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

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS
(ZOOLOGIA)

EFEITO DE VARIÁVEIS AMBIENTAIS E PERTURBAÇÕES ANTRÓPICAS SOBRE O COMPORTAMENTO ANIMAL

Calebe Pereira Mendes

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 Ciências Biológicas (Zoologia).

Dezembro - 2018

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Rio Claro, SP

2018

M538e

Mendes, Calebe Pereira
Efeito de variáveis ambientais e perturbações antrópicas sobre o comportamento animal / Calebe Pereira Mendes. -- Rio Claro, 2018
96 p. : tabs., fotos, mapas

Tese (doutorado) - Universidade Estadual Paulista (Unesp), Instituto de Biociências, Rio Claro
Orientador: Mauro Galetti

1. Zoologia. 2. Comportamento animal. 3. Ecologia. 4. Conservação. 5. Mamíferos. I. Título.

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TÍTULO DA TESE: EFEITOS DE VARIÁVEIS AMBIENTAIS E PERTURBAÇÕES ANTRÓPICAS SOBRE O COMPORTAMENTO ANIMAL

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Dedico este trabalho a Deus e à minha família.

AGRADECIMENTOS

Agradeço primeiramente a Deus, o único, pelo dom da vida, pela saúde e pela oportunidade de ver e testemunhar Sua glória. A Jesus, o Cristo, pela salvação e reconciliação através de Seu santo sangue. E ao Santo Espirito de Deus, por me guiar nas incontáveis noites em que minha mente debatia entre os métodos científicos e teológicos, e por me ensinar como ambos os métodos podem ser conciliados de forma lógica e racional, através de métodos filosóficos aceitos pela comunidade acadêmica. E de fato, como é bela e completa a cosmovisão resultante!

Agradeço a meus pais, Marcos e Rute, e a minha preciosa irmã Débora pelo constante incentivo em buscar o conhecimento. Mesmo estando longe, sempre pude contar com eles. Agradeço a minha família, em Cascavel-PR, Fortaleza-CE, e várias outras localidades do Brasil e do mundo. É uma família geograficamente dispersa, mas muito próxima em união. Agradeço também a Ashwin Naidu, Abhipsha Chatterjee, Alex Ochoa e Betzy por me receberem e tratarem tão bem durante o ano em que morei no Arizona, E.U.A., vocês se tornaram parte de minha família.

Agradeço aos muitos amigos que fazem ou fizeram parte do LABIC e do LEEC, e a muitos outros espalhados por toda a UNESP. Aos amigos e professores Mauro Galetti, Miltinho e John Koprowski que foram particularmente importantes em minha formação. E ao Sérgio, o Super Técnico de Campo, capaz de trabalhar, cozinhar, organizar e ainda fazer piada, tudo ao mesmo tempo!

Agradeço aos membros da República Sedex 10, sem dúvida a república mais pacata e tranquila que já conheci. Se não fosse pelo café preparado de manhã por vocês, talvez esta tese não tivesse sido concluída. Agradeço a comunidade da Igreja Batista do Calvário que me ensinaram muito e me arrastaram em diversas aventuras pelo estado de São Paulo. Sou testemunha do mover de Deus sobre a vida de vocês, e de sua boa conduta como cristãos.

Agradeço também ao Conselho Nacional de Desenvolvimento Científico e Tecnológico - CAPES (processo 168080/2014-1), e a FAPESP (processos 2014/01986-0, 2014/09300-0, 2014/23095-0, 2015/22844-1 e 2015/18381-6). O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001.

“Quanto mais eu estudo a natureza, mais ainda

eu fico maravilhado com a obra do Criador...

Ciência aproxima o homem de Deus”

Louis Pasteur

RESUMO

O comportamento de uma determinada espécies define a forma e intensidade das interações desta para com o ambiente, com muitas espécies ajustando suas estratégias comportamentais de acordo com o contexto físico e biológico ao seu redor. No entanto, o processo de tomada de decisões, intrínseco a qualquer sistema comportamental, requer que o organismo possua a capacidade de obter, interpretar e processar informações úteis. Devido à grande magnitude e novidade evolutiva dos distúrbios antrópicos, estes são capazes de interferir no processo cognitivo das espécies, interferindo na percepção e avaliação das informações necessárias para a tomada de decisões eficientes, e dessa forma, gerando comportamentos não adaptativos, aumento de mortalidade e interferências nas interações ecológicas das espécies. Dessa forma, o objetivo deste trabalho é avaliar a ocorrência de plasticidade comportamental, em diversas espécies modelo, em resposta a fatores ambientais e atividades antrópicas. Foram realizados dois capítulos experimentais e um capitulo de revisão. O primeiro capitulo foi desenvolvido no Arizona, E.U.A., e avaliou o volume de alimentos estocados pelo esquilo-vermelho-de-Graham *Tamiasciurus fremonti grahamensis* em resposta a variáveis climáticas, de relevo e vegetação. Foi observado que apenas as fêmeas responderam a variações no relevo e vegetação, mas não a variáveis climáticas, enquanto os machos não responderam a nenhuma variável testada. O segundo capítulo, desenvolvido em São Paulo, Brasil, avaliou o efeito de perturbações antrópicas variadas sobre a atividade circadiana de 17 espécies de mamíferos florestais. Foi observado que 7 espécies responderam a perturbação antrópica, se tornando em média 20% mais noturnas em áreas perturbadas. Tanto espécies diurnas quanto noturnas se tornaram mais noturnas em resposta a perturbações, sendo que espécies caçadas e perseguidas se tornaram mais noturnas do que espécies não caçadas. O terceiro capitulo foi planejado como uma comparação entre o esquilo-vermelho-de-Graham e um esquilo brasileiro, o *Guerlinguetus brasiliensis*, no entanto, devido à escassez de informações da história natural da segunda espécie, decidiu-se por mapear o atual estado da informação disponível sobre a ecologia de esquilos neotropicais. Assim, foram encontradas 155 publicações, de 15 países, com dados de ecologia para 20 espécies de esquilos que interagiram com 351 outras espécies. Foi observado que esquilos neotropicais tendem a apresentar maiores densidades populacionais em florestas fragmentadas. Também identificamos diversas partes da história natural do táxon que são virtualmente desconhecidas, como por exemplo, a composição da dieta de esquilos dos gêneros *Sciurillus* e *Microsciurus*.

Palavras chave: Zoologia, Comportamento animal, Ecologia, Conservação, Mamíferos.

ABSTRACT

The behavior of a given species defines the style and intensity of its interactions with the environment, with several species adjusting their behavioral strategies in response to the physical and biological circumstances. However, the decision-making process, intrinsic of any behavioral system, requires the organism to is able of obtain, interpret and process useful information. Due to the large scale and evolutive novelty of the anthropic disturbances, they are able to interfere in the cognitive processes of the species, disrupting the perception and evaluation of the information needed to efficient decision-making, and therefore, cause non-adaptative behaviors, increase in mortality and effects on species ecological interactions. This way, the objective of this study is to evaluate the occurrence of behavioral plasticity, in several model species, in response to environmental factors and anthropic activities. Two experimental chapters and one literature revision was performed. The first chapter was developed in Arizona, U.S.A., and evaluate the amount of food cached by the Mt. Graham red squirrel *Tamiasciurus fremonti grahamensis* in response to variables of climate, relief and vegetation. It was found that only the females did respond to relief and variations in relief and vegetation, but not climate, while males did not respond to any examined variable. The second chapter, developed in São Paulo, Brazil, evaluate the effect of general anthropic disturbances on dial activity of 17 forest dwelling mammal species. We detected 7 species becoming more nocturnal in response to anthropic disturbance, with a mean nocturnality increase of 20% in disturbed areas. Both diurnal and nocturnal species become more nocturnal, whereas the poached and persecuted species become more nocturnal than non-poached species. The third chapter was designed as a comparison between the Mt. Graham red squirrel and a Brazilian squirrel, the *Guerlinguetus brasiliensis,* however, due to the scarcity of natural history information, we decided to map the actual state of knowledge availability about neotropical squirrels. This way, we found 155 publications, from 15 countries, with ecological data for 20 squirrel species, which interacted with 352 other species. We recorded an increase of squirrel population densities in fragmented forests. We also identified several areas of the taxa natural history which are virtually unknown, such the composition of diet of the *Sciurillus* and *Microsciurus* genera.

Key words: Zoology, Animal behavior, Ecology, Conservation, Mammals

SUMÁRIO

1. INTRODUÇÃO GERAL

Animais sempre possuem decisões a tomar. Decisões sobre quando estar ativo e quando se recolher ao abrigo (KRONFELD-SCHOR; DAYAN, 2003), sobre onde procurar e quais alimentos consumir (BANACK, 1998), sobre o quanto é aceitável se expor a predadores (VERDOLIN, 2006). A todo momento, existem decisões a serem tomadas, até mesmo sobre se o indivíduo deve continuar uma determinada atividade ou parar e engajar em uma nova atividade. As consequências dessas decisões, por mais simples que sejam, podem se refletir na eficiência de forrageamento, sobrevivência (VERDOLIN, 2006) e no *fitness* dos indivíduos (EGGERS et al., 2006; WEISER; POWELL, 2010). Por este motivo, através da seleção natural, várias espécies se tornaram surpreendentemente eficientes em tomar decisões. Por exemplo, o melro-preto *Turdus merula*, na Grã-Bretanha, é capaz de estimar e comparar o risco de mortalidade por predação e por hipotermia, de modo que durante o inverno, quando hipotermia é a principal ameaça, a ave consome toda sua necessidade energética diária tão cedo quanto possível, mesmo quando o sobrepeso do alimento reduz a agilidade de voo, tornando a ave mais susceptível a predadores. Por outro lado, nas demais estações do ano, quando a demanda energética é reduzida e o risco de hipotermia é baixo, predação se torna a principal ameaça, e a ave se alimenta em duas seções de forrageio ao longo do dia, mantendo sempre um peso reduzido, maior agilidade de voo e menor susceptibilidade a predação (MACLEOD et al., 2005).

No entanto, as espécies tendem a ser eficientes apenas para tomar decisões para as quais estão preparadas através do processo evolutivo, uma vês que possuem o aparato cognitivo necessários para realizar tais decisões, mas costumam ser incapazes ou pouco eficientes em tomar decisões acerca de problemas para qual não foram expostos em seu passado evolutivo (ROBERTSON; REHAGE; SIH, 2013). Por exemplo, girinos de rã-ágil *Rana dalmatina,* engajam em comportamentos defensivos ao detectar pistas químicas da presença de predadores nativos, mas não em resposta a pistas químicas da presença de predadores alopáticos (HETTYEY et al., 2016). Isso acontece porque a cognição (i.e. os processos neurais envolvidos na aquisição, armazenamento e uso de informação) requer complexas estruturas neurais, sendo fisiologicamente cara em termos de energia necessária para manutenção desses estruturas (ISLER; VAN SCHAIK, 2006). Desse modo, é necessário que haja um retorno em fitness para que tais funções evoluam ou sejam mantidas via seleção natural (DUKAS, 2004).

O mecanismo pela qual a capacidade cognitiva afeta o fitness das espécies, é através de seu efeito sobre o repertório comportamental destas (DUKAS, 2004). Visto que o comportamento das espécies define como estas interagem com o meio ambiente, possuir um repertório comportamental plástico e capaz de lidar com situações adversas facilita a sobrevivência da espécie (SOL; LAPIEDRA; GONZÁLEZ-LAGOS, 2013). Por exemplo, a paca *Cuniculus paca* e o tatus *Dasypus* spp. ajustam sua atividade em resposta ao ciclo lunar, reduzindo o forrageamento e evitando as noites de lua cheia, período associado a um maior risco de predação (Prugh & Golden 2014, mas ver Pratas-Santiago et al. 2017). De modo semelhante, o Chapim-azul *Parus caeruleus*, ajusta o período reprodutivo de modo a coincidir com o pico de abundância de alimento, que é estimado a partir da abundância de alimento ao longo do ano anterior (GRIECO; VAN NOORDWIJK; VISSER, 2002).

A plasticidade comportamental também pode ser extremamente importante ao permitir que espécies permaneçam, e por vezes até prosperem, em ambientes alterados por atividade antrópica (SOL; LAPIEDRA; GONZÁLEZ-LAGOS, 2013). Como exemplo, a escrevedeira-dos-caniços *Emberiza schoeniclus* é capaz de alterar a frequência do canto, tornando-o mais agudo e reconhecível em meio a ruído de fontes antrópica, tornando a frequência mais baixa e adequada a ambientes naturais quando o ruído é removido (GROSS; PASINELLI; KUNC, 2010). Outras espécies, como a gaivota-hiperbórea *Larus hyperboreus* obtém incrementos em fitness ao incluir alimentos de fontes antrópicas (i.e. lixo) em suas dietas (WEISER; POWELL, 2010). No entanto, apesar da importância da plasticidade comportamental como um mecanismo capaz de aliviar as consequências das atividades antrópicas sobre a fauna, em muitos casos as pressões antrópicas não podem ser compensadas por alterações comportamentais, excedendo os limites que a espécie é capaz de tolerar (WONG; CANDOLIN, 2015) ou confundindo os próprios meios pela qual essa obtém as informações necessárias para tomar de decisões (ROBERTSON; REHAGE; SIH, 2013).

Devido ao fato de, em termos evolutivos, diversas atividades antrópicas serem novidades, estas são capazes de confundir ou interferir na capacidade de decisão dos animais, gerando decisões não adaptativas e por vezes comportamentos extremamente contraprodutivos (ROBERTSON; REHAGE; SIH, 2013; SCHLAEPFER; RUNGE; SHERMAN, 2002). Por exemplo, filhotes recém eclodidos de diversas espécies de tartarugas marinhas utilizam fototaxia para encontrar o mar, mas acabam confundidos pela presença de iluminação artificial nas praias e morrem por desidratação ou para predadores após vagarem em direção ao continente (WITHERINGTON; MARTIN, 1996). O consumo acidental de plástico, um material sintético, confundido por presas naturais, como águas-vivas, também tem levado a mortalidade em inúmeras espécies marítimas (WILCOX; VAN SEBILLE; HARDESTY, 2015). Distúrbios antrópicos de grandes escalas espaciais, como o aquecimento global (IPCC, 2014) tem degradado a histórica sincronia entre o pico de disponibilidade de alimentos e a data de migração e reprodução de aves (BOTH et al., 2006), e mamíferos (POST; FORCHHAMMER, 2008), gerando reduções nas populações. E a poluição luminosa, altera o padrão de atividade circadiana e a percepção de risco por várias espécies (GASTON et al., 2013).

Dessa forma, considerando a importância da cognição e do comportamento sobre a ecologia e resiliência das espécies animais, assim como as graves consequências das interferências nessas funções, o objetivo geral desta tese é: Avaliar a ocorrência de plasticidade comportamental, em diversas espécies modelo, em resposta a fatores ambientais e atividades antrópicas. Para isso, foram desenvolvidos dois capítulos experimentais e uma revisão.

O primeiro capitulo, denominado “Does caching strategy vary with microclimate in endangered Mt. Graham red squirrels?”, foi desenvolvido entre Abril de 2015 e Abril de 2016, no Arizona, Estados Unidos da América, em colaboração com o Conservation Research Laboratory da University of Arizona. O capítulo tem como objetivo avaliar a ocorrência de plasticidade no comportamento de estoque de alimento do esquilo-vermelho-de-Graham *Tamiasciurus fremonti grahamensis* (Figura 1), em resposta a variáveis climáticas. Diversas variáveis de clima, relevo e vegetação foram medidas, e o volume de alimento estocado pelos esquilos foi estimado. Foi observado plasticidade no volume de alimento estocado por fêmeas, mas não por machos. No entanto, apesar da plasticidade comportamental observado nas fêmeas, ela ocorreu apenas em resposta a variáveis de relevo e vegetação, mas não de clima. Um resultado preocupante visto que a espécie já está sob stress climático, e há previsões para futuro aquecimento da região devido a mudanças climáticas (IPCC, 2014). O manuscrito deste capítulo foi submetido ao periódico Journal of Mammalogy.

Figura 1: Esquilo-vermelho-de-Graham, *Tamiasciurus fremonti grahamensis*, uma subespécie do Esquilo-vermelho-norte-americano endêmico das montanhas Pinalenõ, no Arizona, E.U.A.. A única população desta subespécie compõe o limite sul da distribuição da espécie, e historicamente gira em torno dos 300 indivíduos, embora um incêndio florestal em 2017 os reduziu a cerca de 35 indivíduos. Foto tirada pelo autor.

O segundo capítulo, denominado “Landscape of human fear in Neotropical rainforest-dwelling mammals”, foi desenvolvido no estado de São Paulo e avalia o efeito de perturbações antrópicas diversas nos padrões de atividade circadiana de 17 espécies de mamíferos. Para isso, utilizou-se um extenso banco de dados de câmeras-trap, coletado de 2012 a 2018, ao longo de todo o estado de São Paulo e coletado por diversos membros e colaboradores do LABIC. Mapas de emissividade de luz durante a noite, obtidas por satélite (EOG, 2018), foram utilizadas para gerar um índice de perturbação humana. Através desses dados, observou-se que 7 das 18 espécies de mamíferos florestais testadas se tornaram mais noturnas em áreas perturbadas (Figura 2). Tanto espécies noturnas quanto diurnas ficaram mais noturnas em resposta a perturbação humana, e nenhuma espécie foi observada se tornando mais diurna. A caça e poluição luminosa parecem ser os principais mecanismos afetando os resultados obtidos. O manuscrito do segundo capítulo foi submetido a Biological Conservation.

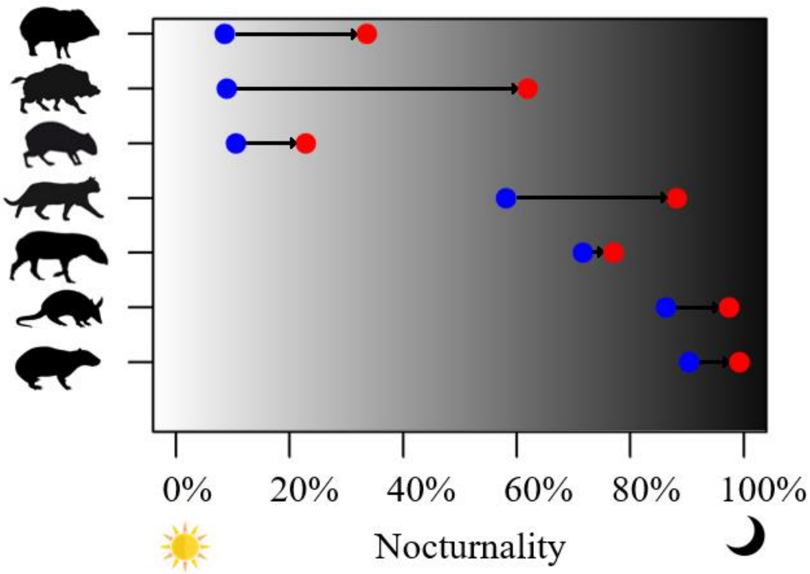

Figura 2: Principal resultado do segundo capítulo, mostrando diferenças no nível de noturnalidade de várias espécies entre áreas preservadas e perturbadas, em azul e vermelho respectivamente.

O terceiro capítulo, no entanto, possui uma temática diferente aos demais, pois foi desenvolvido em resposta a escassez de dados de história natural que impediram o desenvolvimento de um capítulo comparativo entre o comportamento do esquilo-vermelho-de-Graham (endêmico do Arizona, E.U.A.) e do caxinguelê *Guerlinguetus brasiliensis*, que é endêmico do Brasil. Constatada a escassez de informação básicas sobre a história natural de Sciurídeos neotropicais, decidiu-se por realizar uma revisão da literatura, denominada “NEOSQUIRREL: a dataset of the ecological knowledge on Neotropical squirrels”. Esta revisão, não apenas aglomera a pouca informação disponível em um único lugar, facilitando trabalhos futuros, mas também identifica as áreas do conhecimento já estudadas e as que carecem de estudos, apontando as direções prioritárias para estudos futuros. A revisão, submetida para a Mammal Review, sumariza informações provenientes de 155 artigos, publicados entre 1947 e 2017, registrando 649 interações ecológicas entre 20 espécies de esquilos e 351 outras espécies, em 15 países neotropicais (Figura 3). Todos os dados levantados são disponibilizados nos materiais suplementares, na forma de três arquivos .csv e um arquivo de metadata.

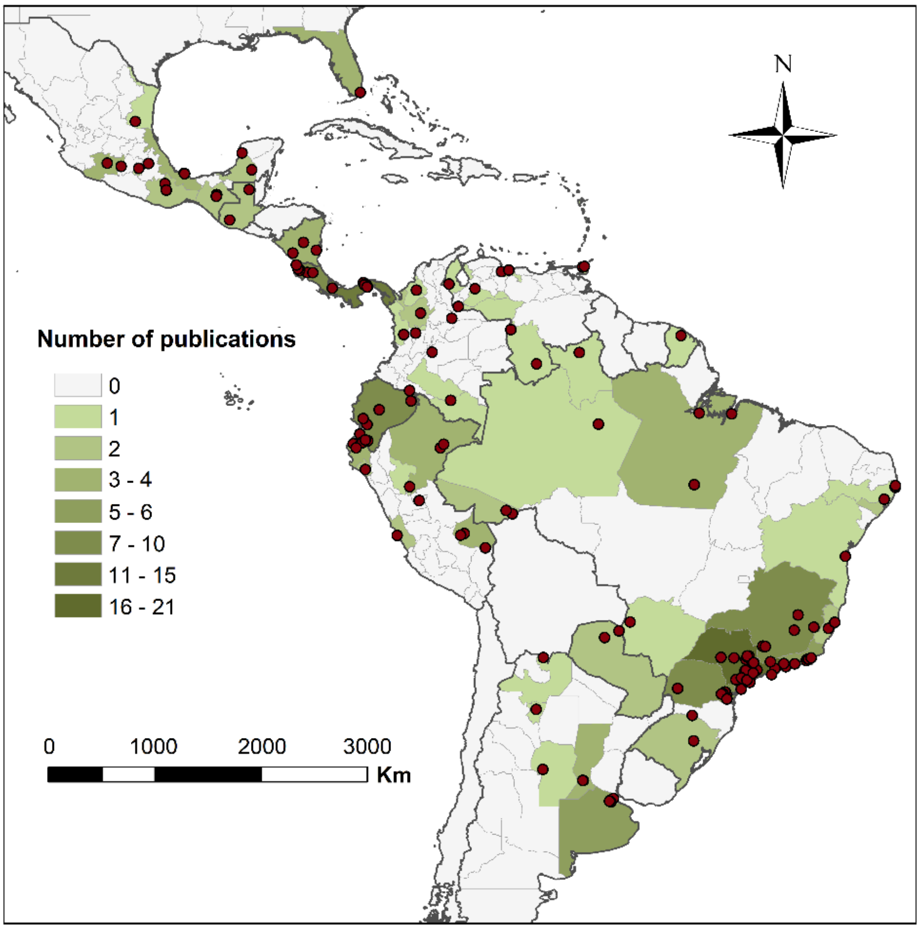

Figura 3: Mapa da localização de todas as interações ecológicas encontradas na literatura envolvendo esquilos neotropicais. É possível ver a concentração de estudos em regiões como São Paulo, enquanto outras regiões com alta biodiversidade, como a Amazônia, possuem poucos dados disponíveis.

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2. DOES CACHING STRATEGY VARY WITH MICROCLIMATE IN ENDANGERED MT. GRAHAM RED SQUIRRELS?

Journal format: Journal of Mammalogy

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2.1 Abstract

Food hoarding is a common behavior used by a variety of animals to cope with periods of low food availability. At the retreating edge of species distribution, the stressful environment and unfavorable climate conditions may impose severe costs on hoarding behavior. Since food hoarding has an evolutionary basis and relict populations are hotspots for evolution and adaptations, we decided to evaluate the occurrence of behavioral plasticity in the amount of food cached by the endangered Mount Graham red squirrel (*Tamiasciurus fremonti grahamensis*). We tested the variation in cache size in response to climate, soil relief, vegetation, food availability and squirrel sex. The number of pits excavated by squirrels to cache cones was affected by mountain slope aspect and density of trees. More pits were excavated in the northest facing slopes. The density of trees negatively affects the cache volume on southwest slopes, but not on northeast slopes. The sex of the resident squirrel also affects the number of pits in the squirrel midden, with males excavating 47% more pits than females. Males and females also presented different responses to the mountain slope aspect, with females excavating more pits on northeastern slopes than on southwestern slopes, whereas male caching did not vary with the slope aspect. Finally, the squirrel's caching behavior did not vary in response to midden microclimate variation, an important finding that suggests a challenge given predicted temperature increases due to climate change.

KEYWORDS: behavioral plasticity, cache size, climate, relict population, *Tamiasciurus fremonti grahamensis*

2.2 Introduction

In response to both predictable and unpredictable variation in food availability, several taxa, including carnivores, rodents, corvids and birds of prey make use of caching behavior to guarantee the food supply during periods of scarcity (Sutton et al. 2016). Caching behavior dampens the variations in food availability, "transferring" part of the resources from the period of abundance to be used during scarcity. The caching behavior also varies considerably in the type of food stored, amount, duration, substrate in which the food is cached, number and spatial distribution of caches (Hadj-Chikh et al. 1996; Sutton et al. 2016).

Squirrels have long served as model organisms in the study of caching behavior, and helped us to understand ecological decisions such as the criteria used by individuals to decide whether to consume or cache a food item (Preston and Jacobs 2009) and how far to move a food item to cache (Tamura et al. 1999). In general, large food items appear to be a preferable option to cache (Preston and Jacobs 2009), as well as items that are less susceptible to spoilage (Hadj-Chikh et al. 1996). More valuable food items are also dispersed farther (Preston and Jacobs 2009). Feeding efficiency is an important factor for rodents, shaping not only their behavior, but also morphology and evolution (Smith 1970; Cox et al. 2012).

The red squirrel (*Tamiasciurus hudsonicus*) uses both larder and scatter-hoarding strategies dependent on cache site conditions (Hurley and Robertson 1990). The cache size is also adjusted to match future energy demand, and differs between sexes because of the energetic investment in mating strategies (Archibald et al. 2013). For red squirrels, cache behavior is important for winter survival, but is also energetically costly and time consuming (Archibald et al. 2013), and exposes the animal to predation risk (Schauffert et al. 2002). The cache size must guarantee winter food security, but should not be large to the point where food is lost to spoilage (Gendron and Reichman 1995). This is also affected by the climate, since the cache depends on cold and moist condition for preservation (Finley Jr 1969; Waite and Strickland 2006).

Since behavioral plasticity has a genetic basis and impacts individual fitness, it also evolves by means of natural selection (Mery and Burns 2010) and may be important for survival of relict populations at the edges of the species distribution (Hampe and Jump 2011). Since relict populations were gradually exposed to what is now a challenging and changing environment and were selected from what once was the core population, they are evolutionary hotspots (Hampe and Jump 2011; Lesica and Allendorf 2015).

Considering that the fine-tuning of cache volume reduces energy waste (Gendron and Reichman 1995), that climate change can interfere in the cost-benefit of food caching behavior (Sutton et al. 2016), and that cache efficiency can limit the distribution of species (Waite and Strickland 2006), our hypothesis is that a larder-hoarding rodent at the distributional limit where the limiting factor is climate, should demonstrate behavioral plasticity in cache size in response to local climatic variation.

The main objective of this study was to evaluate the occurrence of plasticity in the caching behavior of the Mount Graham red squirrel (*Tamiasciurus fremonti grahamensis*), a larder-hoarding rodent at the southern limit of its geographical distribution, in response to climate. Secondarily, we seek to explain the observed behavioral responses. We expect to observe: 1) An effect from climatic variables on cache size, since the cache depends on the correct climate condition to be effective (Finley Jr 1969; Waite and Strickland 2006); 2) An effect of altitude, slope declivity and aspect on cache size, since these relief variables affect midden microclimate (Körner 2007), which in turn affect the cache. 3) A positive effect of food abundance on cache size, because with higher food availability there is a reduction in the cost to find and transport the cones to the cache, therefore, an increase in the overall cost-benefit of the behavior.

2.3 Methods

Study area

The study was in the highest portion of Mount Graham, in the Pinalenõ Mountains, Arizona, USA, between 2770 and 3270 m of altitude, an area with rugged relief, which promotes considerable microclimatic variation in a small geographic area, as part of the Madrean Archipelago. The Madrean Archipelago is a mid-latitude sky island complex, where species of temperate zone are surrounded by a low altitude inhospitable matrix. The montane islands have high biodiversity, each one with its own set of relict populations that have persisted since the end of the last glaciation 10 to 15 k.y.a (Warshall 1995; Koprowski et al.

2013). In fact, with the increase of ~5 degrees in mean annual temperature since the last Glacial maximum, 19 to 23 k.y.a. (Correa-Metrio et al. 2013) and the consequent poleward displacement of the distribution of the species, at least 80 populations inhabiting the Madrean Aquipelago are the actual edges of the respective species (Warshall 1995). In the present study, we chose one of these populations, the squirrel (*Tamiasciurus fremonti grahamensis*), as a model organism.

The Mt. Graham red squirrel is a subspecies of North American red squirrel complex (*Tamiasciurus hudsonicus, T. fremonti*) (Hope et al. 2016) isolated from the species core distribution for at least 11 million years (Granillo and Froehlich 2009). The only population of this subspecies, with around 274 individuals during the study period (Goldstein et al. 2017), is severely threatened by the low habitat quality, insect outbreaks, exotic species and human activities (Granillo and Froehlich 2009). Forest fires are also a threat, and in 2017 a large forest fire reduced the population to about 35 individuals (John Koprowski, personal communication, 2018). In addition to all of these threats, the population is also directly threatened by climate change, as they rely on cold and humid climate for the preservation of cones collected during the fall and consumed during the winter (Finley 1969; Koprowski et al. 2013).

Sampling

To evaluate the effect of climate on squirrel caching behavior, we selected 40 occupied middens distributed along different altitudes, relief, and mountain aspect. We visited each midden 6 times between the fall and winter of 2015 to measure the weather, relief, plant structure and estimate the volume of cones stored by the resident squirrels (response variable). During the visits, we also record the sex of the resident squirrels.

To estimate the volume of cones cached by each squirrel, we took advantage of the fact that the animals do not cache the cones loose in the scale pile but deposit them into holes, here called “pits”, excavated in the scale pile or in the soil (Finley 1969). Thus, we used the number of pits, counted in the last visit before the arrival of the snow, as proxy of the volume of cones cached in each midden. This method follows Gurnell (1984), but in the present study we did not excavate the pits to count the number of cones inside the pits to avoid disturbance of this endangered squirrel's cache. These visits occurred between September and early October. We also used the mean and maximum number of pits counted in all visits to each midden as a possible response variable, but since the results did not change in the results we decided to keep the most parsimonious option (i.e. number of pits at the last visit before the snow). We also recorded the presence of dead logs near middens, because they are occasionally used to store cones (Finley 1969), but since these also had no detectable effects on the results, we excluded the variable from the analysis.

At each visit, we use a handheld weather meter (Kestrel 3000) to measure the air temperature (°C) and relative humidity (%), and a digital soil thermometer (HANNA) to record soil temperature, the temperature inside of the scale pile, and inside of the pits (average value obtained from 3 pits for each midden/visit). Since the middens were not visited simultaneously and to avoid the interference of the daily weather variation and the weather variation along different days, we used data recorded by a meteorological station located at the top of the mountain (MGIO-Mt Graham Summit KAZSAFFO4) and by a data-logger (HOBO U23 Pro v2) buried in the ground as a baseline to allow comparison between middens. The weather station was used as baseline for the air temperature and humidity whereas the data-logger was used for the temperatures of soil, pits and scale piles. By subtracting the baseline measured values from the variables recorded in the middens, the resultant values represent the difference between the midden and the baseline, which is fixed in space, and makes the resultant value comparable.

Autumn is a critical period for the storage of cones because it is warmer and drier compared to the microclimate under the snow over winter, thus we calculated an additional variable called "pit cooling effect". This variable was calculated by subtracting the air temperature from the pit temperature, and represents the cooling effect, mainly by evaporative heat loss, inside the pits.

Longer winters require individuals to depend on the cone reserves for a longer period of time (Sutton et al. 2016); memory and learning are important factors that modulate behavioral plasticity (Mery and Burns 2010). Thus, we decided to test the effect of snow duration on cache size. To do so, we capitalized on the fact that in 2015 all middens were covered by snow on December 12, and so we used the percentage of soil still covered by snow within a 5-m radius of each midden between 10 and 12 of March of 2016 as a proxy for the speed of snowmelt at each midden. We decided to use the data from the 2015-2016 winter with the premise that snow melting speed does not vary randomly along the mountain, but follow patterns defined by relief and microclimatic factors (Abudu et al. 2016). Thus, a total of seven microclimatic variables were tested in the present work (Table 1).

To record the topographic relief variation, we used a GPS unit to record the altitude of each midden, a clinometer to record the declivity and a compass to record the aspect. For vegetation variables, we used a densitometer to record the forest cover for each midden and recorded the arboreal community with diameter at breast height (DBH) ≥ 30 cm by using 4 transects of 30x5 meters, starting from the midden in the four cardinal directions. The density of live conifers in the transects was used as a proxy for the availability of cones near each midden. This proxy is based on the premise that the mean cone production of the conifer species did not varied across the study area, which was tested and confirmed by the Moran's I test for spatial autocorrelation, using data from counted cones for 73 trees of the 3 species that produced cone crops in 2015 (Engelmann spruce: n= 33, p-value= 0.597; Douglas fir n=35, p-value= 0.59; Ponderosa pine: n= 5, p-value= 0.269). From the tree transect data, we also calculated the trees density within the midden vicinity, density of live trees, density of dead trees, density of Englemann spruce, Douglas fir and Ponderosa pine, which are the main tree species that produced cones.

We also estimate the productivity of mushrooms, an alternative food resource used and cached by squirrels (Granillo and Froehlich 2009; Hendricks and Hendricks 2015), and their availability may interfere with the behavior of the animals. Mushroom productivity, recorded in grams, was estimated through 4 transects of 10x1 m, starting from the midden in the four cardinal directions. All mushrooms of all species known to be consumed by the squirrels (Hendricks and Hendricks 2015; Koprowski et al. 2015) were collected and weighed.

To analyze the data, we verified the normality of the variables, using the Shapiro-Wilk test, and normalized the variables when needed. The response variable, number of pits, was log transformed to fulfill normality requirements, and the slope aspect, a circular variable, was tested for uniformity using a Kuiper uniformity test (Pewsey et al. 2013). To allow the slope aspect variable to be analyzed together with the other linear variables, we linearized the variable by dividing along the northeast-southwest axis (bearings 45° and 225°, where north is 0°\360°), and transferring the records in the northwest semicircle to the southwest semicircle. This way, the resultant semicircle can be treated as a linear variable, ranging from bearing 45° to 225°, where 45° represents a place where the soil slope is facing the northeast, whereas 225° represents a place where the soil slope is facing towards southwest. We decided to use a northeast-southwest axis due to visual inspection of the raw data, which pointed to maximal variation.

Due to the large number of explanatory variables, we used Principal Component Analysis (PCA) to identify and delete variables with high similarity, and retained only the most dissimilar variables (Jolliffe 2002). With the PCA results, we selected the dissimilar variables based on the dissimilarity values within the first two dimensions and tested whether selected variables were correlated with other similar variables. Then, we deleted all the correlated variables with smaller dissimilarity values and performed the PCA again, and repeated the process until only the most dissimilar explanatory variables without minimal correlation remain. After use of PCA to select these principal explanatory variables, we used a multiple competing hypothesis approach (Burnham and Anderson 2010) to evaluate the effect of the explanatory variables on the number of pits excavated by the squirrels.

For the competing hypothesis approach, we created a set of Generalized Linear Models (GLM), in which each model uses a different combination of one or two explanatory variables to explain the response variable (number of pits). We created one model for each one of the explanatory variables and for all the possible combinations of these variables, up to two variables per model. We did not test models with more than two variables due to the modest sample size of the dataset. A null model was created by using aleatory generated numbers as an explanatory variable, and the Akaike Information Criterion corrected to small sample size was used to compare the models (Burnham and Anderson 2010). For each model, we calculated the wAICc, a parameter of the relative likelihood, and the ΔAICc, a parameter of relative difference between models. Models with $\Delta AICc < 2$ were considered equally plausible. To reinforce the analysis, we calculated frequency πi, which is a bootstrap method to calculate the frequency in which a model *i* is selected as the best model in the set in 10000 random resamples of the dataset (Burnham and Anderson 2010).

Finally, due to differences in reproductive strategy between males and females (Archibald et al. 2013), we tested the effect of sex of the resident squirrel on the cache size using Student's t-test and linear model regression. All statistics were performed using the R software (R Development Core Team 2016) software, using the "circular", "bbmle", "FactoMineR", "Akima", "plotrix" and "ape" packages.

2.4 Results

From the 40 selected middens, nine were excluded due to migration/death of the resident squirrel during the study. From the 31 remaining, 15 were occupied by males, 11 by females and 5 animals of unknown sex. The 31 remaining middens were evenly distributed in all aspects of the mountain slopes (Kuiper's Test of Uniformity, $p > 0.15$).

The PCA analysis reduced the 19 initial explanatory variables (excluding the resident squirrel sex, which is binary variable) to only 4 principal explanatory variables, which are: air temperature, soil temperature, aspect and the density of trees in the midden vicinity. The other 3 vegetation variables, 2 relief variables, 5 climate variables and 5 food availability variables were removed from the analysis due to their correlation with more dissimilar variables. Based on the four explanatory variables, eleven models were created, including the null model, and compared using an information-theoretic model selection approach (Table 2). The model with highest explanatory power used the aspect and density of trees as predictor variables (Figure 1), with the middens located in the northeastern slopes containing more pits than the middens in the southwestern slopes. At the same time, in the southeastern slopes, the middens surrounded by more trees contained fewer pits than the middens with less trees in its vicinity. This negative correlation between density of trees and number of pits was not observed in the northeast-facing slopes.

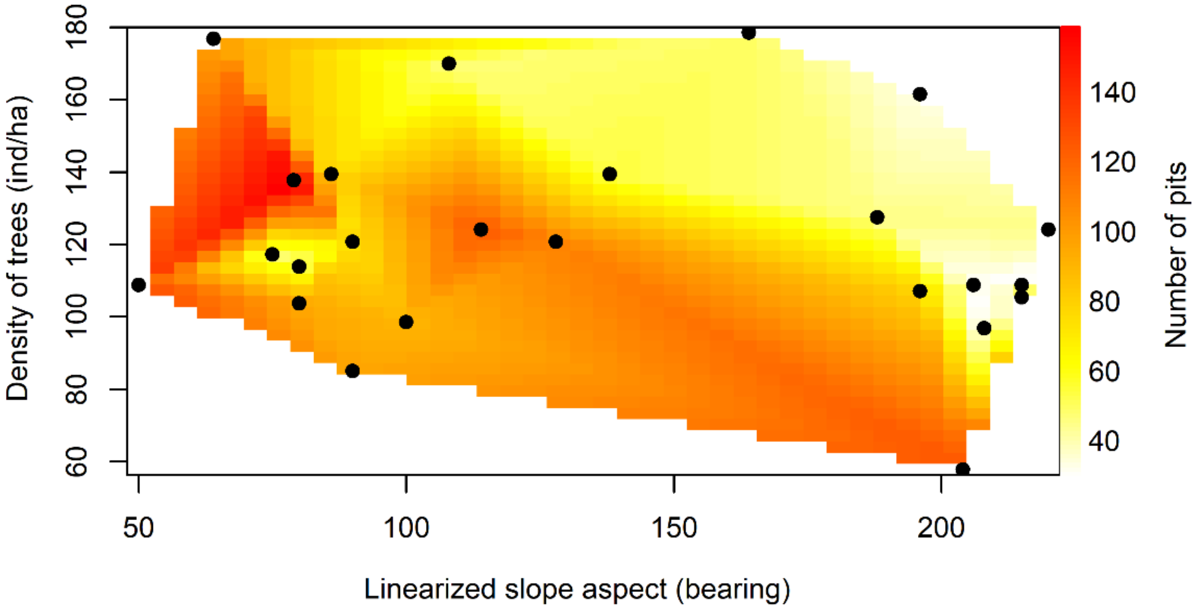

Figure 1: Predicted number of pits excavated by Mt. Graham red squirrels in response to the site aspect (linearized in the x-axis), and density of trees (y-axis), based on the best fitted model. The black points represent the sampled middens.

The best model explained 44% of the observed variation in midden number of pits and received a wAICc = 0.7162, a frequency πi = 0.59, by far the most plausible model. Aspect was clearly the most important variable, since it was also present in the four models with higher explanatory power, while the tree density variable was, by itself, a weak variable and obtained only the sixth position in the rank of better models.

Due to its categorical nature, the resident squirrel sex was not included in the PCA and competing hypothesis analysis, instead, we divided the middens according with the sex of the resident squirrel and compared the two groups with student t-tests. We found differences in the number of pits excavated by males and females (t = 2.5882, df = 23.78, p = 0.016), with males digging 47% more pits than females (Figure 2). We found differences in the aspect of middens of males and females to differ (t = -0.1047, df = 20.196, p-value = 0.054), with more male squirrels being found on slopes with a northeastern aspect and more females in the southwestern facing areas (Figure 3). We found no evidence of an aspect effect on the cache behavior of males (β = -0.0006, t = -0.603, p = 0.556), whereas for females, the effect of the aspect was strong (β = -0.359, t = -7.877, p << 0,05), with females on northeastern slopes digging more pits than the females on the southwest facing areas (Figure 4).

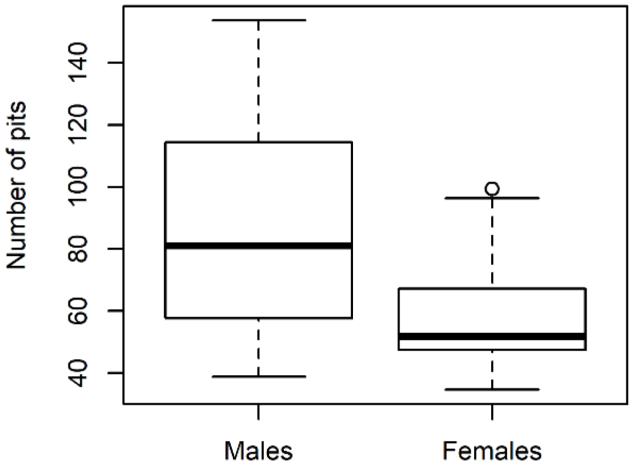

Figure 2: Differences in the number of pits excavated by male and female red squirrels on Mt. Graham, Arizona, USA.

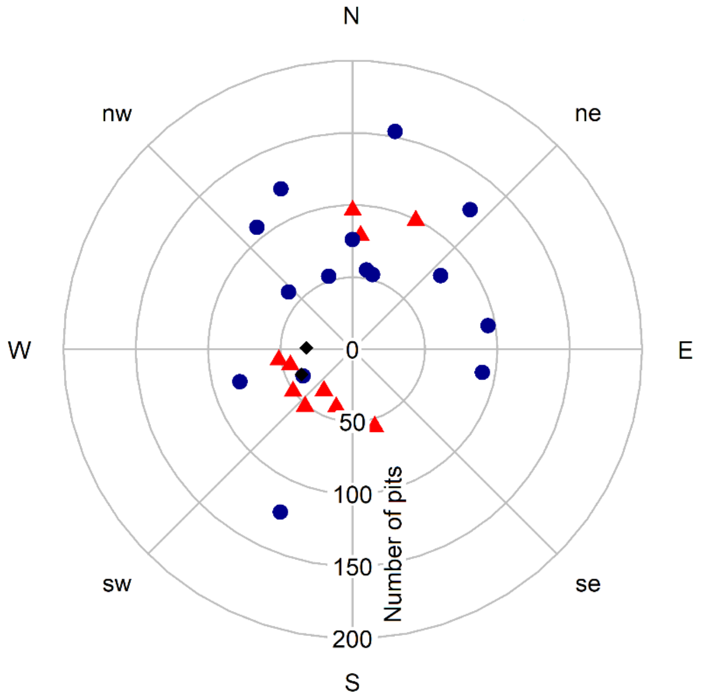

Figure 3: Circular distribution of the sampled middens along the mountain aspect. The radial axis represents the number of pits excavated by each resident squirrel. The distance between points in the picture does not represent geographical distances at the study site. The sex of the resident squirrels is indicated by blue points for males, red triangles for females and black diamonds for squirrels with unknown sex.

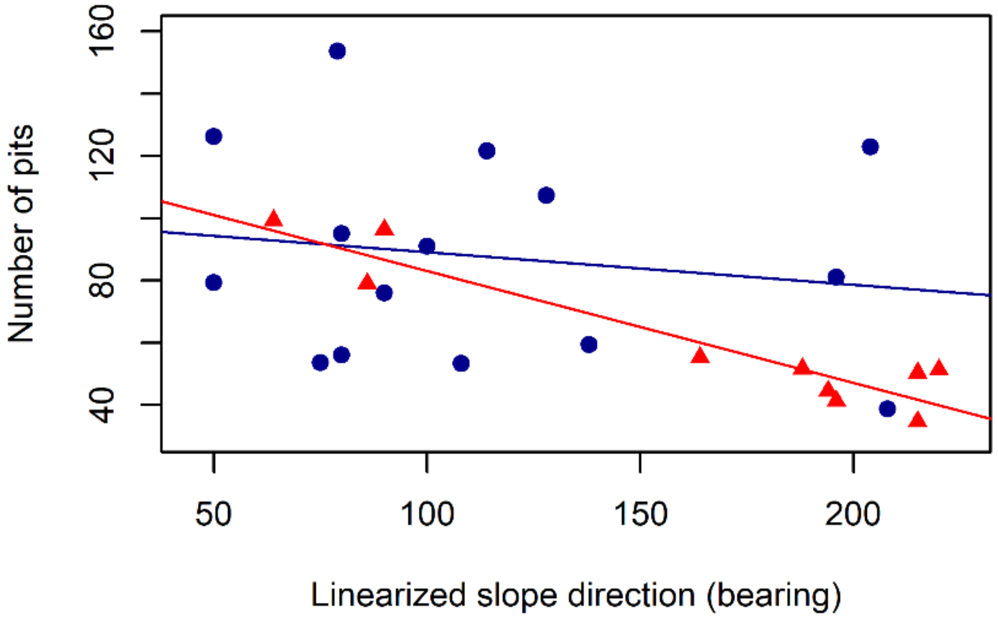

Figure 4: Regression of the number of pits excavated by males (blue points) and females (red triangles) in response of the aspect (linearized in the x-axis). For the description of the linearization process of the aspect variable, see the methods section.

2.5 Discussion

We found evidence to support our hypothesis about the existence of behavioral plasticity in the cache behavior of the Mount Graham red squirrel, so that the number of pits excavated by the animals to cache cones varied according to aspect, density of trees in the midden vicinity and sex of the resident squirrel. We did not support the existence of climate related behavioral plasticity, as squirrels displayed no response to any of the tested climate variables. We found support for the existence of behavioral plasticity in response to topographic relief, with middens in the northeastern slopes of the mountain containing more pits than in the southwestern slopes. This response was caused mainly by females that strongly responded to aspect, whereas males did not. We also found mixed support for the plasticity in response to food availability, but in a pattern opposite to the expected, with squirrels digging more pits in the areas with reduced cone availability.

The absence of a response in the squirrel’s cache behavior to variation in the microclimate along the mountain is surprising given how important microclimate is to cone preservation and thus to squirrel survival. However, several factors could interfere and/or preclude the emergence and maintenance of such behavioral plasticity in response to climate. First, mean air temperature varied only 4.7 °C between middens, it is possible that squirrels simply lack the capability to detect such small variation and thus, do not respond to these small temperature variations, independent of its importance to cone preservation. Even if squirrels are not capable of perceiving these variations in mean temperature, increases of just a degree or two are enough to impact cone preservation (J John Koprowski, personal communication, 2018), and an increase of 4.7 °C is within the expected climate change for 2100 (IPCC 2014).

A second possible explanation is that the squirrels indeed have no plasticity in the caching behavior in response to climate variables. Although we have found plasticity in the squirrel’s cache behavior in response to other environment variables (i.e. mountain relief and density of trees), it did not imply that plasticity should also occur in response to climate (Tuomainen and Candolin 2011). If climate related behavioral plasticity does not exist in Mt. Graham red squirrels even with a increased variation in the cache site climate conditions, then the ability to respond behaviorally to predicted climate change is unlikely (IPCC 2014).

Although we observed an effect of the aspect, density of trees and sex of the squirrel on cache size, it was not possible to completely isolate and precisely estimate the effect of each one of these three variables on the animals' cache behavior. The exact mechanism or environmental cues used by males and females that resulted in the different responses to aspect is unclear but may be unrelated to the microclimate since we ruled out most of the climatic options.

Considering the northeast-southwest axis of the observed pattern and that the density of trees also affected the squirrel cache behavior, it is possible that the mechanism underlying this result may be related to solar radiation. In the northern-hemisphere, the southern mountain slopes receive sunlight at a more perpendicular angle than the northern slopes, whereas the spatial distribution of the forest canopy in the northern slopes also blocks a significant amount of solar radiation from reaching the ground (Courbaud et al. 2003). Density of trees, per se, is an extremely poor predictor of the number of pits, however, it become a good predictor when paired with aspect indicating that the effect of trees density on the squirrel' behavior is also aspect dependent.

Another possible mechanism that underlies the effect of tree density on squirrel cache behavior is related to the individual's perception of predation risk. Differences in boldness, vigilance and risk perception between sexes are a relatively common phenomena (Elgar 1989), and vigilance activities frequently interfere with foraging efficiency (Verdolin 2006). Since avian predation is a significant mortality cause in the Mt. Graham squirrel population (Schauffert et al. 2002; Goldstein et al. 2017) and females have a higher annual survival rate than males (Goldstein et al. 2017), it is possible that tree density has different effects on risk perception for males and females, affecting the cache efficiency and cache size. These two possible explanations are not mutually exclusive.

Our result document the existence of cache related behavioral plasticity in response to variation in the topographic relief and vegetation, but an apparent lack of plasticity in response to climatic variation in an endangered red squirrel. Although the mechanism by which the topographic relief affects the squirrel cache behavior is not clear, we were able to identify possible explanations this key question. Temperature may turn the Pinalenõ Mountains into unsuitable forest for the Mt. Graham red squirrel. Moreover, our results also highlight how complex and unexpected interactions between the environment and the individuals of a population can be (Waite and Strickland 2006). At the distributional edges of a species, behavioral plasticity may not be enough to deal with the harsh environmental conditions or may even be maladaptive and counterproductive (Tuomainen and Candolin

2011). Independent of the occurrence of behavioral plasticity within a population, the environmental conditions may completely disrupt/preclude vital activities, which include food caching (Waite and Strickland 2006; Sutton et al. 2016). In the face of already unavoidable climate change, sky island complexes, such as the Madrean Archipelago (Warshall 1995; Parmesan 2006), can be used as natural laboratories, providing us the opportunity to better understand how species characteristics, including behavior, affects its persistence capability. This may also allow us to develop and improve our conservation methods to protect populations in the edge of its distribution range and in the edge of extinction (Goldstein et al. 2018).

Acknowledgements

We would like to thank to the Conservation Research Laboratory of the University of Arizona, Arizona Agriculture Experiment Station and the USDA Forest Service for the logistic and financial support, to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Universidade Estadual Paulista “Júlio de Mesquita Filho” (UNESP-RC) for provide a one-year studentship at the study site, and to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for a studentship in Brazil.

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Table 1: List of tested explanatory variables with the respective units. The principal explanatory variables, marked by a *, were selected by PCA analysis and were used to create models for subsequent model selection analysis.

Variables	Type	Unit
Altitude	Relief	Meters
Declivity	Relief	Degrees
Aspect*	Relief	Bearing
Soil temperature*	Climate	°C
Air temperature*	Climate	°C
Air humidity	Climate	°C
Pit temperature	Climate	°C
Scale pile temperature	Climate	°C
Pit cooling effect	Climate	°C
Snow melt speed	Climate	Percentage
Forest cover	Forest structure	Percentage
Tree density*	Forest structure	Ind/ha
Live tree density	Forest structure	Ind/ha
Dead tree density	Forest structure	Ind/ha
Live conifer density	Food availability	Ind/ha
Engelmann spruce	Food availability	Ind/ha
Douglas Fir	Food availability	Ind/ha
Ponderosa pine	Food availability	Ind/ha
Mushroom productivity	Food availability	Grams
Resident squirrel sex	Sexual	Binary

Table 2: Results of the model selection for Number of Pits (NP) excavated by Mt. Graham red squirrels. We fitted generalized linear models and calculated the corrected Akaike relative difference (ΔAICc), relative likelihood (wAICc) and a bootstrap model selection frequency (πi) to all models. Models with ΔAICc ≤ 2 were considered as equally plausible. The πi parameter was calculated based on 10.000 permutations.

Models	ΔAICc	Df	wAICc	πi
GLM10: NP ~ Aspect + Density of Trees	0.0	4	0.7161	0.5998
GLM3: NP ~ Aspect	3.4	3	0.1337	0.0610
GLM8: NP ~ Aspect+ Air Temperature	4.2	4	0.0865	0.2200
GLM6: NP ~ Aspect+ Soil Temperature	5.3	4	0.0518	0.0358
GLM5: NP ~ Soil Temperature + Air Temperature	10.3	4	0.0041	0.0584
GLM4: NP ~ Density of Trees	11.5	3	0.0022	0.0014
GLM9: NP ~ Air Temperature + Density of Trees	11.6	4	0.0021	0.0170
GLM7: NP ~ Soil Temperature + Density of Trees	12.4	4	0.0015	0.0035
GLM1: NP ~ Soil Temperature	12.9	3	0.0011	0.0019
GLM2: NP ~ Air Temperature	14.5	3	<0.001	0.0004
GLM0: NP ~ Null (aleatory generated values)	15.1	3	<0.001	0.0008

3. LANDSCAPE OF HUMAN FEAR IN NEOTROPICAL RAINFOREST-DWELLING MAMMALS

Journal format: Biological Conservation

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3.1 Abstract

The landscape of fear has profound effects on the species behavior, with most organisms engaging in risk avoidance behaviors in areas perceived as riskier. Most risk avoidance behaviors, such as temporal avoidance, have severe tradeoffs between foraging efficiency and risk reduction. Human activities are able of affect the species landscape of fear, by increasing mortality of individuals (i.e. hunting, roadkill) and by disruption of the clues used by the species to estimate predation risk (e.g. light pollution). In this study, we used an extensive camera-trapping and night-time light satellite imagery to evaluate whether human activities affect the dial activity patterns of 17 species of rainforest dwelling mammals. We found evidence of nocturnality increase is 7 of 17 analyzed species, which become 20% more nocturnal in high perturbed areas, while no species was observed to become more diurnal in response to human disturbance. This activity shifts toward nocturnality were observed for both diurnal and nocturnal species, with no group being more susceptible to present these shifts. Persecuted species (game and predators) were more susceptible to increase nocturnality.

Because changes in foraging activity may affect species fitness, the behavior of human's avoidance may be another driver of the Anthropocene defaunation.

Key words: Anthropocene, Nocturnality, Night-time light, Poaching, Light pollution

3.2 Introduction

The biosphere is becoming increasingly modified by human activities, where 75% of the world area experiences a measurable amount human pressure (Venter et al., 2016). Activities such the direct removal of natural forests and grasslands to allocate croplands, livestock pastures, urban development and expansion of road networks are the main drivers of the reduction of the natural biomes (Mittermeier et al., 2004). As a consequence, the populations of innumerous species have been also severely impacted by direct and indirect human driven habitat modification and harvesting, with 25% of mammals, 13% of birds and 41% of amphibian species being considered threatened to some degree (IUCN, 2018). Moreover, beyond the direct habitat destruction and species overharvesting, the effects of human activities have also discreet but equally pervasive consequences on population dynamics, caused by changes in the species perception of an area as safe or dangerous (Frid and Dill, 2002).

All species on Earth evolved strategies to avoid predation and are capable of modulate their behavior to achieve the energetic daily demands while avoiding being killed. The oldfield mice *Peromyscus polionotus*, for instance, reduces predation risk by foraging on safer areas, under vegetation shelter and during darker nights, while avoiding open areas especially under the moonlight (Orrock et al., 2004). In a similar way, the paca *Cuniculus paca* and nine-banded armadillo *Dasypus novemcinctus*, also avoid foraging under the bright full moon, when the perceived predation risk is higher, but concentrate the foraging on new moon nights, when the perceived risk is lower (Harmsen et al., 2011). This spatial and temporal variation in risk perception by the species is called the "Landscape of Fear", and have profound effect on the species ecological interactions along both space and time (Laundre et al., 2010). The landscape of fear perceived by elks in response to reintroduced wolves, for instance, is directly related with the herbivory levels along the Yosemite National Park, with higher herbivory levels occurring on areas perceived as safer (Laundre et al., 2010). The landscape of fear perceived by a predator species also affects the distribution of its preys, with higher prey density on areas perceived as risky by the predator (Muhly et al., 2011). Fear have strong effects in foraging efficiency (Verdolin, 2006), and the species' choice about how much risk is acceptable depends on the cost-benefit ratio of forage in dangerous areas and on the amount of information available to estimate the risk (Bouskila and Blumstein, 1992).

It is important to highlight that the landscape of fear is not based on the real predation risk, but in the individual's perceived predation risk. Foraging individuals rarely have complete knowledge about the real predation risk they are exposed, and therefore, they usually estimate the risk based on environmental clues, such light intensity, canopy density, and noise (Orrock et al., 2004; Verdolin, 2006). This happens because it is safer to estimate the risk by indirect methods than rely on directs detection of a predator nearby, when it may be too late. It is also less harmful to overestimate the predation risk and lose some foraging efficiency, than to underestimate the risk, which can lead to death by predation (Bouskila and Blumstein, 1992; Frid and Dill, 2002). These non-lethal interactions between prey-predator can have even bigger effects on ecosystems dynamics than direct prey-predator interactions (Preisser et al., 2015).

Since the wildlife estimate the predation risk mostly based on clues, human activities can easily confound these clues and lead to false risk estimations, causing entire populations to live and behave under a level of risk perception which is disconnected with the reality, creating landscapes of fear that did not correspond to the real distribution of risks (Frid and Dill, 2002). Wildlife can perceive the noise of vehicles, agriculture machinery, airplanes, electric illumination and even the simple presence of humans as predation risk (Frid and Dill, 2002). As consequence, the high risk perception is known to increase stress hormones (Zbyryt et al., 2018), reduce feeding efficiency (Orrock et al., 2004) and reproductive success (Sheriff et al., 2009). Besides, it can also trigger risk avoidance behaviors, such spatial avoidance and temporal avoidance.

Temporal avoidance between the predator and prey is the risk avoidance behaviors in which the prey shift its normal dial activity period to become active on a safer period, thus, reducing the chances of encounter a predator or other threat (Saleni et al., 2007). It is also an alternative and/or complement for spatial avoidance (i.e. avoid risky areas) when the latter is not possible due to low habitat availability, or not enough to avoid conflicts (Martin et al., 2010; Saleni et al., 2007). However, since most species are not cathemeral (i.e. adapted to be equally active at any period of the day), shifts in dial activity period can negatively impact the species capability to navigate in the environment, find food, detect mates and predators (Bennie et al., 2014; Gaston et al., 2014). Despite its drawbacks, temporal avoidance has been showed as a common strategy used by mammals under human disturbed areas (Gaynor et al., 2018).

Here we tested the temporal avoidance behavior of forest-dwelling mammals in response to human-driven disturbances, both direct (e.g. poaching and logging) and indirect (e.g. human presence, noise and light pollution). We expected to: 1) Observe temporal shifts on poached or persecuted species, such ungulates, armadillos and wild felids (Bitetti et al., 2008; Cullen et al., 2000; Espinosa and Salvador, 2017; Gaynor et al., 2018); 2) Confirm temporal shifts to occur towards nocturnality, with species becoming relatively more nocturnal in more disturbed areas (Gaynor et al., 2018); 3) Confirm no temporal avoidance in response to human disturbance by small body sized non-poached species, such opossums (*Didelphis spp*.) and small rodents, and species that increase density in fragmented landscapes, such squirrels (Koprowski, 2005; Mendes et al., 2016), and synanthropes (i.e. which benefit from association with humans), such as dogs (Paschoal et al. 2012).

3.3 Methods

Survey

To detect possible shifts in activity period, we used a large dataset from camera-traps (Bushnell HD Nature View and Reconyx RC55) to record the activity period of mammalian species along a gradient of habitat disturbance and degradation in continuous and fragmented areas of Atlantic Forest in Brazil. A total of 602 sampling stations were used, with each station receiving one camera for a period between 8 and 278 days (47 ± 30.3). The sampling occurred from August 2012 to February 2018 and the stations were spread along 36 Atlantic Forest areas (Figure 1), with sizes ranging from 16 ha to continuous (here defined as forest patches with more than a million hectares). In all sampled sites, the camera-traps were placed around 30 cm above the ground, recording video or a rapid 5 pictures sequence in response to the activation of a passive infrared sensor. To ensure temporal independence between records, we used 60 minutes as a minimum time allowed between two records for the same species at the same camera. This effort resulted on a total of 28567 camera/days, and 40 mammal species recorded (Beca et al., 2017; Brocardo et al., 2018).

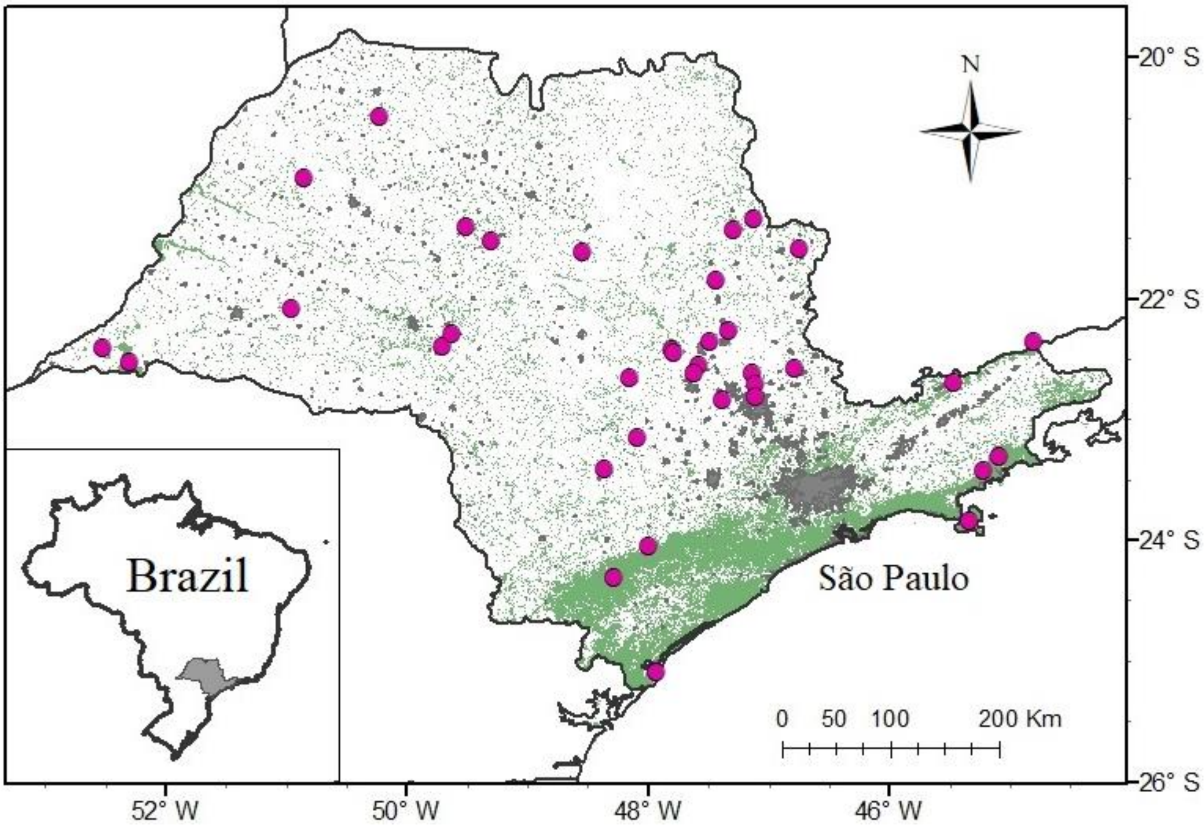


Figure 1: Map of the 36 Atlantic forest sites sampled within the state of São Paulo. The remaining natural forests are in green and the urban areas in gray.

With the data obtained from camera-trapping, we divided the records in two groups based on the time of sunset and sunrise. Records taken when the sun was up in the sky (i.e. after sunrise and before sunset) were considered "day" records, while records taken after the sunset or before the sunrise were considered "night" records. Since the exact timing of sunrise and sunset changes according with latitude and season, we used a solar calendar to guarantee the correct day/night classification along the sampling years. Using the day/night records for each site as a binomial variable, we evaluated the effect of the human disturbance on 17 mammalian species (Table 1), including two exotic species, the domestic dog *Canis lupus familiaris* and the wild pig *Sus scrofa*. Since small sized terrestrial rodents cannot be reliably identified by camera-trap records, we grouped all the records and analyzed it as a single group, here called "Small rodents". Other 22 mammal species could not be evaluated due to the reduced number of records or because the number of sites where these species were recorded was not enough to allow statistical comparisons. Sites where a given species was recorded less than three times were also not include in the analysis of the species.

Human disturbance proxy

Since human disturbance is a multiform phenomenon with a diverse array of pathways in which it can affect the local fauna, we decided to use the anthropogenic night-time light radiance, measured from satellite imagery, in a buffer of 10 km around the sample forest patches as a proxy of human disturbance. Anthropogenic night-time light can be described as the light radiated by a region during the night period, whereas the light is created by human devices, such the lightbulbs used for house and streets illumination. This light radiance is measured by satellites, such the Suomi-NPP satellite, and filtered to remove natural atmospheric and surface light reflections (mainly moonlight and starlight reflections, see Lee et al. 2006). Ephemeral light producing events, such fires, can also be identified and removed by comparing sequences of images or by light spectrum analysis. The spectral radiance unite used in the resulting raster is the microflick, measured in watt/cm²/sr/µm, where “sr” is a square radian, and the “µm” is a micrometer of span in wavelength. More technical information is available on Elvidge et al. (1997) and Lee et al. (2006). However, for reference, in a 10 km buffer in an isolated area of the Amazon Forest (7°48’06”S, 59°03’38”W) the measured mean of spectral radiance is -0.068 microflicks, while in a similar area in the São Paulo metropolitan area (23°33’01”S, 46°38’29”W) the measured mean radiance is 73 microflicks. The night-time light imagery used in this study is available freely by the Earth Observation Group (EOG, 2018), already filtered to remove natural reflections and ephemeral light producing events. Moreover, we used ArcGIS (ESRI, 2016) to calculate and extract the radiance values from the raster.

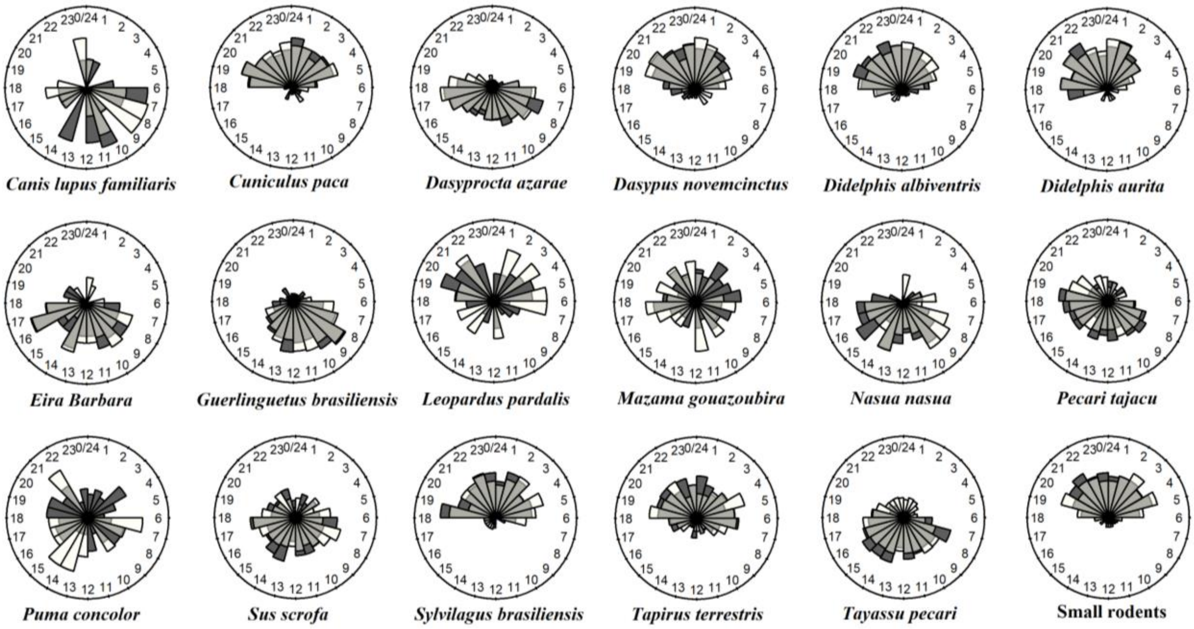
The decision of use night-time lights as a human disturbance proxy was made based in several factors, including: A) Night-time light is commonly used as a proxy of human density, infrastructure density and intensity of light pollution (Elvidge et al., 1997); B) For nocturnal species, light pollution is, per se, a human caused disturbance which can be malefic or benefic according to the species traits (Gaston et al., 2013), although, for diurnal species, it may facilitate the displacement and habitat use during night time (Gaston et al., 2013); C) In our study area, night-time light is negatively correlated with forest cover percentage in the same 10km buffer (Pearson's product-moment correlation, $t = 8.598$, $df = 34$, p-value $<< 0.05$), whereas the matrix on these landscapes (mostly crops and pastures) were also created and maintained by human activities; D) Night-time light is also correlated with the size of the sampled forest in the study sites (Pearson's product-moment correlation, t = -5.333, df = 34, p-value << 0.05), an important information since temporal avoidance behaviors are frequently used in conjunction with spatial avoidance (Martin et al., 2010). Thus, at least in our study areas, night-time light could be safely used not only as a proxy of light pollution and human infrastructure density, but also as a general human disturbance proxy. This approach is also similar to the technique that uses of cellphone coverage area as a proxy of human presence (Macedo et al., 2018).

Data analysis

We used model regressions to evaluate the effect of human disturbance on forest-dwelling mammal temporal avoidance behavior, with the proportion of day/night records as a binomial response variable and the human disturbance proxy (i.e. night-time light, in logarithm with base 10) as explanatory variable. Generalized Linear Models (GLM) were used to test possible linear responses, while Generalized Addictive Models (GAM) for non-linear responses. When both GLM and GAM models were significant, we gave preference to the GLM due to its simplicity and parsimony. Finally, the activity period of all species was also plotted for visual inspection (Figure 2), and useful circular metrics of dial activity, such mean direction and Rho (i.e. a measurement of circular mean and dispersion, respectively) were calculated and reported in the supplementary material (Table S1). All analysis were performed in R (R Core Team, 2016), using the packages “circular” (Agostinelli and Lund, 2017), “GAM” (Hastie, 2017) and ggplot2 (Wickham, 2016).

3.4 Results

We found evidence of activity shifts in response to human disturbance for seven of the 17 evaluated species, where six became more nocturnal in more disturbed areas and one become more nocturnal in areas with intermediate disturbance (Figure 3, table 1). The average increase in nocturnality was 20% from the less disturbed areas to the more disturbed ones. No species was observed to become more diurnal in response to the increase in human disturbance. However, we must clarify that by “becoming more nocturnal”, we did not mean that a diurnal species shifted its peak of activity to the night period. Instead, it means that although the species main activity still occulting during the day, the amount of records taken during the night (i.e. before the sunrise and after sunset) has increased. In a similar way, when a nocturnal animal become “more nocturnal”, it means that the relative amount of records taken during the day has decreased even more.



Canis lupus familiaris · *Cuniculus paca* · *Dasyprocta azarae* · *Dasypus novemcinctus* · *Didelphis albiventris* · *Didelphis aurita*

Eira Barbara · *Guerlinguetus brasiliensis* · *Leopardus pardalis* · *Mazama gouazoubira* · *Nasua nasua* · *Pecari tajacu*

Puma concolor · *Sus scrofa* · *Sylvilagus brasiliensis* · *Tapirus terrestris* · *Tayassu pecari* · **Small rodents**

Figure 2: Circadian activity of the 17 sampled species, plus the group Small rodents. The sites were divided in two groups, with the more disturbed half in dark gray and the more preserved half in white.

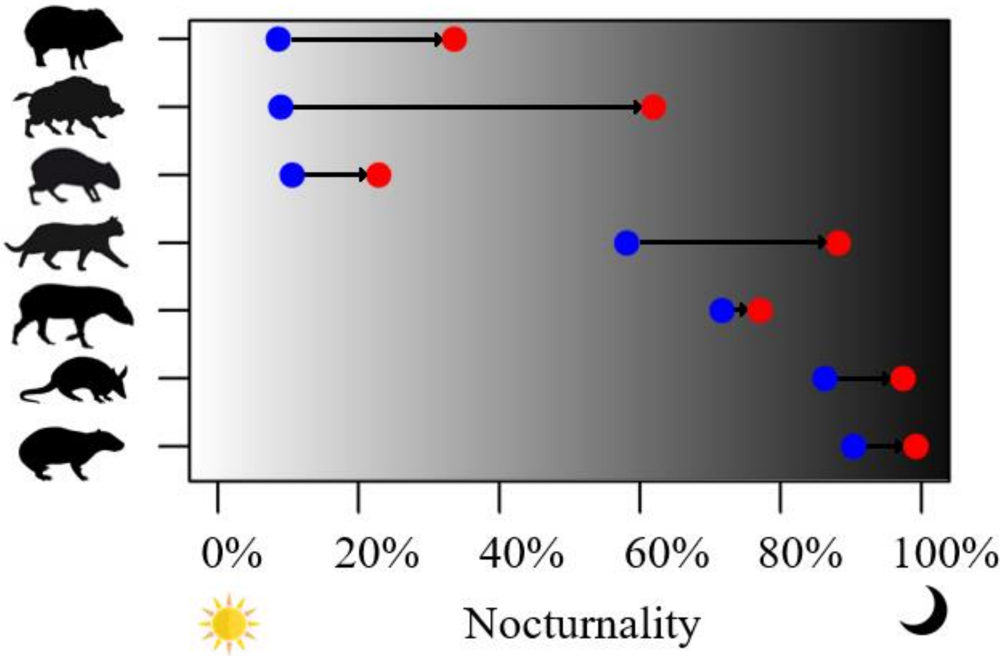


Figure 3: Shifts in nocturnality of forest-dwelling mammals in response to human disturbance, from the most preserved to the most disturbed sites in which each species/group

was recorded, in blue and red respectively. From top to bottom, the species/groups are: Peccaries (*Tayassu pecari* and *Pecari tajacu*), *Sus scrofa, Dasyprocta azarae,* Small cats (*Leopardus guttulus, L. pardalis* and *L. wiedii*), *Tapirus terrestris*, Armadillos (*Dasypus novemcinctus, Cabassous unicinctus, C. tatouay* and *Euphractus sexcinctus)*, and *Cuniculus paca.*

The species which clearly responded to human disturbance are, the paca (*Cuniculus paca* , GLM, $\beta = -0.481$, $z = -2.135$, $p = 0.033$), Azara's agouti (*Dasyprocta azarae*, GLM, $\beta = -0.169$, $z = -3.865$, $p << 0.05$), nine-banded armadillo (*Dasypus novemcinctus*, GLM, $\beta = -0.246$, $z = -1.934$, $p = 0.053$), collared peccary (*Pecari tajacu*, GLM, $\beta = -0.522$, $z = -2.654$, $p = 0.008$), white-lipped peccary (*Tayassu pecari*, GLM, $\beta = -0.8528$, $z = -5.323$, $p << 0.05$), wild pig (*Sus scrofa*, GAM, $\chi^2 = 26.386$, $p << 0.05$) and tapir (*Tapirus terrestris*, GAM, $\chi^2 = 6.1873$, $p = 0.045$). From these species, only the wild pigs and tapirs present a non-linear response to human disturbance, being better described by GAMs. Tapirs did not present a response to human disturbance until areas with high levels of disturbance (with mean radiance above 0.5 microflicks), where it became more nocturnal. Wild pigs become more nocturnal only at intermediary levels of disturbance, where the mean spectral radiance was between 0.2 and 3 microflicks (Figure 4).

The two felid species with sample size big enough to be evaluated, the ocelot (*Leopardus pardalis*, GLM, $\beta = -0.311$, $z = -1.639$, $p = 0.101$) and the puma (*Puma concolor*, GLM, $\beta = 0.212$, $z = 1.628$, $p = 0.104$), did not present activity shift in response to human disturbance. Beyond that, other 8 species did not present detectable activity shifts in response to human disturbance. These species are white-eared opossum (*Didelphis albiventris*, GLM, $\beta = -0.016$, $z = -0.185$, $p = 0.853$), black-eared opossum (*Didelphis aurita*, GLM, $\beta = 0.018$, $z = 0.162$, $p = 0.871$), tayra (*Eira barbara*, GLM, $\beta = 0.069$, $z = 0.147$, $p = 0.883$), Atlantic forest squirrel (*Guerlinguetus brasiliensis*, GLM, $\beta = 0.110$, $z = 0.777$, $p = 0.437$), gray brocket deer (*Mazama gouazoubira*, GLM, $\beta = 0.022$, $z = 0.162$, $p = 0.871$), coati (*Nasua nasua*, GLM, $\beta = 0.086$, $z = 0.448$, $p = 0.654$), Brazilian cottontail (*Sylvilagus brasiliensis*, GLM, $\beta = 0.246$, $z = 1.191$, $p = 0.234$) and domestic dog (*Canis lupus familiaris*, GLM, $\beta = 0.194$, $z = 0.484$, $p = 0.628$). Small rodents also did not respond for human disturbance (GLM, $\beta = 0.145$, $z = 1.525$, $p = 0.127$).

Finally, since ocelots had few records in several sites, being recorded in 20 sites, from which 8 had less than 3 records, we decided to improve the test by merging all records of the genus *Leopardus*, therefore, increasing the model sample size. This group of species, here called "small cats", include *Leopardus guttulus, L. pardalis* and *L. wiedii*. As result, the small cats also present shift toward nocturnality in response to human disturbance, (GAM, χ^2 = 4.154, p = 0.041), mainly in sites with radiance below 0.03 and above 3 microflicks, while being relatively unresponsive in the intermediary disturbances areas. In a similar manner, we also merged all the records of armadillo species to test the general response of the taxa to human disturbance. The group, here called "Armadillos", included the species *D. novemcinctus, Cabassous unicinctus, C. tatouay* and *Euphractus sexcinctus*, and also present shift toward nocturnally in response to human disturbances (GLM, β = -0.2531, z = -2.135, p.value = 0.033) (Figure 4). When all the species within these groups were included in the overall results, 13 of the 24 evaluated species become more nocturnal in response to human disturbance.

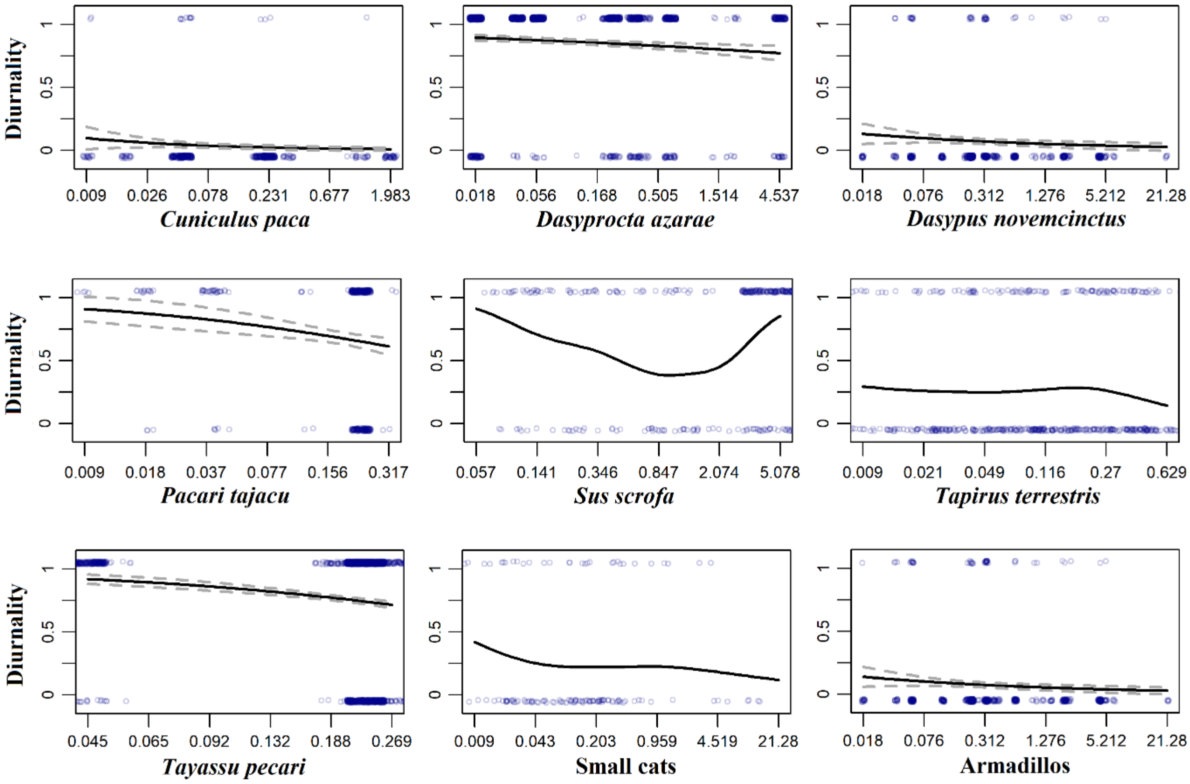


Figure 4: Decrease in diurnality (i.e. increase in nocturnality) of the species which were affected by human disturbance. The human disturbance was measured using the night-time light radiance of each site as a proxy and is recorded in microflicks. The group "Small Cats" contain *Leopardus guttulus, L. pardalis* and *L. wiedii*, while the group "Armadillos" contain *Dasypus novemcinctus, Cabassous unicinctus, C. tatouay* and *Euphractus sexcinctus.*

3.5 Discussion

We found that 56% of tested forest-dwelling mammals changed their activity period and become 20% more nocturnal in more disturbed areas, while no species was observed to become more diurnal in response to human disturbance. This result agrees with other studies that reported decreases in wildlife diurnally as a consequence of human disturbances (Bennie et al., 2014). Surprisingly, the species activity period (i.e. diurnal or nocturnal) did not appear affect its probability to become more nocturnal in response to human disturbance, since 4 of the 8 diurnal species and 3 of the 8 nocturnal evaluated species presented shifts toward nocturnality. However, the fact that we recorded shifts toward nocturnally for both diurnal and nocturnal species indicate that the mechanism behind these changes is probably related to the human diurnal behavior. This assumption is complemented by the fact that the proportion of mammalian species red listed by the IUCN is higher for the diurnal species, which are active in the same periods than humans, than for nocturnal species (Bennie et al., 2014). Moreover, shifts toward diurnally are possible when in response to nocturnal threats, as observed for the bearded pig *Sus barbatus* avoiding the Sunda clouded leopard *Neofelis diardi* (Ross et al., 2013).

The only species which displayed a different response, becoming more nocturnal only in areas with intermediary disturbance levels, was the invasive wild pig. This result is partially expected since wild pigs are legally controlled by hunting and are also a synanthropic species, with a recent domestication historic, hybridization events with domestic lineages and have a diet that include agricultural crops (Barrios-Garcia and Ballari, 2012). Considering the wild pig's large body mass and the absence of top predators, such jaguars, in most of the Atlantic Forest (Paviolo et al., 2016), humans have become their main "predator" (Rosa et al., 2018). Therefore, since the population control occurs in agriculture areas and these areas have a night light radiance higher than the protected state parks, but lower than the urban peripheric regions, it is reasonable to argue that the observed results indeed represent the species landscape of fear. Therefore, the areas with intermediary levels of radiance, between 0.2 and 3 microflicks, might be being perceived as more dangerous than the low radiance protected parks and high radiance urban surroundings. In the other hand, the domestic dog, which is also synanthropes, is not hunted but maintained by rural villages (Paschoal et al., 2012), so did not displayed any activity shift due to human disturbance.

We found that all of the 13 species for which we observed temporal shifts, here including the species within the Small cats and Armadillos groups, are game or persecuted species. In other hand, from the 11 species for which no activity shifts were observed, only two species, the gray brocket deer and the puma, are game or persecuted species. Moreover, the observed differences in the response of mammals to human disturbance are probably related to how the species perceive the human presence and activities as a threat. Although perceived predation risk is mostly estimated and susceptible to disruption (Frid and Dill, 2002), the species are also able of perform accurate estimates, such demonstrated by the Japanese sika deer (*Cervus nippon*), which is capable of differentiate the threat represented by humans during and out of hunting season, increasing its avoidance behavior in the prior (van Doormaal et al., 2015). In this context, the absence of nocturnality shift in the gray brocket deer is particularly surprising since temporal avoidance is a strategy commonly reported for cervids, including close related species such the red brocket deer *Mazama americana* (Bitetti et al., 2008).

Although we have strong evidences pointing to the effects of hunting as a mechanism driving activity shifts in forest-dwelling mammals, since we used a proxy of human disturbance instead of a direct hunting measurement, we cannot exclude other possible human-driven mechanisms which can potentially affect the observed activity changes on the local fauna, such as changes in food availability, habitat reduction and light pollution. Food availability, for instance, can be greatly increased by human activities if a species is capable of consume crops or garbage (Weiser and Powell, 2010), but it can also be decreased if the food sources are destroyed (Galetti and Aleixo, 1998). As consequence, changes on food availability affect the time required to an individual fulfill its daily diet requirements, sometimes forcing individuals to forage on less optimal time periods (van Schaik and Griffiths, 1996). Nevertheless, although some of the evaluated species are known to consume crops (Barrios-Garcia and Ballari, 2012) and to increase population density in fragmented areas, changes on food availability should not be the main driver of the observed results since it have no temporal directionality. Thus, whilst shifts toward diurnally are possible (Hirakawa, 2006), they were not observed in none of the evaluated species.

The reduction of habitat availability is another mechanism with possible effects on the observed activity shifts. Since temporal avoidance is a complement for spatial avoidance strategies (Kronfeld-Schor and Dayan, 2003), it is expected that disturbed populations on small forest patches rely more on temporal avoidance than equally disturbed population on large patches, which have more space available to benefit from spatial avoidance. Considering that, in the study region, night-time light was correlated with forest size and landscape forest cover, the differential reliance on temporal avoidance of the wildlife populations along the disturbance gradient may partially explain the observed results. However, although habitat availability may affect the observed results, it cannot be the main drive of the observed shifts toward nocturnally for two reasons: 1) Forest size and landscape forest cover per se were such a poor predictors of the species activity period that we decided to remove them from the main analysis, preferring to use only the night-time lights, which have an intrinsic human density factor (Elvidge et al., 1997) lacking on the pure habitat availability variables; 2) If habitat availability indeed changes the populations reliance on temporal avoidance, the population still needs a cause for engage in avoidance behavior, such high perceived risk or competition for resources.

Light pollution is another mechanism with strong potential as a driver to the observed activity shifts, and since our human disturbance proxy (i.e. night-time lights) is based on the amount of light emitted to the night sky, it is also a direct measurement of light pollution. Light is the most obvious environmental variable that changes along the dial cycle, ranging from 103000 lux under full sunlight to 0.001 lux in a clear starry night (Gaston et al., 2013). These cycles of light and darkness remained constant along the entire evolutionary history, and most species have evolved sensorial systems optimized to operate during a specific period of the dial cycle (Bennie et al., 2014; Gaston et al., 2013). However, today, more than 80% of earth’s surface is under some degree of light pollution (Falchi et al., 2016), and in intensely light polluted regions, the illuminance provided by the urban skyglow can be brighter than in a clear full moon night (Gaston et al., 2013). In this way, since several nocturnal species shift their activities in response to the moon cycles (Harmsen et al., 2011), the light pollution have illuminance more than enough to cause changes in species activity period (Gaston et al., 2013).

Moreover, similar to the observed results, light pollution also drive the species activities towards nocturnality, by increasing the amount of light available at night time for the navigation of diurnal and cathemeral species, while reducing the activity of species that naturally avoid illuminated night (Gaston et al., 2013). Changes on activity due to the moonlight cycles were reported for some of the evaluated species, including the paca and nine-banded armadillo (Harmsen et al., 2011), puma, red broket deer and opossum species (Pratas-Santiago et al., 2017), but not for tapirs (Oliveira-Santos et al., 2010) and ocelots

(Pratas-Santiago et al., 2016). Thus, light pollution added to hunting, are probably an important driver behind the observed activity shifts in disturbed areas.

Is summary, human disturbance was observed to increase nocturnally in seven of the 17 evaluated forest-dwelling mammal species and in two of the tree evaluated groups of species, with no species becoming more diurnal in response to human disturbance. Nocturnality was increased 20% in average, and among other factors, hunting and light pollution are the most plausible drivers of the observed activity changes. Consequently, human disturbed areas are probably perceived as higher risk areas by the wildlife, or at least by the species observed to change their activity patterns. Beyond the usually reported effects of human disturbance on species community composition and population densities, our results highlight how human presence and activities also affect the species behavior in a landscape scale. The ecological consequences of these behavioral changes are particularly difficult to predict due to the myriad of possible scenarios and possible causal pathways (see Gaston et al. 2013, 2014; Gaynor et al. 2018). Thus, a careful evaluation of how the proximal drivers affect the dial activity of each species is highly recommended, as well as the consequences of these dial shifts in the individual’s survival, stress level and fitness. Finally, since several human settlements are located inside protection parks, we advise the use of night-time light imagery, in a finer resolution, to map the distribution of these settlements and to select areas for conservation action.

Acknowledgements

The resources required to the development of this study were provided by the Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq (process 168080/2014-1) and by the Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (processes 2014/01986-0, 2014/09300-0, 2014/23095-0, 2015/22844-1 and 2015/18381-6).

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Table 1: Main results of the regression analysis, testing the effect of human disturbance on the nocturnality of each species and groups of species. Significative results are in bold. The group "Small Cats" contain *Leopardus guttulus, L. pardalis* and *L. wiedii. T*he group "Armadillos" contain *Dasypus novemcinctus, Cabassous unicinctus, C. tatouay* and *Euphractus sexcinctus*. The group "Small rodents" contain several unidentified species of rodents.

Species *Groups	Model type	Estimate	z value or *χ Square	P value
Canis lupus familiaris	GLM	0.1943	0.484	0.62866
Cuniculus paca	GLM	-0.4814	-2.135	**0.0328**
Dasyprocta azarae	GLM	-0.16869	-0.481	**0.000111**
Dasypus novemcinctus	GLM	-0.2459	-1.934	***0.0531***
Didelphis albiventris	GLM	-0.01599	-0.185	0.853
Didelphis aurita	GLM	0.01813	0.162	0.871
Eira barbara	GLM	0.06913	0.147	0.88279
Guerlinguetus brasiliensis	GLM	0.1102	0.777	0.437
Leopardus pardalis	GLM	-0.311	-1.639	0.101
Mazama gouazoubira	GLM	0.02163	0.162	0.871
Nasua nasua	GLM	0.08634	0.448	0.654
Pecari tajacu	GLM	-0.5218	-2.654	**0.00797**
Puma concolor	GLM	0.2124	1.628	0.104
Sus scrofa	GAM	-	26.386*	**2.796e-07**
Sylvilagus brasiliensis	GLM	0.2456	1.191	0.234
Tapirus terrestris	GAM	-	6.1873*	**0.04534**
Tayassu pecari	GLM	-0.8528	-5.323	**1.02e-07**
Small rodents*	GLM	0.14456	1.525	0.127
Armadillos*	GLM	-0.2531	-2.135	**0.0328**
Small cats*	GAM	-	4.154*	**0.04155**

3.7 Supplementary Material

Table S1: Dial activity patterns of the 17 evaluated species, plus the group "Small rodents". The mean activity hour is a circular statistics version of the arithmetic mean, while the Rho is a circular statistics descriptor of dispersion.

Species	Activity	Mean activity hour	Rho	N. records	N. sites
Canis lupus familiaris	Diurnal	9:31	0,457274	54	16
Cuniculus paca	Nocturnal	23:43	0,615824	482	19
Dasyprocta azarae	Diurnal	12:9	0,433268	1552	18
Dasypus novemcinctus	Nocturnal	23:17	0,641137	629	34
Didelphis albiventris	Nocturnal	23:13	0,645843	862	24
Didelphis aurita	Nocturnal	22:49	0,676625	342	16
Eira barbara	Diurnal	12:29	0,557002	104	18
Guerlinguetus brasiliensis	Diurnal	10:35	0,709208	478	21
Leopardus pardalis	Nocturnal	23:12	0,333667	100	20
Mazama gouazoubira	Cathemeral	1:51	0,098956	87	12
Nasua nasua	Diurnal	12:29	0,567901	168	24
Pecari tajacu	Diurnal	14:39	0,567901	387	13
Puma concolor	Cathemeral	17:48	0,065707	90	18
Sus scrofa	Diurnal	12:21	0,286522	298	16
Sylvilagus brasiliensis	Nocturnal	23:54	0,581279	368	15
Tapirus terrestris	Nocturnal	0:23	0,329588	464	11
Tayassu pecari	Diurnal	13:15	0,398446	1754	8
Small rodents	Nocturnal	0:44	0,645905	1303	27

4. NEOSQUIRREL - A DATASET OF THE ECOLOGICAL KNOWLEDGE ON NEOTROPICAL SQUIRRELS

Journal format: Mammal Review

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4.1 Abstract

1. Squirrels (Sciuridae) with 292 species is the second most diverse family of rodents. They play an important role as seed and spore dispersal and seed predators in all regions where they occur. In Neotropical regions, around 28 species of squirrels are recognized. However, the actual knowledge of neotropical Sciuridae ecology is severely incomplete, lacking the most basic ecological information of most species.

2. We reviewed the literature in English, Spanish and Portuguese, for all squirrel species in the neotropical biogeographic region, summarizing the ecological interactions between squirrels and the local biota, population density records and the number and distribution of publications.

3. We found information for 20 species (71 % of the recognized species), from 15 countries, from 48 publications containing 126 population density records and 155 publications containing 649 ecological interactions. The most studied species were *Guerlinguetus brasiliensis*, *Notosciurus granatensis* and *Sciurus variegatoides*, with 53% of all publications,

whereas for 8 species of *Microsciurus*, we found no publications. The density of neotropical squirrels varied from 0.08 to 100 ind/km^2 and were negatively correlated with forest area

4. Neotropical squirrels were recorded eating 174 plants species, 5 fungi, 4 invertebrates and 1 vertebrate. Palms were a common item in squirrels' diet (30 palm species, 27% of feeding records). Squirrels cached 28 species, from which 15 are palms.

5. Sixty-five species of parasites occur in squirrels, and the most common were *Enderleinellus* lices. Zoonotic parasites, including trypanosomes, *Leptospira ssp*., *Leishmania spp.,* and plague we also reported.

6. Our review reveals the main information gaps in the current knowledge about the ecology of Neotropical Sciuridae, and map the geographic distribution of the available information through the South and Central Americas. Because squirrels usually thrive in small forest fragments they can provide important role as seed dispersers and prey for mesopredators.

Key words: Sciuridae, South America, Central America, rodents, tropical forests.

Word count: 9970

4.2 Introduction

Squirrels (family Sciuridae) contains 292 species divided in 60 genera worldwide, and is the second most diverse family of Rodentia (Koprowski et al. 2016), surpassed only by Muridae (Catzeflis et al. 1992). The family, which contains tree, ground and gliding squirrels, marmots, chipmunks and prairie dogs, occurs in all continents except Antarctic and Australia (Koprowski et al. 2016), although squirrels were introduced to the latter in the 20th century (Long 2003). The family achieves its peak of diversity in the equatorial forested regions, with some countries of Southeast Asia reaching a diversity up to 50 species at a regional scale (Koprowski & Nandini 2008), and up to 8 species being able to share the same forest habitat in Gabon, Africa (Emmons 1980).

Sciuridae is a basal rodent family first appearing most probably in North America during late Eocene, before westward movement into Asia and, around 3.4 million years ago during Great American Interchange, southward to South America (Koprowski et al. 2016). The earliest fossil records are teeth of *Hesperopetes thoringtoni* from 36 m.y.a., but nearly complete specimens of *Douglassciurus jeffersoni* were found in the same period (Koprowski et al. 2016). In most of the post cranial features, *Douglassciurus jeffersoni* is nearly indistinguishable from modern tree squirrels, with the few differences in cranial features still pointing to a very similar lifestyle to modern species (Emry & Thorington Jr. 1984).

Ecologically, squirrels are mainly herbivores, acting both as seed dispersers and seed predators (Thorington et al. 2012), with potential to consume more than 90% of seed production in some situations (Steele et al. 2004). Several species, such as *Sciurus variegatoides* and *Guerlinguetus brasiliensis*, are specialized to consume hard shelled nuts, while others, such as *Sciurus niger* and *Tamiasciurus hudsonicus*, are capable of caching food for long periods. Fungi are present in the diet of several species, which can act as spore dispersers (Hendricks & Hendricks 2015). Although mainly herbivorous, most species consume invertebrates to some degree, and occasionally even small vertebrates such birds, rodents, lizards and small snakes. At least one species, the Malaysian shrew-faced Squirrel *Rhinosciurus laticaudatus*, is a cursorial insectivore (Koprowski et al. 2016).

In contact with humans, squirrels can be considered desirable synanthropes in parks or pets but can also provide food or pelts as game species (Barragán et al. 2007, Bertolino & Lurz 2013). However, they can be also viewed as pests due to the transmission of zoonotic diseases, damage to crops, gardens and infrastructure such as cables and power stations (Signorile & Evans 2007, Montes et al. 2011). Although very resilient to habitat perturbations, habitat loss is a threat for 25 of the 31 squirrel red-listed by the IUCN, in the categories Vulnerable, Endangered or Critically Endangered (IUCN 2017). Persecution due to crop and infrastructure damage threatens at least five species, and although several species are hunted, only one (*Marmota sibirica*) is red-listed primarily due to overexploitation (Clayton 2016). Squirrels have also an enormous invasive capacity, as demonstrated by *Callosciurus erythraeus* and *Sciurus carolinensis*, which are able to successfully colonize new areas from as few as 10 initial individuals, causing considerable environmental damage in the new areas and being extremely difficult to eradicate (Palmer et al. 2007, Bertolino & Lurz 2013).

Squirrels are powerful model organisms with potential to answer a great variety of questions. Due to their diurnal activity (except for the flying squirrels), small living area and caching behavior, squirrels are good models for ecological studies, including subjects such as optimum foraging (Bowers et al. 1993), predator avoidance (Brown et al. 1992), food choice (Summers & Proctor 1999), cache distribution (Steele et al. 2014) and other animal-plant interactions (Visser et al. 2011, Mendes et al. 2016). Because of the great diversity and relatively recent diversification, squirrels also provide insight into biogeographic studies (Mercer & Roth 2003), even when the limited fossil record is considered. Squirrels have long been used for physiological studies due to their small size and easy maintenance in captivity, with special attention to studies on bioenergetics, torpor, hibernation, thermal regulation (Humphries et al. 2005), and neurophysiology of memory (Smulders et al. 2010). Evolutionary studies have also benefited by the use of sciurids as models since tree squirrels are considered living fossils (Emry & Thorington Jr. 1984), and thus, gliding squirrels are the only gliding mammal taxa for which the ancestral form remains extant, which permits better understanding of the function and evolution of each morphological feature related to the evolution of gliding locomotion (Thorington et al. 1997).

Although tropical regions are hotspots of squirrel diversity, most of our current knowledge about sciurids was obtained from less than 10 species inhabiting the northern hemisphere temperate zones, with most of the tropical and subtropical species producing few or no studies (Koprowski & Nandini 2008). For 136 arboreal and 44 gliding squirrels species, only nine species (*Sciurus carolinensis*, *S. vulgaris*, *S. niger*, *S. aberti*, *Tamiasciurus hudsonicus*, *Glaucomys sabrinus*, *G. volans*, *Callosciurus erythraeus* and *Pteromys volans*) comprise more than 50% of the publications available and none of these species occur naturally in the neotropical biogeographic region (Koprowski & Nandini 2008). In South America, the taxonomy of the group is also unclear, with the number of recognized forms being usually around 3 genera with 16 species (Thorington et al. 2012) and 7 genera with 19 species (Vivo & Carmignotto 2015). In such absence of information, it is not possible to assume that Neotropical squirrels behave similarly to the well-studied Nearctic species, which inhabit very different environments, or to the Paleotropic species, which are also poorly studied and phylogenetically more distant than the Neoarctic forms (Koprowski & Nandini 2008, Thorington et al. 2012). Neotropical squirrels are expected be less seasonal than its Nearctic counterparts, with long reproductive season and two or more litters per year (Heaney & Thorington 1978) and possibly caching less food, what may affect its hole as seed dispersers. Further ecological differences may occur due to the plants in which the squirrels rely as main food source, with some Neotropical squirrels being reported as heavily associated with palms (Emmons 1984).

Here we examine the current status of the knowledge about the ecology of Neotropical squirrels, identifying the main information gaps and highlighting the species and regions with great knowledge deficits. The main objective of this field synopsis is to gather in one place the few and dispersed information available about the ecology of neotropical squirrels, facilitating the access to the data and pointing the direction for future studies. Although we focus on species interactions (i.e. diet, seed dispersal, mutualism, agonistic interactions, predation and parasitism), we also examine information about their density across the neotropical region.

We decided to include all squirrel species of the entire Neotropical biogeographic region because it makes more biological sense than use of the artificial borders of countries. This way, our study includes a total of 28 squirrel species, from 20 countries, ranging from latitude 25ºN to 35ºS. Ground and flying squirrels do not occur in Neotropical regions and were not considered, although some species occur in the transition between the Neotropical and Nearctic biogeographical regions, in Mexico (Koprowski et al. 2016).

4.3 Methods

To review the available knowledge on neotropical squirrel ecology, we searched the Google Scholar library using the scientific names, synonyms and popular names for all the 28 squirrel species described to occur in the neotropical region (Vivo & Carmignotto 2015, Koprowski et al. 2016). We decided to use Google Scholar because it is by far the largest online library available today, with around three times the size of ISI Web of Science, including gray literature of difficult access (Orduña-Malea et al. 2014). All documents found, including books, journal papers, thesis, dissertations, and scientific notes were recorded. We searched the content of the literature in English, Spanish or Portuguese, and although it is possible that some manuscripts were published in other languages (such as French due to French Guyana), we did not expect these manuscripts to be abundant. Moreover, a simple abstracts in any of the three searched languages, together with the scientific names would be enough for the Google Engine to locate many of the manuscripts in other languages, but we found no manuscripts in other language than English, Spanish and Portuguese. We are also not aware of any academic periodical in Latin America that uses any other language instead of the country’s official languages.

The documents found in the search were than searched for ecological interaction data. Here, we defined ecological interaction data as any information about a species of squirrel interacting with other living organisms (i.g. feeding upon a seed, mobbing a bird of prey, being predated by a felid, etc.), including conspecifics (i.g. nest sharing). From these documents, we recorded the interacting species to the most precise taxonomic level possible, the type of interaction, the geographic coordinates and the publication year. Vague taxonomic information of the interactions, such "eat fruits and seeds" or "parasitized by fleas" were not included as a record. When the coordinates were not specified, a common problem in older publications, we recorded the description of the study site and when possible, obtained the coordinates from the Google maps database. Since population density and individual home ranges are important for population dynamic studies, we also considered studies containing this information as records, but for methodological simplicity, we analyzed these records (here and after "density records") apart from the ecological interaction records. Both the ecological interaction and population density records are provided as supplementary material (Supplementary material Table S1, S2 e S3).

Since there is no consensus about the number of squirrel species in the Neotropics, we decided to use the same classification used by the sixth volume of the Handbook of Mammals of the World (Koprowski et al. 2016) for the Central American species, and the classification proposed by Vivo and Carmignotto (2015) for the South American species, which resulted in a total of 28 squirrel species (Table 1). The species names were recorded as originally published, and later, converted to the classification system used in this study. In situations where a publication cites a squirrel species that was later divided into two or more species, if these species are not sympatric, we attributed the ecological record to the new species that occurred in the study site, but when the new species were sympatric, we recorded the squirrel only by the genus. To avoid inflating the list of species with which the squirrels interacted by citing the same species more than once by synonymous names, we also reviewed the classification of these species, for the most recent taxonomic classification in use and removed synonyms. For this, we accessed online taxonomic databases, such the catalogueoflife.org online database, and searched by each species name found to interact with neotropical squirrels, standardizing the synonyms and correcting mistyped names. As additional information, when provided, we recorded the forest area (in km²) from the manuscripts, however, the authors frequently reported the area of the study site, but not the entire area available to the squirrels, with several study sites being connected with continuous forests. To correct this, we verified whether the study sites were connected with larger forest patches, correcting the information. Forests with more than 10,000km² were considered as continuous habitat. Finally, for further analysis, we divided the Neotropical region into ecoregion, following the map shapefiles provided online by The Nature Conservancy (The Nature Conservancy 2009). Linear models were used to test possible relationship between variables, which were log-linearized when required.

4.4 Results and Discussion

4.4.1 Population estimates

We found 48 publications that contained 126 population densities for 16 squirrel species (Supplementary material, Table S1). These records came from 72 sites, from 9 of the 20 countries within the neotropical biogeographic region in which squirrels occur (Supplementary material, Figure S1). Squirrels do not occur naturally in Uruguay, Chile and in the Caribbean insular countries, with the exception of Trinidad and Tobago (Vivo & Carmignotto 2015, Koprowski et al. 2016). We also found records from a population of the neotropical *Sciurus aureogaster* in Florida Keys, Florida, U.S.A., where it is considered an exotic invasive species (Palmer et al. 2007). Most of the densities (117) were recorded in Moist Broadleaf Forests, for 16 squirrel species. In Argentina, seven records of *Callosciurus erythraeus,* are located in the Pampas biome, although the squirrels inhabit the forest patches, both natural or plantations, within the biome. Other seven densities, from four species (*C. erythraeus, Guerlinguetus brasiliensis*, *Sciurus aureogaster* and *Simosciurus nebouxii*) were recorded from other ecoregions, such as dry broadleaf forests, coniferous forests, flooded grasslands and savannas, xeric shrublands, and mangroves (Supplementary material Table S1), however, some of these results may also be artefacts of the mapping scale, with the squirrels inhabiting other forest habitat patches within these ecoregions.

The species with most records were *Guerlinguetus brasiliensis* and *Hadrosciurus spadiceus*, with 40 and 21 records, from 14 and 11 publications respectively. These two squirrels sum 48.4% of the records and 52% of the publications found, while for other 12 species, no population density data was found. The estimated densities range from 0.16 to 89 ind/km² (mean 14.8) for *Guerlinguetus brasiliensis* and 0.8 to 15.2 ind/km² (mean 4.7) for *Hadrosciurus spadiceus*. This large differences in densities are most probably caused by variation on habitat characteristics, not explored on this review, but may also be caused by imprecisions in the transect sampling methods, since some squirrels are known to engage in defensive behaviors which make then extremely difficult to detect by the observer (Mendes & Cândido-Jr 2014).

Linear-transect was the most common estimation method, used for 114 of the records. Other methods used were Point-transect (10 records) and with one record each, capture-mark-recapture and capture-mark-observation, the last used only to estimate home-range. Only 73 of these records (58%) provided absolute density estimations (reported in individuals per km^2 or ind/ha), while 52 records (41.2%) presented only relative density estimations, reported in individuals/10 km of transect. The point-transect method appear to overestimate the densities, with records as high as 1530 ind/km^2 for *Callosciurus erythraeus* (Benitez et al. 2013) and the lowest density record being 323 ind/km^2 (Benitez et al. 2013), while the maximum density found for any other method was only 100 ind/km^2 (Estrada & Coates-Estrada 1985). However, since we found no species sampled by both methods, it is not possible to safely compare the results.

Using all the records collected by the line-transect method, reported in absolute density values and paired with forest area information ($n = 61$), we observed a negative relation between squirrel densities and forest area ($\beta = -0.255$, $R^2 = 0.20$, $t = -4.039$, $p << 0.05$), with higher squirrel densities occurring in smaller forest patches (Figure 1-A). At the species level, only *Guerlinguetus brasiliensis* have records enough to perform a regression ($n = 23$), and again, higher densities where observed on smaller forest patches ($\beta = -0.258$, $R^2 = 0.25$, $t = -2.902$, $p = 0.008$) (Figure 1B). Since 37% of the records available to regression are for *G. brasiliensis*, we repeated the regression without this species ($n = 38$) and confirmed that the result was consistent ($\beta = -0.2982$, $R^2 = 0.15$, $t = -2.756$, 0.009) (Figure 1-C), indicating that higher squirrel densities in smaller forest patches is indeed a pattern occurring in several Neotropical species. We also tested for possible latitude effects by using only records from moist broadleaf forests ($n = 62$), to reduce the effect of habitat structure, and we found no effect of latitude on squirrel densities ($\beta = 0.021$, $R^2 = 0.009$, $t = 1.261$, $p = 0.21$). The same result was obtained when using records only from *G. brasiliensis* ($n = 23$) to reduce possible effects of variation between squirrel species ($\beta = 0.014$, $R^2 = -0.013$, $t = 0.46$, $p = 0.64$).

The higher density of squirrels in smaller forest patches is a pattern already known for Nearctic and Palearctic squirrels, but was unknown for Neotropical species (Koprowski 2005). The mechanism that allow a higher abundance of squirrels in small forest fragments is still not clear, but some possible explanations are higher food productivity at forest edges, lower predation or competition with other rodents (Koprowski 2005, Mendes et al. 2016).

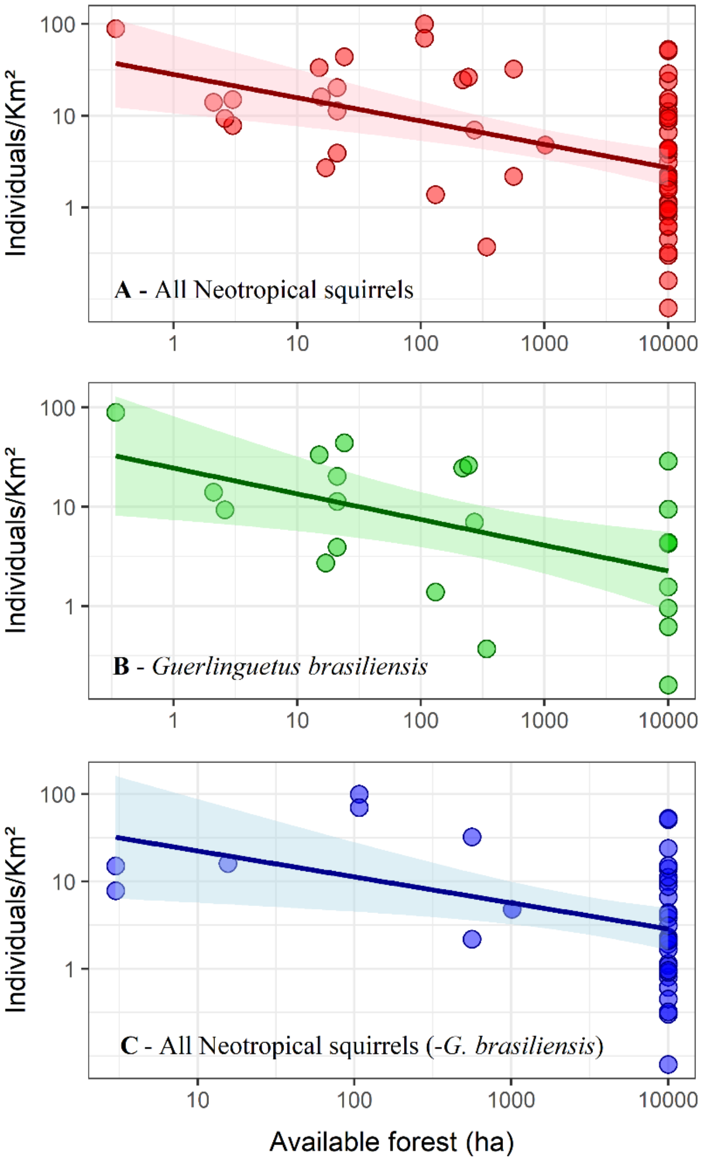

Figure 1: Effect of forest area on Neotropical squirrel density, using the records of all species available (A), and for the species with most records, Guerlinguetus brasiliensis (B), showing higher densities at smaller forest areas. Since 37% of the records are from G. brasiliensis, we repeated the regression excluding the species to avoid possible bias (C), but the results remain constant.

4.4.2 Ecological interactions

We found a total of 155 publications (Supplementary material, Table S2 and S3) that contained ecological interactions for 20 of the 28 species of Neotropical tree squirrels, from

14 countries (Figure 2). No publication containing ecological information was found from Belize, Bolivia, El Salvador, Guyana, Honduras and Suriname, countries in which squirrels are known to occur (Koprowski et al. 2016). A deep and extensive search into the old and gray literature from these countries may probably reveal some ecological records about the local squirrels, however, we decided to not increase the search effort in these countries to avoid geographical bias, keeping the results comparable between countries. We also found ecological interaction records from the *Sciurus aureogaster* population in Florida Keys, Florida, U.S.A.

The 8 species for which we found no data, all from the same genus, are *Microsciurus boquetensis, M. isthmius, M. otinus, M. sabanillae, M. santanderensis, M. similis, M. simonsi and M. venustulus*. These species are distributed through Costa Rica, Panamá, Colombia and Ecuador, in a region with extremely high levels of biodiversity and endemism, including the south range of the so-called Mesoamerica biodiversity hotspot and most of the Chocó-Darién-Western Ecuador biodiversity hotspot (Vivo & Carmignotto 2015). Although highly biodiverse, scientific research in the Chocó regions is challenging due to the rough terrain, lack of basic infrastructure and the presence of guerrilla groups (Suman 2007). It is also important to highlight that the taxonomy and phylogenetic relationships of *Microsciurus* still are not completely resolved, and thus, the genus is very susceptible to changes in number of recognized species. However, even if the entire genus *Microsciurus* were treated as one unique “species”, it would still be a very poorly studied squirrel taxa in the Neotropics, for which we found only 7 publications and 11 interactions in the literature.

In absolute numbers, Brazil is the country with the most publications with 58 documents (37.4%) found for 14 squirrel species, followed by Peru (18 publications, 11.6%) and Mexico (15 publications, 9.7%). In Brazil, the distribution of the publications is very irregular, with São Paulo alone counting 22 publications (40% of Brazilian publications), followed by Minas Gerais and Paraná with only 7 publications each, whereas in the large and biodiverse states of Amazonas and Pará, within the Amazon forest biome, we found only 1 and 3 publications respectively (1.7 and 5.2% of Brazilian publications). For the other 9 states in which squirrels are known to occur, not even one publication contained ecological data. These nine states are Amapá, Ceará, Mato Grosso, Paraíba, Rio Grande do Norte, Rondônia, Santa Catarina, Sergipe and Tocantins. This irregular distribution of publications through Brazilian territory appear to follow the overall distribution of scientific research in the country, in which most of the scientific production, research and development investments, universities and researchers are located in the Southeastern regions, mainly in São Paulo state (Sidone et al. 2016).

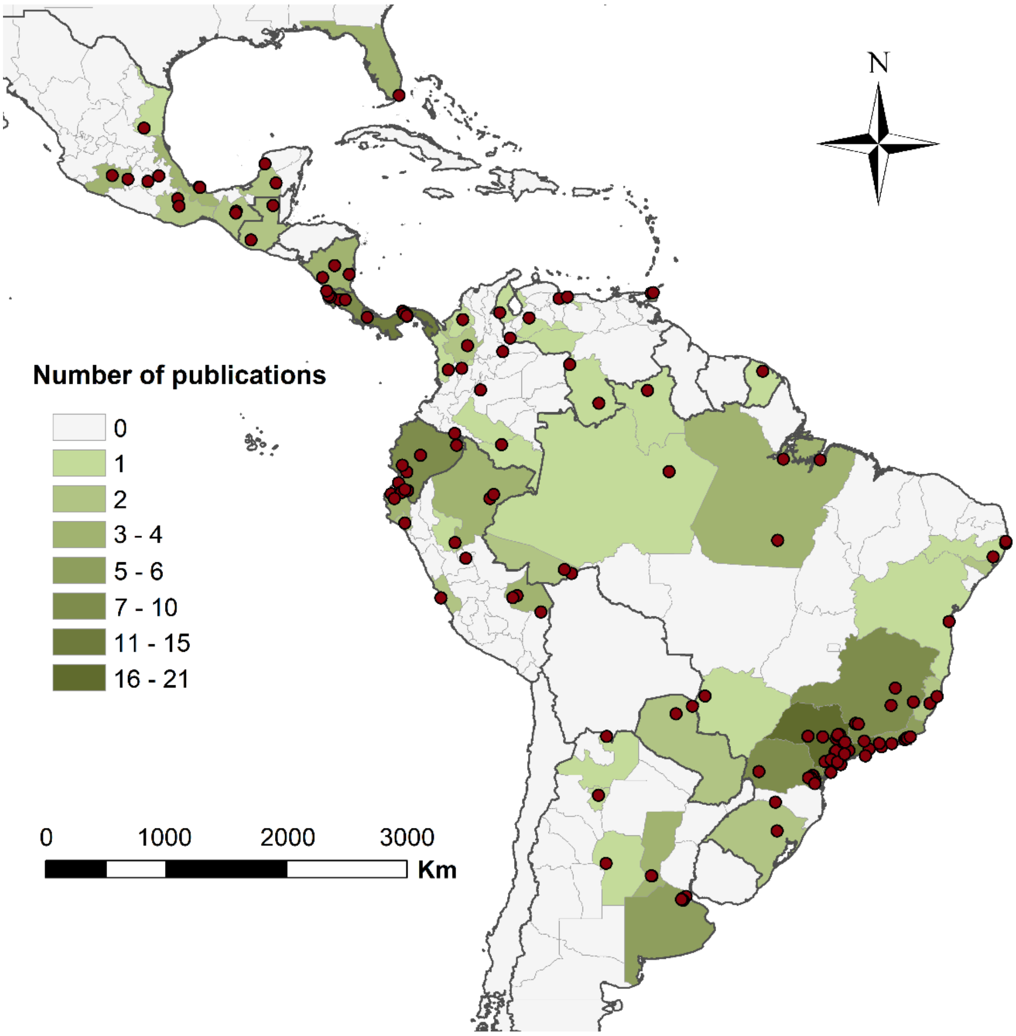

Figure 2: Distribution of the study sites of the publications containing ecological records for Neotropical squirrel species found in Google Scholar database. The color of the countries/states represents the number of publications for that state/country. The bigger countries were divided in states to reduce the variation in the size of the polygons.

In Peru, the publications are mainly in the north and east side of the country, in which the departments of Loreto, Madre de Dios and Piura have 3, 3 and 4 publications respectively. In the capital Lima, we found 2 publications about the ecology of an introduced population of *Simosciurus nebouxii* (Jessen et al. 2010). We found no publications about squirrel ecology in 19 departments of Peru. In Mexico, the publications for 3 squirrel species (*Sciurus aureogaster, Sciurus deppei and Sciurus yucatanensis*) were found in 8 states along the southern half of the country, being Tamaulipas the northernmost of these states where data were found for a native neotropical squirrel. With exception of the invasive population of *Sciurus aureogaster* in Florida Keys, the northernmost ecological interaction found in the literature for a native neotropical squirrels was a *Sciurus deppei* being parasite by a tick (*Ixodes tamaulipas*) (Kohls & Clifford 1966).

In other countries, fewer publications have been produced. In Argentina, the number of publications is low (8) because squirrels occur naturally only in the extreme north of the country (Vivo & Carmignotto 2015). However, 6 of these publications are about the ecology of the invasive *Callosciurus erythraeus,* a species from eastern and southeast Asia, introduced in 1970 (Guichón & Doncaster 2008). These publications are mainly from Lujan and Buenos Aires, and highlight the overall concern about the species, which is proved to cause damage to forest species (Pedreira et al. 2017) and is increasing its area of occurrence (Benitez et al. 2013). In Panamá, the small area of the Panamá Canal Zone, which include the internationally recognized research areas of Barro Colorado Island and the Parque Nacional Soberanía, are the study site for 11 publications with ecological data for 2 squirrel species (*Notosciurus granatensis* and *Sciurus yucatanensis*). Although small in area, Costa Rica has 8 publications for 3 squirrel species (*Sciurus deppei, Sciurus variegatoides* and *Syntheosciurus brochus*), with 6 publications from Guanacaste province. Finally, in Ecuador, five of the 9 publications found are focused on parasitology, since the species *Notosciurus granatensis* and *Simosciurus nebouxii* are hosts of *Yersinia pestis* (Macchiavello 1957), *Leishmania ecuatorensis* (Grimaldi Júnior et al. 1992) and *Rhodnius ecuadoriensis* (Grijalva et al. 2012), which is a vector of the Chagas disease. However, with exception of one record of a plant species, *Gustavia angustifolia,* being used as food by one squirrel species, *Simosciurus stramineus* (Cornejo 2015), we found no other data about the diet of squirrels in Ecuador.

The documents found with ecological data were published between 1947 and 2017, and increased after the year 2000 from 1.04 to 5.61 publications per year (Figure 3). This increase in the amount of publications may represent an increase in the amount of studies about Sciurid ecology, but may also be an artefact caused by the better indexation of newer documents by the Google Scholar engine (Orduña-Malea et al. 2014).

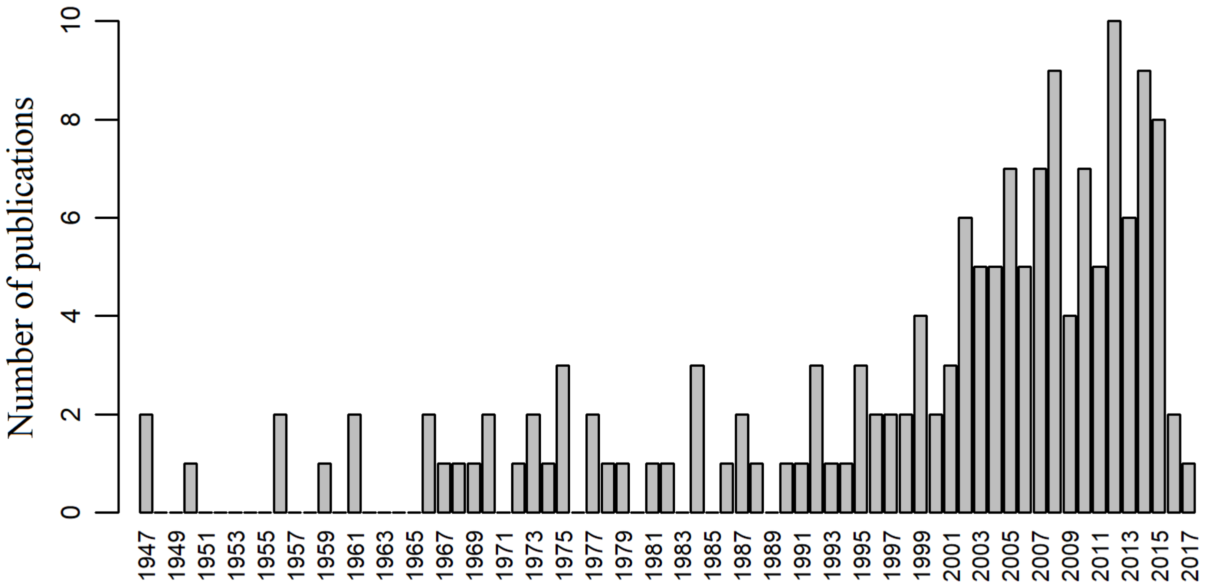

Figure 3: Year of publication of studies found in the Google Scholar database containing ecological records for Neotropical squirrel species.

4.4.3 Squirrel species

The three most studied species, *Guerlinguetus brasiliensis, Notosciurus granatensis* and *Sciurus variegatoides*, account for 53% of the publications, with the other 47% divided between 17 species (Figure 4), and 8 species for which we found no publication containing ecological interactions. In these publications, we found records of 649 ecological interactions between 20 squirrel species and at least 351 other species, from 123 families (Supplementary material, table S3). From these 351 species, 223 are plants, 109 are animals and 5 are fungi species.

As a clear reflection of the higher number of studies, *Guerlinguetus brasiliensis* and *Notosciurus granatensis* are also the squirrels with higher numbers of documented interspecific interactions, with 89 and 54 species respectively. Indeed, the number of publications explains 75% of the variation in the number of species known interactions with the squirrel species (Linear model with log transformed variables: $\beta = 1.068$, $t = 7.458$, $p << 0.05$) (Supplementary material, Figure S2). Although it is not possible to use these data to estimate the number of species expected to interact with these squirrel species, the existence of a severe gap in the basic ecological knowledge of most species it is very clear. Beyond the 8 species without any ecological interaction data, only 8 squirrel species are documented to interact with more than 10 other species, and for 8 squirrel species (*Guerlinguetus aestuans,* *Hadrosciurus pyrrhinus*, *Microsciurus alfari*, *Microsciurus flaviventer*, *Microsciurus mimulus*, *Sciurillus pusillus*, *Sciurus richmondi* and *Simosciurus stramineus*) we found 5 or less interactions in the literature.

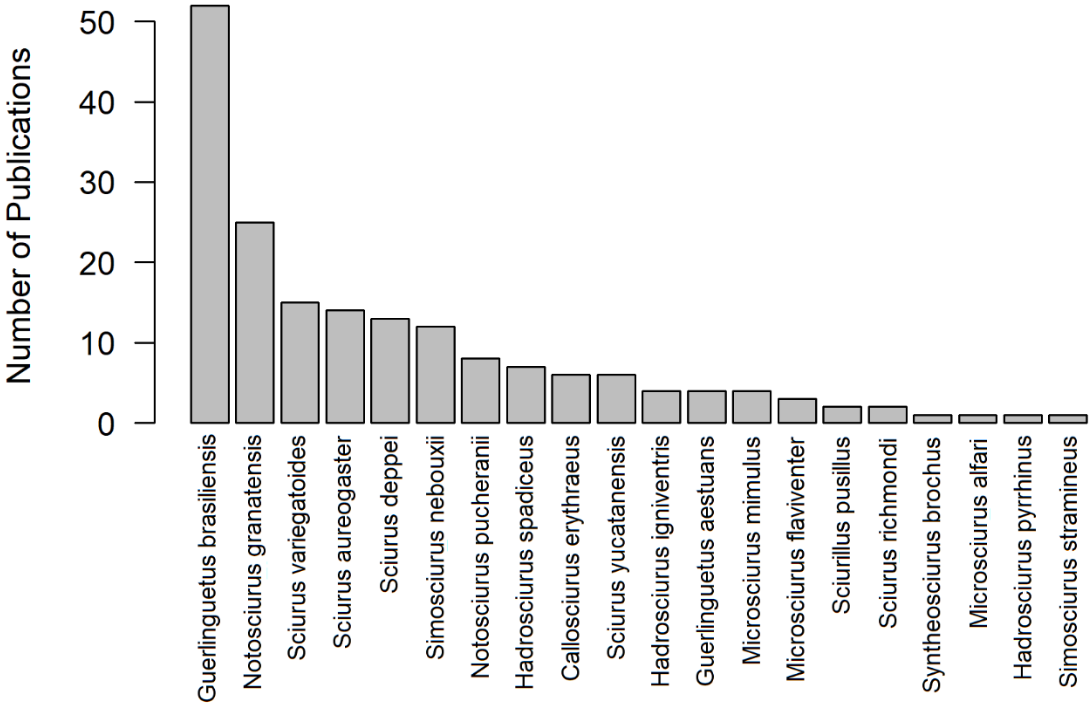

Figure 4: Number of publications found in the Google Scholar database containing ecological records for Neotropical squirrel species. We found no ecological records for the species *Microsciurus boquetensis, M. isthmius, M. otinus, M. sabanillae, M. santanderensis, M. Similis, M. simonsi* and *M. venustulus*.

A possible explanation for the absence of ecological data for eight species of *Microsciurus* is related to the difficulty in locating and observing these small bodied rodents (~100 grams) in the high and dense forest canopy. These small squirrel species are generally bark gleaners (Jessen et al. 2013a, 2016), consuming plant exudates and insects, that are also difficult to identify. Other similar sized species are also affected by the lack of basic ecological data, such as for *Sciurillus pusillus* for which we found no diet information. The four diet items found for a species of the *Microsciurus* genus were fruits and seeds of the palm *Oenocarpus bataua* consumed by *Microsciurus mimulus* (Franco-Quimbay & Rojas-Robles 2014) and anecdotal information of 3 mushroom species being consumed by *Microsciurus flaviventer* (Vasco-Palacios et al. 2008). The very limited and isolated distribution range of most *Microsciurus* species may also be a factor negatively affecting the number of studies.

Beyond the amount of research, the ecological data available for different squirrel species were also affected by the type of the studies, with more interactions described for squirrels that were the target of natural history studies rather than for squirrels for which studies tested hypotheses. This happened because natural history studies usually contain extensive lists and tables with the species with which the squirrels interacted (i.e. consumed, dispersed, was predated by, used for nest building, etc.). Natural history studies were found for most of the well-studied species (Heaney & Thorington 1978, Glanz 1984, Alvarenga & Talamoni 2006, Della-Flora et al. 2013), as well as for exotic squirrels in Argentina (Pedreira et al. 2017) and in Florida Keys, United States (Brown & McGuire 1975).

To better explore the gaps in the information available about ecology of neotropical squirrels, we divided the interactions into: a- Diet: interactions in which a species was eaten by the squirrels; b- Dispersal: interactions in which seeds or fruit were dispersed or cached by squirrels; c- Resources: interactions with species which provide non-diet resources for the squirrels, d- Non-aggressive interactions: non-aggressive interactions between a squirrels and other vertebrates; e- Agonistic interaction: non-lethal agonistic interaction with other vertebrate, such as territorial defense and mobbing; f- Parasitic: interactions with species that are internally or externally parasites of squirrels; g- Predation: interactions with species that prey upon squirrels (Figure 5).

4.4.4 Diet

For feeding interactions, we found 301 records from 68 publications, in which 187 species were consumed by 15 squirrel species. Plants were the main source of food for these rodents, with 287 (95%) of the records and 174 species consumed. From the 57 plant families consumed, Arecaceae was the most recorded, with 82 records for 30 palm species, followed by the family Fabaceae with only 20 records for 13 species (Supplementary material, Figure S3). We found no feeding data for 13 squirrel species, including *Hadrosciurus pyrrhinus, Microsciurus alfari, Sciurillus pusillus, Sciurus richmondi* and *Sciurus yucatanensis*. Moreover, we found in the literature less than 5 consumed species for the squirrels species *Guerlinguetus aestuans, Hadrosciurus igniventris, Hadrosciurus spadiceus, Microsciurus flaviventer, Microsciurus mimulus, Notosciurus pucheranii* and *Simosciurus stramineus* whereas for the well-studied *Guerlinguetus brasiliensis* and *Notosciurus granatensis*, we found 61 and 31 species consumed, respectively).

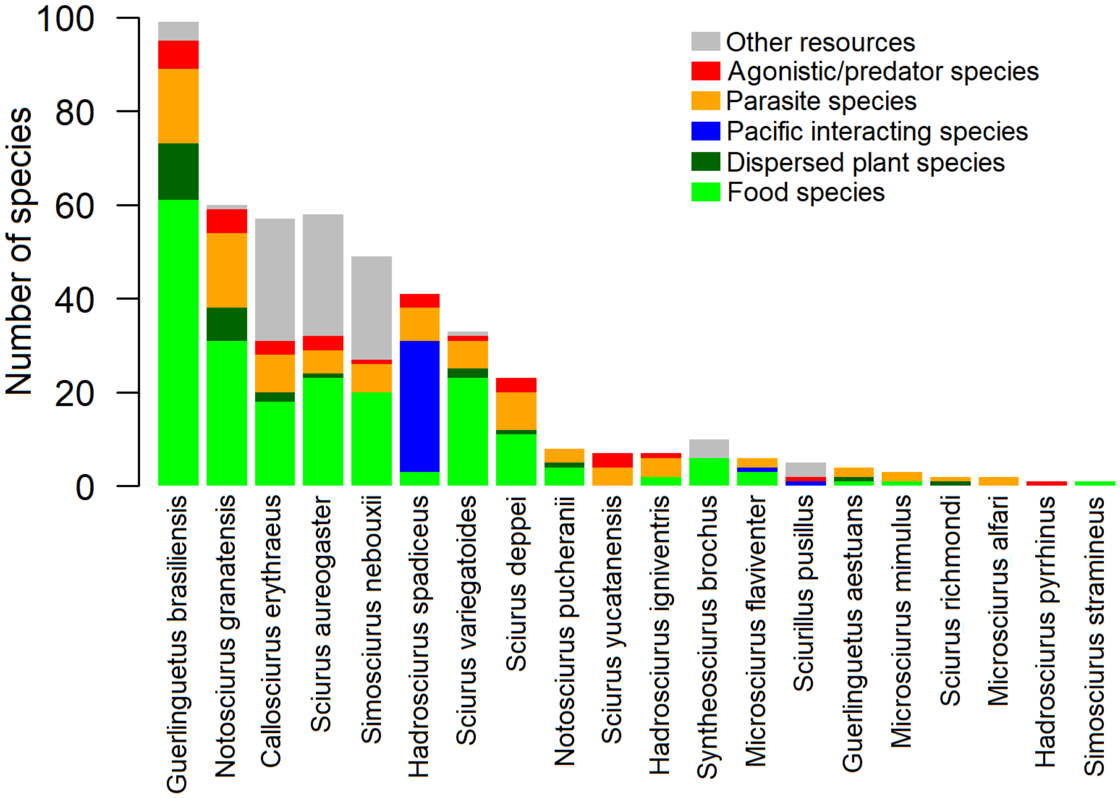

Figure 5: Number of species confirmed to interact with Neotropical squirrel species, according to the interaction type. The division of interaction type follows the method described in the manuscript. For instance, although parasitic interactions are agonistic by nature, here they are considered a group apart. The interaction group "Other resources" contain mostly plants species used by the squirrels as nest site or nest building material.

Although fungi is consumed by several squirrels species around the world (Koprowski et al. 2016), we found records of only 5 species of fungi (*Lentinula raphanica*, *Trogia buccinalis*, *Hydropus cavipes*, *Pleurotus sp.* and a non-identified *Tricholomataceae*) being consumed by 3 species of squirrels (*Guerlinguetus brasiliensis*, *Microsciurus flaviventer* and *Notosciurus granatensis*) (Heaney & Thorington 1978, Bordignon & Monteiro-filho 1999, Vasco-Palacios et al. 2008). A similarly small number of records was found to consumed invertebrates, in which we found only four beetle species (*Speciomerus giganteus*, *Pachymerus cardo*, *Golofa* sp. and *Revena rubiginosa*) eaten by 4 squirrel species (*Guerlinguetus brasiliensis, Hadrosciurus igniventris, Sciurus variegatoides* and *Simosciurus* *nebouxii*) (Silvius 2002, Negrão & Valladares-Pádua 2006, Gálvez & Jansen 2007). Although neotropical squirrel species are known to consume fungi and invertebrates to some degree, the precise identification of the consumed species is usually not provided, being described simply as “fungi”, “mushroom” or “larva”.

Invertebrates appear to be an common food source for squirrels, which in some situations prefer to prey upon beetle larva infested rather than uninfested fruits (Alves et al. 2018). It is very probable that the difficulty in observing when squirrels are consuming infested fruits contribute to the underestimation of invertebrates as a food source for these rodents (Silvius 2002). Another factor that should underestimate the importance of invertebrates as a food source for neotropical squirrels is that the content of the diet of the bark gleaner species remains unknown, and bark gleaners consume a significant amount of insects (Emmons 1980, Jessen et al. 2013a, 2016). The consumption of small vertebrates by squirrels also occurs (Koprowski et al. 2016), but is nearly unexplored for neotropical species, and we found only one record of a *Simosciurus nebouxii* consuming the eggs of *Phytotoma raimondii*, an endangered bird in Peru (Nolazco & Roper 2014). The consumption of lichen was recorded only for *Guerlinguetus brasiliensis* (Alvarenga & Talamoni 2006), and at least 6 species (*Sciurus aureogaster, Sciurus deppei, Sciurus yucatanensis*, *Notosciurus granatensis, N. pucheranii* and *Callosciurus erythraeus*) were reported to damage crops of corn, cocoa and trees (Best 1995, Best et al. 1995, Mollineau et al. 2008, Ramos-Lara & Cervantes 2011, Pedreira et al. 2017).

4.4.5 Seed dispersal

For seed-dispersal interactions, we found only 46 records, from 23 publications, of 28 plant species being dispersed by 9 squirrel species (Supplementary material, Figure S4-A). It is important to highlight that by “dispersal”, we mean simply that a propagule was moved away from the mother tree, but does not account for the viability of these propagules or whether the squirrel did so intentionally (caching behavior) or not, since this information was not provided in most publications. However, it was possible to attribute the dispersal of at least 13 plant species to the cache behavior of four squirrel species (*Guerlinguetus brasiliensis, Notosciurus granatensis, Notosciurus pucheranii* and *Sciurus aureogaster*).

The plant family with the most records of dispersal is Arecaceae, with 15 palm species and 31 records (Supplementary material, Figure S4-B). The well-studied *Guerlinguetus brasiliensis* and *Notosciurus granatensis* were recorded to disperse 12 and 7 plant species respectively, whereas the other 7 squirrel species for which dispersion interactions were found (*Callosciurus erythraeus, Guerlinguetus aestuans, Notosciurus pucheranii, Sciurus aureogaster, Sciurus deppei, Sciurus richmondi, Sciurus variegatoides*) were documented to disperse less than 3 species each. The potential of small bark gleaner species for seed dispersal is an open subject for future studies, since the available data for these species is not enough to confirm whether these species eat seeds. Only one study was found about endozoochoric seed dispersal by squirrels, and surprisingly, it is about the exotic *Callosciurus erythraeus* in Argentina, which was recorded to disperse viable seeds of two exotic tree species, *Morus alba* and a unidentified species of *Casuarina* (Bobadilla et al. 2016). Although rodents are generally not considered endozoochoric seed dispersers, they do have this capability (Wells et al. 2009, Duron et al. 2017), and squirrels are no exception (Penner et al. 1999, Bobadilla et al. 2016). Nevertheless, we found no publication that evaluated the subject for native neotropical species.

Further explorations of the diet information were severely hindered by the imprecision of the descriptions found in the literature. Beyond the publications with vague diet description, the publications with identification of the consumed species often did not specify which vegetal tissues were consumed. We found 141 records in which a squirrel is described to consume the fruit of an identified species, however, it is not clearly stated whether the seeds within the fruit were consumed or not. In a similar way, the seed dispersal information is also not fully reliable since the authors rarely report if the squirrels indeed displayed caching behavior or simply carried the seeds out of the observers' sight (possibly consuming the seeds when out of sight). With the exception of one study (Bordignon & Monteiro-Filho 2000), we found no manuscript that reported dispersal distances and whether the cached seeds germinated or not. As a result, it is not possible to access the degree to which Neotropical squirrel acts as seed dispersers. This information is critical to our understanding about degraded forest dynamics, considering the importance of seed mortality and dispersal in tree recruitment (Steele et al. 2004), the squirrels persistence and higher densities in smaller forest patches, which affect seed predation in a landscape scale (Mendes et al. 2016).

4.4.6 Resources

For other non-alimentary resources, we found 97 records for 8 species. At least 47 species of plants, of 31 families, were used as nest sites or nest materials by five squirrel species (*Guerlinguetus brasiliensis, Sciurillus pusillus, Sciurus aureogaster, Simosciurus* *nebouxii* and *Syntheosciurus brochus*). No data about the trees used as nest site were found for the other 23 neotropical squirrel species. The use of structures built by other organisms as a nest site was observed for two species, the Amazon pygmy squirrel, *Sciurillus pusillus*, which builds its nest inside abandoned termite nests (Jessen et al. 2013b), and *Syntheosciurus brochus*, which built its nest in a cavity excavated by woodpeckers (Giacalone et al. 1987). Leaves, twigs and bark of 38 plant species were used by 7 squirrel species to build dens and line the nests. Bark stripping, a behavior capable of cause severe damage to trees (Pedreira et al. 2017), may be performed to gather nest materials (Mendes & Cândido-Jr 2014) or feeding (Snyder 1992), and was reported for five squirrel species (*Guerlinguetus brasiliensis, Notosciurus granatensis, Sciurus aureogaster, Sciurus variegatoides* and the exotic *Callosciurus erythraeus*), on least 30 tree species. Geophagy, the consumption of clay, was recorded for *Hadrosciurus spadiceus* in a Peruvian forest (Brightsmith & Munoz-Najar 2004).

4.4.7 Non-aggressive interactions

Records of non-aggressive interactions were found for only three squirrel species. *Hadrosciurus spadiceus*, is known to participate in mixed bird flocks in the Brazilian Pantanal, together with at least 38 birds species, including the nuclear species *Cantorchilus guarayanus* (Della-Flora et al. 2013). The squirrel *Microsciurus flaviventer* is also known to participate in mixed bird flocks, but with the exception of the nuclear species, the bird *Thamnomanes caesius*, the author did not specified which other bird species participate in the mixed flock (Buitron-Jurado & Tobar 2007). The neotropical pigmy squirrel, *Sciurillus pusillus,* was observed to simultaneously share nests, built inside an abandoned termite nest, with conspecifics in the Peruvian Amazon forest (Jessen et al. 2013b). Nest sharing is known to occur in temperate species such as *Glaucomys volans* (Layne & Raymond 1994), *Sciurus niger* and *Sciurus carolinensis* (Koprowski 1996), but until now, the behavior was believed to be associated with thermoregulation in response to extreme cold weather, which is improbable in the warm Amazon forest. A female *S. deppei* was reported to hybridize with a male *S. yucatanensis* in captivity, in Yucatán, with 5 offspring being produced and reaching maturity, however the fertility of the offspring was not confirmed (Best et al. 1995).

4.4.8. Agonistic interaction

Agonistic interactions were recorded for only two squirrel species. *Guerlinguetus brasiliensis* was observed mobbing a *Leopardus wiedii* (Solórzano-Filho 2006), and also responded to playbacks of the owl *Glaucidium brasilianum* with aggressive mobbing behavior (Cunha et al. 2013). The squirrel *Hadrosciurus spadiceus*, was reported to fight with a pygmy marmoset, *Cebuella pygmaea*, which was foraging for insects in the squirrel's vicinity. After observing each other for about 3 minutes and a brief mutual physical aggression, the squirrel was repelled by the marmoset (Canizo & Calouro 2011).

4.4.9 Parasitic interactions

A total of 133 records of parasitic interactions were found, in which 16 squirrel species were hosts of at least 65 species of parasites (Supplementary material, Figure S5). Most of the studies found were focused on ectoparasites, with 97 records (72.9%) of 44 ectoparasite species found for 16 squirrel species. The lice genus *Enderleinellus* was the most diverse and commonly reported in the literature, with 9 species and 24 records found in 9 squirrel species. Since most records of ectoparasites came from taxonomic revisions of the parasite genus, the exact location of where the parasite-squirrel interaction was observed is usually not provided, with only vague descriptions such "Costa-Rica", "Mexico" and "São Paulo-Brazil".

For endoparasites, only 12 records (9%) of 9 species were found, from only 7 squirrel species (*Guerlinguetus aestuans, Guerlinguetus brasiliensis, Hadrosciurus spadiceus, Notosciurus granatensis, Sciurus aureogaster, Sciurus yucatanensis* and the exotic *Callosciurus erythraeus*). With the exception of one record of a tapeworm, species not identified but found in a *Sciurus yucatanensis*, the other 8 endoparasite species were nematodes. For the other 21 squirrel species, we found not even one endoparasite record in the literature.

Records for unicellular parasites were also found in the literature, with 24 records (18%) of 12 unicellular parasite forms, and a heavy bias toward species with zoonotic potential. At least four squirrel species (*Guerlinguetus brasiliensis, Hadrosciurus igniventris, Hadrosciurus spadiceus* and *Notosciurus granatensis*) are hosts of at least tree species of trypanosomes (*Trypanosoma cruzi, Trypanosoma lewis* and *Trypanosoma rangeli*) (Perruolo 1974, Revollo et al. 1998, Lainson et al. 2005). *Notosciurus granatensis* is a host of *Leishmania equatorensis* (Grimaldi Júnior et al. 1992) and *Endotrypanum* spp. (Katakura et al. 2003), whereas *Hadrosciurus spadiceus* is a host of *Giardia sp*. (Lainson et al. 2005). The mosquito species *Lutzomyia evansi*, a vector of Leishmaniosis, was reported to feed on *Notosciurus granatensis* in northern Colombia (Adler et al. 2003). The squirrel *Simosciurus* *nebouxii* was reported with four serovars of *Leptospira* (Montes et al. 2011) and the exotic *Callosciurus erythraeus* is host of *Leptospira interrogans* (Gozzi et al. 2013).

Plague, *Yersinia pestis*, was also found in *Simosciurus nebouxii*, with human transmission confirmed in Loja department, Ecuador (Macchiavello 1957). In laboratory conditions, the squirrel *Guerlinguetus brasiliensis* was vulnerable to plague infections (Mello 1968), although we found no data about such interaction in natural conditions. Other zoonotic diseases of economic importance, due to a threat to livestock, were found in the literature, with the squirrels *Sciurus variegatoides* and *Notosciurus granatensis* killed by *Besnoitia darlingi* infection (Schneider 1967), whereas *Hadrosciurus spadiceus* is a host of *Eimeria damnosa* (Lainson et al. 2005). The overall small amount of data available for squirrel parasites is surprising when considering the potential threat to human healthy and economic damage that these diseases may cause.

4.4.10 Predation

Finally, we found 32 records of 16 vertebrate species that prey upon 12 squirrel species in the Neotropics. We found records of only four species of bird of prey (*Spizaetus tyrannus*, *Spizaetus ornatus*, *Micrastur semitorquatus* and *Parabuteo unicinctus*) preying upon five squirrel species (*Guerlinguetus brasiliensis*, *Notosciurus granatensis, Sciurus deppei, Sciurus yucatanensis* and the exotic *Callosciurus erythraeus*) (Aprile & Chicco 1999, Naveda-rodríguez 2004, Whitacre & Jenny 2012, Rocha et al. 2017). In northeastern Peruvian Amazonia, a Neotropical pygmy squirrel, *Sciurillus pusillus*, was captured and almost killed by a white-throated toucan, *Ramphastos tucanus*, which displayed a normal predatory behavior towards the squirrel. However, due to the observer presence, the toucan dropped the squirrel and flew away (Amasifuén & Heymann 2016). Capuchin monkeys (*Sapajus apella*) also preyed upon squirrels (Galetti 1990). Six species of mesopredators, three canids (*Canis latrans, Lycalopex sechurae, Canis familiaris*) and three felids (*Leopardus wiedii, Leopardus pardalis* and *Felis catus*), preyed upon six squirrel species (*Callosciurus erythraeus, Guerlinguetus brasiliensis, Hadrosciurus spadiceus, Notosciurus granatensis, Sciurus aureogaster* and *Simosciurus nebouxii*), and a squirrel *Hadrosciurus spadiceus* was eaten by a jaguar, *Panthera onca* (Emmons 1987). Only tree species (*Guerlinguetus brasiliensis, Microsciurus alfari and Microsciurus flaviventer*) were reported killed by roadkill, but this information is probably underestimated and a more specific search in road ecology literature should provide more roadkill records. For 14 squirrel species, we found no data about predators or mortality causes.

4.5 Conclusion

In general, the available amount of information about the ecology of neotropical sciurids is limited and strongly biased toward the few relatively well-studied species. For comparison, while the most studied species, *Guerlinguetus brasiliensis*, is known to eat at least 63 species (including seeds, fruits, fungi and insects), we found records of only 4 species (3 of which are anecdotal records) being consumed for the entire genus *Microsciurus*, which comprises 11 squirrel species. Even for the well-studied species, severe gaps exist in our ecological knowledge, including aspects of diet (i.e. the consumption of animal matter and fungi), predators (i.e. what species prey upon these squirrels? what is the predation mortality rate?), and seed dispersal (i.e. what plant species are effectively dispersed? dispersal distance, occurrence of endozoochoric seed dispersal).

The absence of basic ecological information is particularly severe for the small bark gleaning species, including the genus *Microsciurus* and *Sciurillus,* for which the diet is unknown. The relative small body size and high canopy habits appear to increase the difficulty in the collection of data for these species, however, other factors such as the small distribution of most *Microsciurus* species and the isolation of these areas may also negatively affect the amount of studies performed on these species. Since the distribution of scientific research in a country is strongly affected by the distribution of the universities as well as research and development investment (Sidone et al. 2016), the squirrel species that inhabit scientifically active regions are also the target of more studies than species inhabiting more distant regions. This appears to be the case of *Callosciurus erythraeus* in Buenos Aires, Argentina, and *Guerlinguetus brasiliensis* in southeastern Brazil (Sidone et al. 2016). Exotic squirrel populations such as *Callosciurus erythraeus* and *Sciurus aureogaster* (in Florida, U.S.A), also received more attention due to the ecological threat that they represent for the native ecosystems. A similar situation happened for species which are hosts of zoonoses, such as *Notosciurus granatensis* and *Simosciurus nebouxii* in Ecuador, which have received attention as a potential zoonotic threat to humans. Nevertheless, the zoonotic potential and the parasitic interactions of most of neotropical squirrel species, including the Ecuadorian species, is still poorly understood.

The lack of basic ecological knowledge may, per se, correlate to the general lack of interest in the development of studies about neotropical sciurids, despite the taxon's enormous potential as a model group. In order to use neotropical squirrel species as model organisms for advanced ecological studies, it is imperative to describe the natural history of species, but natural history studies have become severely devalued in recent decades (Tewksbury et al. 2014). The low efficiency of capture and manipulation methods in use may also be a problem since most methods were never designed specifically to the squirrels and to the neotropical fieldwork reality (Bovendorp et al. 2017, but see Koprowski 2002).

In the future, with a complete taxonomic revision and an extensive exploration of the natural history of these neotropical squirrels, the taxon may provide unparalleled opportunities for new studies. The genus *Microsciurus* may become particularly useful model for biogeographical and niche partitioning studies due to the high similarity between sympatric species and high diversity over a small geographical range, improving our understanding about how similar species are able to coexist. Ecological comparisons between *Sciurillus* and other worldwide pygmy squirrel may add valuable information to the already existing morphometric comparisons, such how environment and life style affects species body size and proportions, being relevant for evolutionary studies due to the unusual phylogenetic history of *Sciurillus*. Finally, forest dynamic studies will benefit by being able to access the real impact of these widespread arboreal rodents on plant recruitment, which is highly relevant when considering that squirrels are active seed predators and dispersers in the Central and South American forests since the Great American Interchange, around 3.4 million years ago (Koprowski et al. 2016).

Acknowledgements

We would like to thank to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), the Universidade Estadual Paulista (UNESP-RC), the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for provide the resources required to the development of this study.

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4.7 Supplementary material

(Provided as an external .csv file)

Table S1: List of the 48 publications found in the literature containing 128 population density data for neotropical squirrel species at 72 sites. The densities were converted to ind/km² and notes are provided about some manuscripts. The squirrel species names are provided following Vivo and Carmignotto (2015) and as reported by the authors. The Source column contains the information reference, presented as a DOI (when available) or as the document title plus the information needed to find and access it (when the DOI is unavailable).

(Provided as an external .csv file)

Table S2: List of the 155 publications found in the literature containing ecologic data about neotropical squirrel species. Publications with more than one study site received an ID for each site and were repeated in the list, totalizing 195 sites. The ID column is connected with the ID column in the Interaction table (Table S3). The Source column contains the information reference, presented as a DOI (when available) or as the document title plus the information needed to find and access it (when the DOI is unavailable).

(Provided as an external .csv file)

Table S3: List of ecological interactions recorded for neotropical species in the literature. The entries of the column ID is connected with the ID column of the Location/Source table (TableS1).

Figure S1: Distribution of the study sites of the publications containing population density data for neotropical squirrel species found in Google Scholar database. The color of the countries/states represents the number of publications for that state/country. The bigger countries were divided in states to reduce the variation in the size of the polygons. The point color represents the squirrel genera (de Vivo 2015): *Guerlinguetus* in dark green, *Hadrosciurus* in purple, *Microsciurus* in pink, *Notosciurus* in red, *Sciurus* in yellow, *Callosciurus* in light blue, *Sciurillus* in light green, *Simosciurus* in dark blue and *Syntheosciurus* in brown.

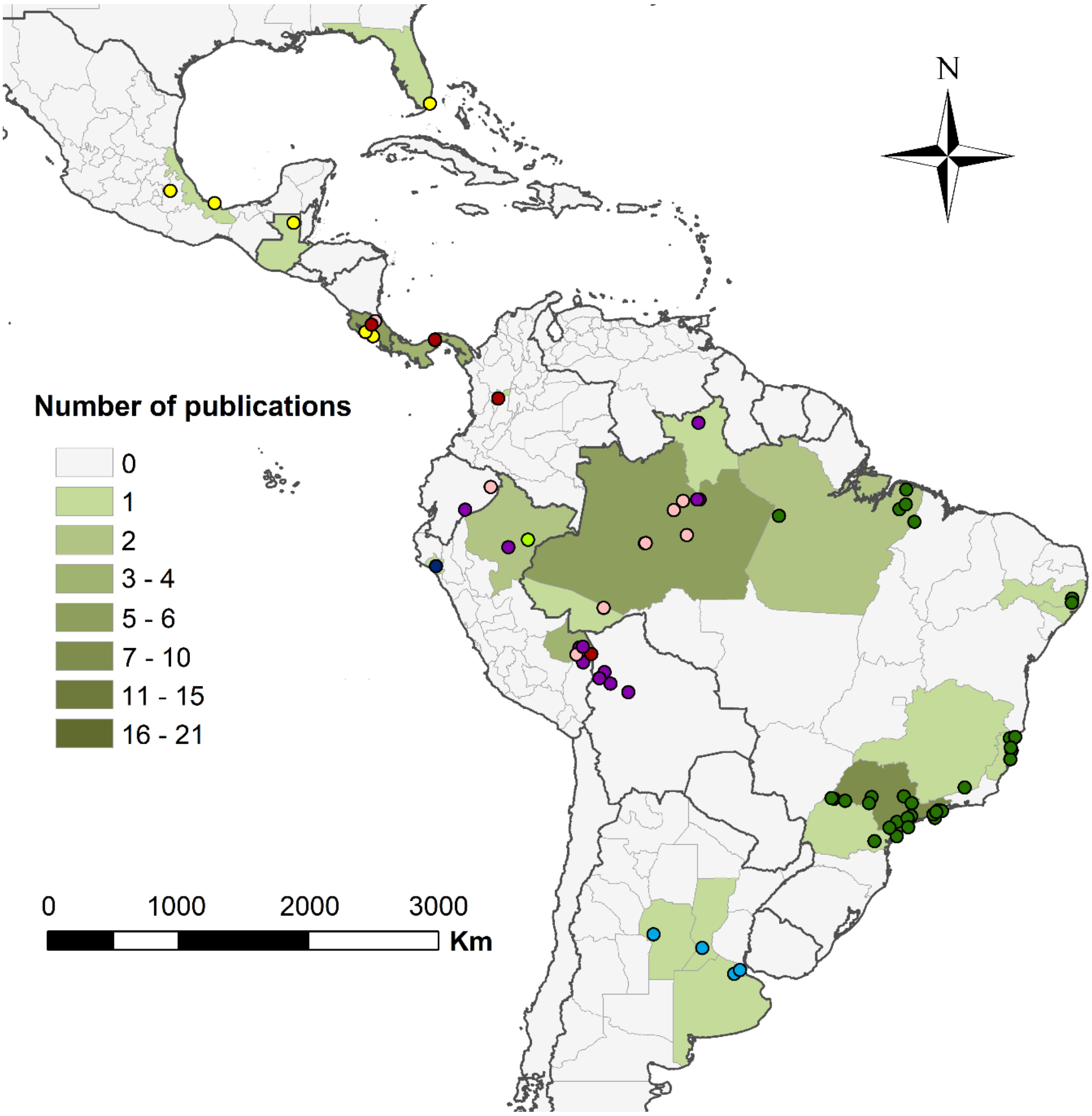


Figure S2: Regression of the number of species known to interact with a squirrel species as a function of the number of published studies found for the squirrel species. Each point represents a neotropical squirrel species.

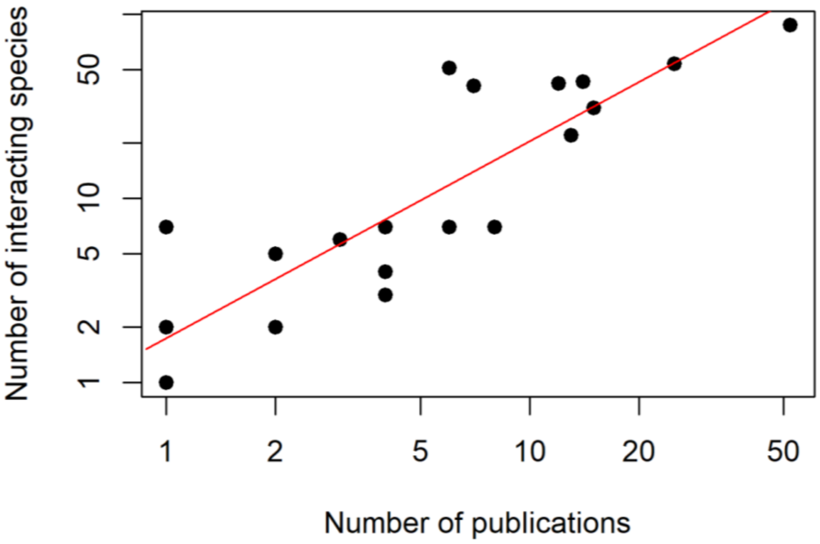


Figure S3: Number of species confirmed to be consumed by neotropical squirrels.

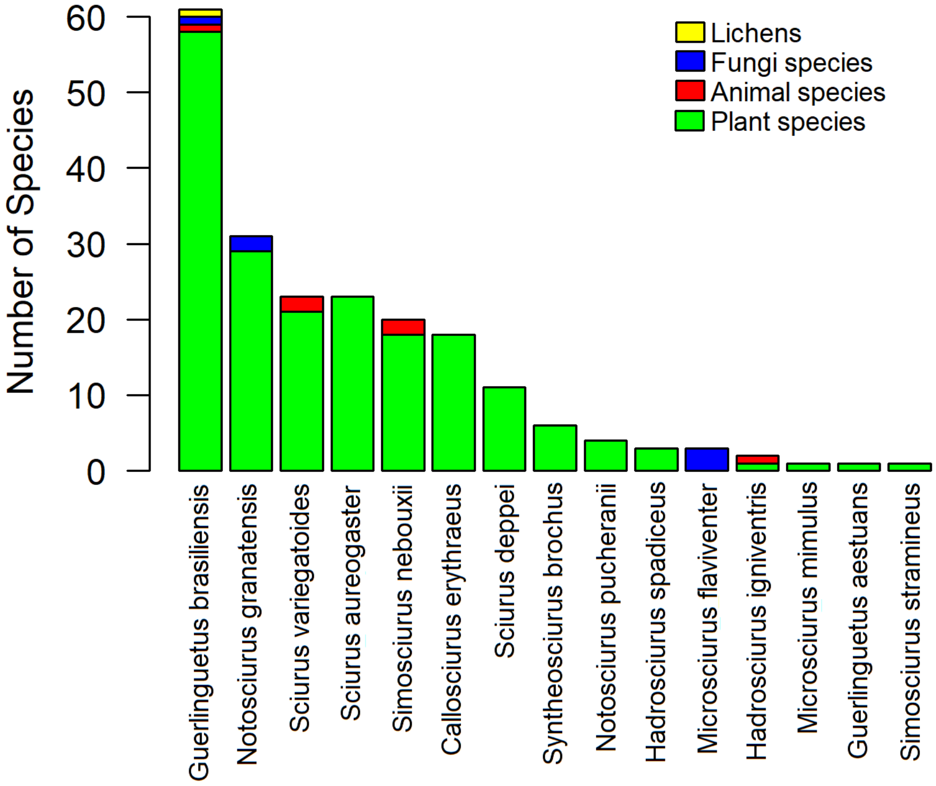


Figure S4: Number of confirmed plant species dispersed by neotropical squirrels (A) and the families of these dispersed plants (B).

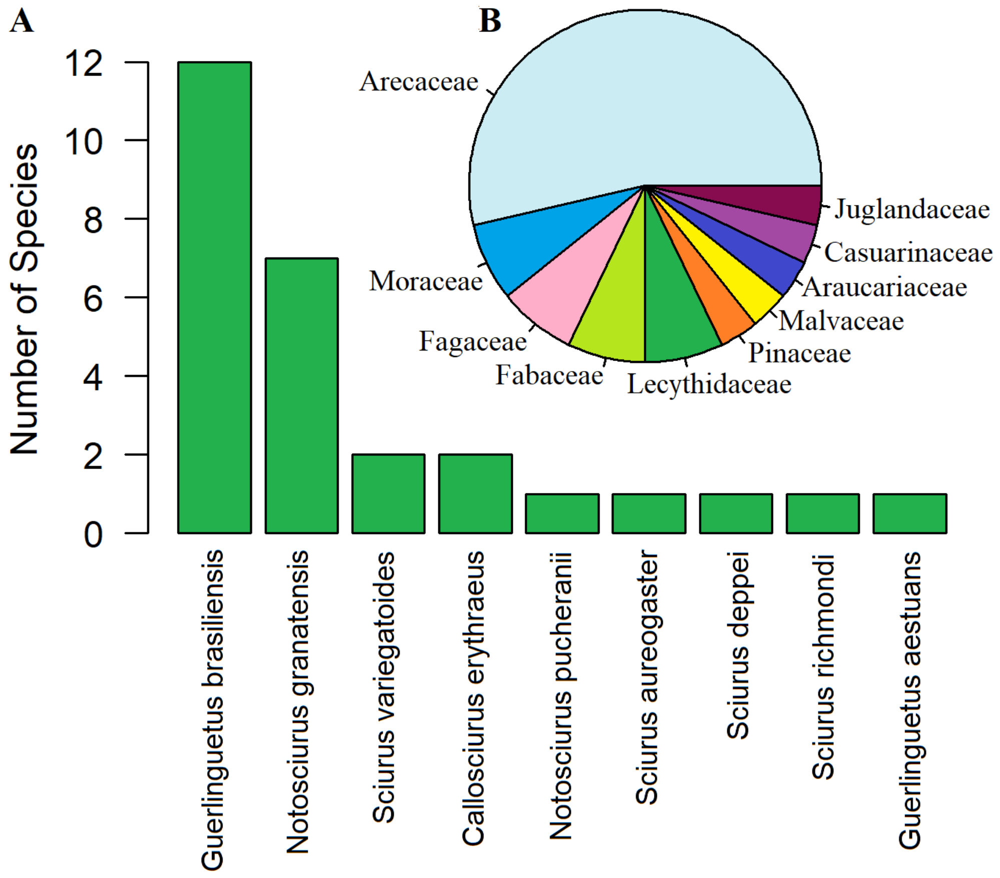
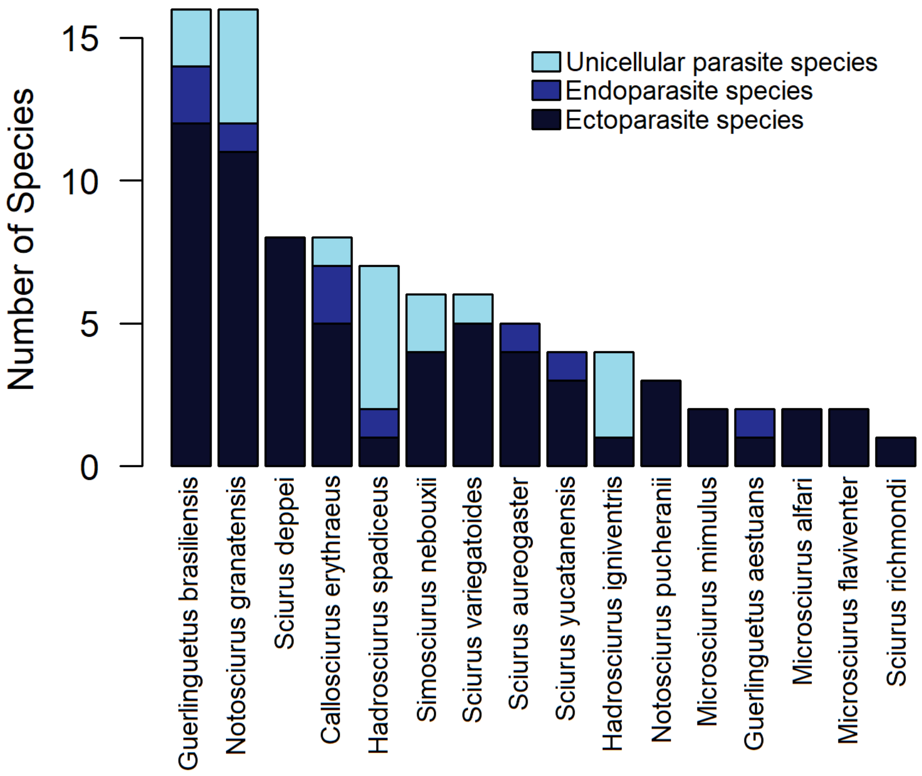


Figure S5: Number of parasite species reported for neotropical squirrels.



5. CONCLUSÃO GERAL

De modo geral, todos os objetivos propostos pela tese foram atingidos. No primeiro capítulo, foi demonstrando que apesar da existência de plasticidade comportamental no comportamento de cache do esquilo-vermelho-de-Graham, esta plasticidade é relacionada à estrutura do habitat, mas não a fatores climáticos, que tem sido afetados pelas mudanças climáticas. Este resultado é particularmente preocupante para a conservação e sobrevivência dessa subespécie ameaçada de esquilos-vermelhos, visto que há previsões de acréscimos de até 4.7 graus na temperatura média da região até 2100.

O segundo capítulo, demonstrou a ocorrência de plasticidade comportamental em resposta a perturbações antropogênicas variadas em pelo menos sete espécies de mamíferos florestais (*Cuniculus paca, Dasyprocta azarae, Dasypus novemcinctus, Pecari tajacu, Tayassu pecari, Sus scrofa e Tapirus terrestris*), com fortes evidências de similares respostas para outras cinco espécies (*Leopardus guttulus, L. wiedii, Cabassous unicinctus, C. tatouay* e *Euphractus sexcinctus*). Essa plasticidade foi detectada na forma de alterações no padrão de atividade circadiana, com as espécies se tornando em média 20% mais noturnas em áreas perturbadas. Essas alterações na atividade circadiana foram observadas tanto em espécies noturnas quanto diurnas, e principalmente em espécies alvo de caça ilegal e perseguição. Em conjunto, caça ilegal e poluição luminosa são os mais plausíveis mecanismos por traz das alterações na atividade circadiana observadas.

Por fim, no terceiro capitulo, foi possível reunir um total de 155 publicações contendo dados de 20 espécies de esquilos Neotropicais, em 15 países. A partir dessas publicações, foi possível identificar 182 itens alimentares, 28 espécies dispersas por esquilos, 47 espécies utilizadas para fins não alimentares (e.g. construção de ninhos), 39 espécies que interagem com esquilos de forma não agonística, 65 parasitas e 16 espécies que predam esquilos. Também registramos um aumento na densidade de esquilos em áreas florestais fragmentadas, o que demonstra a resiliência e o potencial de algumas espécies para serem dispersores de sementes em paisagens fragmentadas. Identificou-se também a necessidade de mais informações sobre a dieta, especialmente para as espécies de menor porte (i.e. *Sciurillus pusillus* e *Microsciurus spp.*), a capacidade de dispersão de sementes, parasitos e de potencial zoonótico para a maior parte das espécies de esquilos neotropicais.