



Norwegian University
of Life Sciences

Master's Thesis 2022 60 ECTS

Faculty of Environmental Sciences and Natural Resource Management
(MINA)

Effect of Season and Habitat on Two Congeneric Bat Species in a Fragmented Forest Landscape, Central Amazon, Brazil

Ingrid Johanne Müller Tangvik

Master of Science in Ecology

Acknowledgements

This thesis was written as a part of my MSc in Ecology at the Faculty of Environmental Science and Natural Resource Management at the Norwegian University of Life Sciences. I chose this project hoping it would take me to the Amazonian rainforest, but due to the COVID-19 pandemic lasting far longer than initially expected, this became a desk-based project instead. Despite of this, I have learned so much about both the Amazon and the amazing taxon of bats, and I am very happy with my choice of topic. It has inspired me for future projects and contributed to my understanding of nature and its conservation needs.

The writing of this thesis has been a very educational process, but at times also quite challenging. Luckily, I have received a lot of well wishes and good energy from the people around me. I would therefore like to thank my supervisor Torbjørn Haugaasen for excellent feedback, guidance, and encouragement. Thank you to my co-supervisor Giulliana Appel for help throughout the writing process, with feedback on statistical analysis, and for providing me with the dataset to make my thesis possible. I am also very grateful for the help I received with my statistical analysis from Louise R. Bauer-Nilsen. I also wish to thank my supportive family, friends, and partner for motivating me and cheering me on throughout this project. Lastly, I want to show my gratitude for my aunt, Bente Tangvik, for great help with my thesis and invaluable encouragement and support.

Norwegian University of Life Sciences

Ås, December 2022

Ingrid Johanne Müller Tangvik

Abstract

Bats are an important component of Amazonian mammal communities but are often ignored during conventional biodiversity assessments. Much is therefore unknown regarding spatial and temporal forest use and the partitioning between different bat species in the community. The objectives of this study were to examine how different habitats and seasons affects the activity of two congeneric species (*Pteronotus alitonus* and *P. rubiginosus*), and how temporal partitioning of activity in these two species varies with habitat types and seasonality. I used ultrasound recordings of the two *Pteronotus* species collected in three different habitat types (continuous primary forest, secondary forest and 10ha forest fragments) and during two seasons (wet and dry) in the Biological Dynamics of Forest Fragments Project in central Amazon, Brazil. The activity levels of both *Pteronotus* species were found to significantly increase during the wet season, suggesting that insect abundance during the wet season would positively affect bat activity levels. Furthermore, *P. alitonus* had highest activity levels in secondary forest, whereas *P. rubiginosus* exhibited high activity levels in both primary and secondary forest. Forest fragments were least favoured by both species. The same patterns were found in spatial hourly activity. The results suggest that *P. alitonus* and *P. rubiginosus* spatially and temporally partition activity and that this partitioning may be a way to reduce competition.

Table of contents

Acknowledgements	i
Abstract	iii
Introduction	1
Methods	4
Study site.....	4
Study species.....	5
Data collection and data (pre)processing.....	6
Statistical analysis.....	7
Results	8
Data summary.....	8
Effect of season and habitat on bat activity levels.....	9
<i>Effect of season on bat activity</i>	9
<i>Effect of habitat on bat activity</i>	10
<i>Comparison of activity levels</i>	11
Effect of season and habitat on hourly bat activity levels.....	12
<i>Effect of season on hourly bat activity levels</i>	12
<i>Effect of habitat on hourly bat activity levels</i>	13
Discussion	14
Seasonal effect on bat activity.....	14
Habitat selection.....	15
Seasonal effect on hourly activity.....	17
Hourly activity in different habitats.....	19
Conclusion	21
References	22
Supplementary information	v

Introduction

The Amazon is the largest tropical rainforest on the planet, and it harbours a disproportionate amount of the global biodiversity (Garda et al., 2010). New species are regularly discovered, for instance seven new species of *Callicebinae*, titi monkeys, have been discovered since 2002 (Boubli et al., 2019; Gusmão et al., 2019) and several new insect species has been described just this year (Bento et al., 2022; Nakahara et al., 2022; Nascimento et al., 2022). However, the Amazon is threatened by deforestation, fragmentation, and anthropogenic disturbances (Gibson et al., 2011). Fragmentation of forest is acknowledged as one of the major threats to wildlife, especially in the tropics, and it has a profound impact on species composition (Faria, 2006). Bird assemblages appear affected by fragmentation (Barlow et al., 2007) and other disturbances such as fires (Barlow et al., 2002) and logging (Barlow et al., 2006), and insectivorous birds disproportionately so (Barlow et al., 2007; Canaday, 1996). However, it remains unclear how forest fragmentation affects less studied taxa such as bats. For instance, Meyer & Kalko (2008) found that insectivorous bats were highly sensitive to edge-effects, even when fragments were near primary forest. In contrast, Estrada & Coates-Estrada (2002) reported that most bat species seem to tolerate anthropogenic impacts and found that fragmented habitats had the same species richness as the natural continuous forest. It is argued that the variable results can be caused by the distance between fragments and the distance of fragments to primary forest, but little evidence of this has been found (Faria, 2006). It therefore remains unclear to what extent fragmentation affects insectivorous bats (Barros et al., 2014).

The Amazon rainforest is a highly seasonal biome defined by precipitation levels, dividing the year into a wet and dry season. The resource abundance and composition in the forest change dramatically between the two seasons (Klingbeil & Willig, 2010). Phenological patterns in the forest are highly predictable, with flowering and fruiting correlating with seasonality (Haugaasen & Peres, 2005) and serving as reliable food sources. There is a strong link between seasonality and arthropod abundance, which also exhibit peak activity levels during the wet season (Develey & Peres, 2000; Jahn et al., 2010). Bats are the most species-rich mammal taxon in the Amazon, and most species are insectivorous (Fleming et al., 1972). Insectivorous bats follow insect activity patterns and time important life cycle events, such as reproduction, to correlate with insect abundance (Klingbeil & Willig, 2010; Meyer et al., 2004; Russ et al., 2003). Bat activity patterns can often differ between species which could be related to the activity of preferred prey (Meyer et al., 2004), but may also reflect an effort to avoid competition through temporal and spatial partitioning (Pavan et al., 2018).

Aerial insectivorous bat activity is highly dependent on environmental conditions (Thies et al., 2006). Bats are for example found to be less active at low humidity levels and heavy rainfall due to disruption of echolocation calls and direct interference with flight (Appel et al., 2019; Voigt et al., 2011). In addition, rainfall and temperature can affect thermoregulation and insect abundance (Agosta et al., 2005; Barros et al., 2014). If declining resources result in poor foraging success, or the cost of flight is too high to attain a positive energy balance, insectivorous bats cease to forage (Thies et al., 2006). Predation and prey availability can also influence activity by causing trade-off situations between foraging and predation risk (Thies et al., 2006). Structural environmental conditions are also found to affect bat activity levels (Appel et al., 2021; Rocha et al., 2020; Thies et al., 2006; Yoh et al., 2022). Highly cluttered vegetation is found to be limiting for some echolocating bats (de Oliveira et al., 2015; Pavan et al., 2018), whilst open spaces like riparian zones might increase risk of detection by predators (de Oliveira et al., 2015). Despite of this, both cluttered vegetation and riparian zones are favoured for foraging due to high degree of insect mass (Barros et al., 2014; de Oliveira et al., 2015; Janzen, 1968). Bat assemblage composition and foraging patterns may therefore be different between different habitats.

In this study, I take advantage of a large-scale fragmentation experiment – the Biological Dynamics of Forest Fragmentation Project (BDFFP) in central Amazonia – where it is possible to study primary forest, secondary forest, and forest fragments in the same area. I used ultrasound recordings of two *Pteronotus* species (*P. rubiginosus* and *P. alitonus*), to assess their habitat use and foraging patterns. They are both small, insectivorous bats with overlapping distribution ranges in northern Amazonia, but the *P. rubiginosus* distribution stretches far south from the sympatric areas (Bruno & Falcão, 2022; Pavan et al., 2018). Previous studies suggest that these species show some spatial and temporal niche division in sympatric areas, but these trends are not fully verified (Bruno & Falcão, 2022; Pavan et al., 2018). For instance, de Oliveira et al. (2015) found that *P. rubiginosus* preferred heavily cluttered areas, namely continuous primary forest. Contrastingly, Pavan et al. (2018) suggested that the echolocation call of *P. rubiginosus* is more suited to less cluttered habitat than *P. alitonus*, and that the latter preferred highly cluttered forests as there are no reports of this species in open areas.

The main objective of this study was to assess how the behavioural patterns of the two sister-species of aerial insectivorous bats are affected by habitat type and seasonality and use this to assess the niche partitioning between these species. More specifically, I examined (1) how habitat and season affected bat activity, and (2) how temporal partitioning of activity

between these two species varied with habitat and seasonality, and (3) compared the activity patterns between the two species. This was done by analysing bat activity within primary forest, secondary forest, and forest fragments during the wet and dry season. I hypothesize that the effect of habitats on behaviour is greater than seasonality since heavy rain and humidity is likely to affect both species similarly. I also predict that the general activity level of the two bat species is higher during the wet season than the dry season since insect abundance peaks during the wet season. I continue the assumption of Pavan et al. (2018) that *P. rubiginosus* is more active in secondary forest and *P. alitonus* in primary forest. Additionally, I hypothesize that *P. rubiginosus* tackle fragmented habitats better than *P. alitonus*, since the natural distribution range of *P. rubiginosus* is much wider, and the species is seemingly more adaptable to different habitats.

Methods

Study site

The study was conducted in central Amazonia, Brazil – more specifically at the Biological Dynamics of Forest Fragments Project (BDFFP) ~80 km north of Manaus (Fig. 1). The BDFFP is one of the longest-running investigations of habitat fragmentation (Yoh et al., 2022). The study area contains lowland *terra firme* rainforest with eleven 10ha primary forest fragments surrounded by a second growth matrix (Laurance et al., 2016). The fragments were originally surrounded by cattle ranches, but the ranches were abandoned, and secondary forest reclaimed the pastures (Yoh et al., 2022).

The dry season in this area is characterized by precipitation less than 100mm/month and lasts from July to November, while the rainy season lasts from November to June, with precipitation of 300mm/month (Ferreira et al., 2017). Sunset and sunrise occur at around 18:00h and 06:00h during both seasons. The study area is situated at 80-160masl (Farneda et al., 2018).

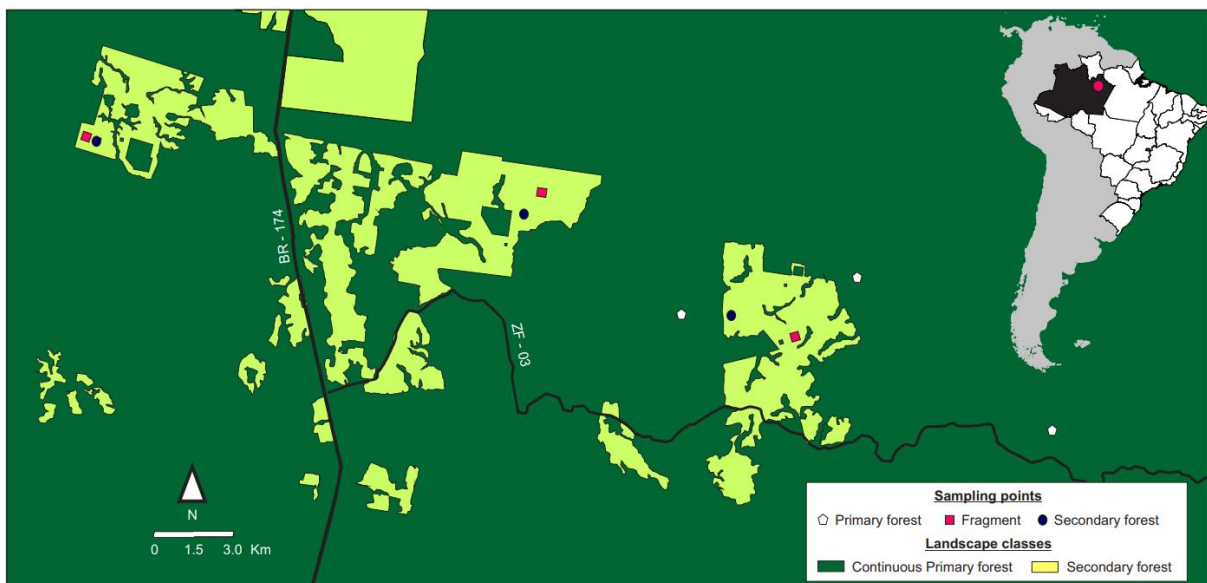


Figure 1 Location of study area in the Biological Dynamics of Forest Fragments Project (BDFFP) with the distribution of sampling points in continuous forest, secondary forest and 10 ha fragments. Continuous forest is represented in dark green and secondary forest (matrix) in light green. The map in the upper right corner shows the location of the BDFFP in the central Amazon of Brazil. The map was produced using QGIS.

Study species

This study focuses on *Pteronotus alitonus* (Fig. 2) and *Pteronotus rubiginosus* (Fig. 3), two closely related insectivorous bat species, native to the Americas (Pavan et al., 2018; Thoisy et al., 2014). Both species measure ~ 6 cm in body length and weigh ~ 20 gram, with *P. rubiginosus* being slightly larger in size (Pavan et al., 2018). *Pteronotus rubiginosus* has a wider distribution range than *P. alitonus*, with sympatric appearances in Guyana, Surinam, French Guyana, and the northern parts of the Brazilian Amazon (Bruno & Falcão, 2022; López-Baucells et al., 2018; Pavan et al., 2018; Thoisy et al., 2014). Both species derive from *P. parnellii*, a complex of species where *P. rubiginosus* was thought to be a subspecies and *P. alitonus* was yet unidentified (Clare et al., 2013; Thoisy et al., 2014). Through phylogenetic and morphometric analysis, *P. rubiginosus* was recognized as its own species, and *P. alitonus* suggested as a species under the name “*Pteronotus* species 1” in 2016 (Pavan & Marroig, 2016). In a comparative study between *P. rubiginosus* and *P. alitonus*, discernible differences in call frequency, morphological traits and molecular variations were found, and *Pteronotus alitonus* was formally described as a new species (Pavan et al., 2018). *Pteronotus rubiginosus* has an echolocation call frequency with maximum energy of 55 kHz and *P. alitonus* has max. energy of 60 kHz, which is easily separated from each other (López-Baucells et al., 2016; Pavan et al., 2018). Interestingly, the morphologic differences between *P. rubiginosus* and *P. alitonus*, for instance skull size, were greater in areas with sympatric occurrence than in allopatric areas (Pavan et al., 2018).



Figure 2 *Pteronotus alitonus*. © Manuel Ruedi, Museum Geneva



Figure 3 *Pteronotus rubiginosus*. © Manuel Ruedi, Museum Geneva

Data collection and data (pre)processing

The acoustic sampling took place at six camps in the BDFFP, namely Porto Alegre, Cabo Frio, Colosso, Florestal, 41 and Dimona. There were three sites in each forest type (continuous primary forest, secondary forest and 10ha fragments). In the 10ha fragments, the recorders were placed in the middle of the fragment, at least 100m from the edge to secondary forest. The sites were visited during the wet season of 2018 and 2019, and in the dry season of 2019.

The data was collected using an automatic ultrasound recorder (Song Meter SM2Ba+) with an omnidirectional ultrasonic SMS-US microphone (Wildlife Acoustics, Inc., USA) which was installed at each sampling site. The equipment was placed 1.5m above ground, and passively registered bat activity in real time. The recorders had a full spectrum resolution of 16bit, a high-pass filter set at 12 kHz, with an additional adaptive trigger level relative to noise floor of 18 SNR. Bat activity was recorded continuously between 17:30 and 06:30. The recorders were installed to register five nights at each visit, but due to battery malfunctions some locations only have four recording nights. Total sampling effort was 138 nights, with 1,794 recording hours. The number of recordings per season and habitat is presented in Table 1.

Table 1 Number of recording nights in wet and dry season and in three habitat types (continuous primary forest, secondary forest, and forest fragments) in BDFFP – central Amazonia.

Recording nights				
	Continuous forest	Secondary forest	Forest fragments	<i>Total</i>
Wet season	30	18	23	71
Dry season	23	23	21	67
<i>Total</i>	53	41	44	138

The recordings were split into five-second segments, with a bat pass defined as a sequence of minimum two recognizable search phase calls per species in each segment (Appel et al., 2019; Torrent et al., 2018). Bat passes on each five-second segment were visually identified with the identification key of López-Baucells et al. (2016) using Kaleidoscope Viewer Version Software (Wildlife Acoustics, Inc. Maynard, Massachusetts, USA). The sum of segments with bat passes per night (nightly activity) and per hour (hourly activity) were used to calculate bat activity.

Statistical analysis

To test the effects of habitat type and season on species-specific bat activity levels, I used Generalized Linear Mixed Models (GLMM) with a negative binomial distribution due to overdispersion (Bolker, 2022). The `glmmTMB` package in R was used for these analyses (Bolker, 2022; Magnusson et al., 2020). The response variable is bat-passes per night with habitat and season as predictor variables. To run the plot for the GLMM model, the `sjPlot` package (Lüdecke, 2022) was used. To compensate for the difference in sampling nights, I put a weight on the sampling nights during the seasons corresponding to the imbalance. This imbalance was then included in the analysis using “offset” in the GLMM model. To compensate the temporal and spatial correlation, we used the sampling night nested within each research camp as a random variable in GLMM models. The GLMM-results did not provide comparisons between all habitats. I therefore used `lsmeans` with Tukey method to test the comparisons (Lenth, 2016).

To test correlogram of the model residuals of each GLMM model and to see if the models procedure were successful, I used the `DHARMA` package (Residual Diagnostics for Hierarchical (Multi-Level/Mixed) Regression Models) (Hartig & Lohse, 2022). All models procedures were successful and not overdispersed and without outliers (Fig. S1).

Kolmogorov-Smirnov 2-sample test is commonly used to test differences between hourly bat activity of different species or habitat types (see: Presley et al. (2009); Rocha et al. (2020)). I therefore used this to test hourly activity levels for season and habitat (Lilliefors, 1967; Massey, 1951). Activity was pooled into hourly intervals, and then tested for each species between seasons (wet versus dry), and between all habitats (continuous vs. secondary, continuous vs. fragmented and secondary vs. fragmented).

Figures 4-8 were made using `ggplot2` package and `tidyverse` package in R (Wickham, 2016; Wickham et al., 2019). For Figs. 4 and 7, the habitat-data were pooled for each species, and for Figs. 5 and 8, the seasonal data were pooled for each species. All analysis was conducted in R Studio (R version 4.0.3).

Results

Data summary

In total, there were 6680 recordings of the target *Pteronotus* species. *Pteronotus alitonus* had an average of 15.79 bat passes per night ($SD \pm 2.94$) in continuous forest, 28.61 passes ($SD \pm 5.61$) in secondary forest and 10.78 passes ($SD \pm 1.79$) in fragmented forest (Table S1). *Pteronotus rubiginosus* had an average of 16.51 bat passes per night ($SD \pm 5.15$) in continuous forest, 22.11 passes ($SD \pm 5.32$) in secondary forest and 9.41 passes ($SD \pm 1.65$) in fragmented forest (Table S1). Seasonal activity for *P. alitonus* averaged 16.40 bat passes per night ($SD \pm 2.00$) in the wet season and 22.28 passes per night ($SD \pm 5.27$) in the dry season (Table S1). Contrastingly, *P. rubiginosus* had an average of 21.10 bat passes per night ($SD \pm 3.92$) during the wet season and 6.89 passes per night ($SD \pm 1.14$) during the dry season (Table S1).

Effect of season and habitat on bat activity levels

Effect of season on bat activity

Pteronotus alitonus showed higher activity levels during the wet season than the dry season (Table S1), with the median number of passes per night being nine in the wet season and six in the dry season (Fig. 4A). The variance in activity levels were greater during the dry season than the wet season, and this is portrayed by the larger variance around the median (Fig. 4A). The GLMM predicted more activity during the wet season than the dry season in both continuous forest and forest fragments, but the opposite for secondary forest (Table S2). The activity level of *P. alitonus* in continuous forest is estimated to increase five times from the dry season to the wet season (Table S2). The change from the dry season to the wet season significantly impacted activity levels ($P=7.26^{e-07}$; Table S2).

Pteronotus rubiginosus had similar activity pattern with higher activity during the wet season than the dry season (Fig. 4B). Median passes per night were four passes in the dry season and eight passes in the wet season. Total number of bat passes were almost six times higher during the wet season than during the dry season (Table S1). The change from the dry season to the wet season was significant for *P. rubiginosus* ($P=9.99^{e-10}$; Table S2), and the GLMM predicted more activity in all habitats during the wet season.

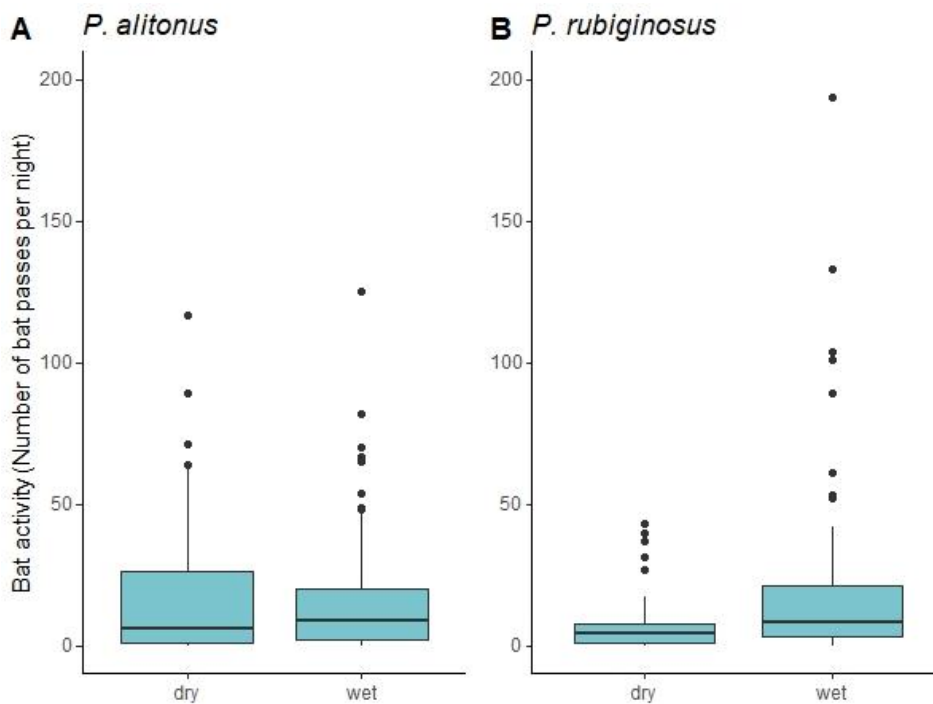


Figure 4 Observed seasonal activity levels for (A) *P. alitonus* and (B) *P. rubiginosus* in central Amazonian fragmented landscape. The horizontal black line is the median number of bat passes per night and the black dots are outliers. Boxes represent the middle 50 % of observations and the vertical black lines are the upper 25% and lower 25% of data points.

Effect of habitat on bat activity

Pteronotus alitonus had a higher activity level in secondary forest compared to primary forest and forest fragments (Fig. 5A), and this difference was significant ($P=0.048$; Table S2). Median bat passes per night were six in primary forest, 15 in secondary forest and six in forest fragments (Fig. 2A). Noticeably, the activity in secondary forest during the dry season was higher than in all other habitat types regardless of season (Table S2).

Pteronotus rubiginosus had lower activity in fragmented forest compared to the other habitat types (Fig. 5B & Table S1). However, the GLMM did not uncover any significant differences in activity levels between habitats (Table S2). Median bat passes per night were five in primary forest, nine in secondary forest and five in forest fragments (Fig. 2B).

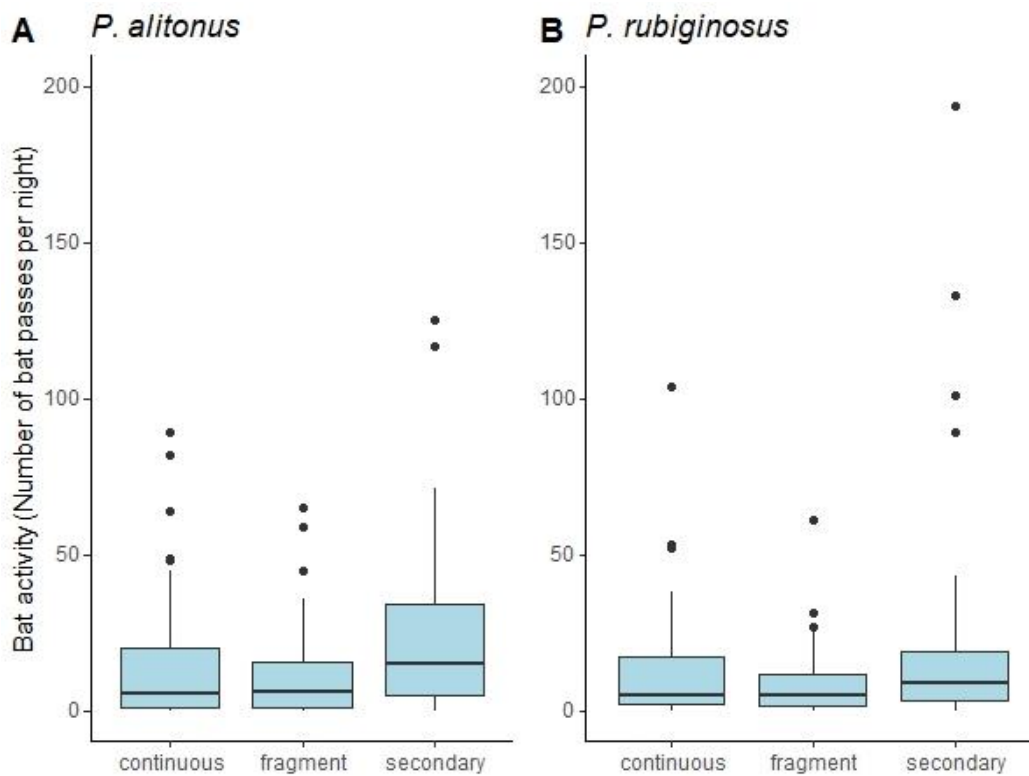


Figure 5 Observed bat activity (bat passes per night) in primary forest (continuous), secondary forest (secondary) and 10ha forest fragments (fragment) for (A) *P. alitonus* and (B) *P. rubiginosus*. The horizontal black line is the median number of bat passes per night and the black dots are outliers. Boxes represent the middle 50 % of observations and portray variance in bat activity level and the vertical black lines are the upper and lower 25% of data points.

Comparison of activity levels

Both *Pteronotus* species have higher activity levels during the wet season than the dry season in primary forest (Fig. 6). In forest fragments, activity is low compared to in the two other habitats, with little distinction between the seasons (Fig. 6). Despite this, activity during the wet season is higher than during the dry season also in the forest fragments. For secondary forest, *P. alitonus* have much higher activity levels than *P. rubiginosus* during dry season, even though *P. rubiginosus* is equally active in the two seasons (Fig. 6). *Pteronotus alitonus* also exhibits more variance in activity patterns in both habitat and season (Fig 6).

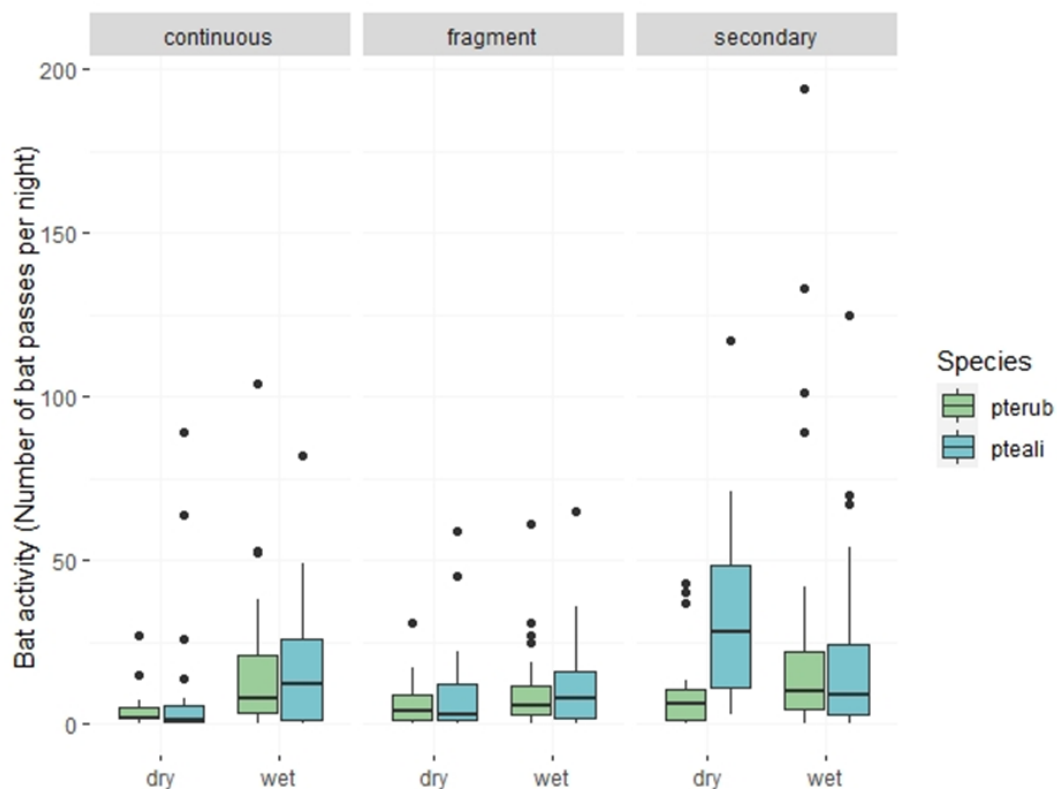


Figure 6 Observed spatial and seasonal (dry and wet season) activity patterns for *P. alitonus* (*pteali*) and *P. rubiginosus* (*pterub*) in a central Amazonian landscape consisting of primary forest (continuous), secondary forest (secondary) and 10ha forest fragments (fragment). Bat activity is measured as bat passes per night. Boxes represent the middle 50 % of observations and portray variance in bat activity level. The horizontal black line is the median number of bat passes per night and the black dots are outliers. Boxes represent the middle 50 % of observations and the vertical black lines are the upper and lower 25% of data points.

Effect of season and habitat on hourly bat activity levels

Effect of season on hourly bat activity levels

Pteronotus alitonus activity levels did not significantly change between the seasons (Table S4) but exhibited highest activity early in the night during the dry season and late in the night during the wet season (Fig. 7A). In contrast, *P. rubiginosus* activity drops dramatically during the dry season, with no hourly interval containing more than 100 bat passes (Fig. 7B), and the difference was significant (Kolmogorov-Smirnov 2-sample test; $P=1.25e^{-06}$; Table S4). Interestingly, the peak activity time for *P. rubiginosus* at 21.00h in the wet season, is also the peak activity time for *P. alitonus* during dry season (Fig. 7). *Pteronotus alitonus* exhibited earlier onset of activity and later withdrawal of activity during both seasons compared to *P. rubiginosus* (Fig. 7). The onset of activity by *P. rubiginosus* is earlier during the wet season than during the dry season (Fig. 7).

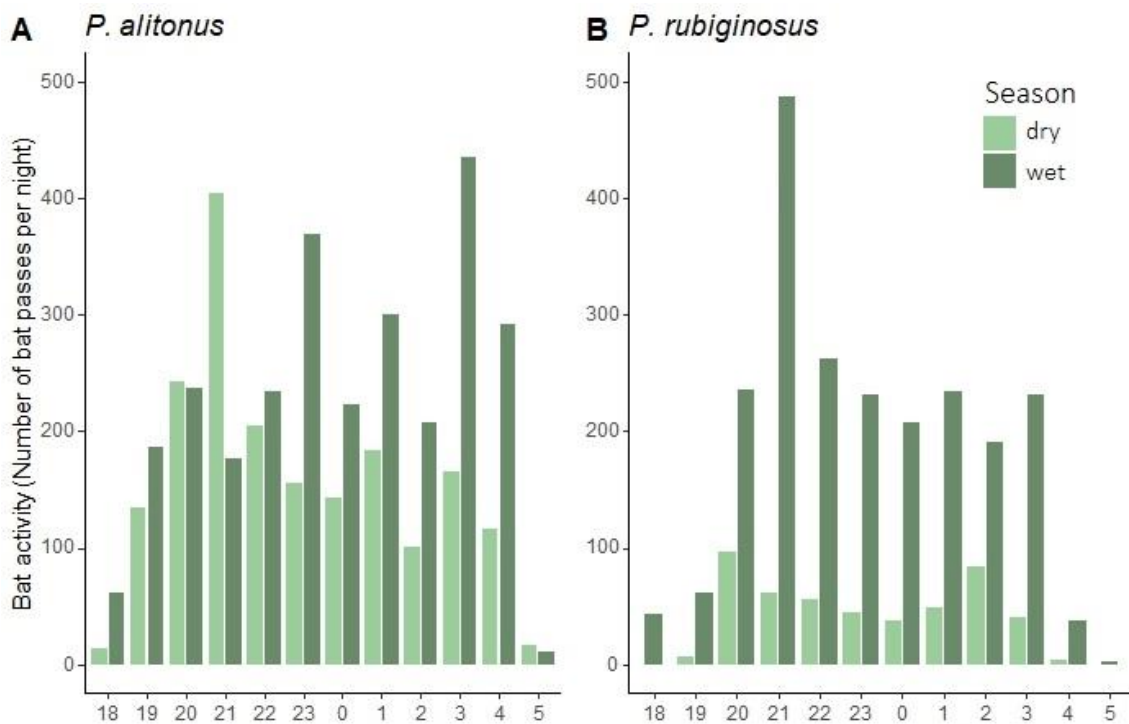


Figure 7 Observed hourly activity (bat passes per night) following 24-hour clock for (A) *P. alitonus* and (B) *P. rubiginosus* in dry season (light green) and wet season (dark green) in central Amazonian fragmented landscape.

Effect of habitat on hourly bat activity levels

Pteronotus alitonus has higher activity levels in secondary forest than in the two other habitat types (Fig. 8A). The change in activity levels was significant both between continuous forest and secondary forest (Kolmogorov-Smirnov 2-sample test; $P=4.662e^{-05}$) and between secondary forest and forest fragments (Kolmogorov-Smirnov 2-sample test; $P=9.245e^{-07}$) (Table S4).

For *P. rubiginosus* the overall activity is more even distributed in all habitat types (Fig. 8B), and even though the highest activity peaks occur in continuous primary forest there were no significant differences in activity levels among habitats (Table S4).

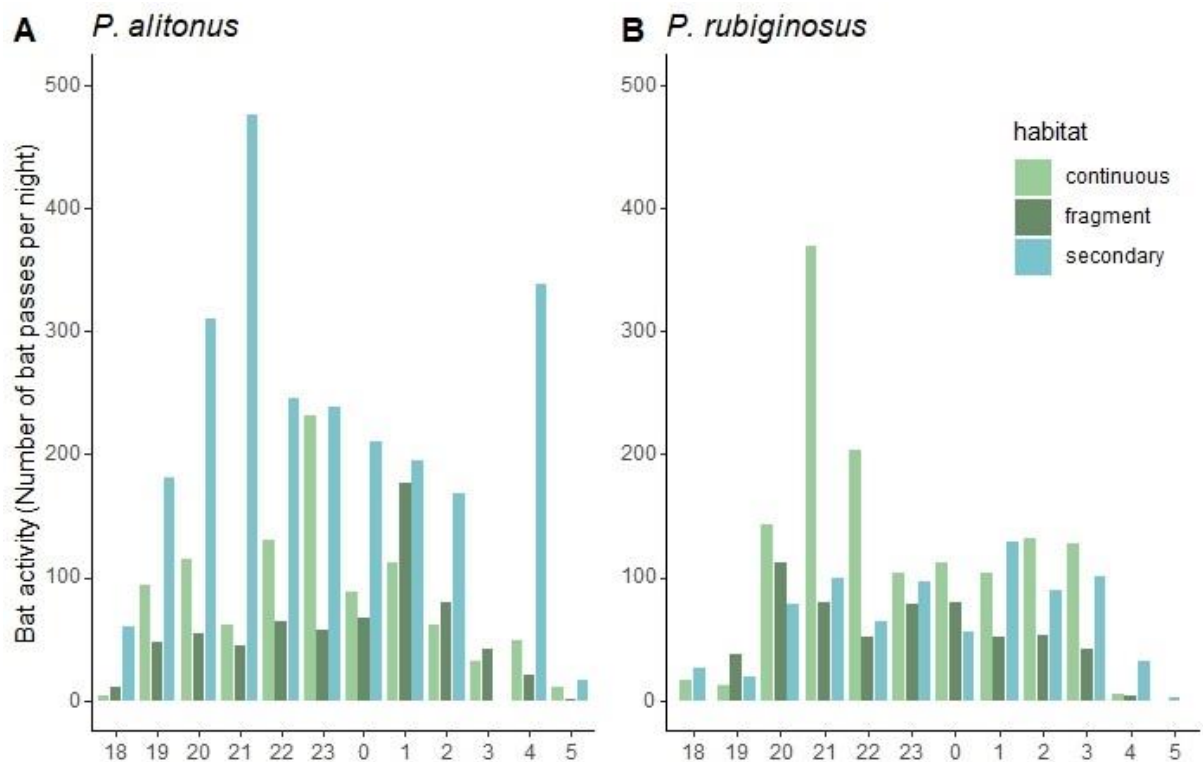


Figure 8 Observed hourly activity (bat passes per night) following 24-hour clock for (A) *P. alitonus* and (B) *P. rubiginosus* in three habitats: continuous primary forest (continuous), secondary forest (secondary), and 10ha primary forest fragments (fragment) in central Amazonia.

Discussion

Seasonal effect on bat activity

The activity levels significantly increased during the wet season for both *Pteronotus* species. Tropical insects exhibit seasonal abundance fluctuation, with most species favouring the wet season (Wolda, 1980). Vegetation in tropical areas have evolved toxins to limit herbivory, but young leaves have yet to develop such a defence mechanism (Wolda, 1978). New leaves, together with fruit and seeds, start to evolve at the beginning of the wet season (Wolda, 1978). In the BDFFP, pioneer tree species are found to flower in the transition between the dry season and the wet season, and to fruit during the wet season (Bentos et al., 2008). This provides the insects with abundant vegetational resource availability and thereby support high insect abundance at this time (Racey, 1982; Wolda, 1978). Insectivorous bats take advantage of this abundance, and correlate energy demanding reproduction to increased food availability and stability (Klingbeil & Willig, 2010; Meyer et al., 2004; Russ et al., 2003). *Pteronotus* species are proposed to be seasonally monestrous, having only one gestation per year, with the birth period occurring at onset of the wet season (Fleming et al., 1972; Pavan & Tavares, 2020). The lactating females will increase their activity levels during the wet season to meet the increased energy demands of lactation (Racey, 1982; Rydell, 1993). The elevated activity levels during the wet season are therefore likely related to bat reproduction (Fleming et al., 1972; Racey, 1982; Rydell, 1993).

Even though tropical insect abundance fluctuates seasonally, and they appear more abundant in the wet season, insects are unlikely to be in shortage for foraging bats at any time of year. In fact, many Amazonian trees flower during the dry season (Haugaasen & Peres, 2005) and many Amazonian palms flower throughout the year (Henderson et al., 2000). If insect mass is scarce during the dry season, it could be argued that the bat activity levels would be higher due to prolonged foraging activity to obtain enough food. This further suggests that higher bat activity in the wet season is due to reproduction rather than differences in insect abundance. However, evidence of larger home ranges during the dry season due to less food availability (Fleming et al., 1972) could contribute to fewer bat detections by the recorders, and thereby provide fewer measurements of activity. This supports the hypothesis that low activity during the dry season could be caused by reduced insect mass. Further studies are therefore needed to fully understand the observed trends.

Pteronotus alitonus has significantly higher activity than *P. rubiginosus* in secondary forest during the dry season. Fruit availability is found to be less seasonal in the secondary forest of the BDFFP (Ferreira et al., 2017). Some arthropod taxa (Coleoptera and Hemiptera) favour secondary habitat (de Aquino et al., 2022), suggesting these taxa could be preferred prey species by *P. alitonus*. The difference in activity levels between the *Pteronotus* species in this habitat can also indicate a spatial partitioning to limit food resource competition. Moreover, if the seasonal trends are less distinctive in secondary forest, it could be speculated that *P. alitonus* gives birth earlier in this habitat, which suggest that the elevated activity could be due to lactating females' increased energy demand. The dry season data were obtained in 2019, and it is possible that the activity trend observed would be less distinctive with data collected over several years.

Bat activity in different habitats

Pteronotus alitonus used secondary forest significantly more than the other habitats, whereas *P. rubiginosus* did not show a distinctive preference for any of the habitat types. This contrasts my assumption of *P. rubiginosus* favouring secondary forest and *P. alitonus* favouring primary forest due to the structure of their echolocation calls. Echolocation is a mean for bats to orient through the environment and to localize and hunt prey, in limited or no light, using ultrasonic signals (Fenton et al., 2012; Schnitzler & Kalko, 2001). The composure of the echolocation calls is individual for all echolocating bat species (Fenton et al., 2012), and some species have shown geographical variances to their calls (López-Baucells et al., 2018). During foraging, vegetational structure has the biggest impact on echolocation, but the signals can also be disrupted by rain and humidity (Schnitzler & Kalko, 2001; Sleep & Brigham, 2003; Thies et al., 2006). The structure of the call can be limiting in some environments and is often adapted to preferred habitat and prey (de Oliveira et al., 2015). Bats that forage in high-cluttered habitats usually have short, broad-banded, and high frequented calls (Sleep & Brigham, 2003), which is true for both species of this study. Both *P. alitonus* and *P. rubiginosus* are found to be more active in the understory than in the canopy (Gomes et al., 2020), suggesting a preference for prey inhabiting cluttered habitats. Previous studies done on *P. rubiginosus* found that the species was well suited for foraging in highly cluttered habitats, with evidence of efficient hunting, following prey within centimetres of obstacles (de Oliveira et al., 2015; Goldman & Henson, 1977). Additionally, de Oliveira et al. (2015) found that *P. rubiginosus*' activity level were higher in areas with a high degree of clutter than in more open habitats and suggested that

the species followed insect mass regardless of habitat structure. The difference in the frequency of the echolocation calls of the two species is relatively small, and therefore unlikely to be adapted to different sized prey (Pavan et al., 2018). It can therefore be argued that *P. alitonus*' preference for secondary forest in sympatric areas with *P. rubiginosus* could be an adaptation to avoid resource competition for food resources. Furthermore, it suggests that *P. rubiginosus* follows insect mass rather than habitat type, which supports my hypothesis of this species being more adaptable to different habitats.

The elevated use of secondary forest can also be due to the second growth age. The secondary forest of the BDFFP is mostly > 30 years old and therefore quite structurally complex (Bobrowiec & Gribel, 2010; Rocha et al., 2017; Yoh et al., 2022). The second growth forest can therefore host many of the same species as the continuous primary forest, in addition to provide sufficient cover for the bats to not be detectable for predators (Silva et al., 2020). Second growth will in time mature to have low contrast with surrounding primary growth and fragments, which enables the support of species rich assemblages (Rocha et al., 2017). In the study area (BDFFP), bat assemblages are found to vary across the landscape despite of low structural contrast between continuous forest, secondary forest, and forest fragments (Rocha et al., 2017). The second growth in the matrix is thought to have two successional paths, and thereby also two different vegetative structures, with one of the structures more diverse and comparable to primary forest than the other (Bobrowiec & Gribel, 2010). The different camp sites in this study were pooled into habitat type, but for future studies it could be interesting to see if preference for secondary forest varies along with successional pathway of the secondary growth.

The mean activity level was lowest in forest fragments for both species, with *P. alitonus* having a slightly higher activity level than *P. rubiginosus* (Table S1). This contradicts my assumption that *P. rubiginosus* would be better at tackling forest fragments. Forest fragments are less structurally complex than primary forest and more exposed to edge effects (Rocha et al., 2017; Yoh et al., 2022). Forest fragments also host less diverse community assemblages than other habitats (Yoh et al., 2022). Insect composition is found to be affected by fragmentation and edge effects, with soil-foraging and social insects being negatively impacted (Caitano et al., 2020). Insectivorous bats are adversely affected by insect decline, and bat species composition in forest fragments is found to be less diverse than in primary and secondary forest (Vleut et al., 2012). Availability of sufficient roost sites is also scarce in fragmented forests (Rocha et al., 2017). Furthermore, canopy openness is found to increase

predation risk (Vleut et al., 2012), especially in combination with high moonlight intensity (Appel et al., 2021). Despite the secondary forest functioning as a buffer for edge effects in the fragments (Rocha et al., 2017), the *Pteronotus* species in this study do not equate forest fragment habitats to primary or secondary forest. Additionally, *Pteronotus* species are found to reduce their activity pattern on moonlit nights in forest fragments and secondary forest, but not in primary forest (Appel et al., 2021), suggesting that predator avoidance during the night can be a determinant for the variation of hourly activity. The low activity levels in this habitat are therefore likely due to a combination between low prey availability, unsuitable roost sites and predation risk. However, other measurable factors such as temperature, canopy cover, landscape metrics, vertical stratification and moonlight intensity may also affect spatial and temporal bat activity levels (Appel et al., 2021; de Oliveira et al., 2015; Ferreira et al., 2017; Gomes et al., 2020; López-Baucells et al., 2018; Yoh et al., 2022). *Pteronotus alitonus*' higher activity levels than *P. rubiginosus* in forest fragments, combined with preference for secondary forest, suggests that *P. alitonus* prefers prey species inhabiting less cluttered environments.

Seasonal effect on hourly bat activity

Pteronotus rubiginosus showed significantly higher hourly activity levels during the wet season than during the dry season, and emergence time was earlier in the wet season. Emergence time in insectivorous bats is correlated to increased insect abundance which occurs shortly after sunset (Reichard et al., 2009). Due to insect abundance being highest during the wet season (Jahn et al., 2010), it is likely that prey availability is high from sunset to sunrise. Dusk is favoured foraging time for many bat species due to availability of diurnal insects in addition to nocturnal species (Pavey et al., 2001). Bat assemblages are known to partition their temporal activity to avoid resource competition (Beilke et al., 2021). Temporal partitioning is probable for the two *Pteronotus* species of this study seeing as the difference between echolocation call frequency is too small to be specialized to different prey (Pavan et al., 2018), and their prey preference thereby likely overlaps.

Pteronotus rubiginosus exhibited an even distribution of activity levels throughout the night during the wet season, except for peak activity at 21.00h. Emergence time in bats is also influenced by predation risk (Fenton & Fleming, 1976; Rodriguez-Duran & Lewis, 1985), with the highest predation risk occurring when travelling to and from roosts sites, especially when travelling in great numbers (Fenton & Fleming, 1976; Rodriguez-Duran & Lewis, 1985). Avoidance behaviour in bats is common and contribute to change in temporal activity (Lima &

O’Keefe, 2013). It is known that bats emerge later when predators are present at the entrance of roosts (Lima & O’Keefe, 2013). Several owl species have bats as part of their diet (Carvalho et al., 2011; Ibañez et al., 1992). Opportunistic bat predation by owls has been reported in the study area in the BDFFP, with two occurrences shortly after dusk, on the same night, in secondary forest and in a forest fragment (Rocha & López-Baucells, 2014). Owl activity is correlated with sunrise and sunset, with peak activity 1-3 hours before sunrise and 1-3 hours after sunset (Delaney et al., 1999). Even though predation risk greatly influences bat behaviour (Lima & O’Keefe, 2013), insectivorous bats are usually opportunistic prey for most predators (França & Lima, 2012; Nyffeler & Knörnschild, 2013; Rocha & López-Baucells, 2014). Still, it could be speculated that the evenly distributed activity level of *P. rubiginosus* during the night is due to increased predation risk and the exhibited activity pattern is a trade-off between risk and obtaining a positive energy balance.

The constant activity level during the night exhibited by *P. rubiginosus* during the wet season can also be caused by individuals at different reproductive stages, which are known to emerge at different times (Reichard et al., 2009). Lactating females emerge earlier, often before sunset, with extended foraging time (Rydell, 1993), likely due to empty energy reserves from lactation (Duvergé et al., 2000). Juvenile bats still suckling emerge later than adult females, whereas weaned juveniles with higher energy demand tend to emerge earlier, despite of higher predation risk (Reichard et al., 2009).

The activity level for *P. rubiginosus* during dry season is low, never reaching more than 100 bat passes per night. Peak activity occurs 2 hours after sunset and 3 hours before sunrise, with activity distribution following a U-curve between peaks. The bat activity peaks correlate with insect abundance during dusk and dawn and suggests an optimal foraging strategy in a season with limited food resources (Fenton & Thomas, 1980). Additionally, females are pregnant during the later parts of the dry season (Fleming et al., 1972). Pregnant females tend to leave roosts later than other bats (Duvergé et al., 2000) and forage for a shorter period (Rydell, 1993), which likely is predator avoidance behaviour due to reduced flight ability along with progressing pregnancy (Reichard et al., 2009). Predation risk is seemingly also higher due to limited resources, and some opportunistic bat predators are found to have more bats in their diet during the dry season than the wet season (Motta-Jr. & Taddei, 1992). Still, the activity peaks correlate with predator activity, which suggest *P. rubiginosus* is making a trade-off between obtaining a positive energy balance and predation risk.

Pteronotus alitonus also significantly increase activity levels during the wet season, except in secondary forest where the activity levels are higher during the dry season. Notably *P. alitonus* activity is higher during the early hours of the night in dry season and during the late hours in the wet season. The increased activity for *P. alitonus* during the latter hours of night during the wet season can be suggested as temporal partitioning of resources between the two *Pteronotus* species, as *P. rubiginosus* activity peaks during the earlier hours of night and prey preference in the two species likely overlap. The early onset of activity by *P. alitonus* during the dry season could also be explained by preying on diurnal insects or a wider home range forcing them to emerge earlier to forage with limited resources.

Hourly bat activity in different habitats

Pteronotus alitonus was significantly more active in secondary forest than in continuous forest and forest fragments. There was a steadily increase in activity until peak activity at 21.00h and then a steady decrease until a new peak at 04.00h. No activity was recorded at 03.00h, likely due to a fault in the dataset. The activity pattern follows the expected insect abundance rhythm and predator avoidance behaviour. Continuous forest was used less than secondary forest, but more than forest fragments. Activity around sunset was higher in primary forest than in secondary forest and forest fragment, suggesting that *P. alitonus* takes advantage of the diversity of both diurnal and nocturnal insects present at this hour in complex vegetation. The lowest activity levels for *P. alitonus* were found in forest fragments, and only at 01.00h were there more than 100 bat passes per night. Activity like this can be related to heavy rain stopping, since bats tend to stop all foraging in heavy rain only to continue when rain has ceased (Appel et al., 2019; Thies et al., 2006). But for this instance, seasonal data is pooled so this remains speculations.

Pteronotus rubiginosus has similar activity levels in all three habitats, but exhibits the highest activity peaks in continuous forest, occurring at 20.00h, 21.00h and 22.00h. Emergence time in continuous forest for *P. rubiginosus* is later than for *P. alitonus*, and the activity stops earlier. *Pteronotus rubiginosus* is slightly bigger in size than *P. alitonus* (Pavan et al., 2018) which makes it a more favourable prey. This can in turn cause a stronger predator avoidance behaviour. In secondary forest, activity level is low with more than 100 bat passes occurring only at 01.00h. The lower activity in this habitat highly contrasts the activity level of *P. alitonus* which strongly suggests a spatial partitioning between the species. The activity levels in forest

fragments are the lowest of all habitat types. Only at 19.00h is the activity level higher in forest fragments than in continuous and secondary forest. This could be explained by less insect diversity and activity in fragmented forest and *P. rubiginosus* therefore needs to exploit the insect abundance right after sunset. However, as the number of observations of *P. rubiginosus* during wet season was much higher than in the dry season, the activity shown in habitat is likely somewhat unbalanced since the seasonal data are pooled.

Conclusion

The results of this study found indications of spatial and temporal partitioning between *P. alitonus* and *P. rubiginosus*. The former exhibited clear preference for secondary forest, whereas the latter had no clear habitat preference. Neither species seem to thrive within forest fragments, adding to evidence that forest fragments support fewer species and underline the need for conservation and protection of primary forest. The results also highlight the need for protection of secondary growth, as mature secondary growth can host a good portion of primary growth species. Seasonality in the Amazon seems to affect closely related insectivorous bat species similarly but can also coerce spatial distribution to avoid competition. Documenting hourly activity levels enables a more careful insight of temporal distribution of activity, providing evidence of minor differences in emergence time and seasonal effect on activity levels.

References

- Agosta, S. J., Morton, D., Marsh, B. D., & Kuhn, K. M. (2005). Nightly, seasonal, and yearly patterns of bat activity at night roosts in the Central Appalachians. *Journal of Mammalogy*, *86*(6), 1210–1219. <https://doi.org/10.1644/05-MAMM-A-012R1.1>
- Appel, G., López-Baucells, A., Magnusson, W. E., & Bobrowiec, P. E. D. (2019). Temperature, rainfall, and moonlight intensity effects on activity of tropical insectivorous bats. *Journal of Mammalogy*, *100*(6), 1889–1900. <https://doi.org/10.1093/jmammal/gyz140>
- Appel, G., López-Baucells, A., Rocha, R., Meyer, C. F. J., & Bobrowiec, P. E. D. (2021). Habitat disturbance trumps moonlight effects on the activity of tropical insectivorous bats. *Animal Conservation*, *24*(6), 1046–1058. <https://doi.org/10.1111/acv.12706>
- Barlow, J., Haugaasen, T., & Peres, C. A. (2002). Effects of ground fires on understory bird assemblages in Amazonian forests. *Biological Conservation*, *105*(2), 157–169. [https://doi.org/10.1016/S0006-3207\(01\)00177-X](https://doi.org/10.1016/S0006-3207(01)00177-X)
- Barlow, J., Mestre, L. A. M., Gardner, T. A., & Peres, C. A. (2007). The value of primary, secondary and plantation forests for Amazonian birds. *Biological Conservation*, *136*(2), 212–231. <https://doi.org/10.1016/J.BIOCON.2006.11.021>
- Barlow, J., Peres, C. A., Henriques, L. M. P., Stouffer, P. C., & Wunderle, J. M. (2006). The responses of understory birds to forest fragmentation, logging and wildfires: an Amazonian synthesis. *Biological Conservation*, *128*(2), 182–192. <https://doi.org/10.1016/J.BIOCON.2005.09.028>
- Barros, M. A. S., Pessoa, D. M. A., & Rui, A. M. (2014). Habitat use and seasonal activity of insectivorous bats (*Mammalia: Chiroptera*) in the grasslands of southern Brazil. *Zoologia*, *31*(2), 153–161. <https://doi.org/10.1590/S1984-46702014000200006>
- Beilke, E. A., Blakey, R. v., & O’Keefe, J. M. (2021). Bats partition activity in space and time in a large, heterogeneous landscape. *Ecology and Evolution*, *11*(11), 6513–6526. <https://doi.org/10.1002/ece3.7504>
- Bento, M., Jameson, M. L., & Seidel, M. (2022). New species and illustrated key of *Macraspis* (*Scarabaeidae, Rutelinae, Rutelini*) from the Amazon biome of Brazil. *ZooKeys*, *2022*(1124), 161–189. <https://doi.org/10.3897/zookeys.1124.91156>
- Bentos, T. v, Mesquita, R. C. G., & Williamson, C. G. (2008). Reproductive phenology of Central Amazon pioneer trees. *Tropical Conservation Science*, *1*(3), 186–203.
- Bobrowiec, P. E. D., & Gribel, R. (2010). Effects of different secondary vegetation types on bat community composition in Central Amazonia, Brazil. *Animal Conservation*, *13*(2), 204–216. <https://doi.org/10.1111/j.1469-1795.2009.00322.x>
- Bolker, B. (2022). *Getting started with the glmmTMB package*.
- Boubli, J. P., Byrne, H., da Silva, M. N. F., Silva-Júnior, J., Araújo, R. C., Bertuol, F., Gonçalves, J., Melo, F., Rylands, A., Mittermeier, R., Silva, F., Nash, S., Canale, G. & Alencar, R., Rossi, R., Carneiro, J., Sampaio, I., Farias, I., Schneider, H., & Hrbek, T.

- (2019). On a new species of Titi Monkey (*Primates: Plecturocebus* Byrne et al., 2016), from Alta Floresta, southern Amazon, Brazil. *Molecular Phylogenetics and Evolution*, 132, 117–137. <https://doi.org/10.1016/j.ympev.2018.11.012>
- Bruno, M., & Falcão, F. (2022). *Pteronotus rubiginosus* (Wagner, 1843): first record of the bat family *Mormoopidae* in the Southeastern Region of Brazil. *Check List* 18(5): 1017-1022, 18(5), 1017–1022. <https://doi.org/10.15560/18.5.1017>
- Caitano, B., Chaves, T. P., Dodonov, P., & Delabie, J. H. C. (2020). Edge effects on insects depend on life history traits: a global meta-analysis. *Journal of Insect Conservation*, 24(2), 233–240. <https://doi.org/10.1007/S10841-020-00227-1>
- Canaday, C. (1996). Loss of insectivorous birds along a gradient of human impact in Amazonia. *Biological Conservation*, 77(1), 63–77. [https://doi.org/10.1016/0006-3207\(95\)00115-8](https://doi.org/10.1016/0006-3207(95)00115-8)
- Carvalho, L. F. A. da C., da Cunha, N. L., Fischer, E., & Santos, C. F. (2011). Predation on broad-eared bat *Nyctinomops laticaudatus* by the Spectacled Owl *Pulsatrix perspicillata* in southwestern Brazil. *Revista Brasileira de Ornitologia*, 19(3), 417–418. www.ararajuba.org.br/sbo/ararajuba/revbrasorn
- Clare, E. L., Adams, A. M., Maya-Simões, A. Z., Eger, J. L., Hebert, P. D. N., & Fenton, M. B. (2013). Diversification and reproductive isolation: cryptic species in the only New World high-duty cycle bat, *Pteronotus parnellii*. *BMC Evolutionary Biology*, 13, 26. <https://doi.org/10.1186/1471-2148-13-26>
- de Aquino, K. K. S., Baccaro, F. B., Appel, G., Henriques, A. L., Dineli Bobrowiec, P. E., & Borges, S. H. (2022). Forest fragments, primary and secondary forests harbour similar arthropod assemblages after 40 years of landscape regeneration in the Central Amazon. *Agricultural and Forest Entomology*, 24(2), 178–188. <https://doi.org/10.1111/afe.12481>
- de Oliveira, L. Q., Marciente, R., Magnusson, W. E., & Bobrowiec, P. E. D. (2015). Activity of the insectivorous bat *Pteronotus parnellii* relative to insect resources and vegetation structure. *Journal of Mammalogy*, 96(5), 1036–1044. <https://doi.org/10.1093/jmammal/gyv108>
- Delaney, D. K., Grubb, T. G., & Beier, P. (1999). Activity patterns of nesting Mexican Spotted Owls. *The Condor*, 101(1), 42–49. <https://doi.org/10.2307/1370444>
- Develey, P. F., & Peres, C. A. (2000). Resource seasonality and the structure of mixed species bird flocks in a coastal Atlantic Forest of Southeastern Brazil. *Journal of Tropical Ecology*, 16(1), 33–53. <https://doi.org/10.1017/S0266467400001255>
- Duvergé, P. L., Jones, G., Rydell, J., & Ransome, R. D. (2000). Functional significance of emergence timing in bats. *Ecography*, 23(1), 32–40. <https://doi.org/10.1111/J.1600-0587.2000.TB00258.X>
- Estrada, A., & Coates-Estrada, R. (2002). Bats in continuous forest, forest fragments and in an agricultural mosaic habitat-island at Los Tuxtlas, Mexico. *Biological Conservation*, 103(2), 237–245. [https://doi.org/10.1016/S0006-3207\(01\)00135-5](https://doi.org/10.1016/S0006-3207(01)00135-5)

- Faria, D. (2006). Phyllostomid bats of a fragmented landscape in the north-eastern Atlantic Forest, Brazil. *Journal of Tropical Ecology*, 22(5), 531–542. <https://doi.org/10.1017/S0266467406003385>
- Farneda, F. Z., Rocha, R., López-Baucells, A., Sampaio, E. M., Palmeirim, J. M., Bobrowiec, P. E. D., Grelle, C. E. V., & Meyer, C. F. J. (2018). Functional recovery of Amazonian bat assemblages following secondary forest succession. *Biological Conservation*, 218, 192–199. <https://doi.org/10.1016/J.BIOCON.2017.12.036>
- Fenton, M. B., Faure, P. A., & Ratcliffe, J. M. (2012). Evolution of high duty cycle echolocation in bats. In *Journal of Experimental Biology* (Vol. 215, Issue 17, pp. 2935–2944). <https://doi.org/10.1242/jeb.073171>
- Fenton, M. B., & Fleming, T. H. (1976). Ecological interactions between Bats and nocturnal Birds. *Biotropica*, 8(2), 104. <https://doi.org/10.2307/2989629>
- Fenton, M. B., & Thomas, D. W. (1980). Dry season overlap in activity patterns, habitat use, and prey selection by sympatric african insectivorous bats. *Biotropica*, 12(2), 81. <https://doi.org/10.2307/2387723>
- Ferreira, D. F., Rocha, R., López-Baucells, A., Farneda, F. Z., Carreiras, J. M. B., Palmeirim, J. M., & Meyer, C. F. J. (2017). Season-modulated responses of Neotropical bats to forest fragmentation. *Ecology and Evolution*, 7(11), 4059–4071. <https://doi.org/10.1002/ece3.3005>
- Fleming, T. H., Hooper, E. T., & Wilson, D. E. (1972). Three Central American Bat communities: structure, reproductive cycles, and movement patterns. *Ecology*, 53(4), 556–569.
- França, F. G. R., & Lima, R. A. de. (2012). First record of predation on the bat *Carollia perspicillata* by the false coral snake *Oxyrhopus petolarius* in the Atlantic Rainforest. *Biotemas*, 25(4). <https://doi.org/10.5007/2175-7925.2012v25n4p307>
- Garda, A. A., da Silva, J. M. C., & Baião, P. C. (2010). Biodiversity conservation and sustainable development in the Amazon. *Systematics and Biodiversity*, 8(2), 169–175.
- Gibson, L., Lee, T. M., Koh, L. P., Brook, B. W., Gardner, T. A., Barlow, J., Peres, C. A., Bradshaw, C. J. A., Laurance, W. F., Lovejoy, T. E., & Sodhi, N. S. (2011). Primary forests are irreplaceable for sustaining tropical biodiversity. *Nature*, 478(7369), 378–381. <https://doi.org/10.1038/nature10425>
- Goldman, L. J., & Henson, O. W. (1977). Prey recognition and selection by the constant frequency bat, *Pteronotus p. parnellii*. *Behavioral Ecology and Sociobiology*, 2(4), 411–419. <https://doi.org/10.1007/BF00299509>
- Gomes, D. G. E., Appel, G., & Barber, J. R. (2020). Time of night and moonlight structure vertical space use by insectivorous bats in a Neotropical rainforest: An acoustic monitoring study. *PeerJ*, 8. <https://doi.org/10.7717/peerj.10591>
- Gusmão, A. C., Messias, M. R., Carneiro, J. C., Schneider, H., de Alencar, T. B., Calouro, A. M., Dalponte, J. C., Mattos, F. de S., Ferrari, S. F., Buss, G., de Azevedo, R. B., Santos Júnior, E. M., Nash, S. D., Rylands, A. B., & Barnett, A. A. (2019). A new species of

- Titi Monkey, *Plecturocebus* Byrne et al., 2016 (*Primates, Pitheciidae*), from Southwestern Amazonia, Brazil. *Primate Conservation*, 33. <https://www.researchgate.net/publication/338332881>
- Hartig, F., & Lohse, L. (2022). *Residual diagnostics for hierarchical (multi-level / mixed) regression models*. <http://florianhartig.github.io/DHARMA/>
- Haugaasen, T., & Peres, C. A. (2005). Tree phenology in adjacent Amazonian flooded and unflooded forests. *Biotropica*, 37(4), 620–630. <https://doi.org/10.1111/J.1744-7429.2005.00079.X>
- Henderson, A., Fischer, B., Scariot, A., Whitaker Pacheco, M. A., & Pardini, R. (2000). Flowering phenology of a palm community in a central Amazon Forest. *Brittonia* 2000 52:2, 52(2), 149–159. <https://doi.org/10.2307/2666506>
- Ibañez, C., Ramo, C., & Busto, B. (1992). Notes on food habits of the Black and White Owl. *The Condor*, 94(2), 529–531. <https://doi.org/10.2307/1369226>
- Jahn, A. E., Levey, D. J., Mamani, A. M., Salidas, M., Alcoba, A., Ledezma, M. J., Flores, B., Vidoz, J. Q., & Hilarion, F. (2010). Seasonal differences in rainfall, food availability, and the foraging behavior of Tropical Kingbirds in the southern Amazon Basin. *Journal of Field Ornithology*, 81, 340–348.
- Janzen, D. H. (1968). Differences in insect abundance and diversity between wetter and drier sites during a tropical dry season. In *Schoener Ecology* (Vol. 49, Issue 1).
- Klingbeil, B. T., & Willig, M. R. (2010). Seasonal differences in population-, ensemble- and community-level responses of bats to landscape structure in Amazonia. *Oikos*, 119(10), 1654–1664. <https://doi.org/10.1111/j.1600-0706.2010.18328.x>
- Laurance, W. F., Camargo, J. L. C., Fearnside, P. M., Lovejoy, T. E., Williamson, G. B., Mesquita, R. C. G., Meyer, C. F. J., Bobrowiec, P. E. D., & Laurance, S. G. W. (2016). An Amazonian Forest and its fragments as a laboratory of global change. In L. Nagy, B. R. Forsberg, & P. Artaxo (Eds.), *Interactions Between Biosphere, Atmosphere and Human Land Use in the Amazon Basin*. Springer Berlin Heidelberg. <https://doi.org/10.1007/978-3-662-49902-3>
- Lenth, R. v. (2016). Least-squares Means: The R Package lsmeans. *Journal of Statistical Software*, 69(1), 1–33. <https://doi.org/doi:10.18637/jss.v069.i01>.
- Lilliefors, H. W. (1967). On the Kolmogorov-Smirnov Test for Normality with Mean and Variance Unknown. *Journal of the American Statistical Association*, 62(318), 399–402. <https://doi.org/10.1080/01621459.1967.10482916>
- Lima, S. L., & O’Keefe, J. M. (2013). Do predators influence the behaviour of bats? *Biological Reviews*, 88(3), 626–644. <https://doi.org/10.1111/BRV.12021>
- López-Baucells, A., Rocha, R., Bobrowiec, P., Bernard, E., Palmeirim, J., & Meyer, C. (2016). *Field guide to Amazonian bats*. Instituto Nacional de Pesquisas da Amazônia. www.tropicalconservation.net
- López-Baucells, A., Torrent, L., Rocha, R., Pavan, A. C., Bobrowiec, P. E. D., & Meyer, C. F. J. (2018). Geographical variation in the high-duty cycle echolocation of the cryptic

- Common Mustached Bat *Pteronotus* cf. *Rubiginosus* (*Mormoopidae*). *Bioacoustics*, 27(4), 341–357. <https://doi.org/10.1080/09524622.2017.1357145>
- Lüdecke, D. (2022). *sjPlot: Data Visualization for Statistics in Social Science*. <https://strengjacke.github.io/sjPlot/>
- Magnusson, A., Skaug, H., Nielsen, A., Berg, C., Kristensen, K., Maechler, M., van Bentham, K., Bolker, B., Sadat, N., Lüdecke, D., Lenth, R., O'Brien, J., & Brooks, M. (2020). *Generalized linear mixed models using template model builder*. <https://orcid.org/0000-0001-9683-9262>
- Massey, F. J. (1951). The Kolmogorov-Smirnov test for goodness of fit. *Journal of the American Statistical Association*, 46(253), 68–78. <https://doi.org/10.1080/01621459.1951.10500769>
- Meyer, C. F. J., & Kalko, E. K. v. (2008). Assemblage-level responses of phyllostomid bats to tropical forest fragmentation: land-bridge islands as a model system. *Journal of Biogeography*, 35, 1711–1726.
- Meyer, C. F. J., Schwarz, C. J., & Fahr, J. (2004). Activity patterns and habitat preferences of insectivorous bats in a West African forest-savanna mosaic. *Journal of Tropical Ecology*, 20(4), 397–407. <https://doi.org/10.1017/S0266467404001373>
- Motta-Jr., J. C., & Taddei, V. A. (1992). Bats as prey of Stygian Owls in Southeastern Brazil. *Journal of Raptor Research*, 24(4), 259–260.
- Nakahara, S., Piovesan, M., Baine, Q., MacKenzie, E. C., Gallice, G., Barbosa, E. P., & Kleckner, K. (2022). A new species of *Caeruleptychia* Forster, 1964 from the Amazon basin (*Lepidoptera: Nymphalidae: Satyrinae: Satyrini*). *Tropical Lepidoptera Research*, 33(1), 23–36. <https://doi.org/10.5281/zenodo.7246643>
- Nascimento, R. F. O., Esposito, M. C., & Carvalho-Filho, F. S. (2022). Two new species and new records of *Sepsidae* from the Brazilian Amazon. *Journal of Medical Entomology*, 59(5).
- Nyffeler, M., & Knörnschild, M. (2013). Bat predation by spiders. *PLoS ONE*, 8(3), 58120. <https://doi.org/10.1371/journal.pone.0058120>
- Pavan, A. C., Bobrowiec, P. E. D., & Percequillo, A. R. (2018). Geographic variation in a South American clade of mormoopid bats, *Pteronotus* (*Phyllodia*), with description of a new species. *Journal of Mammalogy*, 99(3), 624–645. <https://doi.org/10.1093/jmammal/gyy048>
- Pavan, A. C., & Tavares, V. da C. (2020). *Pteronotus gymnonotus* (*Chiroptera: Mormoopidae*). *Mammalian Species*, 52(990), 40–48. <https://doi.org/10.1093/mspecies/seaa003>
- Pavan, A. C., & Marroig, G. (2016). Integrating multiple evidences in taxonomy: species diversity and phylogeny of Mustached Bats (*Mormoopidae: Pteronotus*). *Molecular Phylogenetics and Evolution*, 103, 184–198. <https://doi.org/10.1016/j.ympev.2016.07.011>

- Pavey, C. R., Burwell, C. J., Grunwald, J.-E., Marshall, C. J., & Neuweiler, G. (2001). Dietary benefits of twilight foraging by the insectivorous bat *Hipposideros speoris*. *Biotropica*, *33*(4), 670–681. <https://www.jstor.org/stable/3593169>
- Presley, S. J., Willig, M. R., Castro-Arellano, I., & Weaver, S. C. (2009). Effect of habitat conversion on temporal activity patterns of phyllostomid bats in lowland Amazonian rain forest. *Journal of Mammalogy*, *90*(1), 210–221. <http://jmammal.oxfordjournals.org/>
- Racey, P. A. (1982). Ecology of bat reproduction. In Thomas H. Kunz (Ed.), *Ecology of Bats* (1st ed.). Plenum Publishing Corporation.
- Reichard, J. D., Gonzalez, L. E., Casey, C. M., Allen, L. C., Hristov, N. I., & Kunz, T. H. (2009). Evening emergence behavior and seasonal dynamics in large colonies of Brazilian Free-tailed bats. *Journal of Mammalogy*, *90*(6), 1478–1486. <https://doi.org/https://doi.org/10.1644/08-MAMM-A-266R1.1>
- Rocha, R., & López-Baucells, A. (2014). Opportunistic predation by Crested Owl *Lophotrix cristata* upon Seba's Short-tailed bat *Carollia perspicillata*. *Revista Brasileira de Ornitologia*, *22*(1), 35–37. <https://doi.org/10.1007/bf03544230>
- Rocha, R., López-Baucells, A., Farneda, F. Z., Ferreira, D. F., Silva, I., Acácio, M., Palmeirim, J. M., & Meyer, C. F. J. (2020). Second-growth and small forest clearings have little effect on the temporal activity patterns of Amazonian phyllostomid bats. *Current Zoology*, *66*(2), 145–153. <https://doi.org/10.1093/cz/zoz042>
- Rocha, R., López-Baucells, A., Farneda, F. Z., Groenenberg, M., Bobrowiec, P. E. D., Cabeza, M., Palmeirim, J. M., & Meyer, C. F. J. (2017). Consequences of a large-scale fragmentation experiment for Neotropical bats: disentangling the relative importance of local and landscape-scale effects. *Landscape Ecology*, *32*(1), 31–45. <https://doi.org/10.1007/s10980-016-0425-3>
- Rodriguez-Duran, A., & Lewis, A. R. (1985). Seasonal predation by merlins on Sooty Mustached Bats in western Puerto Rico. *Biotropica*, *17*(1), 71. <https://doi.org/10.2307/2388382>
- Russ, J. M., Briffa, M., & Montgomery, W. I. (2003). Seasonal patterns in activity and habitat use by bats (*Pipistrellus* spp. and *Nyctalus leisleri*) in Northern Ireland, determined using a driven transect. *Journal of Zoology*, *259*, 289–299.
- Rydell, J. (1993). Variation in foraging activity of an aerial insectivorous bat during reproduction. *Journal of Mammalogy*, *74*(2), 503–509. <https://doi.org/10.2307/1382411>
- Schnitzler, H. U., & Kalko, E. K. V. (2001). Echolocation by insect-eating bats. *BioScience*, *51*(7), 557–569. [https://doi.org/10.1641/0006-3568\(2001\)051\[0557:EBIEB\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2001)051[0557:EBIEB]2.0.CO;2)
- Silva, I., Rocha, R., López-Baucells, A., Farneda, F. Z., & Meyer, C. F. J. (2020). Effects of forest fragmentation on the vertical stratification of neotropical bats. *Diversity*, *12*(2). <https://doi.org/10.3390/d12020067>
- Sleep, D. J. H., & Brigham, R. M. (2003). An experimental test of clutter tolerance in bats. *Journal of Mammalogy*, *84*(1), 216–224. <https://academic.oup.com/jmammal/article/84/1/216/2373169>

- Thies, W., Kalko, E. K. V., & Schnitzler, H. U. (2006). Influence of environment and resource availability on activity patterns of *Carollia castanea* (phyllostomidae) in Panama. *Journal of Mammalogy*, 87(2), 331–338. <https://doi.org/10.1644/05-MAMM-A-161R1.1>
- Thoisy, B. de, Pavan, A. C., Delaval, M., Lavergne, A., Luglia, T., Pineau, K., Ruedi, M., Rufray, V., & Catzeflis, F. (2014). Cryptic diversity in Common Mustached Bats *Pteronotus cf. parnellii* (Mormoopidae) in French Guiana and Brazilian Amapa. *Acta Chiropterologica*, 16(1), 1–13. <https://doi.org/10.3161/150811014X683228>
- Torrent, L., López-Baucells, A., Rocha, R., Bobrowiec, P. E. D., & Meyer, C. F. J. (2018). The importance of lakes for bat conservation in Amazonian rainforests: an assessment using autonomous recorders. *Remote Sensing in Ecology and Conservation*, 4(4), 339–351. <https://doi.org/10.1002/rse2.83>
- Vleut, I., Levy-Tacher, S. I., Galindo-González, J., de Boer, W. F., & Ramírez-Marcial, N. (2012). Tropical rain-forest matrix quality affects bat assemblage structure in secondary forest patches. *Journal of Mammalogy*, 93(6), 1469–1479. <https://doi.org/10.1644/12-MAMM-A-005.1>
- Voigt, C. C., Schneeberger, K., Voigt-Heucke, S. L., & Lewanzik, D. (2011). Rain increases the energy cost of bat flight. *Biology Letters*, 7(5), 793–795. <https://doi.org/10.1098/rsbl.2011.0313>
- Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. <https://ggplot2.tidyverse.org>.
- Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T., Miller, E., Bache, S., Müller, K., Ooms, J., Robinson, D., Seidel, D., Spinu, V., ... Yutani, H. (2019). Welcome to the Tidyverse. *Journal of Open Source Software*, 4(43), 1686. <https://doi.org/10.21105/joss.01686>
- Wolda, H. (1978). Seasonal fluctuations in rainfall, food and abundance of tropical insects. *The Journal of Animal Ecology*, 47(2), 369. <https://doi.org/10.2307/3789>
- Wolda, H. (1980). Seasonality of tropical insects. *The Journal of Animal Ecology*, 49(1), 277. <https://doi.org/10.2307/4289>
- Yoh, N., Clarke, J. A., López-Baucells, A., Mas, M., Bobrowiec, P. E. D., Rocha, R., & Meyer, C. F. J. (2022). Edge effects and vertical stratification of aerial insectivorous bats across the interface of primary-secondary Amazonian rainforest. *PloS One*, 17(9), e0274637. <https://doi.org/10.1371/journal.pone.0274637>

Supplementary information

Table S1. Total number of bat passes (mean \pm SD) in space and time for the two aerial-insectivorous bat species studied. Dry = dry season; Wet = wet season.

	<i>Pteronotus alitonus</i>			<i>Pteronotus rubiginosus</i>		
	Dry	Wet	Total	Dry	Wet	Total
Continuous forest	229 (9.957 \pm 4.61)	892 (18.58 \pm 3.71)	1121 (15.79 \pm 2.94)	99 (4.30 \pm 1.22)	1073 (22.35 \pm 7.48)	1172 (16.51 \pm 5.15)
Secondary forest	1044 (45.39 \pm 13.22)	759 (18.96 \pm 3.95)	1803 (28.61 \pm 5.61)	225 (9.7 \pm 2.63)	1168 (29.2 \pm 8.08)	1393 (22.11 \pm 5.32)
Fragments	220 (10.48 \pm 3.41)	416 (10.95 \pm 2.07)	636 (10.78 \pm 1.79)	138 (6.57 \pm 1.65)	417 (10.97 \pm 2.36)	636 (10.78 \pm 1.79)
Total	1493 (22.28 \pm 5.27)	2067 (16.40 \pm 2.00)	3560 (18.46 \pm 2.24)	462 (6.89 \pm 1.14)	2658 (21.10 \pm 3.92)	3120 (16.16 \pm 2.63)

Table S2. Summary of Generalized Linear Mixed Models examining bat activity of each species in terms of season, habitat, and their interaction. Reference category used in the model is dry season and continuous primary forest. This is chosen in R by alphabetical ordering. Sampling night and camp sites were specified as random effects, and data from 138 nights were used for this analysis. Bold values indicate $P < 0.05$.

Species	Fragment			Secondary			Wet season			Fragment ~ wet season			Secondary ~ wet season		
	Estimate	z	P	Estimate	z	P	Estimate	z	P	Estimate	z	P	Estimate	z	P
<i>Pteronotus alitonus</i>	-0.165	-0.34	0.74	0.832	1.98	0.048	1.689	4.95	7.26e⁻⁰⁷	-0.7699	-1.58	0.114	-1.48	-3.22	0.00128
<i>Pteronotus rubiginosus</i>	0.417	0.98	0.327	0.682	1.71	0.0868	1.938	6.11	9.99e⁻¹⁰	-0.688	-1.43	0.151	-0.455	-0.97	0.329

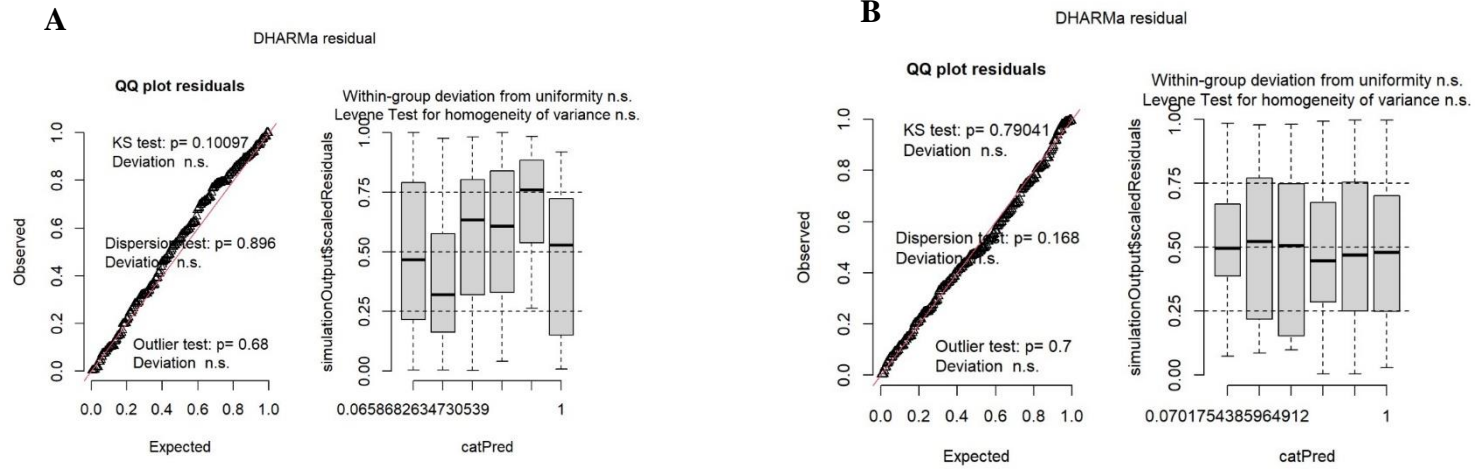
Table S3. Summary of lsmeans contrasts in Generalized Linear Mixed Models comparing habitats, using Tukey method. Bold values indicate $P < 0.05$.

Species	Continuous ~ fragment			Continuous ~ secondary			Fragment ~ secondary		
	Estimate	t-ratio	P	Estimate	t-ratio	P	Estimate	t-ratio	P
<i>Pteronotus alitonus</i>	0.5498	1.357	0.3657	-0.0899	-0.264	0.9622	-0.6397	-2.423	0.0430
<i>Pteronotus rubiginosus</i>	-0.0735	-0.254	0.9652	-0.4541	-1.737	0.1943	-0.3806	-1.472	0.3068

Table S4. Results of Kolmogorov-Smirnov 2-sample tests comparing the hourly bat activity between wet and dry season and between habitat types in Amazonian fragmented landscape. Bold values indicate $P \leq 0.05$.

Species	Continuous ~ fragment		Continuous ~ secondary		Secondary ~ fragment		Wet ~ dry	
	D	P-value	D	P-value	D	P-value	D	P-value
<i>Pteronotus alitonus</i>	0.32246	0.1738	0.66667	4.662e⁻⁰⁵	0.78804	9.245e⁻⁰⁷	0.21905	0.362
<i>Pteronotus rubiginosus</i>	0.40	0.08152	0.26364	0.4603	0.16364	0.942	0.68025	1.25e⁻⁰⁶

Figure S1. Result of DHARMA test of GLMM models for A) *P. alitonus* and B) *P. rubiginosus*.





Norges miljø- og biovitenskapelige universitet
Noregs miljø- og biovitenskapelige universitet
Norwegian University of Life Sciences

Postboks 5003
NO-1432 Ås
Norway