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ANIMAL SCIENCE

Seasonal analysis of the diet and trophic niche breadth of *Molossus rufus* É. Geoffroy, 1805 (Mammalia: Chiroptera) in a tropical urban environment

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Abstract: This study aimed to analyze the diet and trophic niche breadth of an insectivorous bat very common in an urban environment in southern Brazil. To analyze the feeding habit of *Molossus rufus*, it was necessary to collect fecal samples in urban colonies in the city of Maringá, State of Paraná. Samplings occurred from November 2018 to September 2019, including rainy and dry seasons. The fragments found in the samples were identified up to the level of order or family whenever possible. Percentage of volume and frequency of occurrence, Feeding Index (IAi%), PERMANOVA, and trophic niche breadth were used. Thus, a total of 140 samples were obtained, 92 for the rainy season and 48 for the dry season. In terms of percentage of volume, the diet consisted mainly of Hymenoptera (46.9%) in the rainy season and Coleoptera (39.2%) in the dry season. The results for standardized trophic niche breadth were Ba = 0.36 for the rainy season and Ba = 0.37 in the dry season, indicating a low food diversity. In conclusion, there was a food preference on the part of *M. rufus* according to the season evaluated, with Hymenoptera and Coleoptera being the most relevant dietary items.

Key words: Bat, insectivory, feeding habits, seasonality, southern Brazil.

INTRODUCTION

The degradation of the natural environment has caused changes in the habitats and behavior of wild species (Ellington & Gehrt 2019, Frick et al. 2020, Lynch et al. 2021). Several mammals in Brazil are present in urban areas; this is possible because they have adapted to the urban environment (Pacheco et al. 2010). Bats use shelters in urban constructions, such as roofs or abandoned buildings, since the destruction of natural shelters, such as caves and forests, has become frequent with the advance of urbanization (Pacheco et al. 2010).

In urban centers, there are several species of insectivorous bats. Usually, these animals consume a variety of arthropods that birds cannot prey on because they have nocturnal habits, and most birds are diurnal (Reis et al. 2017). Bats present different foraging strategies, for example, vespertilionids and emballonurids obtain most of their food in mid-flight, capturing insects up to the approximate height of the treetops, whereas molossids generally perform their foraging above this stratum (Reis et al. 2007). How these animals perform the foraging and the types of habitats occupied can indicate, in a considerable way, the composition of the diet (Emiliano et al. 2017).

Many insects are urban pests and possible vectors of infectious agents. In common with frugivorous bats playing an important role in seed dispersal, as demonstrated in Faustino et al. (2021) and Jacomassa et al. (2021), insectivorous

bats are relevant because they consume insects in abundance. One of the important ecosystem services provided by insectivorous bats is the regulation function, which means suppressing the abundance of these arthropods in a given environment and acting on effective biological pest control (Boyles et al. 2011, Cleveland et al. 2006). Certain species of insectivorous bats can consume insects in an amount equivalent to their body weight in just one night (Reis et al. 2017). This means that large numbers of individuals in food activity contribute to the removal of thousands of insects from the environment per night (Reis et al. 2017, Kurta et al. 1989).

These animals have a high potential to control agricultural pests, which Lepidoptera, Hemiptera, and Coleoptera are some of the main orders of insects that cause losses in agriculture (Williams-Guillén et al. 2008, Leelapaibul et al. 2005). However, these insects are also common in urban areas, as observed in Garcia (2007). Some insect species can be disease vectors (Papavero & Guimarães 2000) and insectivorous bats can be essential to reduce their population in cities. Some Diptera, for example, are transmitters of important public health pathogens, such as *Aedes aegypti*, a vector of dengue and yellow fever viruses (Galati et al. 2015). There is evidence that the occurrence of these insects may be seasonal (Vasconcellos et al. 2010), which indicates the possibility of seasonality also in the food consumption of insectivorous bats.

The availability of food resources in the environment, at different times of the year, maybe related to rainfall, because, according to Barclay (2008), the increase in the volume of rain or temperature can affect the spatial and temporal distribution of insects. In this way, it becomes relevant to evaluate the trophic niche breadth of bats relative to seasonality.

Molossus rufus É. Geoffroy, 1805 is one of the most common insectivorous species found in an urban environment in Brazil. These Molossidae bats are known as acrobatic bats, given their ability to perform maneuvers during flight (Gregorin & Taddei 2002, Nolte et al. 2009). They are large bats, with a body mass from 21 to 43 g and forearms from 46 to 53 mm long (Gregorin & Taddei 2002, Reis et al. 2017). Food habit records include orders such as Coleoptera, Hymenoptera, Hemiptera, Orthoptera, Lepidoptera, Diptera, Odonata (Fenton et al. 1998, Freeman 1981, Marques 1986, Howell & Bruch 1974, Pine 1969), and for southern Brazil, Emiliano et al. (2017) reported high consumption of Coleoptera and Lepidoptera by *Molossus* spp.

Few studies describe the diet of tropical insectivorous bats. Thus, the goal of this study was to describe the diet and the trophic niche breadth of *M. rufus* in a tropical urban environment in southern Brazil, taking into account the seasonality and to understand the importance of *M. rufus* in the suppression of insects that can cause some damage to society's lifestyle.

MATERIALS AND METHODS

Study area

Sampling was conducted in six urban shelters for insectivorous bats in Maringá (23°25'16.12" S, 51°55'59.16" W), municipality of the State of Paraná, Southern Brazil, from November 2018 to September 2019. The area was located within the boundaries of the Atlantic Forest; the type of vegetation in the municipality is Semideciduous Seasonal Forest (Instituto Brasileiro de Geografia e Estatística [IBGE] 2004). The climate, according to the Köppen classification, is Cfa, subtropical mesothermal, humid, without a rigorous dry season, and with hot summers (Maack 2012).

In the study area, the dry season was from April to September 2019, with the lowest rainfall in August (5.6 mm); and the highest

rainfall volumes from November 2018 to March 2019, peaking in March (153.2 mm), (Figure 1) (data provided by the Sistema de Tecnologia e Monitoramento Ambiental do Paraná [SIMEPAR] 2020).

Sampling

For both seasons (rainy and dry), fecal samples were collected from three to five colonies of *M. rufus* in the roofs of urban residences. Eight days of sampling were made per season, with a maximum of 15 fecal samples taken per day. To obtain fecal samples, we used the methodology used by Pokhrel & Budha (2014), adapted for the roofs of residences. Plastic (smooth and previously cleaned, 1 m × 1 m) was used for 24 h inside the shelter, under the colony, to collect only fresh samples. Before the collection of feces, individuals were captured from each colony for identification and confirmation of the species present at the site. Individuals were identified with the help of the identification keys of Gregorin & Taddei (2002) and Reis et al. (2017) and later released. Fecal samples of bats were placed in Eppendorf® tubes containing 70% alcohol and stored in a refrigerator until analysis.

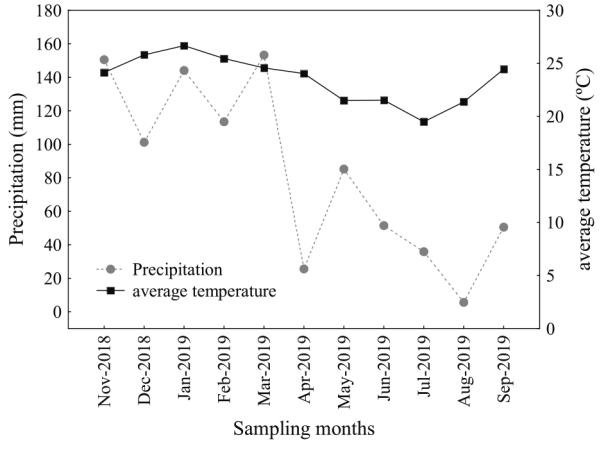

Figure 1. Ombrothermic diagram for a tropical urban environment in southern Brazil, from November 2018 to September 2019 (SIMEPAR 2020).

All procedures used in this study, referring to the management of animals, followed the guidelines for capture, handling, and care of mammals, according to Sikes and American Society of Mammologists (2016) and with the authorization of the Ethics Committee on the Use of Animals of the University State of Maringá (Process 4182101018).

It is known that there is a difference in the diet between male and female bats, especially among reproductive distinct stages (Wilkinson & Barclay 1997). In this study, however, the chosen methodology did not favor this type of information collection.

Analyses

The insect identification was made by morphological analysis of the fragments found in bat feces according to Whitaker et al. (2009), with the determination at the order or family level, using stereomicroscope. Insect identification was restricted to hard parts that can be visually identified. Each fecal sample was examined separately, obtaining the percentage of volume and the frequency of each identified item. The volume of each food item was determined using a gridded dish, thus obtaining the volume in mm^3, which was later transformed into mL (Hellawell & Abel 1971). Insect fragments that cannot be confidently distinguished were treated as unidentified insects (U. I.).

To characterize the species diet, the Feeding Index (IAi%) was calculated to distinguish the relative importance of each item in the species' diet (Kawakami & Vazzoler 1980), using the following formula:

$$IAi = \left(Fi\,x\,Vi\right) / \left[\Sigma\left(Fi\,x\,Vi\right)\right]$$

where IAi = feeding index; i = is each food item; F = occurrence frequency (%); V = volume (%). IAi is an index that varies from 0 to 1.

Seasonal difference in diet species composition, considering the rainy season and dry, was evaluated using a Permutational Multivariate Analysis of Variance, PERMANOVA (Anderson et al. 2008), with a Bray Curtis similarity matrix generated from the data of volume of food items. The analysis was performed using PRIMER software version 6.0 (Anderson et al. 2008).

To determine the relative level of specialization in the bat diet, the standardized trophic niche breadth was calculated using the standardized Levins index (Hurlbert 1978), which ranges from 0, when the species consumed only one type of food, to 1, when the species consumed several food types.

$$Ba = \left[\left(\Sigma jPij2 \right) - 1 - 1 \right] \left(n - 1 \right) - 1$$

where Ba = standardized trophic niche breadth; P_{ij} = is the proportion of the food item in the species' diet; n = refers to the total of food items.

To relate the food items found in the bats' diet with the insect families that cause damage to agriculture, three references were used: Chagas et al. (2016) and Albuquerque et al. (2002), which addresses groups of insects that damage agriculture in the region of the municipality of Maringá, and, Garcia (2007), that identify the insects considered pests in an urban environment, as they cause damage to ornamental trees in cities.

RESULTS

In the total of 140 samples collected, 92 were during rainy season, and 48 were in the dry season. Eight insect orders were identified, six of which were present in the rainy season sampling and seven that were present in the dry season. In terms of volume percentage, the *M. rufus* diet consisted mainly of Hymenoptera (46.9%), for the rainy season, and Coleoptera (39.2%), for the dry season (Table I). The order Coleoptera (73.9% and 95.8%, for the rainy and dry season, respectively) was the food item most frequently consumed in both seasons, followed by the orders Hemiptera (69.6%) in the rainy season

Table I. Percentage of volume, frequency of occurrence, and Feeding Index (IAi%) of the items found in the diet of *Molossus rufus*, for the rainy and dry seasons, in a tropical urban environment in southern Brazil, from November 2018 to September 2019.

	Rainy season (92 samples)			Dry season (48 samples)			Total values (140 samples)		
Order	**Volume (%)**	**Frequency (%)**	**IAi (%)**	**Volume (%)**	**Frequency (%)**	**IAi (%)**	**Volume (%)**	**Frequency (%)**	**IAi (%)**
Hymenoptera	46.9	68.5	46	24.1	54.2	17.2	40.4	63.6	36.6
Hemiptera	27.6	69.6	27.5	28.9	70.8	27	28.0	70.0	27.9
Lepidoptera	0.4	55.4	0.4	5.1	77.1	5.2	1.8	62.9	1.6
Coleoptera	24.6	73.9	26	39.2	95.8	49.5	28.7	81.4	33.4
Isoptera	0.001	1.1	0	0.01	2.1	0	0.003	1.4	0
Trichoptera	0.01	1.1	0	-	-	-	0.01	0.7	0
Diptera	-	-	-	0.4	18.8	0.1	0.1	6.4	0.01
Neuroptera	-	-	-	0.006	4.2	0	0.002	1.4	0
U. I.*	0.5	28.3	0.2	2.3	35.4	1.1	1.0	30.7	0.4

*** U. I.: unidentified insects.**

and Lepidoptera (77.1%) in the dry season (Table I).

According to the Feeding Index (IAi%), the most relevant items consumed were Hymenoptera (46%), in the rainy season and Coleoptera (49.5%), in the dry season (Table I). The trophic niche breadth did not vary between the seasons, for the rainy season, Ba = 0.36 and for the dry season, Ba = 0.37, which indicates the high specialization of *M. rufus*, regardless of seasonality. PERMANOVA identified significant differences in the specie diet composition according to the seasons evaluated (F = 3.69; P = 0.023). Considering that Trichoptera was recorded only in the rainy season, in addition to Diptera and Neuroptera that were recorded only in the dry season.

Five families (Hymenoptera: Formicidae; Hemiptera: Pentatomidae and Cicadellidae; Coleoptera: Chrysomelidae and Curculionidae) were identified as insect pests (Figure 2). Regarding the frequency of occurrence of these taxa, Formicidae (47.8%) was the most frequent in the diet during the rainy season and Chrysomelidae (79.2%), which is associated with damage to local agriculture, was the food item most consumed during dry season (Table II).

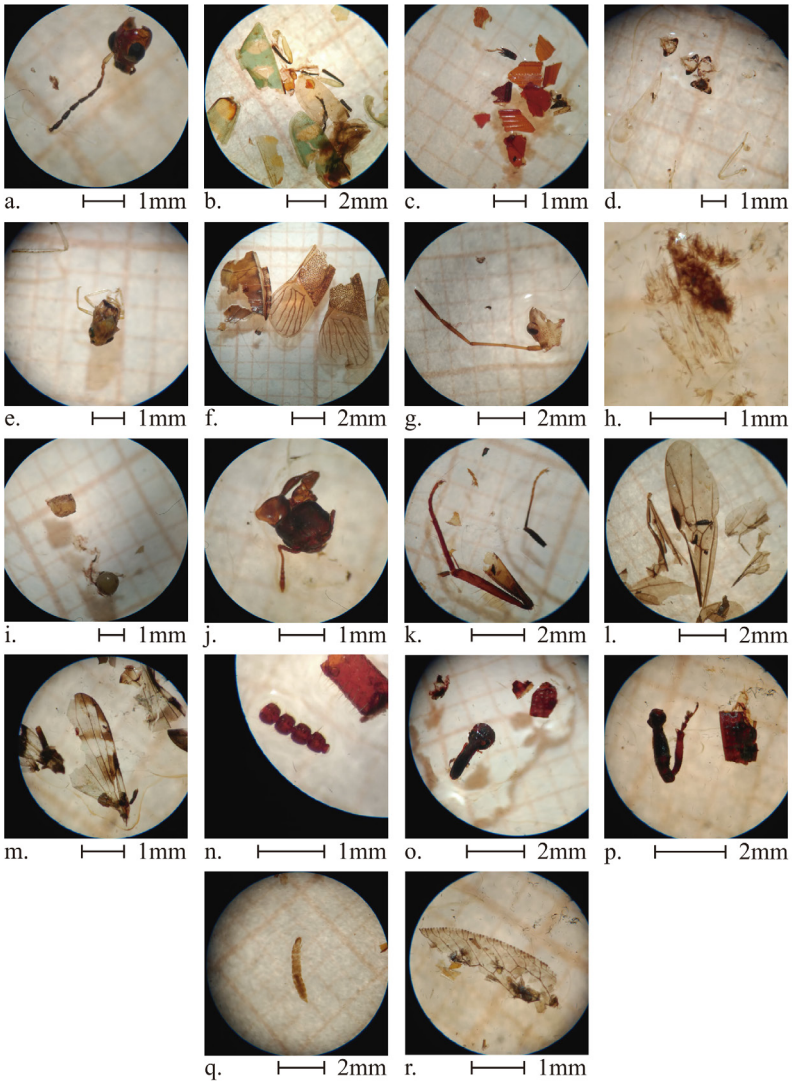

Figure 2. Food items were found in fecal samples of *Molossus rufus*, in a tropical urban environment in southern Brazil, from November 2018 to September 2019. a and b - Coleoptera: Chrysomelidae (*Diabrotica* sp.); c - Coleoptera; d and e - Hemiptera: Cicadellidae; f and g - Hemiptera: Pentatomidae; h and i - Lepidoptera (scale and eggs); j, k and l - Hymenoptera: Formicidae; m - Diptera; n - Isoptera; o and p - Coleoptera: Curculionidae; q - Trichoptera (larva); r - Neuroptera.

Table II. Percentage of volume (%V) and frequency of occurrence (%F) of taxa identified in the diet of *Molossus rufus* for the rainy and dry seasons, in a tropical urban environment in southern Brazil, from November 2018 to September 2019. Percentages of volume refer to the total of each order.

		Rainy season		Dry season		Total values	
Taxa		%V	%F	%V	%F	%V	%F
Hymenoptera							
	Formicidae	88.2	47.8	77.8	39.6	86.4	45.0
Hemiptera							
	Pentatomidae	69.6	37.0	-	-	49.0	24.3
	Cicadomorpha	-	-	38.4	39.6	11.4	13.6
Coleoptera							
	Chrysomelidae	13.3	23.9	44.8	79.2	0.4	2.9
	Curculionidae	-	-	1.0	8.3	41.9	42.9

DISCUSSION

The composition of the *M. rufus* diet revealed the most consumed food items during the two seasons, with a reduced number of samples in the dry season. The most consumed orders by *M. rufus* were Hymenoptera, Coleoptera, and Hemiptera, also evidenced by Marques (1986), who analyzed the diet of the species in a house roof in northern Brazil, and Gnocchi et al. (2019), in a study in the Atlantic Forest of southeastern Brazil.

The high frequency of occurrence observed for Lepidoptera fragments in the samples may be associated with the fact that parts of these insects can remain in the digestive tract of bats and be detected for several days (Whitaker et al. 2009). Thus, even low consumption could lead to their permanence until complete elimination. Occasional food items can also be observed in the diet of *M. rufus*, as occurred for Trichoptera, which had only one record. The order is commonly found in the diet of bats, with records for Vespertilionidae (Whitaker 2004). Intensely fragmented food items were frequent throughout the study, a fact that may be related to the morphology of the snout of *M. rufus*, which, according to Nolte et al. (2009), allows the food to be kept longer in the mouth until swallowing, thus being chewed more often.

Considering seasonality, the most important food item for the rainy season was Hymenoptera, and for the dry season, it was Coleoptera. These orders are among those with the highest species richness on the planet (Miličić et al. 2021). With this, urban environments, characteristically altered and with lower richness (McIntyre 2000), tend to favor tolerant species belonging to these groups. Seasonal changes such, as low temperatures, can influence bat activity reducing the foraging activity (Wojciechowski et al. 2007). However, the range breadth of *M. rufus* did not change seasonally. *M. rufus* contributed to the suppression of insects that can cause damage to plants, including ornamental tree species (Chagas et al. 2016, Garcia 2007, Albuquerque et al. 2002).

There was a shift in the consumption of Hemiptera by *M. rufus*, relative to the composition of the families between the two seasons, changing the consumption of Pentatomidae for Cicadellidae. This may be related to the availability of some types of insects in the urban environment, as in the population fluctuation

of Cicadellidae in a citrus orchard in the State of São Paulo (Yamamoto et al. 2001). Population fluctuations can influence the availability of predators. Insectivorous bats that inhabit places where food availability is limited, their diet remains similar to the abundance of insects available in the environment, that is, a more restricted diet (Whitaker 2004).

The constant consumption of the two main families found in the diet, Formicidae (in this case, winged ants) and Chrysomelidae, indicates that *M. rufus* contributes to the control of these arthropods in the environment. The consumption of the family Chrysomelidae was marked by the presence of *Diabrotica* sp., which can be considered a pest. *Diabrotica speciosa*, at its larval stage, reduces the efficiency of the root system of corn, reducing the height and the dry weight of the plant, while the adult insect consumes the leaf area, mainly legumes, such as beans and soybeans, but also grasses, such as corn and rice (Marques et al. 1999). This insect is considered a pest in agriculture for damaging foods of high economic value (Teodoro et al. 2014). There is no evidence that insects of this genus can cause major economic impacts in urban centers; however, the family Chrysomelidae is capable of affecting species used in urban afforestation (Garcia 2007), in addition to small ornamental plants. Thus, this consumption represents an ecosystem service that the bat provides by removing this insect from the environment.

Our results showed that the feeding habit of *M. rufus* in an urban tropical environment in southern Brazil was composed mainly of Hymenoptera in the rainy season and Coleoptera in the dry season, in addition to having specialized diet, with a narrow trophic niche breadth. This leads us to infer about the important ecosystem service that insectivorous bats provide in urban centers, through feeding and thus reducing the abundance of the population of insects that can cause some damage to society's resources, as in the reduction of possible vectors of agricultural pests.

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