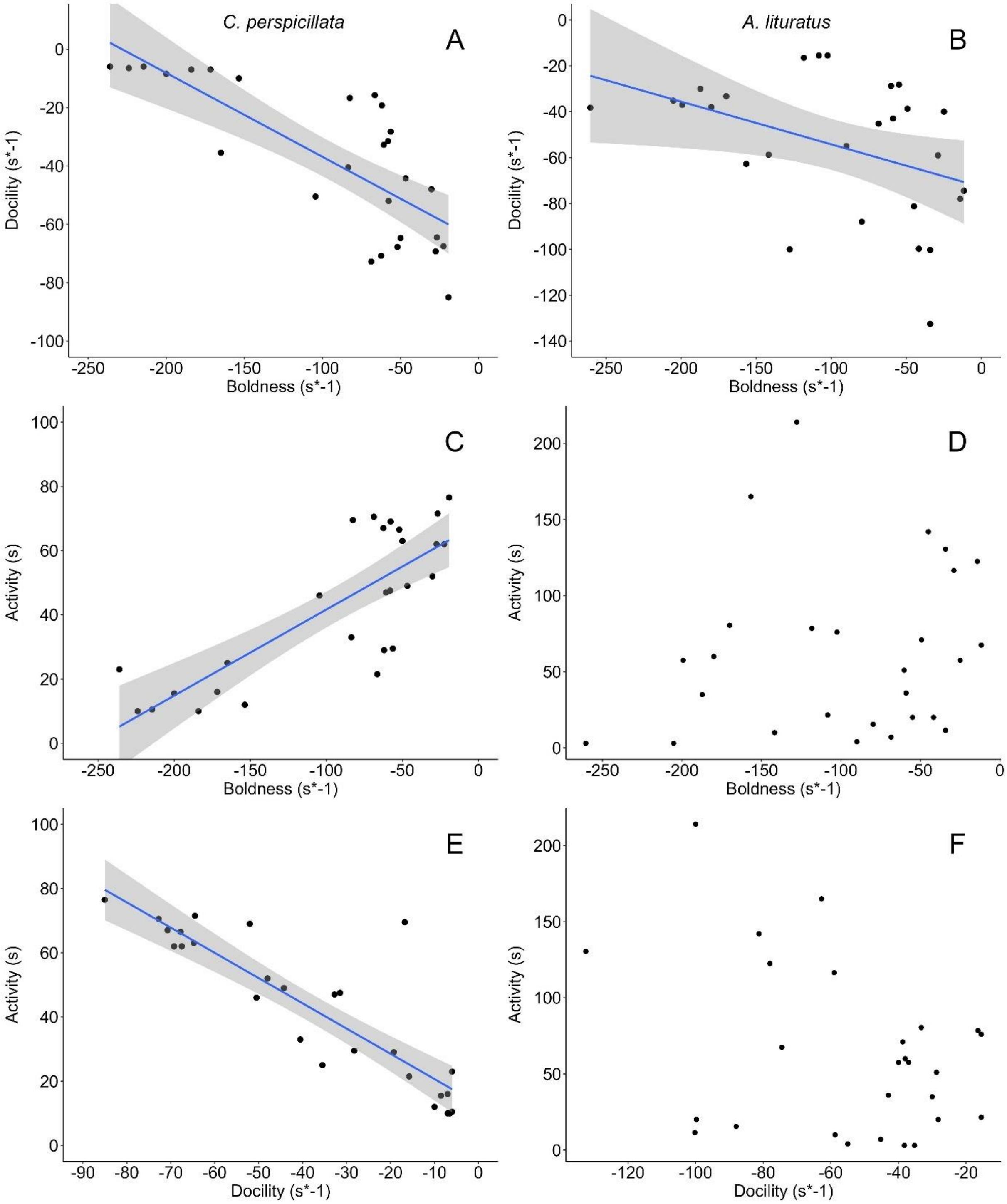

Fig. 1. Among-individual variation in docility, boldness and activity in the fruitbats *Carollia perspicillata* and *Artibeus lituratus*. The straight line in A, B, C and E panels indicates a significant association between the personality traits and the hatched area indicates its 95% standard error, whereas the panels D and F illustrate independent variation of these behavioral responses. To enhance the comprehension of the biological meaning of boldness

and docility, latency to feed and time struggling during the handling-bag test were multiplied by -1 (e.g. zero latency is the highest boldness value and zero seconds of struggling time is the highest docility value).

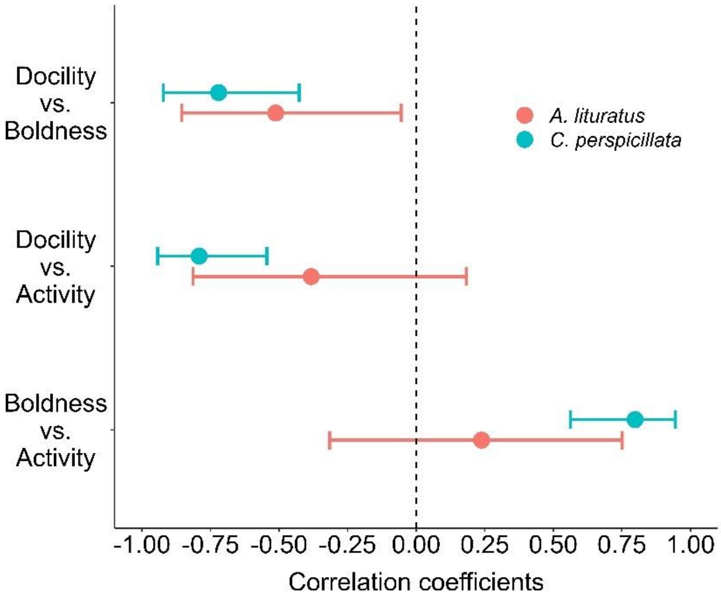

Fig. 2. Behavioral syndromes resulting from among-individual correlation between docility, boldness and activity in the fruitbats *Carollia perspicillata* and *Artibeus lituratus*. The dots indicate the median and the whiskers delimit the respective 95% credible interval of correlation coefficients obtained from multivariate linear mixed-effects models by the Markov chain Monte Carlo method. Significant associations between the personality traits were assumed when the 95% credible interval does not overlap 0 (vertical dashed line).

4 DISCUSSION

The three behavioral axes studied showed repeatability at individual level for *A. lituratus* and *C. perspicillata*, corroborating our first prediction, so that we can consider them personality traits in both species. This result is in line previous studies on insectivorous bats

(WEBBER; WILLIS, 2020; BOYER et al., 2020) and numerous evidence from other taxa (BELL et al., 2009), and reinforces that intraspecific variation in behavioral decisions has an expressive relevance to understand ecological and evolutive aspects of populations (MACKINLAY; SHAW 2023).

We found evidence of behavioral syndromes for both species studied, whereas previous studies on bats report mixed evidence supporting behavioral syndromes (WEBBER; WILLIS 2020; KUO et al., 2023) or not (BOYER et al., 2020; WANG et al., 2020). We highlight, however, that our second hypothesis was not fully supported as we found different behavioral syndromes for each species, given the correlation between boldness and docility for both, and the correlation of docility with activity and boldness only for *C. perspicillata*. The part of the behavioral syndrome common to the two model species was composed by boldness and docility, where the more aggressive the individuals, the bolder they were. One possible explanation for this is related to responses to predators, where bolder animals need to be more aggressive to thrive in the face of predators or competitors, while less bold animals tend to adopt evasive behaviors to avoid risks (DINGEMANSE; WOLF, 2010; LE COEURS et al., 2015; RÉALE et al., 2010). Predation plays a crucial role in determining the fitness of individuals within a population and more aggressive individuals can have a competitive advantage when facing predators due to their increased chances of survival and reproduction (DINGEMANSE; WOLF, 2010; WOLF; WEISSING, 2012). On the other hand, shyer individuals may reduce their risk of predation by avoiding direct confrontations, but it can reduce their opportunities of obtaining resources or reproductive partners (DINGEMANSE; WOLF, 2010; LE COEURS et al., 2015; RÉALE et al., 2010).

Carollia perspicillata and *A. lituratus* exhibited a common correlation between boldness and docility, which can be attributed to the underlying mechanisms driving personality traits in animals, such as genetic inheritance, environmental factors, and lifetime experiences (DOUGHERTY; GUILLETTE, 2018; WOLF; WEISSING, 2012). For example, bold individuals are more likely to display higher levels of aggression, which can be favorable when facing threats or competition, such as defending their territories (ZWOLAK; SIH, 2020; WOLF; WEISSING, 2012). This territorial defense can be crucial to ensuring access to food resources, such as feeding areas or breeding sites. Furthermore, the correlation between boldness and docility is also evident when these bats face predators. Bolder individuals tend to react more aggressively when faced with threats (MACKINLAY; SHAW, 2022; MOIRON et al., 2020; WOLF; WEISSING, 2012).

There were differences in the behavioral syndromes between the two species as activity was correlated to docility and boldness only in *C. perspicillata*. Potential factors related to this difference include some ecological and behavioral particularities of each species, which can be attributed, in part, to their different evolutionary histories. Over time, these species may have faced different selective pressures due to variations in the ecology of their habitats (LARSEN et al., 2013; PAVAN et al., 2011). *Carollia perspicillata*, being dominant in fragmented areas, may have experienced more intense selective pressure for the development of specific behaviors aimed at dealing with increased predation risk in these areas (ANDRADE et al., 2013; FLEMING; HEITHAUS 1981; LAURINDO et al., 2017; PAVAN et al., 2011). In contrast, *Artibeus lituratus*, although also capable of exploiting fragmented habitats, may have evolved in environments where selection for behavioral flexibility was more relevant (ANDRADE et al., 2013; GALETTI; MORELLATO, 1994; LARSEN et al., 2013; MEDINA et al., 2007). These differences in selective pressure over the evolutionary history of each species may have shaped their behavioral responses in distinct ways, resulting in the observed variation in activity and docility levels between them. Consequently, higher activity levels would demand a selection for greater docility to enable these individuals to deal with potential threats associated with habitat fragmentation. In contrast, *A. lituratus* is dominant either in continuous forest or fragmented areas (ANDRADE et al., 2013; GALETTI; MORELLATO, 1994; MEDINA et al., 2007).

The species demonstrated individual variation in activity levels independently of docility and boldness, which results in a greater flexibility of individual behavioral profiles. This high plasticity at population level may translate into greater adaptability, allowing *A. lituratus* to occupy habitat patches within a wide disruption degree, making it more resilient to environmental changes (ANDRADE et al., 2013; GALETTI; MORELLATO, 1994; MEDINA et al., 2007).The observed differences in behavioral syndromes between the two species may also be associated to divergences in their foraging habits. *Artibeus lituratus* exhibits lower fidelity to specific locations and a broader foraging rang, influenced by food availability, particularly of *Ficus ssp.* (GALETTI; MORELLATO, 1994; MEDINA et al., 2007). In contrast, *C. perspicillata*, due to a lower flight capacity and specialized feeding preferences for *Piper* ssp., displays greater fidelity to specific foraging areas, favored by the edge effect in fragmented habitats (ANDRADE et al., 2013; FLEMING; HEITHAUS 1981; LAURINDO et al., 2017).

Our study revealed that two species of frugivorous bats present different behavioral syndromes, which shed light on the understanding of the ecological functions provided by each of them, since their behavioral profiles potentially modulate several stages of zoochoric seed dispersal, such as selecting the feeding location and dispersal distance (BREHM et al., 2019; HUNTER JR et al., 2022; ZWOLAK; SIH, 2020). Moreover, by indicating that activity is repeatable in frugivorous bats, our results indicate a way by which a personality trait may affect seed dispersal quality at individual level, a neglected aspect of plant-animal interaction, which deserves further studies (ZWOLAK; SIH, 2020). Although our study was based on males only, we argue that our results are robust enough to shed light on the remarked expression of personality traits and behavioral syndromes in our study species.

Future studies should investigate intersexual differences regarding these aspects as well as how they vary between different reproductive stages of males and females.

5 CONCLUDING REMARKS

Our data indicate that behaviors related to docility, boldness and activity are linked to personality in *C. perspicillata* and *A. lituratus*, presenting relatively high degrees of repeatability at the individual level. We also found that some of these behaviors may be associated with each other forming species-specific behavioral syndromes. The structure of these behavioral syndromes suggests that *C. perspicillata* has less intraspecific variation in behavioral profiles than *A. lituratus*, since the three behavioral aspects studied are being selected apparently in an associated way in the former but not in the last. The behavior responsible for this difference in the composition of behavioral syndromes between species is activity, which may be linked to selective forces of divergent natures acting on each of them regarding movement ecology. The results of this study are particularly important to demonstrate the need to evaluate the biological performance of these species in different ecological scenarios from the perspective of personality and behavioral syndromes.

Data Availability

Data that support the findings of this study are available at: https://github.com/pedrohmiguel2/Personality-and-behavioral-syndromes-in-two-species-of-fruit-bats-Chiroptera-Phyllostomidae-

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SUPPLEMENTARY MATERIAL

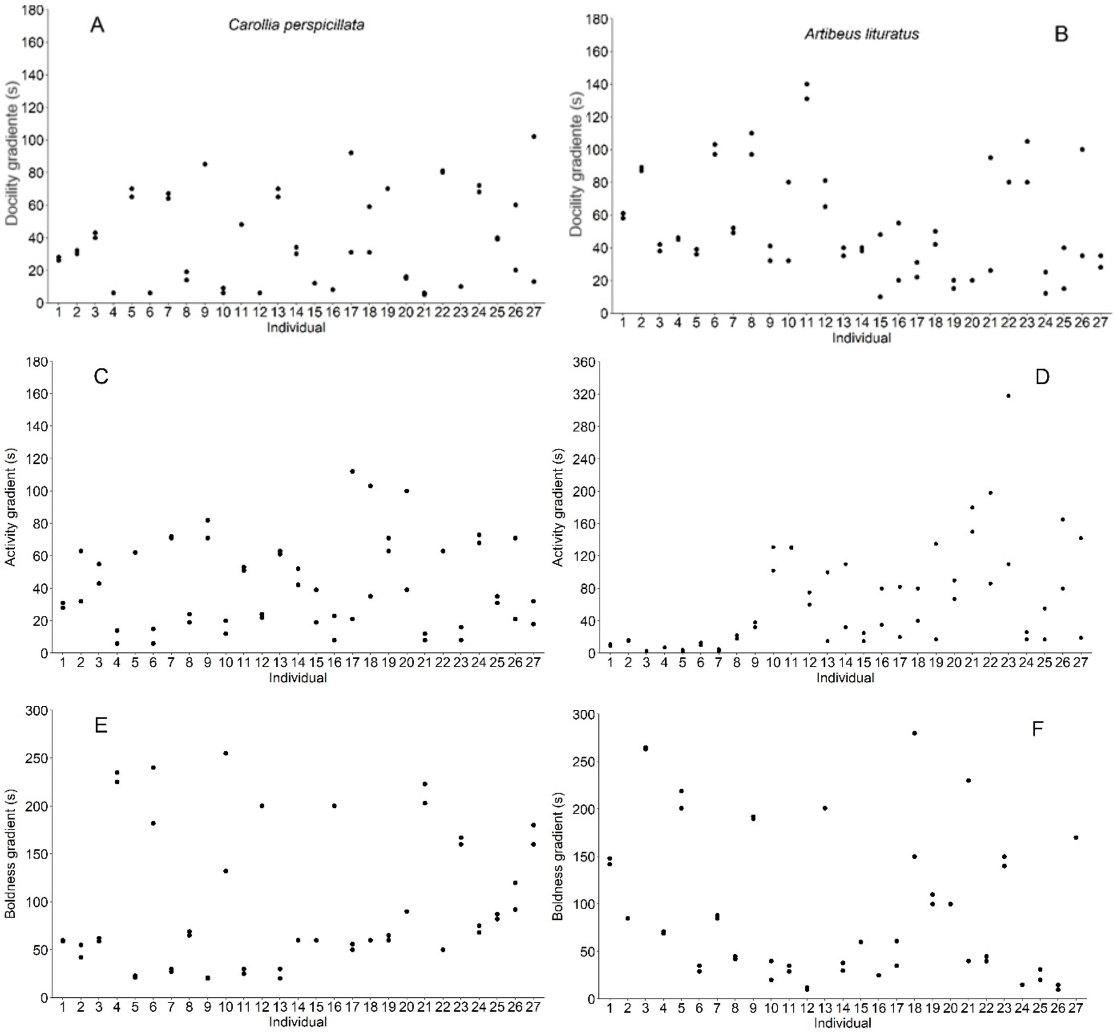


Fig A. Individual variation in docility, activity and boldness in *Carollia perspicillata* (left, A, C and E) and *Artibeus lituratus* (right, B, D, F) measured respectively by the time struggling in the bag-handling test, where zero seconds of struggling time is the highest docility value, time moving in the flight tent and latency to start feeding in face of an observer, where latency 0 corresponds to the highest boldness value.

CAPÍTULO 2: Investigating the effects of habitat loss in the Atlantic Forest on the behavior and physiology of three species of Neotropical fruit bats.

Tipo de manuscrito: artigo

Highlights

- We investigated the effects of habitat loss on the behavior and physiology of three species of Neotropical fruit bats.
- Habitat loss was associated with higher levels of physiological stress, measured by the N/L index.
- We did not find a significant relationship between forest cover and bat docility.
- Behavioral plasticity and ecological adaptability can influence the uniform responses of species to habitat loss.

Abstract

Habitat loss is considered the most significant form of environmental degradation resulting from human activities, potentially leading to various adverse effects such as increased extinction risk, reduced species richness, and higher predation rates among sensitive species. The Atlantic Forest serves as a prime example of such degradation, characterized by fragmented and diminished remnants. Habitat loss can profoundly influence animal behavior, with consistent behavioral variations observed within species. These variations impact environmental perception and responses to risks. Behavioral adaptations in animals can mitigate the negative impacts of habitat loss but may also correlate with physiological changes, such as an increase in neutrophils relative to lymphocytes, known as the N/L ratio, which often indicates physiological stress. Fruit bats are an ideal model for studying these dynamics due to their ecological role as seed dispersers and their sensitivity to habitat alterations. Studies on Neotropical fruit bats have demonstrated the presence of distinct personalities, with correlations to habitat loss observed in some studies. This research aims to investigate the effects of habitat loss on behavior and physiology, specifically through the N/L ratio. The study was conducted in the Cantareira-Mantiqueira region, examining gradients of forest cover ranging from 11% to 91%. Bats were captured using mist nets placed in flight corridors. Behavior was assessed using the handling bag test, and body condition was evaluated using the scaled mass index. For N/L analysis, blood smears were prepared from 10 µl samples per individual and examined under oil immersion microscopy at 100x magnification. Differential leukocyte counts were conducted by identifying and counting lymphocytes, neutrophils, eosinophils, and monocytes among 100 leukocytes, from which the N/L ratio was derived. Structural equation modeling was employed to explore relationships between behavior, N/L ratio, body condition, and habitat loss. A total of 226 fruit bats belonging to *Artibeus lituratus* (n = 63), *Carollia perspicillata* (n = 67), and Sturnira lilium (n = 96) were analyzed. Results indicate that forest cover significantly influences bat stress levels as indicated by the N/L ratio and body mass. Higher forest cover was associated with lower physiological stress levels, suggesting that more intact habitats provide better nutritional conditions for bats. However, no direct significant relationship was found between forest cover and docility behavior, despite a positive association between behavior and the N/L ratio. These findings suggest that habitat loss may indirectly impact bat behavior through changes in physiological stress levels. Species-specific responses indicate varying behavioral and physiological adaptations that facilitate survival in altered environments, underscoring the importance of behavioral flexibility and foraging strategies for species conservation amidst habitat loss. Previous studies have noted a negative correlation between habitat loss and the N/L ratio, although the absence of a significant link between habitat loss and behavior suggests potential behavioral adaptations of these bats to varying forest cover levels. The positive correlation between habitat loss and body condition may reflect shifts in food resource availability or physiological responses to habitat degradation. The association between the N/L ratio and behavior underscores the interconnectedness of physiology and behavior in these fruit bat species. The observed behavioral adaptability of fruit bats to different forest cover levels implies their capacity for flexible responses to environmental changes.

Keywords: Habitat loss, Fruit bats, Physiological stress, Docility, Animal behavior

1 INTRODUCTION

Deforestation and forest fragmentation represent critical forms of environmental degradation resulting from human activities. These processes entail the reduction of original vegetation, leading to several adverse effects such as heightened extinction risks, decreased species richness, and increased predation rates for vulnerable species (EWERS; DIDHAM, 2006; FAHRIG, 2003). The remnant small habitat fragments can lead to reduced genetic diversity, altered species interactions, increased vulnerability to environmental changes and often contribute to population decline due to limited resources and increased isolation of remaining patches (BENNETT; SAUNDERS, 2011; BERNARD; FENTON, 2007; BENDER et al., 1998). Connectivity between forest fragments, land use, and total habitat extent within surrounding areas directly influence spatial distribution, diversity and species behavior (CORNELIUS et al., 2017; ESTAVILLO et al., 2013; SIH et al., 2004).

The Atlantic Forest biome is a pivotal scenario for conservation studies, being considered one of the global biodiversity hotspots due to its high concentration of endemic species and severe degradation (BICUDO DA SILVA et al., 2020; MARQUES et al., 2021; MYERS, 2003), with only approximately 12 to 16% of its original cover (MYERS et al., 2000; RIBEIRO et al., 2009; VANCINE et al., 2024). Most (i.e. 80-97%) of these forest fragments are smaller than 50 hectares, and approximately 50-60% of the remaining vegetation lies within 90 meters of fragment edges (RIBEIRO et al., 2009; VANCINE et al., 2024). Moreover, these areas are in landscapes heavily altered by human activities, marked by intense agriculture, inadequate protection, and reduced connectivity (BICUDO DA SILVA et al., 2020; MARQUES et al., 2021; VANCINE et al., 2024). These conditions underscore the biome's vulnerability and complexity, making it a crucial model for investigating the impacts of habitat loss on animal behavior (BICUDO DA SILVA et al., 2020; VANCINE et al., 2024).

Behavior is a complex response mediated by the nervous system and influenced by the endocrine system. The nervous system processes sensory information and coordinates immediate responses, while the endocrine system releases hormones that can impact long-term behavior. Thus, the link between behavior and stress occurs through stress hormones such as corticosterone and cortisol, which are primary responses to stress (SCHWABL, 1995; SILVERIN, 1998; COCKREM; SILVERIN, 2002; COCKREM, 2007). The release of these stress hormones increases neutrophil counts and decreases lymphocyte counts (DAVIS et al., 2008). Consequently, the neutrophil-to-lymphocyte ratio (N/L ratio) has been proposed as a proxy for measuring stress levels in vertebrates (DAVIS et al., 2008; JOHNSTONE et al., 2012). These physiological changes allow for adjustments in both physiological and behavioral responses to adverse environmental conditions, thereby explaining behavioral plasticity within populations (CARERE et al., 2003).

Animals can adapt their behavior to mitigate risks, especially during exploration and dispersal movements, when they encounter threats such as predation risk and competition for resources (CORNELIUS et al., 2017; DILL, 1990). In this way, animal movement within landscapes, including fragmented ones, can be shaped by adaptations to environmental conditions (SIH et al., 2004; SPIEGEL; CROFOOT, 2016). Gathering and utilizing information about these risks enhance the safety of animal movements, enabling avoidance of high-risk areas and exploration of more favorable environments (DINGEMANSE et al., 2012; DOSMANN; MATEO, 2014; SIH et al., 2012). This ability of using environmental information can result from genetic inheritance and direct experiences with the environment (MORTELLITI; BREHM, 2020; STAMPS; GROOTHUIS, 2010; WIROWSKA et al., 2024), both potentially contributing for consolidating divergent behavioral strategies among conspecifics. For instance, proactive individuals are typically less docile, more exploratory, and prone to risk-taking, whereas reactive individuals exhibit lower activity levels and greater shyness (KOOLHAAS, 2008; WIROWSKA et al., 2023). This behavioral diversity reflects adaptive strategies for coping with environmental challenges and directly influences individual reproductive success and survival (SANTICCHIA et al., 2018; SIH et al., 2012; VILLEGAS-RÍOS et al., 2018), especially when resource abundance is lower as in fragmented areas. The increased competition for limited resources in degraded habitats may lead to reduced docility as animals can adopt more aggressive or bold strategies to secure resources (SANTICCHIA et al., 2018; VILLEGAS-RÍOS et al., 2018).

The maintenance of behavioral diversity within populations can be explained by the 'extended pace-of-life syndrome' hypothesis (BELL, 2007; BREHM; MORTELLITI, 2024; RÉALE et al., 2010), suggesting that different individuals adopt varied strategies to allocate resources (BREHM; MORTELLITI, 2024; CARTER et al., 2013). Behaviors such as activity, boldness, and docility are usually part of these individual-level trade-offs (DAMMHAHN et al., 2018; RÉALE et al., 2010; SHAPIRO et al., 2021). According to that hypothesis, more active, bold, and less docile individuals are expected to achieve faster growth rates and greater reproductive success, potentially at the cost of increased mortality and exposure to parasites (BARBER; DINGEMANSE, 2010; BIRO; STAMPS, 2008).

To investigate the ecological significance of animal behavior, researchers often conduct measurements in captivity, which offers numerous advantages, primarily the ability to control testing conditions (CAMPBELL et al., 2009; HERBORN et al., 2010). While measurements in controlled environments are generally extrapolable to natural settings, studying processes such as habitat loss solely through laboratory analyses may yield contentious data, particularly because behavioral changes can occur as wild individuals adapt to captivity (BUTLER et al., 2006). Therefore, field studies are very important as they provide reliable assessments of how animals interact with their natural habitats and respond to environmental stressors.

Although animal behavior is a fundamental topic for ecology (MERRICK; KOPROWSKI, 2017; WAGNON et al, 2024), the relationships between animal behavior and habitat loss remain poorly studied (HALE et al., 2020; WAGNON et al, 2024). This knowledge gap becomes even more evident in some taxa, such as bats (BOYER et al., 2020; MENZIES et al., 2013; SHAPIRO et al., 2021; WEBBER; WILLIS, 2020). Chiroptera is the second largest order of mammals, encompassing over 1450 bat species distributed worldwide (SIMMONS; CIRRANELO, 2022). They play crucial ecological roles, including pollination, seed dispersal, and insect population control (FLEMING et al., 1972; REIS et al., 2007). Half of the 204 species within the Phyllostomidae family are primarily frugivorous and potential seed dispersers, exhibiting varying levels of dietary specialization (FENTON; SIMMONS, 2014). However, there remains a research gap concerning behavioral studies on Neotropical fruit bats. Given their ecological significance in seed dispersal (HOWE; MIRITI, 2004; MEDELLÍN; GAONA, 1999), studies utilizing this group as a model are crucial for analyzing ecological degradation processes such as habitat loss (EWERS; DIDHAM, 2006; FAHRIG, 2003).

We sought to address this gap by investigating the relationship between forest cover as a proxy for habitat loss, docility behavior measured by the handling test duration, and physiological stress measured by the N/L ratio in three neotropical bat species —*Sturnira lilium*, *Carollia perspicillata*, and *Artibeus lituratus*. Our first hypothesis posits that habitat loss affects the docility behavior of these three bat species. Specifically, we predict that in areas with greater habitat loss, characterized by reduced forest cover, these three species will exhibit lower docility, potentially reflecting increased competition for resources and other environmental pressures resulting from habitat loss.

Our second hypothesis is that habitat loss correlates with N/L ratio levels as decreasing habitat area can intensify resource competition and expose bats to more adverse environmental conditions. Therefore, we predict that the extent of habitat loss will be positively associated with higher N/L ratio levels, indicating higher physiological stress.

Third, we hypothesized that physiological stress, indicated by N/L ratio levels, correlates with docility-related behaviors across the three fruit bat species. We predict that individuals with higher N/L ratio levels will exhibit lower docility, reflecting a direct link between increased physiological stress and corresponding changes in behavior. This prediction is based on the understanding that elevated N/L ratios are often associated with heightened stress responses, which can manifest as altered behavioral patterns. The reduced docility may serve as a coping mechanism, where individuals under greater physiological stress become less tolerant of handling or social interactions, potentially as a way to conserve energy or avoid further stress.

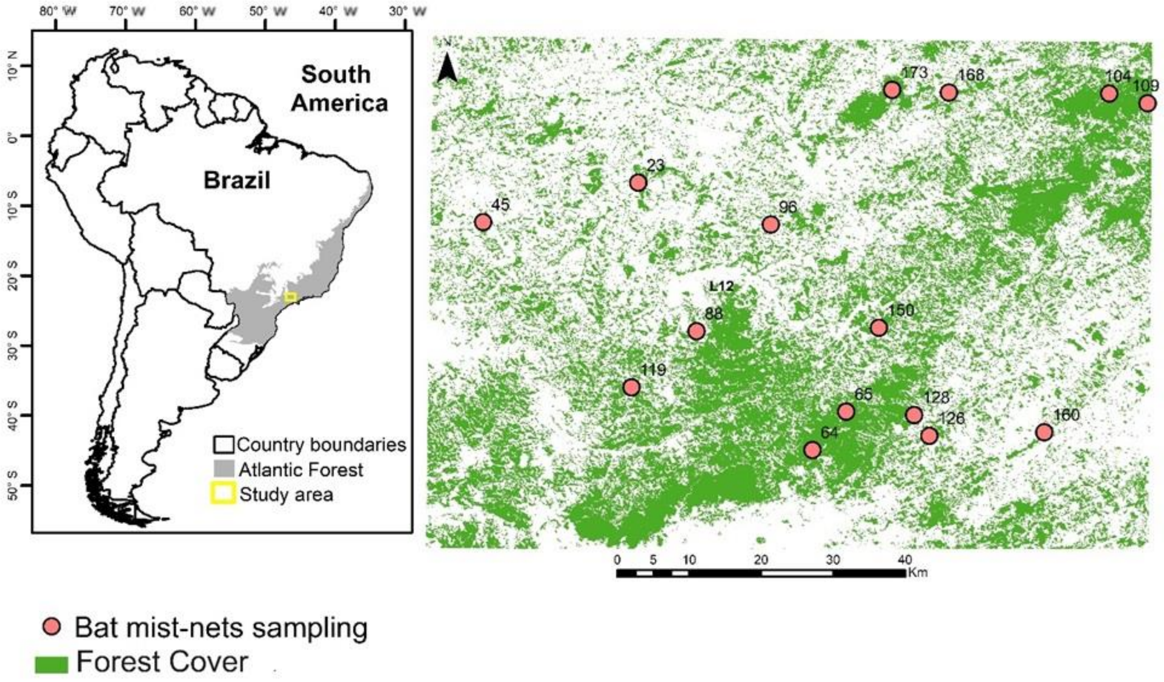
2 MATERIAL AND METHODS

2.1 Study area

The study areas are located in the Cantareira-Mantiqueira region (23°14'22.38"S and 46°27'36.89"W, 700 - 1,200 m a.s. l.), state of São Paulo, southeast of Brazil. This geological formation is part of the Planalto Paulistano and Planalto Atlântico regions, which are significant due to their unique topographical and ecological characteristics within the Atlantic Forest biome. Ecologically, the Cantareira-Mantiqueira region presents a rich mosaic of habitats due to its varied topography and climate. It features mountainous relief and exposed slopes, with average annual rainfall around 1600 mm. The climate includes hot, rainy summers and cold, dry winters, with significant fire occurrences approximately every 3-5 years, primarily during the dry winter months. The average monthly temperature ranges between 18 to 20°C, rarely exceeding 22°C in the forest interior. The vegetation is typical from the Atlantic Forest, predominantly humid tropical broadleaf forest with altitudinal variations (HUECK, 1972).

Within the Cantareira-Mantiqueira region, we selected 17 sampling sites using mapped landscapes that generated a gradient of percentage of forest cover in 2km buffers, from 11 to 91% (Figure 1). Forest cover data were obtained using Geographic Information System (GIS) software, specifically QGIS 3.16 (QGIS Development Team). The forest cover maps were derived from high-resolution satellite imagery provided by the Landsat 8 Operational Land Imager (OLI) and processed using supervised classification techniques. In supervised classification, representative samples of different land cover types (e.g., forest, water, urban area) were used to train the classification algorithm, which then categorized all pixels in the imagery. The resulting data layers were analyzed to calculate the percentage of forest cover within each buffer zone

Figure 1. Location of the study area and the 17 landscapes



2.2 Bat capture and data collection

We carried out two nights of bat capture each sampling using six mist nets (dimensions 12 × 3m; mesh 16 × 16mm) installed in places with greater probability of capture, such as flight corridors formed by vegetation, trails, roads and edges of forest patches. Sampling took place for six hours after sunset, aiming to obtain a more diverse sample of the fruit bat assemblage in the area, as this period encompasses peak activity times for different bat species (TREVELIN et al., 2017).

Nets were inspected every 30 minutes, and as soon as the bats were removed from the mist nets, they were individually placed in cloth bags of the same dimensions (20 cm x 20 cm) to quantify their docility. This is a widely used method in behavioral ecology (CAREAU et al., 2015; MARTIN; REALE, 2008), which involves quantifying the movement time, that is, the time the animal struggles from the moment it is placed in the cloth bag over a period of 180 seconds. The calculation of docility began as soon as the handler started placing the bat into the bag. This quantification was always performed by the same person to avoid sampling bias. Individuals who moved for longer were considered less docile (CAREAU et al., 2015; MARTIN; REALE, 2008; MIGUEL et al., 2024).

All bats were identified to species level in the field using a combination of taxonomic keys (REIS et al., 2007). A total of 326 bats from 18 species were captured and analyzed. Among them, 96 were *Sturnira lilium*, 67 were *Carollia perspicillata*, and 63 were *Artibeus lituratus*, accounting for 70% of the captures. Therefore, analyses were restricted to these three species due to their higher relative abundance, which constituted the majority of the captured individuals.

2.3 N/L ratio

After the handling bag test, a 10 µl blood sample was collected from the antebrachial vein using a 26-gauge sterile needle as described by Voigt and Cruz-Neto (2009). placed in a microcapillary tube, subsequently placed on a glass slide and then spread over the surface with the clean edge of another slide used to obtain blood smears. After air drying, the smears were fixed with methanol, subsequently stained with Giemsa (20%) and posteriorly analyzed in the lab under an oil immersion microscope at 100x magnification. We calculated differential leukocyte counts by identifying and counting in 100 leukocytes the relative number of lymphocytes, neutrophils, eosinophils and monocytes (PAKSUZ et al., 2009). From these data the N/L ratio was calculated.

2.4 Body condition

The body mass of each individual was recorded using a spring balance (Pesola, LIGHT LINIE, accuracy 0.1 g, Switzerland) to the nearest 0.1 g, and forearm length was measured using a digital caliper (Digimess 100.041A, accuracy 0.02 mm, Brazil). To estimate the body condition of each individual, we used the Scaled Mass Index (SMI) (PEIG; GREEN 2010). This index is derived from the following equation:

Scaled Mass Index (SMI): $\hat{M}_i = M_i[\frac{L_0}{L_i}]^{b_{SMA}}$

M_i represents the body mass of individual i;

L_i is a linear measurement of the body of individual i;

L_0 is a reference value for L (for example, the arithmetic mean for the population under analysis);

^{b}SMA is the scaling exponent estimated by the standardized principal axis (SMA) regression of lnM on lnL;

$\hat{M}_i$ is the estimated body mass for individual i when the linear body measurement is standardized to L_0.

The SMI was developed by Peig and Green (2009) as an alternative approach to normalizing body mass taking into account the scaling relationship between animal mass and size. This index has been suggested as a more accurate way of estimating reserves of fat, protein and other body components compared to traditional body condition index methods.

2.5 Statistical analyses

The statistical analysis carried out in this study involved structural equation modeling (SEM). We chose structural equation modeling because it differs from other modeling approaches by testing direct and indirect effects on pre-assumed causal relationships. Path analysis performed through SEM can explain the causal relationships between variables, providing a more comprehensive and detailed view of the underlying processes. A common function of path analysis is mediation, which assumes that a variable can influence an outcome both directly and indirectly through another intermediate variable. This approach allowed us to better understand the underlying mechanisms and interrelationships between the variables of interest (FAN et al., 2016).

In the SEM, the response variables were docility, represented by the time each bag struggled in the bag, N/L ratio and body condition, while the explanatory variable was forest cover. Additionally, bat species and sex were included as covariates in the analysis to account for their potential effects on the response variables. These covariates were included because species differences can significantly influence behavioral and physiological traits, and sex differences can impact both body condition and immune responses. Including these factors allowed us to control for their variability and focus on the effects of habitat loss. Prior to the analyses, docility, N/L ratio, body condition and forest cover were stardardized to mean = 0 and stardard deviation = 1. The structural equation model (SEM) was fitted using the maximum likelihood (ML) estimator and the NLMINB optimization method. The model included a total of 12 parameters and was based on 194 captures for the three study species (*Carollia perspicillata*, *Artibeus lituratus* and *Sturnira lilium*). This analysis was conducted in the R statistical environment (version 4.3.2), using the following packages: lavaan (ROSSEEL 2012), sem (Fox et al., 2022) and semPlot (EPSKAMP, 2022).

2.6 Ethical Note

This study received ethical approval from the Coordination of the Animal Use Ethics Committee (CEUA number 18/2021) and adhered to the ASAB/ABS guidelines. Following the completion of the study, all captured bats were released at their original capture location.

3 RESULTS

The model tested presented a chi-square value of 168.387 with 3 degrees of freedom, resulting in a p-value < 0.001 (Table 1). The comparative fit index (CFI) was 0.299 and the Tucker-Lewis fit index (TLI) was -1.803.

The root mean square error of approximation (RMSEA) was 0.533, with a 90% confidence interval ranging from 0.466 to 0.603. The RMSEA measures the discrepancy per degree of freedom for the model; values less than 0.08 indicate a reasonable fit, and values closer to 0.05 or below are considered good. The high RMSEA value indicates poor fit. The p-value for the null hypothesis that RMSEA is less than or equal to 0.05 was 0.001, suggesting that the probability of RMSEA being 0.05 or lower is very low. Conversely, the p-value for the null hypothesis that RMSEA is greater than or equal to 0.080 was 1.000, indicating a very high probability that the RMSEA exceeds the acceptable threshold for a good fit.

The standardized quadratic residual index (SRMR) was 0.163 (Table 1). The SRMR is an index of the average discrepancy between the observed and predicted correlations; values less than 0.08 are generally considered a good fit. The SRMR value of 0.163 suggests a poor fit of the model to the data, indicating substantial differences between the observed and predicted values.

Table 1: Table 1 displays the structural equation model (SEM) fit measures, providing a comprehensive assessment of the fit of the model to the observed data. It includes the Comparative Fit Index (CFI) and the Tucker-Lewis Fit Index (TLI), which indicate how well the model fits the data, with values close to 1 being desirable. The Root Mean Square Error of Approximation (RMSEA) provides a measure of model fit, with a 90% confidence interval for additional precision. p-values associated with the RMSEA indicate the statistical significance of the model fit, with values lower than 0.05 considered indicative of a good fit. The Standardized Quadratic Residual Index (SRMR) evaluates the standardized mean discrepancy between the observed data and the values estimated by the model.

Index	Valor
Estimate	ML
Optimization method	NLMNB
Number of parameters	12
Number of observations	194
Chi-square	168.387
Degrees of freedom	3
Chi-square p-value	<0.001
Comparative Fit Index (CFI)	0.299
Tucker-Lewis Index (TLI)	-1.803
Root Mean Square Error of Approximation (RMSEA)	0.533
90% Confidence Interval RMSEA (Lower)	0.466
90% Confidence Interval RMSEA (Upper)	0.603
P-value H0: RMSEA $\leq$ 0.050	<0.001
P-value H0: RMSEA $\geq$ 0.080	1.000
Standardized Root Mean Square Residual (SRMR)	0.163
Loglikelihood (Modelo ajustado)	-784.489
Akaike (AIC)	1592.978
Bayesian (BIC)	1632.192
Sample-Size Adjusted Bayesian Information Criterion (SABIC)	1594.178

The standardized estimates of the regression parameters in SEM (Std.all), which allow for comparison across variables by converting them to a common scale and show the relative strength of each predictor's effect, are presented in Table 2. The forest cover variable demonstrated a significantly negative effect on the N/L ratio (Estimate = -0.480, $p < 0.001$, Std.all = -0.480), indicating that an increase in vegetation cover is associated with a decrease in the N/L ratio of the bats (Figure 3). Furthermore, sex presented a significant negative effect on the N/L ratio (Estimate = -0.284, $p = 0.022$, Std.all = -0.142), indicating an influence of the sex of the bats on the N/L ratio (Figure 4). The N/L ratio was higher for females (0.466 ± 0.320, range: 0.021-1.333) compared to males (0.348 ± 0.311, range: 0.022-1.354).

Table 3 details the covariance estimates between variables and the variances of endogenous variables in the SEM model. The covariance between behavior and the N/L ratio was significantly positive (Estimate = 0.236, $p = 0.004$, Std.all = 0.236), indicating an association between these variables in the context of the study (Figure 5). The relationship between forest cover and docility was not significant (Estimate = 0.096, $p = 0.160$, Std.all = 0.101), suggesting that changes in forest cover do not directly affect the docility of the bats (Figure 6).

The variances of endogenous variables, such as N/L ratio (Estimate = 0.736, $p < 0.001$, Std.all = 0.739) and behavior (Estimate = 0.942, $p < 0.001$, Std.all = 0.944), were also significant. This indicates substantial variability in these measures within the analyzed sample. The significance of these variances suggests that the N/L ratio and behavior exhibit considerable variation among the bats studied, which points to the influence of factors not included in the model. Therefore, while the model captures part of this variability, it also reveals the presence of other factors affecting these variables.

Table 2. Standardized estimates of regression parameters in the SEM model. Each estimate includes the estimated value of the parameter, its standard error, z value and the associated p value. The Std.lv and Std.all measures represent the standardized factor loadings to indicate the magnitude of the influence of each independent variable on the dependent variables in the model.

Relationship	**Estimate**	**Std. Error**	**Z-value**	**P-value**	**Std.lv**	**Std.all**
N/L ratio ~						
Forest cover +	-0.480	0.062	-7.759	0.001	-0.480	-0.480
Sex	-0.284	0.124	-2.290	0.022	-0.284	-0.142
Behavior ~						
Forest cover +	0.032	0.081	0.396	0.692	0.032	0.032
Sex	0.242	0.143	1.692	0.091	0.242	0.120
Body condition ~						
Forest cover +	0.195	0.070	2.768	0.006	0.195	0.195
Sex	0.114	0.141	0.809	0.419	0.114	0.057
Behavior ~						
Species+	-0.058	0.085	-0.683	0.494	-0.058	-0.048
N/L ratio	0.236	0.081	2.919	0.004	0.236	0.236

Table 3 Estimates of covariances between variables and the variances of endogenous variables in the SEM model. For each estimated covariance, the value of the covariance estimate, its standard error (Std.Err), the z value and the corresponding p value. The Std.lv and Std.all measures are used to standardize the covariances, facilitating the interpretation of the strength of the relationships between the variables within the model.

Relationship	***Estimate***	***Std. Error***	***Z-value***	***P-value***	***Std.lv***	***Std.all***
Covariance						
Behavior ~~ Body condition	0.096	0.068	1.406	0.160	0.096	0.101
Variances						
N/L ratio	0.736	0.075	9.849	0.001	0.736	0.739
Behavior	0.942	0.096	9.849	0.001	0.942	0.944
Body condition	0.952	0.097	9.849	0.001	0.952	0.957

Figure 2: The Structural Equation Model (SEM) showing the relationships between different variables: species, sex, forest cover within a radius of 2 km (Forest cover), neutrophil/lymphocyte ratio (N/L ratio), docility and body condition. The arrows connecting the boxes represent the relationships between these variables, with green arrows indicating positive relationships and red arrows indicating negative relationships. Dashed arrows represent covariances (non-causal relationships), while solid arrows represent direct and causal effects. The numbers next to the arrows are the standardized estimates of the regression coefficients and covariances.

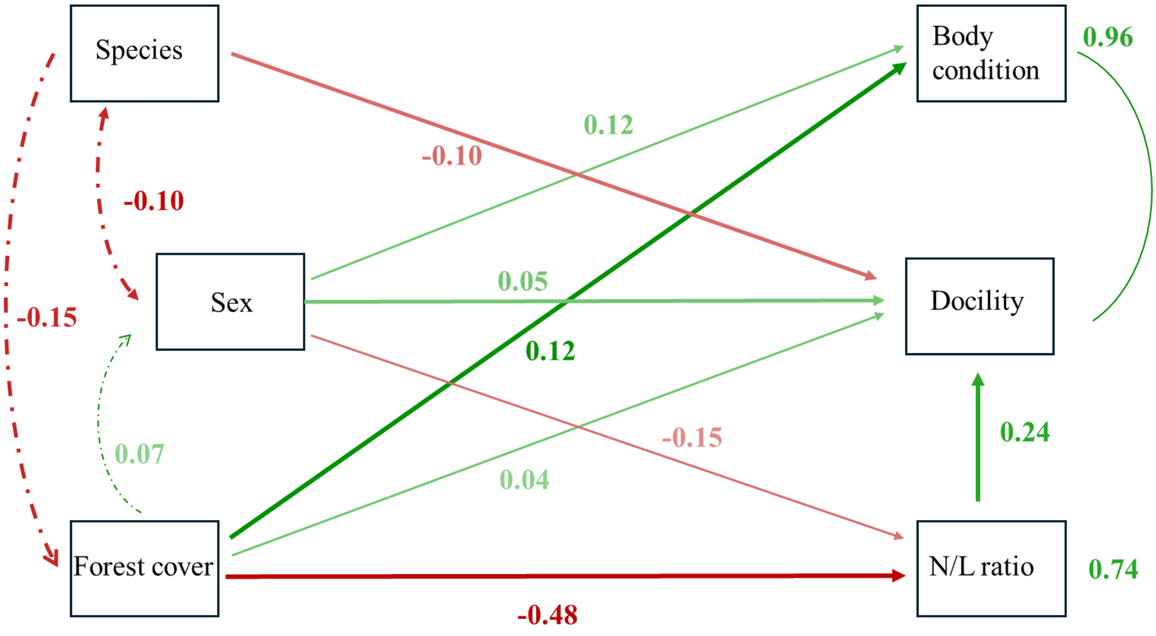


Figure 3: Relationship between forest cover within a 2 km radius and the N/L ratio in the bats in the study. Each point represents an individual bat sample. The blue line represents the linear trend of the relationship, showing a significant negative correlation ($p < 0.05$).

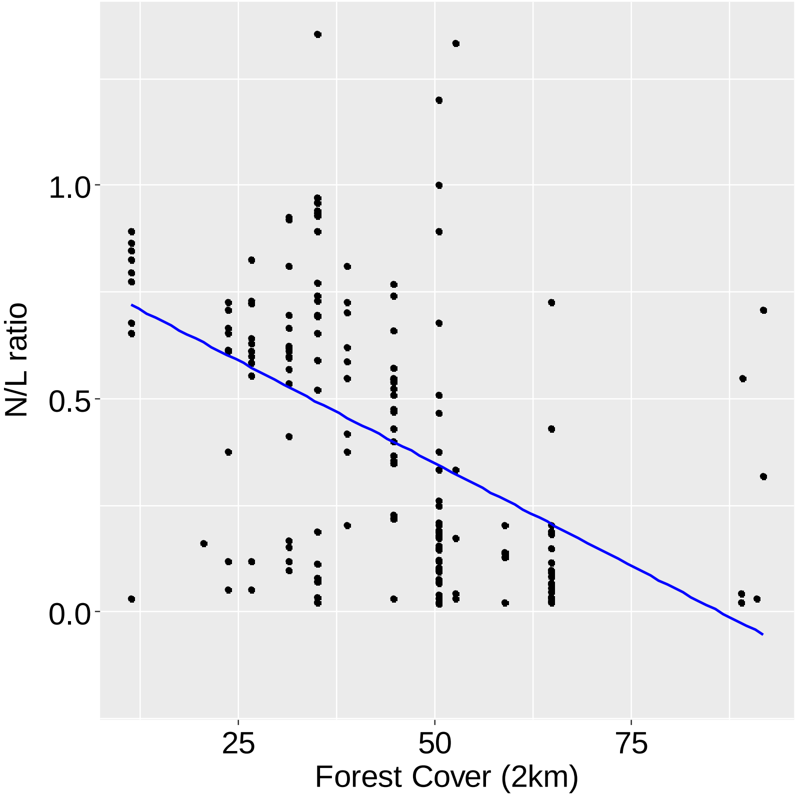


Figure 4: Comparison of the N/L ratio between female and male bats. The box plots show the median (thick line), quartiles (box) and extreme values (outer lines) of the N/L ratio for each group. It can be seen that females have a slightly higher median N/L ratio than males. The red asterisk (*) indicates a significant difference ($p < 0.05$) between the sexes.

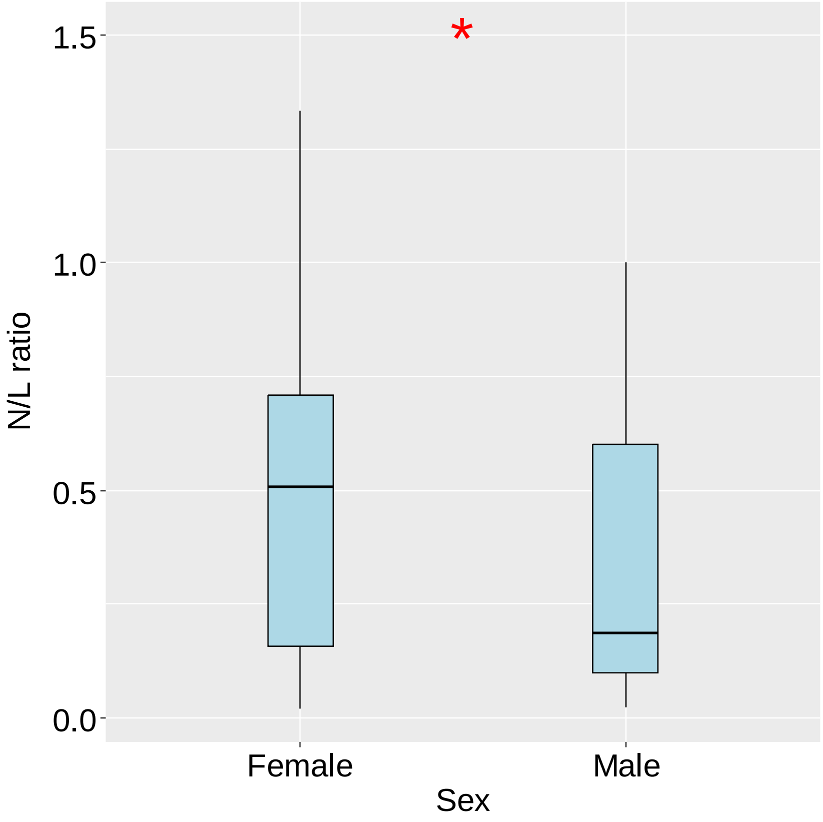


Figure 5: Relationship between N/L ratio and docility (measured in seconds) in bats. Each black dot represents an individual observation, while the blue line indicates the linear trend. A significant positive correlation is observed ($p < 0.05$).

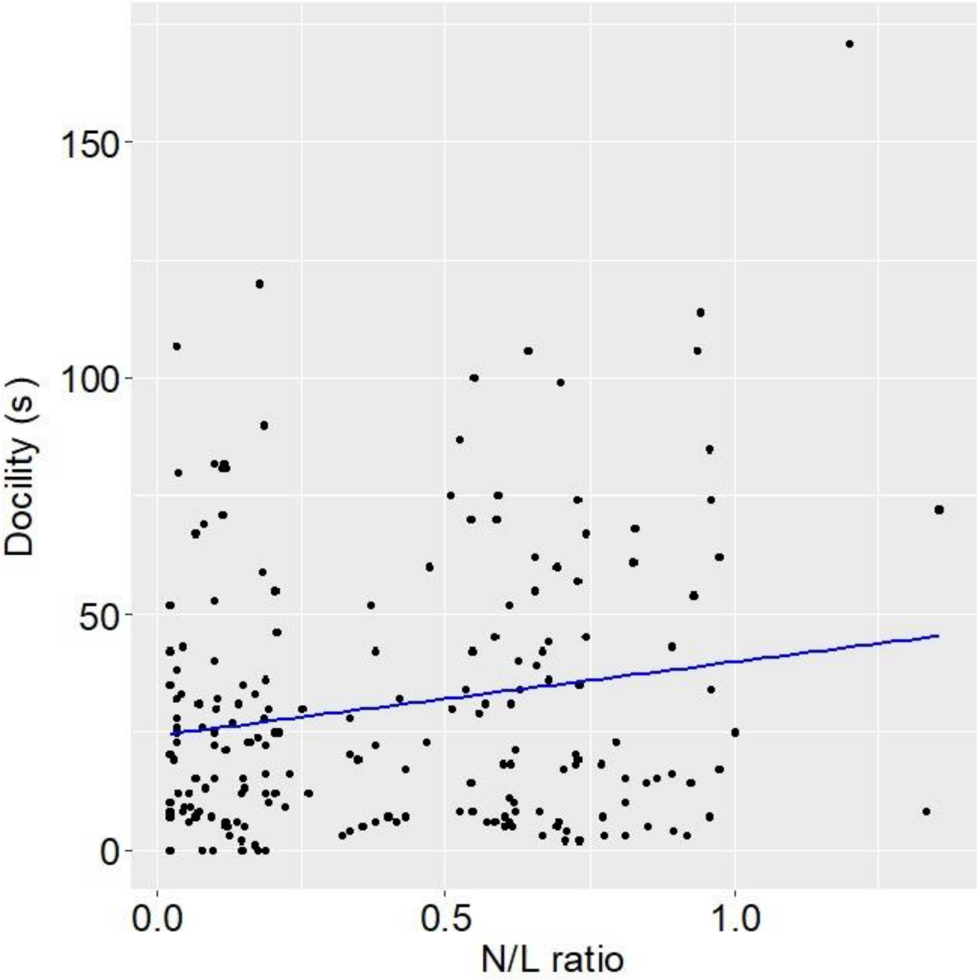
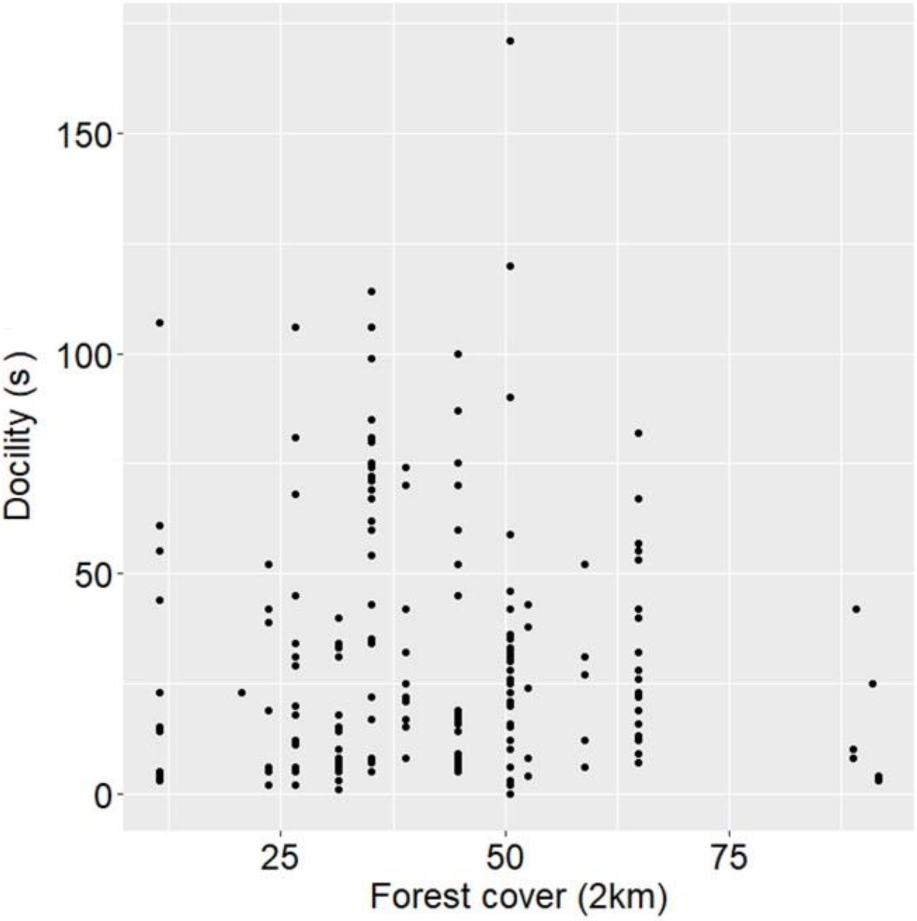


Figure 6: Relationship between forest cover within a 2 km radius and docility (measured in seconds) in bats. Each black dot represents an individual bat observation. No trend line is visible, suggesting that there is no clear correlation between forest cover and bat docility in this sample.



4 DISCUSSION

This study investigated the relationship between habitat loss, behavior, and physiology in three species of Neotropical fruit bats (*C. perspicillata*, *A. lituratus* and *S. lilium*). Our results indicate that increased habitat loss, as characterized by reduced forest cover, is significantly associated with a higher N/L ratio, which correlates with decreased docility, in line with our predictions. However, the data did not support a direct relationship between forest cover and behavior changes. Additionally, sex was found to significantly influence the N/L ratio. The positive covariance between docility and the N/L ratio suggests evidence of a link between behavioral modifications and physiological stress. While our model exhibited poor fit indices, these findings suggest that habitat loss has adverse effects on bat stress levels and behavior, emphasizing the essential role of forest cover in maintaining the health and facilitating adaptive behavioral responses in bat populations.

Our results did not support the hypothesis that bats would exhibit different levels of docility in response to habitat loss, with a reduction in docility-related behaviors in areas with lower forest cover (VAN OERS; SINN, 2013; SHAPIRO et al., 2021). Previous studies have suggested that habitat loss can lead to behavioral changes due to increased stress and reduced resources, which may result in less docile behavior (LAGOS et al., 1995; ORROCK et al., 2004). However, in our study, we did not observe a direct significant effect of forest cover on docility behavior. This difference might be due to species-specific differences in behavioral plasticity or the presence of other environmental factors that were not measured in our study (HENRY JOUARD, 2007; MIGUEL et al., 2019; KALKO; HANDLEY JR, 2001; SILVA et al., 2020). It is also possible that the behavioral adjustments of the bats in our study are more complex and not solely driven by habitat loss. Additionally, the time scale of habitat loss and subsequent adaptation might play a important role, as immediate behavioral responses could differ from long-term adjustments (METTKE-HOFMANN et al., 2002). Animals, and specifically bats, may exhibit rapid responses to environmental stress, such as behavioral or physiological changes, while long-term adaptations may involve more profound changes in behavioral ecology, survival strategies, and social interactions (HALE et al., 2020; WAGNON et al, 2024).

Our results showed a significant negative relationship between forest cover on the N/L ratio, indicating that habitat loss is linked to elevated physiological stress. This supports the hypothesis that physiological stress is influenced by habitat conditions, even if the direct behavioral response was not observed. The positive covariance between docility and the N/L ratio suggests a link between behavioral changes and physiological stress. The positive association between body condition and forest cover suggests with greater forest cover provide better nutritional conditions for bats. This is probably because habitats with greater forest cover ensure more food resources and, consequently, improve the nutritional status of bats, as in other mammals (KLINGBEIL; WILING, 2010; MEYER et al., 2007; STRUEBIG et al., 2008). Our findings support the forested probably environments offer richer and more stable food sources, which contribute to the body condition of fruit bats. Although our results did not find a direct significant effect of forest cover on docility behavior, it is possible that better body condition, resulting from higher forest cover, indirectly influences behavior by reducing physiological stress. This highlights the interplay between environmental conditions, physiological health, and behavioral responses in bats. The significant positive covariance between docility and the N/L ratio further supports this link, suggesting that bats that are likely to be better nourished experience lower stress levels and exhibit more docile behavior.

Although the direct relationship between forest cover and behavior was not significant, the positive and significant covariance between behavior and N/L ratio suggests that bat behavior can be indirectly influenced by forest cover through its impact on stress levels. The association between lower forest cover and higher N/L ratio reinforces the idea that habitat loss increases stress levels, which can lead to less docile and more cautious behaviors. This is in line with previous studies demonstrating the N/L proxy relationship for stress and habitat loss, such as in the review by Davis et al. (2008) and specifically with bats in Miguel et al. (2019). Therefore, this study reinforces that physiological stress can modulate animal behavior meaning that stress may affect the way animals express their behavior, making them more reactive or cautious in response to environmental changes (CARBILLET et al. 2019).

In the review by Davis et al. (2008), researchers reported the relationship between leukocyte profiles and stress hormones in mammals is evident. They demonstrated how the N/L ratio can be influenced by different factors, such as environmental degradation. This highlights the importance of the N/L ratio as a valuable indicator of the stress response in mammals. By understanding these relationships, we gain significant insights into understanding the physiological mechanisms underlying the stress response.

In Miguel el al. (2019), the authors analyzed the relationship of several physiological variables, including the N/L ratio in relation to habitat loss of four species of fruit bats, including *C. perspicillata*, *A. lituratus* and *S. lilium*. They also found a negative relationship between forest cover and the N/L ratio. This finding suggests that Neotropical fruit bats may have adaptive mechanisms that help them cope with habitat loss. Although these changes in the N/L ratio reflect a physiological response to environmental stressors, the authors conclude they do not necessarily imply a complete failure of the species to maintain their overall health in altered environments. Furthermore, those authors emphasizedthe importance of animal behavior research in understanding not only animals' direct responses to environmental changes, but also the hierarchical effects and underlying mechanisms that enable allow them to survive and even thrive in altered environments.

Carbillet et al. (2019) analyzed variation in behavior and N/L ratio in wild deer populations under different environmental conditions. They found that deer in low-quality forest areas had a significantly higher N/L ratio, suggesting that habitat quality influences physiological stress responses. This finding aligns with our results, where we observed a relationship between habitat loss and increased N/L ratios in Neotropical fruit bats, indicating that both species may experience heightened stress levels in degraded environments.

The absence of a direct relationship between behavior and habitat loss (Figure S1 and Table S1, supplementary material) suggests that, despite variations in ecological traits among species, all exhibited similar behavioral responses to habitat degradation. The lack of significant differences in docility between species indicates that, although their ecological characteristics may differ, these bats share comparable adaptive strategies to cope with environmental disturbances. This behavioral consistency may be a sign of significant plasticity, allowing them to maintain stable levels of docility regardless of varying environmental pressures.

In Ebay et al. (2023), the authors explored how land-use changes affect the behavior of the bat *Pteropus alecto* and increase the risk of Hendra virus spillover. Over 23 years of data collection, the study showed that these bats altered their foraging and roosting patterns, increasingly occupying urban and agricultural areas due to nectar shortages and habitat loss. This shift led to greater contact with susceptible hosts. The discussion highlighted the crucial role of native habitat restoration and land-use management in reducing the risks of viral transmission, proposing a framework for understanding and preventing pandemics in regions impacted by environmental changes. These findings on behavioral adjustments in P. alecto complement our observations on the behavioral plasticity of the three Neotropical bat species, suggesting that adaptive responses to habitat loss may be widespread among different bat species.

This pattern of behavioral plasticity is further supported by the distinct foraging strategies of *C. perspicillata*, *A. lituratus*, and *S. lilium*. *C. perspicillata* predominantly forages in the understory, while *A. lituratus* prefers the canopy (KALKO; HANDLEY JR, 2001), and *S. lilium* occupies intermediate forest strata, often favoring areas with dense understory vegetation (HENRY; JOUARD, 2007; SILVA et al., 2020). This vertical segregation in habitat use reflects the ecological niches of these species and highlights their adaptations to different forest strata (HENRY; JOUARD, 2007; KALKO; HANDLEY JR, 2001; SILVA et al., 2020). Despite these ecological distinctions, their consistent behavioral responses to habitat loss suggest shared adaptive mechanisms that enable them to respond similarly to environmental stressors.

Our results indicate that while there is no direct relationship between behavior and habitat loss, understanding species-specific traits such as foraging strategies and habitat preferences can offer insights into why this relationship was not significant. Although our study does not specifically measure how different forest strata are impacted by habitat loss, the observed lack of significant differences in docility among species suggests that despite their varying foraging strategies and habitat preferences, the bats exhibit a similar level of behavioral response to habitat disturbance. This could imply that the vertical stratification and habitat use differences among the species may not significantly alter their overall docility in response to habitat loss. Thus, the vertical stratification and differing habitat use among the species likely contribute to the complex and species-specific responses to habitat loss, obscuring a direct relationship between behavior and habitat loss (HENRY JOUARD, 2007; KALKO; HANDLEY JR, 2001; SILVA et al., 2020). In addition to differences in the preferred habitat features, the studied species also exhibit significant variations in feeding patterns. *Carollia perspicillata* prefers fruits from *Piper* (Piperaceae), *Solanum* (Solanaceae) and *Vismia* (Hypericaceae), and typically transports these fruits to temporary feeding sites in the understory layer of the forest (CASTILLO-FIGUEROA, 2020; MELLO et al., 2004; KALKO; HANDLEY JR, 2001). In contrast, *Artibeus lituratus*, primarily consumes fruits from *Ficus* (Moraceae), whitch are often found the canopy layer (ENRÍQUEZ-ACEVEDO et al., 2020; FLOREZ-MONTERO et al., 2022; SILVEIRA et al., 2024) and *Sturnira lilium* prefers Solanaceae fruits (HENRY and JOUARD, 2007; MUYLAERT et al., 2014). These distinct food preferences reflect the ecological specialization of each species to exploit specific resources in different parts of the forest, highlighting their adaptation to various forest layers (CASTILLO-FIGUEROA, 2020; FLOREZ-MONTERO et al., 2022; HURTADO-MATERON; MURILLO-GARCÍA, 2023).

Another important factor that may be related to our failure to find significance between behavior and habitat loss is the possible difference in plasticity between species. For example, in Atlantic Forest the *Artibeus lituratus* demonstrates a clear preference for areas of continuous forest compared to fragmented areas (LANGLOIS et al., 2023).

In this study, each individual was tested and immediately released, which restrains us from exploring whether the behavioral responses we found accrues from within individual variation or among individual variation (i.e. personality). The study of animal personalities involves consistent behavioral traits across time (DINGEMANSE et al., 2020; RÉALE et al., 2007), which can constitute behavioral syndromes when multiple personality traits are correlated (CAREAU; GARLAND, 2012; SIH; BELL, 2008). Miguel et al. (2024) explored these concepts in two of the studied species, *C. perspicillata* and *A. lituratus* and reported that docility, boldness, and activity were consistent over time and identified species-specific behavioral syndromes. Therefore, is reasonable to suppose that the docility measured should must have consistent repeatable at individual level whether the individuals studied were tested multiple times, leading us to infer that habitat loss may affect the expression of personality traits within populations. Moreover, *C. perspicillata* displayed syndromes that combined boldness with docility, boldness with activity, and docility with activity. In contrast, *Artibeus lituratus* showed a syndrome combining boldness with docility. Additionally, significant correlations were found between these traits and the results from the bag test, a field methodology easily replicable in natural settings. The study underscores the importance of considering these selective pressures in understanding the behavioral adaptations of fruit bats.

Although differences between the three species in the study are well-documented (BARCLAY; HARDER, 2003; REGOLIN et al., 2020; SILVEIRA et al., 2024), we anticipated finding a significant pattern in how habitat loss impacts them. Specifically, we expected habitat loss to affect all studied species in a similar manner, thereby making the "species" variable significant in our model. However, the lack of significant results for the "species" variable suggests that the impact of habitat loss might be relatively similar across the species studied. This outcome implies that, despite differences in traits such as foraging strategies and habitat preferences, these species may exhibit comparable overall responses to habitat loss. This suggests that factors like behavioral plasticity and ecological adaptability could play a role in this uniform response. Thus, the absence of a significant "species" effect highlights the complexity of ecological responses and suggests a need for further investigation into how different species adapt to habitat loss. However, our results did not support this expectation.

5. CONCLUSION

The analysis of variance in response variables revealed substantial variability in the collected data, highlighting the importance of considering heterogeneity in fruit bat responses to changes in forest cover and/or habitat loss (MEYER et al., 2007; STRUEBIG et al., 2008; KLINGBEIL; WILLIG, 2010). The study's results partially support the proposed hypotheses, showing that habitat loss is associated with an increase in physiological stress, measured by the neutrophil-to-lymphocyte ratio. However, no clear relationship was found between habitat loss and specific changes in bat docility.

The absence of a significant direct impact of habitat loss on docility suggests that, despite environmental changes, bats may maintain consistent levels of docility. This may be attributed to adaptive mechanisms that allow bats to adjust their behaviors in a way that maintains relative stability in their docility, even under adverse conditions. Additionally, the analysis revealed that the body condition of bats is positively associated with vegetation cover, indicating that less degraded habitats are beneficial for bats' overall health.

These findings underscore the need for conservation strategies that prioritize the preservation of habitat integrity and prevent further habitat loss. Ensuring the protection of remaining forest areas is essential for mitigating the adverse effects of habitat degradation on bat health and docility, thereby contributing to the sustainability of bat populations in fragmented. environmentsations

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SUPPLEMENTARY MATERIAL

Figure 1: The figure illustrates the relationship between forest cover within a 2 km radius (in percentage) and docility (measured through the handling bag test, in seconds) for three species of bats: *Artibeus lituratus, Carollia perspicillata* and *Sturnira lilium*. Each species is represented by different colors: *Artibeus lituratus* in red, *Carollia perspicillata* in green, and *Sturnira lilium* in blue. The points on the graph represent individual observations of docility in relation to forest cover for each specific species.

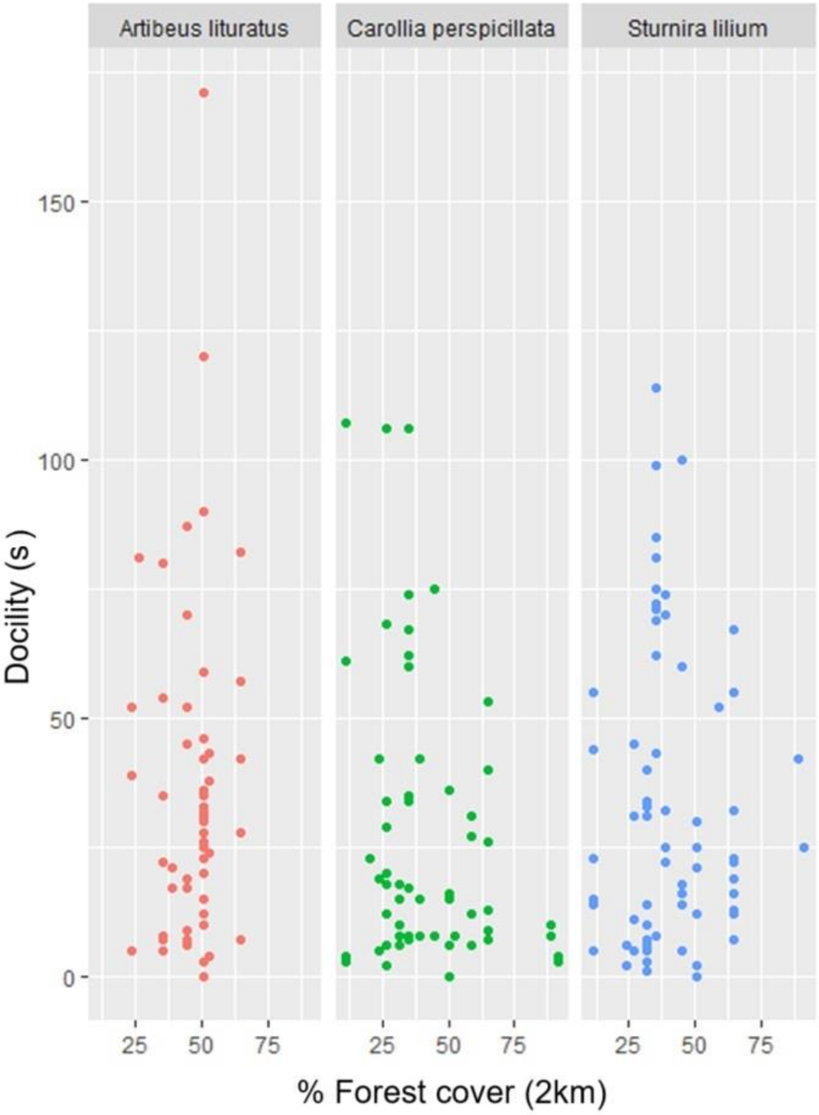

Table 1: Results of the mixed linear model evaluating the predictors of docility behavior in frugivorous bats. The predictors include forest cover, species, and the interactions between forest cover and species. The "Estimates" column presents the estimated coefficients for each predictor, along with confidence intervals (CI) and p values, which indicate statistical significance.

Docility			
Predictors	*Estimates*	*CI*	*p*
(Intercept)	23.34	-14.18 – 60.87	0.221
Forest cover	0.28	-0.48 – 1.04	0.465
species [*Carollia perspicillata*]	19.45	-22.65 – 61.56	0.363
Species [Sturnira lilium]	7.29	-34.78 – 49.35	0.733
Forest cover x species [*Carollia perspicillata*]	-0.67	-1.52 – 0.18	0.120
Forest cover x species [*Sturnira lilium*]	-0.31	-1.17 – 0.54	0.467
Random Effects			
σ^2	828.89		
$\tau_{00\ \text{especie}}$	17.09		
ICC	0.02		
N_{specie}	3		
Marginal R^2 / Conditional R^2	0.043 / 0.062		

CAPÍTULO 3: Habitat Loss and Behavior Influence the Occurrence of Coronaviruses in Bats from a Neotropical Biodiversity Hotspot

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Habitat Loss and Behavior Influence the Occurrence of Coronaviruses in Bats from a Neotropical Biodiversity Hotspot

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Highlights

- We analyzed the influence of habitat loss and docility behavior on the occurrence of viruses from the Coronaviridae family in bats.
- The research was conducted in the Atlantic Forest region, a highly fragmented biodiversity hotspot.
- Higher forest cover was associated with increased occurrence of coronaviruses in bats, suggesting a link between denser populations and viral transmission.
  Docile bats showed a higher likelihood of coronavirus infection, possibly due to increased social interactions and gregariousness habits.

Abstract

Habitat degradation caused by human activities threatens global biodiversity and can influence the spread of pathogens, such as viruses, by altering landscapes and consequently disrupting ecological dynamics. Bats are important natural hosts for a wide variety of mammalian viruses with zoonotic potential. Certain bat behaviors, such as docility, are indicators of their gregariousness and can also impact the dynamics of virus transmission. However, there is still a lack of knowledge regarding the relationships between bat behavior, habitat fragmentation, and viral occurrence. This study analyzed the influence of habitat loss and docility on the presence of viruses of the *Coronaviridae* family in bats within the Atlantic Forest, a highly fragmented global biodiversity hotspot. Using a binomial logistic model, we examined the percentage of forest cover within a 2 km radius and the docility of bats as predictors of viral infections. Overall, 15% of the 326 captured bats tested positive for coronavirus, and viral occurrence increased with the percentage of forest cover and with bat docility. This suggests that areas with greater forest cover may support denser bat populations, facilitating viral transmission, while more docile bats are more likely to be infected by coronaviruses, likely due to increased interaction with other individuals. This study demonstrates that there is a higher occurrence of coronaviruses in bats in areas with greater forest cover within the Atlantic Forest, possibly due to increased population density and social interaction in these habitats. The relationship between docility and viral infection highlights the importance of integrating behavioral and ecological analyses into conservation strategies and the monitoring of zoonoses.

Keywords: Atlantic Forest, docility, habitat fragmentation, *Coronaviridae*, zoonoses.

1 INTRODUCTION

The loss of natural habitats is recognized as one of the primary landscape changes, driven largely by human activities, demands and occurs when forest areas are diminished, leading to the formation of isolated and fragmented forest remnants within the landscape (BROOKS et al., 2002; FAHRIG, 2003). This process is a major threat to global biodiversity (BETTS et al., 2014; FRANKLIN et al., 2002; LINDENMAYER; FISCHER, 2007), and it is a widespread phenomenon observed in all regions of the world (BENDER et al., 1998; FAHRIG, 2003). Habitat loss reduces local species diversity (BENDER et al., 1998; ORTEGA-ÁLVAREZ; MACGREGOR-FORS, 2009), excludes species sensitive to environmental changes, and favors those better adapted to altered habitats (BROWN et al., 2001; ESTAVILLO et al., 2013). Given the rapid pace at which forest remnants are being degraded, it is crucial to understand the effects of this process on pathogen spread, including potential differences across species and environments (ALROY, 2017; MULLU, 2018).

Emerging and reemerging infectious diseases, which include both newly identified diseases and those reappearing in more virulent or resistant forms, have garnered significant attention from researchers since the late 20th century (BECKER et al., 2022; GIBB et al., 2020). Among the various types of pathogens, viruses are particularly notable for their ability to persist in natural reservoirs without causing obvious symptoms, making them especially challenging to manage (TAYLOR et al., 2001). These viruses are maintained in nature through reservoir species, and their transmission to different hosts, including humans, can result in unexpected disease outbreaks (DOBSON, 2005). This phenomenon is of particular concern for public health, as cross-species virus transmission can lead to the emergence of new strains with pandemic potential (DOBSON, 2005; GIBB et al., 2020).

Coronaviruses, members of the *Coronaviridae* family and *Orthocoronavirinae* subfamily are enveloped viruses with a positive-sense single-stranded RNA genome and encompass a diversity of viruses that share common genetic and structural characteristics (ANTHONY et al., 2017; TANG et al., 2008; WOO et al., 2006; WOO et al., 2012). These viruses have been identified in a wide range of hosts, including mammals and birds, and are frequently associated with respiratory diseases (HU et al., 2015; LOH et al., 2015; WOO et al., 2012). Thus, they are further divided into four genera: *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus*. The former two infect mainly humans and other mammals, while the latter two can be found mainly in birds and in two mammal species (BARBOSA et al., 2019; MIHINDUKULASURIYA et al., 2008). The ability of coronaviruses to infect multiple host species, combined with their high mutation rates and genetic recombination potential, makes them particularly concerning in the context of emerging infectious diseases (MURRAY; DASZAK, 2013; OLIVAL et al., 2017). Recent studies have increasingly focused on identifying and characterizing coronaviruses in bats, given that these animals are recognized as significant natural reservoirs of these viruses (TANG et al., 2008; WOO et al., 2006; WOO et al., 2012).

Several factors contribute to the emergence of new pathogens in humans, including environmental and climate changes, human activities, urban expansion, and the evolution of viruses themselves (GOTTDENKER et al., 2014; WANG, 2009). Bats, like other mammals, host a diverse array of viruses, with associations varying by species, geographic location, and even dietary habits (CALISHER et al., 2006). The global COVID-19 pandemic, caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) from the *Coronaviridae* family, has intensified interest in bats as reservoirs of emerging viruses and their interactions with human activities (BOLATTI et al., 2022; BRUSSEL; HOLMES, 2022).

Bats, belonging to the order Chiroptera, are among the most abundant and diverse groups of mammals, comprising 18 families, 202 genera, and representing 1,423 of the more than 6,400 known mammalian species (SIMMONS; CIRRANELLO, 2020; UPHAM et al., 2020). In Brazil alone, there are 9 families, 68 genera, and 181 species known (GARBINO et al., 2022). Bats have a broad geographic distribution, being absent only from the poles and some oceanic islands (NOWAK et al., 1994; KUNZ; LUMSDEN, 2003). These mammals play crucial roles in maintaining ecosystems due to their varied feeding habits, which include pollination, seed dispersal, and the control of insect populations, other invertebrates, and small vertebrates (VOIGT; KINGSTON, 2015; KUNZ; LUMSDEN, 2003).

Bats harbor a large and diverse array of viruses, some of which have crossed species boundaries to infect new hosts, occasionally leading to disease outbreaks (EBY et al., 2022; OLIVAL et al., 2017). The social dynamics of bat populations, including the formation of large colonies often comprising multiple species, as well as their extensive daily foraging movements, create ideal conditions for viral transmission (IRVING et al., 2021; KACPRZYK et al., 2017).

The presence of native vegetation, particularly dense forests, has significant implications for bat conservation and potentially for the transmission of viruses. In bats, changes in species interactions and survival probabilities can directly affect the dynamics of viral transmissions (CARLSON et al., 2022; FRICK et al., 2020; MUYLAERT et al., 2022). For instance, Streicker et al. (2016) demonstrated that the dispersal of male vampire bats facilitated the spread of the rabies virus in Peru, highlighting how host movement can influence pathogen spread. Despite this, there remain gaps in understanding the relationship between environmental degradation and the diversity of viruses associated with bats (LETKO et al., 2020).

Monitoring viruses within bat populations is essential for understanding potential viral evolutions that could harm ecosystems, including human populations (IRVING et al., 2021; EBY et al., 2022). Specifically, virus spread can serve as an indicator of broader environmental changes that lead to stress in bat populations, which act as hosts (GORBALENYA et al., 2020; KUMAR et al., 2017; SIMMONDS et al., 2017). Additionally, the behavior of these hosts can affect pathogen dynamics within bat communities (GERVASI et al., 2017; IRVING et al., 2021). Given that most bat-transmitted viruses can spread to other hosts, it is crucial to understand the interactions that drive viral transmission in these animals (BOLATTI et al., 2022; MUYLAERT et al., 2022).

Viral transmission among bats can occur directly through close contact, such as bites, scratches, or exposure to infected saliva, urine, and feces (BROOK; DOBSON, 2015; SCHRENZEL et al., 2003; WONG et al., 2007). Indirect transmission is also possible when bats come into contact with surfaces or food contaminated by excretions from infected individuals (ABDELGAWAD et al., 2014; SANO et al., 2015; SCHRENZEL et al., 2003). Therefore, specific behavioral traits, particularly those influencing social interactions, can significantly impact the likelihood of both direct and indirect pathogen transmissions.

Docility, defined as an animal's tendency to remain calm and submissive during restraint, confinement, and interactions with conspecifics, predators, and environmental stimuli (BOON et al., 2007; CAREAU et al., 2015; MARTIN; RÉALE, 2008), is particularly relevant. Neotropical bats generally exhibit gregarious behavior, leading to frequent and prolonged social interactions. As a result, docility may facilitate virus spread due to increased social contact (BOON et al., 2007; CAREAU et al., 2015; SIMMONS; CIRRANELLO, 2020; GARBINO et al., 2022). Docile bats may have a higher risk of contracting viruses, as their non-aggressive interactions with other bats could increase the likelihood of direct or indirect pathogen exposure. Moreover, less docile bats might be less averse to modified environments, potentially increasing their exposure to contaminated surfaces, although this behavior can vary across different species (DINGEMANSE et al., 2002; TCHABOVSKY et al., 2024).

To expand knowledge about the various factors that can influence the prevalence of viruses in populations, it is essential to go beyond the traditional parasite-pathogen model and identify environmental degradation events, such as habitat loss, that can influence the dynamics of viral transmission. Although coronaviruses are not new to science, they are still underrepresented in terms of ecological and epidemiological studies (LUIS et al., 2015; SCHOUNTZ, 2014; WONG et al., 2007). Thus, the objective of this study was to analyze whether the occurrence of viruses of the *Coronaviridae* family in bats is affected by habitat loss and docility behavior.

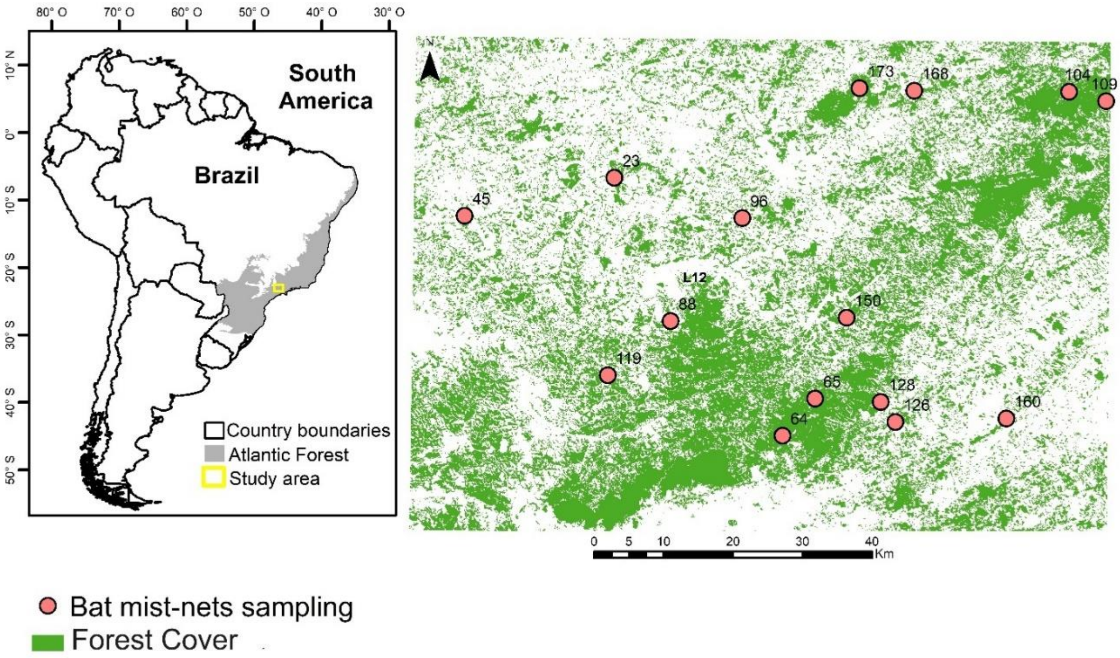
Our hypothesis is that the occurrence of these viruses in bats is influenced both by habitat loss and docility behavior of bats. We predict that the greater the habitat loss (decreased forest cover), the greater the chance of viruses of the *Coronaviridae* family occurring in bats, since habitat degradation can reduce the resources available to bats, such as food and roost sites. The scarcity of these resources can lead to physiological stress, which in turn can weaken the bats' immune systems, making them more susceptible to viral infections. In addition, habitat loss can force bats to concentrate in smaller, more densely populated areas, facilitating the transmission of viruses between individuals. We also predict that less docile bats will be more likely to be infected by viruses, since reduced docility can lead to a greater number of aggressive encounters between bats, increasing the likelihood of virus transmission during these interactions.

2 MATERIAL AND METHODS

2.1 Study area

The study areas are located within the Atlantic Forest biome in the Cantareira-Mantiqueira region of São Paulo state, Brazil. Ecologically, the region presents a rich variety of habitats due to its diverse topography and climate. The region has mountainous relief and steep slopes, with an average annual rainfall of approximately 1600 mm. The climate of the area includes hot and humid summers and cold and dry winters. The average monthly temperature varies between 18 and 20°C, rarely exceeding 22°C within the forest. The vegetation is typical of the Atlantic Forest, predominantly a tropical moist broadleaf forest with variations in altitude (HUECK, 1972). In the region, we selected sampling sites using mapped landscapes that generated a gradient of percentage forest cover in 2 km radius. Forest cover data were obtained using Geographic Information System (GIS) software, specifically QGIS 3.16 (QGIS Development Team). Forest cover maps were created from high-resolution satellite imagery provided by the Landsat 8 Operational Land Imager (OLI) and processed using supervised classification techniques. In supervised classification, representative samples of different land cover types (e.g. forest, water, urban) were used to train the classification algorithm, which then categorized all pixels in the images. The resulting data layers were analyzed to calculate the percentage of forest cover within each buffer zone (Figure 1).

Figure 1. Location of the study area and the 17 sampled landscapes within the Atlantic Forest in southeastern Brazil. The map on the left shows the general location of the Atlantic Forest biome (in gray) within Brazil, highlighting the study area (in yellow). The map on the right displays the specific sampled landscapes (red dots) where the captures of bats



2.2 Bat sampling

Two nights of sampling were conducted in 17 landscapes that form a forest cover gradient from 11% to 91%. To capture the bats, six mist nets (12 x 3 m, 16 x 16 mm mesh) were placed in each area in locations such as trails, roads, and forest edges. The mist nets remained open for 6 hours starting at dusk (TREVELIN et al., 2017), totaling 4320 h.m^2 of sampling effort.

The mist nets were checked every 30 minutes and, once captured, the bats were placed individually in cloth bags measuring 20 cm by 20 cm to assess their docility. This method is widely recognized in behavioral ecology (CAREAU et al., 2015; MARTIN; REALE, 2008) and involves measuring activity time, that is, the period in which the animal struggles from the moment it is placed in the cloth bag for 180 seconds. This measurement was always performed by the same person to avoid sampling bias. Individuals that moved for longer time were considered less docile (CAREAU et al., 2015; MARTIN; REALE, 2008; MIGUEL et al., 2024). All bats were identified to the species level in the field using a combination of taxonomic guides (REIS et al., 2007).

2.3 Virus collection

Two swabs were used to collect material from the oral mucosa and two from the rectal mucosa. The oral mucosa was rubbed for a few seconds with a swab, covering the upper and lower gums and the internal mucosa of the buccinator muscle to the oropharynx region. To obtain material from the rectal mucosa, the swab was inserted into the animal's rectum, posterior to the sphincter, covering the mucosa in a 360º circular motion, rotating the rod to increase the volume of material collected. The swabs were placed in 1.5mL micro tubes and stored frozen in the field in liquid nitrogen vapor (Cryo Shipper – MVE Biological Solutions) until deposition in the laboratory's biobank at -80°C for posterior viral genomic extraction.

2.4 Viral detection and sequencing

For the extraction of viral RNA, 0.5ml of MEM medium was added to each tube containing the swab and then homogenized in a vortex for 15 seconds and centrifuged at 8000 rpm for two minutes at 4ºC. The extraction of viral RNA was carried out using the QIAamp Viral RNA Mini Kit® kit applied to specific QIACube equipment (Qiagen, USA) according to the manufacturer's instructions and stored at -80°C. From the extracted viral RNA, complementary DNA (cDNA) was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems™) and stored at -20°C. This library of viral cDNA was tested for the presence of viruses from the *Orthocoronavirinae* subfamily using a pan-Coronavirus nested-PCR assay targeting the nsp12 genomic region of the RNA-dependent RNA polymerase gene (RdRP) (Chu et al., 2011).

Positive samples are currently being purified using QIAquick Gel Extraction Kit (Qiagen) and submitted to Sanger sequencing with the 3730 XL DNA Analyzer (Applied Biosystems) using the set of primers from the nested-PCR reactions.

2.5 Phylogenetic analysis

The phylogenetic analyses of the virus sequences were performed based on the amino acid (aa) sequences coded by the RdRp, which were aligned using ClustalW by MEGA7 (KUMAR et al., 2016). The best fit models for aa substitution were inferred using ModelTest-NG. The Bayesian Markov Chain Monte Carlo (MCMC) analyses were done using BEAST v.1.10.4 (SUCHARD et al., 2018). The phylogenetic tree was summarized using Tree Annotator and edited in FigTree v1.4.4. Evolutionary distances were estimated between the aa sequences obtained in this study, and the most closely related sequences available in GenBank applying the Maximum Composite Likelihood model (TAMURA et al., 2004) and JTT matrix-based model with 1000 bootstrap replicates using MEGA7 (KUMAR et al., 2016).

For the RdRp aa analyses, a data set was composed of 38 available GenBank sequences representing Alpha and Beta genera, and 5 sequences obtained so far in this study. The Bayesian analyses were performed using the Whelan and Goldman model plus gamma distribution (WAG + G), under a lognormal relaxed clock and constant population size, with the MCMC chain, running for 10,000,000 steps sampling every 5000 steps. All tests for viral identification were conducted in partnership with the Laboratory of Virology and Applied Biotechnology of the State University of Campinas (UNICAMP), coordinated by Prof. Dr. Clarice Weis Arns.

2.6 Statistical analysis

To analyze the impact of forest cover (a proxy for habitat loss) and docility behavior with coronavirus infected bats, we used a logistic regression model with a binomial response variable.

The logistic regression model was adjusted to explore the relationship between the presence or absence of the virus as a binary variable (0 = absent, 1 = present), and the predictor variables forest cover (%) and docility. The choice of the logistic regression model is due to the fact that the response variable is binary, allowing modeling of the probability of coronavirus occurrence based on the explanatory variables. The analysis was conducted using R software (version 4.3.2) and the “stats” package.

3 RESULTS

A total of 326 bats from 18 different species were captured and analyzed, and 49 of these bats from 9 species were found to be infected (Table 1). In the Maximum Likelihood analyses, all 5 aa sequences obtained so far formed a monophyletic group within the *Alphacoronavirus* genus, with robust bootstrap (100) and posterior probability values (0.81) (Figure 2). The most captured species were *Sturnira lilium*, *Carollia perspicillata* and *Artibeus lituratus* performing 69% of all captured bats. The mean movement time in the docility test for infected bats was 20.4 ± 19.4 seconds, ranging from 0 to 171 seconds.

The results of the model showed that both predictors had significant effects on viral occurrence. The estimated coefficient for forest cover was significant, indicating that the probability of viral occurrence increased with the percentage of forest cover (Table 2, Figure 3). The estimated coefficient for docility behavior was also significant, with the probability of viral occurrence decreasing with the movement time of the docility test (Table 1, Figure 4).

Table 1: Number of bat captures by species and the number of individuals that tested positive for coronavirus infection.

Taxon	**Captured individuals**	**Infected individuals**
Phyllostomidae Family		
Subfamily *Desmodontinae*		
Desmodus rotundus (E. Geoffroy, 1810)	26	6
Diphylla ecaudata (Spix, 1823)	6	0
Subfamily *Glossophaginae*		
Anoura caudifer (E. Geoffroy, 1818)	9	1
An*oura geoffroyi* (Gray, 1838)	10	1
Glossophaga soricina (Pallas, 1766)	6	0
Subfamily *Phyllostominae*		
Mimon bennettii (Gray, 1838)	5	0
Subfamily *Carolliinae*		
Carollia perspicillata (Linnaeus, 1758)	67	12
Subfamily *Stenodermatinae*		
Artibeus fimbriatus (Gray, 1838)	7	0
Artibeus lituratus (Olfers, 1818)	63	4
Artibeus planirostris (Spix, 1823)	5	0
Platyrrhinus lineatus (E. Geoffroy, 1810)	8	2
Sturnira lilium (E. Geoffroy, 1810)	96	21
Vespertilionidae Family		
Histiotus velatus (I. Geoffroy, 1824)	4	1
Myotis albescens (E. Geoffroy, 1803)	1	0
Myotis nigricans (Schinz, 1821)	2	0
Myotis riparius (Handley, 1960)	1	0
Myotis ruber (E. Geoffroy, 1806)	5	0
Myotis ssp.	5	2

Figure 2: Phylogenetic tree of the Coronavirus RdRp genomic region using the Bayesian method based on 5 sequences from this study and 38 representative sequences of *Alphacoronavirus* and *Betacoronavirus* genera, and subgenera within *Alphacoronavirus* genus available in GenBank. The nodes show posterior values higher than 0.8. The *Alphacoronavirus* subgenera sequenced thus far in this study are highlighted in red.

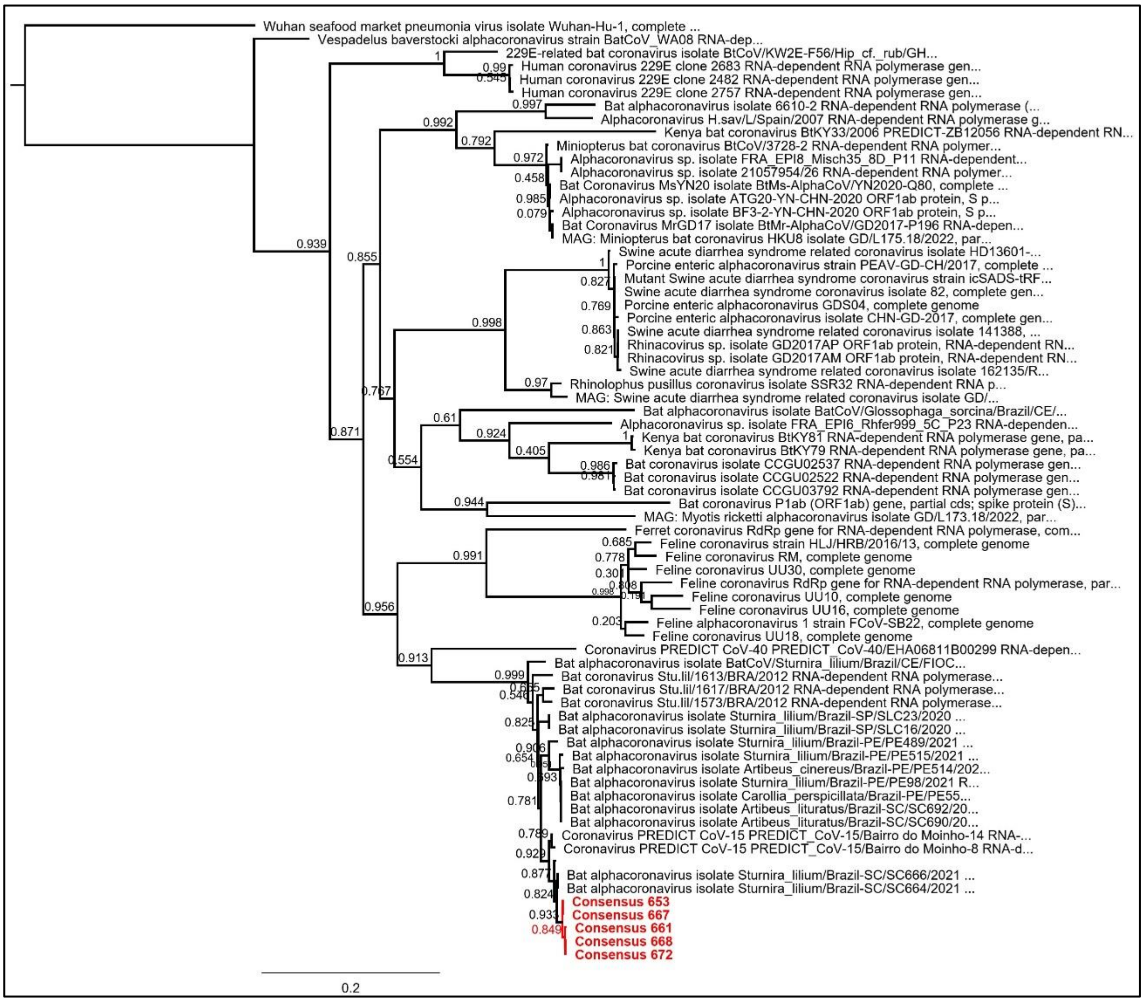


Table 2: Binomial logistic regression model for predicting coronavirus occurrence in studied bats.

	Estimate	*Std. error*	*z-value*	*P*
Intercept	-2.204	0.495	-4.447	<0.001*
Forest cover	0.020	0.002	2.180	0.029*
Docility	-0.016	0.002	-2.120	0.034*

Figure 3: Relationship between the percentage of forest cover within a 2 km radius and the occurrence of coronaviruses in bats (n = 326). The line represents the regression fit accompanied by its standard error in gray shading.

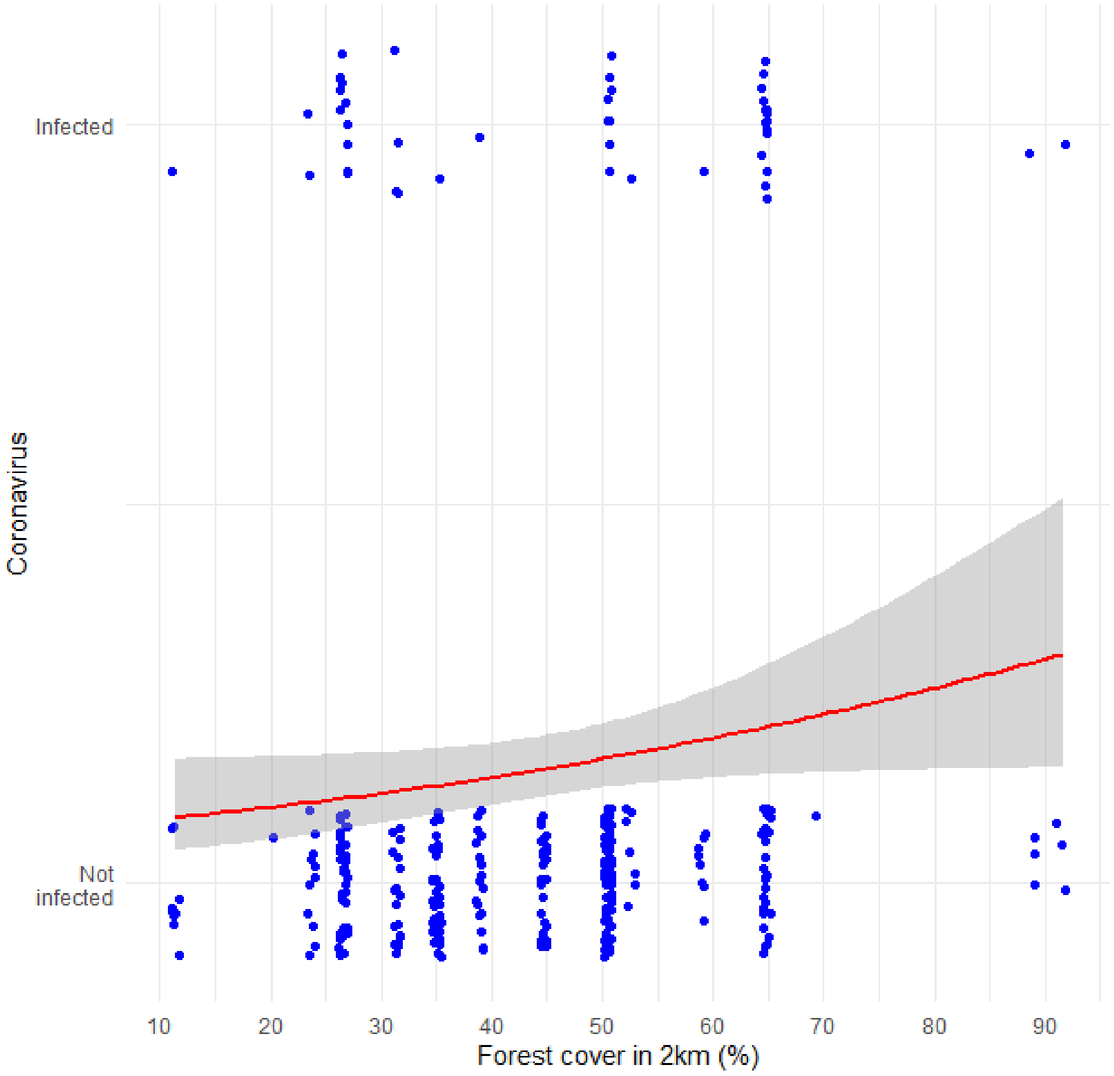
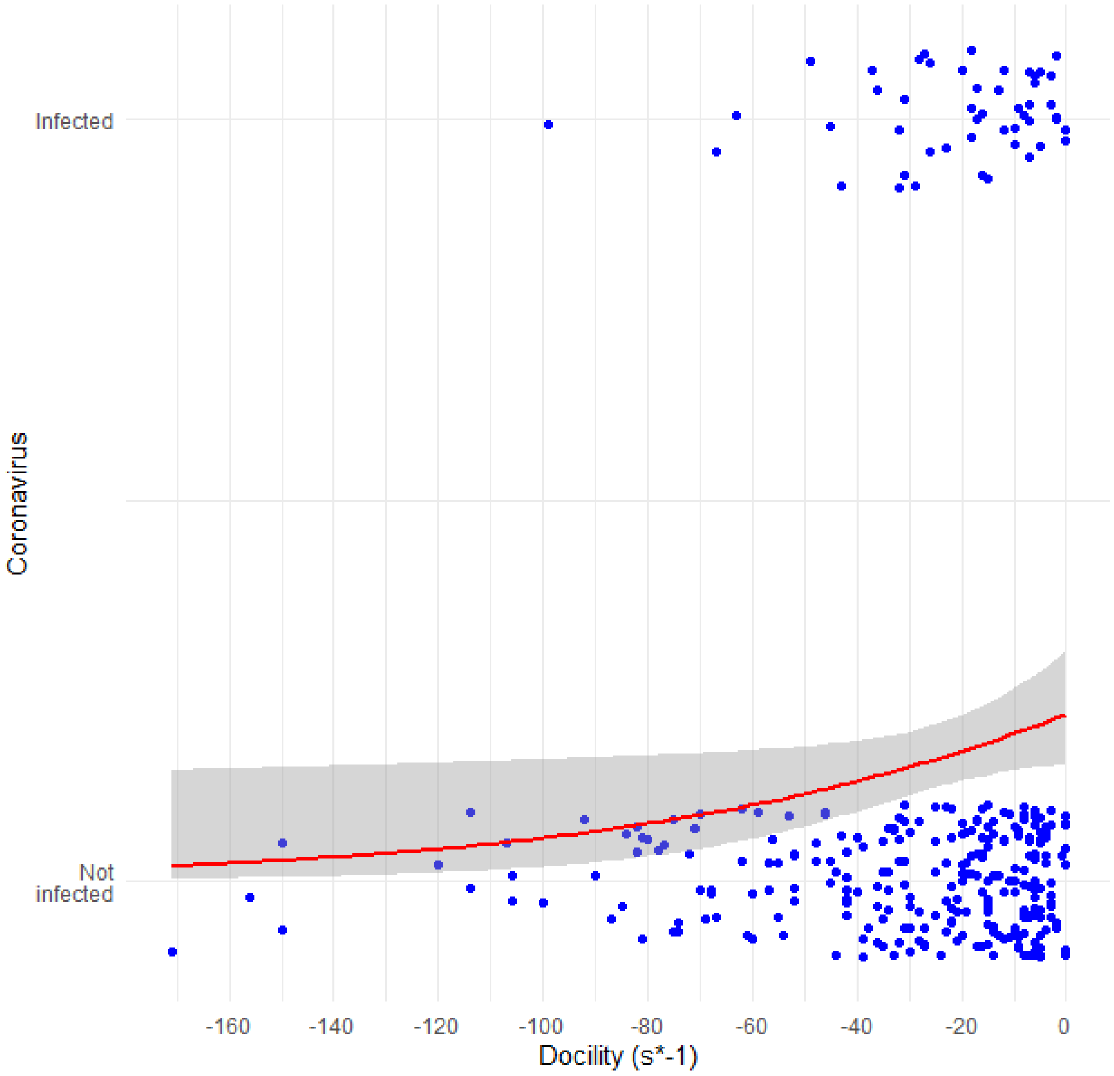


Figure 4: Relationship between movement time in the docility test (measured in seconds) and the occurrence of coronaviruses in bats (n = 326). The line represents the regression fit accompanied by gray shading of its standard error. To facilitate understanding of the biological meaning of docility, movement time during the bag handling test was multiplied by -1 (i.e., zero seconds of movement time is the highest docility value).



4 DISCUSSION

The results confirmed our hypothesis that the occurrence of viruses from the *Coronaviridae* family in bats in the Atlantic Forest is influenced by habitat loss and docility behavior. We found a negative relationship between movement time in the docility test and viral occurrence, suggesting that bats displaying less movement time in the test (indicative of higher docility) are more likely to be infected by a coronavirus. This may indicate that more docile bats, despite probably avoiding aggressive interactions and situations that would require active defense, may be more susceptible to close contact with other individuals during activities such as resting or feeding, facilitating virus transmission.

Contrary to what we expected, we found a positive relationship between forest cover and the occurrence of the virus family analyzed in bats, suggesting that the greater the forest cover, the greater the viral occurrence in bats. However, it is possible that even in areas with greater forest cover, the structure of habitats within these areas is fragmented to varying degrees, creating situations in which bats concentrate in more favorable fragments within a larger forest matrix. This could lead to an increase in local density, similar to what occurs in smaller forest fragments, resulting in greater contact between individuals and, consequently, greater viral transmission (GOTTDENKER et al., 2014; HU et al., 2015; SALMIER et al., 2017). In environments with greater forest cover, habitats are expected to be more stable and with more abundant resources, thus supporting larger and denser bat populations, which facilitates viral transmission (BRÄNDEL et al., 2020; COSSON et al., 1999; MEDELLÍN et al., 2000), resulting in a greater number of infected individuals.

Throughout evolutionary history, many viruses have evolved in different bat species (BROOK; DOBSON, 2015; LUIS et al., 2015; WONG et al., 2007). To establish themselves as reservoirs in their hosts, these viruses need to create a balanced interaction between the host's immune response and their own replication (CHATTU et al., 2018; SCHOUNTZ, 2014). It is possible that, over time, bats and several families of viruses such as those of the *Coronaviridae* family have adapted in such a way as to create a relationship that restricts the manifestation of diseases, but also attenuates antiviral responses (LUIS et al., 2015; SCHOUNTZ, 2014; WONG et al., 2007). This immunological balance may have direct implications on the behaviors observed in bats (AUSTAD et al., 1991; BAKER;

SCHOUNTZ, 2018; SCHOUNTZ, 2014). For example, less docile bats (which move more) may have an immune response associated with a greater capacity to regulate viral replication, resulting in a lower viral load (HAYMAN, 2019; LI et al., 2005; SUBUDHI et al., 2019). Thus, bats with higher metabolism have a more robust immune response, which may reduce the chances of viral occurrence (BAKER; SCHOUNTZ, 2018; SCHOUNTZ, 2014). This may contribute to explaining our findings, in which lower docility was associated with a lower occurrence of coronavirus infections. Thus, it is reasonable to assume that less docile individuals have more effective immunological mechanisms to deal with infections, which suggests a complex interaction between behavior, physiology and viral infection dynamics in bats.

Bats are important natural hosts for a wide variety of mammalian viruses with zoonotic potential for humans and other animals (CALISHER et al., 2006; WONG et al., 2007). Maintaining viral replication at relatively low levels is a peculiar characteristic of bats, which allows them not to develop severe symptoms and not be harmed by most of these viruses (IRVING, et al., 2021; LETKO, et al., 2020). However, our results indicate that environmental factors, such as habitat loss, and behavioral factors, such as docility, can influence the occurrence of viral infections in bats. Exposure to environmental stressors can impact the immune system of bats, potentially altering the dynamics of viral transmission. These findings suggest that habitat integrity and bat behavior play important roles in maintaining viral balance. Habitat preservation and understanding individual behaviors may therefore be crucial for conservation and zoonosis control strategies.

Our results indicate that environmental factors, such as habitat loss, and behavioral factors, such as docility, can influence the occurrence of viral infections in bats. These findings suggest that habitat integrity and bat behavior may also play important roles in maintaining viral balance. Viral distributions and range shapes refer to the way viruses spread geographically and the extent of their presence in different regions; these distributions are a consequence of speciation, extinction, and historical displacements (EBY, et al., 2022; GASTON, 1998; IRVING, et al., 2021). The accumulation of virus species may be related to the historical expansion and contraction of bat species' distribution ranges, with potentially strong distributional effects on virus transmission (LETKO, et al., 2020; MAGANGA, et al., 2014; MUYLAERT, et al., 2022).

On a considerably smaller temporal and spatial scale, our results reinforce this idea by showing that forest cover and docility behavior have a significant impact on the occurrence of coronaviruses in bats. Previous studies with rodents have shown that docility, measured through the handling pouch test, is related to the movement of individuals within the landscape, affecting their dispersal and interaction with the environment (BOON et al., 2007; BREHM et al., 2019; MELLA et al., 2016). These findings highlight the importance of considering environmental changes and behavioral characteristics when assessing the risk of viral transmission in general, especially in highly fragmented habitats such as the Atlantic Forest.

In the article published by Luís et al. (2013), the authors compared bats and rodents as viral reservoirs, highlighting that bats have a higher rate of viruses per species. They attributed this result to ecological factors, such as sympatry and environmental degradation, which can impact virus transmission. In our results, we found an association between habitat loss measured by forest cover and viral occurrence in bats, but, unlike the study cited, the relationship we found was that the lower the habitat loss, the higher the viral occurrence. Although the bats in our study were more likely to be contaminated by viruses in more preserved landscapes, the integrity of these habitats can help mitigate the risks of transmission of these zoonotic diseases by maintaining more robust ecological relationships. This would reduce the chance of spillover, which is when the virus mutates to the point where pathogens can be transmitted from one host species to a new species (JOHNSON et al., 2020; LI et al., 2019; RUIZ-ARAVENA et al., 2022). Studies indicate that preserving the integrity of ecosystems can reduce the risk of spillover by limiting interactions between wild and human species, as well as by keeping wildlife populations in balance (ALVES et al., 2020; BARBIER, 2021; SÁNCHEZ et al., 2022).

The abundance of resources in areas with greater forest cover can lead to the formation of larger colonies, where the high density of bats results in physical proximity that facilitates contact and exposure to contaminated excretions, creating ideal conditions for viral spread (ALURALDE; DÍAZ, 2021; CARVALHO et al., 2021; GLOZA-RAUSCH et al., 2008; TANG et al., 2006). This is particularly relevant in habitats with greater forest cover, where our results indicated that the occurrence of viruses from the *Coronaviridae* family is higher. However, our study did not include detailed analyses of landscape diversity to identify the location of specific resources for bats. Thus, this density effect may be exacerbated by the presence of resources concentrated in areas with greater forest cover, where social interactions are more frequent (MAGANGA, et al., 2014).

Although docility variation was evaluated as a behavioral response at the population level, it is known that this behavior can be understood as part of the personality of these animals (MIGUEL et al., 2024; WEBBER; WILLIS 2020), that is, it is a consistent characteristic at the individual level over time. In this context, Webber and Willis (2020) found that personality traits, such as sociability and exploratory behavior, play a significant role in the probability of transmission of infections among *Myotis lucifugus* bats (WEBBER; WILLIS, 2020). Furthermore, the research revealed that bat behavior not only affects the acquisition of pathogens, but also the intensity of infection (WEBBER; WILLIS, 2020), suggesting that individual variation in behavior is a critical factor in the spread of diseases.

The arguments presented above suggest that certain individuals, due to their personality, may have a greater potential to spread viruses, being more predisposed to explore new habitats and interact with different food sources, also increasing their chances of contact with pathogens. In addition, there may be the presence of behavioral syndromes as found by Miguel et al. (2024), that is, bats may present a correlation between different personality axes, such as docility, activity, and aggressiveness. Therefore, this individual variation may influence the way these individuals relate socially, further increasing the risk of disease spread involving specific individuals.

Nonetheless, Bat-CoV sequences detected in this study thus far belong to the Alphacoronavirus genus, grouping together with previously reported Bat-CoV sequences, possibly reiterating the high circulation of this Bat-CoV genus in bats (RUIZ-ARAVENA et al., 2021; BUENO et al., 2022). Yet, data about the diversity of viruses from the *Orthocoronavirinae* subfamily in the New World, especially Neotropical bats, remain limited.

The relevance of these findings becomes even more apparent when considering the global context of emerging infectious diseases, emphasizing the interconnectedness of human, animal, and environmental health (DESTOUMIEUX-GARZÓN et al., 2018; MACKENZIE; JEGGO, 2019). Recent studies highlight the role of wildlife, particularly bats, as reservoirs for zoonotic viruses that can spill over to humans, as exemplified by the ongoing COVID-19 pandemic caused by SARS-CoV-2 (HASSELL et al., 2017; WHO, 2020). Changes in land use, deforestation, and habitat loss have been increasingly linked to zoonotic disease emergence, as these environmental disturbances disrupt ecological balances and increase human-wildlife interactions (BEGEMAN et al., 2023; PLOWRIGHT et al., 2021). Therefore, preserving habitat integrity is critical not only for biodiversity conservation but also for reducing the risk of zoonotic disease transmission.

5 CONCLUDING REMARKS

This study contributes to the understanding of the relationships between ecology and behavior in the dynamics of viral occurrence in bats in the Atlantic Forest. By identifying that environments with greater forest cover are associated with a higher occurrence of coronaviruses, our findings suggest that factors such as population density and social structure of bats in preserved habitats may play crucial roles in the spread of pathogens. Furthermore, the association between docility and viral infection offers new directions for future research, suggesting that behavior can significantly influence the health of populations. These results indicate the need to integrate behavioral and ecological approaches in conservation and monitoring strategies for zoonoses.

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CONCLUSÃO GERAL

Esta tese buscou responder a três questões principais: (1) se as espécies de morcegos frugívoros *C. perspicillata* e *A. lituratus* apresentam personalidade e síndromes comportamentais; (2) como a perda de habitat afeta um proxy de estresse e a docilidade de espécies de morcegos frugívos; e (3) de que forma a perda de habitat influencia a ocorrência de patógenos virais, como os coronavírus, em morcegos da Mata Atlântica.

Primeiramente, nossos dados indicam que os comportamentos relacionados à docilidade, ousadia e atividade são personalidade em *C. perspicillata* e *A. lituratus*. Observamos que as síndromes comportamentais são menos variáveis em *C. perspicillata* do que em *A. lituratus*, e que isso possivelmente se deve ao comportamento de atividade, que pode estar sujeito a diferentes forças seletivas.

Segundamente os resultados da pesquisa dão suporte parcial às suposições feitas, demonstrando que a diminuição da cobertura florestal está ligada a um aumento no nível de estresse fisiológico, medido pelo proxy da razão neutrófilos e linfócitos. Contudo, não foi identificada uma relação clara entre a perda de habitat e mudanças específicas no comportamento de docilidade dos morcegos frugívoros. A falta de um impacto direto significativo da diminuição da cobertura florestal na docilidade sugere que, apesar das alterações no ambiente, os morcegos conseguem manter níveis consistentes de docilidade. Isso pode ser explicado pela presença de mecanismos adaptativos que permitem aos morcegos ajustar seus comportamentos de modo a manter uma relativa estabilidade na docilidade, mesmo em condições provavelmente desfavoráveis.

Por fim, nosso estudo sobre a ocorrência de coronavírus na Mata Atlântica revelou que ambientes com maior cobertura florestal estão associados a uma maior ocorrência viral, sugerindo que a densidade populacional e a estrutura social dos morcegos em habitats preservados são fatores importantes na disseminação de doenças. A descoberta da associação entre comportamento e infecção viral sugere que integrar abordagens comportamentais e ecológicas pode ser fundamental para estratégias de conservação e monitoramento de zoonoses. Essas estratégias podem ajudar a identificar fatores de risco no comportamento de morcegos que contribuam para a ocorrência viral, sendo cruciais para a vigilância e prevenção do spillover de patógenos para humanos.