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Medium and large-sized mammals in an Atlantic Forest fragment of Brazil: recording of threatened species

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Abstract: Deforestation and habitat fragmentation affect to a great extent larger wild mammals, which require large areas to establish their populations. These mammals can have important functions in the structure and dynamics of tropical forests, acting as seed dispersers, herbivory regulators, and umbrella species. In the present paper, we characterize the community of medium and large wild mammals in a semi-deciduous seasonal forest fragment, a denominated IB Forest (Institute of Biosciences) in Edgardia Experimental Farm, UNESP, Botucatu, São Paulo State, Brazil. By adopting sand plots on three trails as the main method, we identified the occurrence of nine species, besides the occurrence of one species by direct visualization. Some of these are in national red lists — *Leopardus pardalis* and *Puma concolor* are listed as threatened and *Sapajus* cf. *nigritus* as Near Threatened in São Paulo State; *Puma concolor* is also listed as Vulnerable at the national level. Thus, we emphasize the importance of this forest remnant as a wildlife refuge, which makes it necessary to monitor the occurrence of these animals in the area and conserve similar remnants in the region.

Keywords: Conservation, deforestation, habitat fragmentation, IB forest, inventory, Mammalia, red lists, São Paulo State, tropical forests.

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INTRODUCTION

The Atlantic Forest is the second largest rainforest in South America (Metzger 2009), and one of the biomes with the greatest rates of endemism (Myers et al. 2000; Ribeiro et al. 2009). This rainforest is also one of the most threatened tropical ecosystems on the planet suffering constant area loss (Myers et al. 2000). Most of the deforestation in the Atlantic Forest occurred about 70 years ago (Viana & Tabanez 2000), presenting nowadays only 11–16 % of the original cover (Ribeiro et al. 2009). The fragmentation process in the Atlantic Forest reduced the biome area into small, disturbed, and isolated remnants surrounded by agro-mosaic matrices, human settlements, and roads (Ribeiro et al. 2009; Lira et al. 2012). Such fragmentation impairs the maintenance of natural mammal assemblies by limiting their population sizes and results in changes in species composition (Galetti et al. 2017).

Less than 10% of the areas with original vegetation of the Atlantic Forest has 100 or more hectares (Ranta et al. 1998), and most of the other remnants have less than 10 hectares (Canale et al. 2012). In this forest, 298 mammal species are known, of which 90 are endemic (Paglia et al. 2012). Considering that large mammal species usually require larger areas (Chiarello 2000) and have lower population densities (Damuth 1981), it is clear that fragmentation and natural habitat loss is more pervasive for threatened large-sized species, which already have a greatly reduced population.

Thus, loss of natural vegetation areas through human occupation is one of the greatest threats to terrestrial mammals (Costa et al. 2005). The mammals have important functions in tropical ecosystems (Carvalho et al. 2014), with many of them being efficient seed dispersers (Andreazzi et al. 2009) and plant biomass regulators through restricting the population size of herbivores (Estes et al. 2011). Moreover, many large mammals, particularly carnivores, are considered umbrella species (Roberge & Angelstam 2004). Thus, by conserving such mammals, a large number of other species that may occur in the same natural area are also conserved. In this context, it is relevant to evaluate the occurrence of such mammals in the Atlantic Forest remnants, since these remnants are highly compromised because of their poor connectivity and small size (Ribeiro et al. 2009).

The largest remaining forest cover of the entire eastern Brazilian region is located in São Paulo State (Galindo-Leal & Câmara 2003; Ribeiro et al. 2009). In this state, the environmental protection area (EPA) called Corumbataí-Botucatu-Tejupá belongs to the basin of Capivara River (Jorge 2000). In this EPA, there is an experimental farm composed of fragments of natural vegetation with different disturbance influences. We characterize the community of medium and large-sized mammals in this Atlantic Forest fragment of São Paulo State.

MATERIALS AND METHODS

Study site

The Edgárdia Experimental Farm (EEF) is located in the basin of Capivara River, municipality of Botucatu in São Paulo State (-22.791 t o-22.833 ^{0}S & -48.437 to -48.375 ^{0}W) (Jorge 2000) (Fig. 1), altitude about 475m and with climate conditions characterized by two different seasons over the year — a rainy season (from September to March) and a dry season (from April to August) (Alves et al. 2012). This farm is part of the Corumbataí-Botucatu-Tejupá EPA and suffers different degrees of fragmentation and disturbance over time (Ortega & Engel 1992) since inside the EEF and its surroundings there are agro-pastoral activities, erosion areas, roads, and buildings (Jorge 2009). Despite this, fragments of natural vegetation represent a considerable area of the EEF and can be used as a refuge for wildlife species such as mammals.

EEF has five fragments of Atlantic Forest, which in total is about 740ha. These fragments are classified as semi-deciduous forest and consist of primary, secondary, and riparian forests. Primary forests were modified by wood-selective extractions, while secondary forests were modified by fires (Ortega & Engel 1992; Jorge & Sartori 2002). It is known that, since 1978, such fragments are intercalated or separated with agriculture crops, pasture, and natural regeneration areas (Jorge & Sartori 2002). More specifically, matrices that stand out inside and/or around the EEF are areas of rice cultivation, orchards, bamboo plantations, asphalted and rural roads, besides rural buildings, areas of bare soil under erosive processes, and pasturelands (Jorge 2009). We performed the study in one of the fragments of the EEF called IB Forest. This fragment is located at the border of the farm (Fig. 2), has about 90.86ha, and belongs to the Instituto de Biociências of the Unesp Campus de Botucatu Faculdade de Medicina (UNESP), Botucatu.

Data collection

The occurrence of medium and large mammals was recorded from footprint tracks on three tracks in the IB Forest. In each track (T), we placed 12 sand plots distributed in three sets. Each set presented four 50cm×50cm sand plots 50m apart (Fig. 3). Plots from

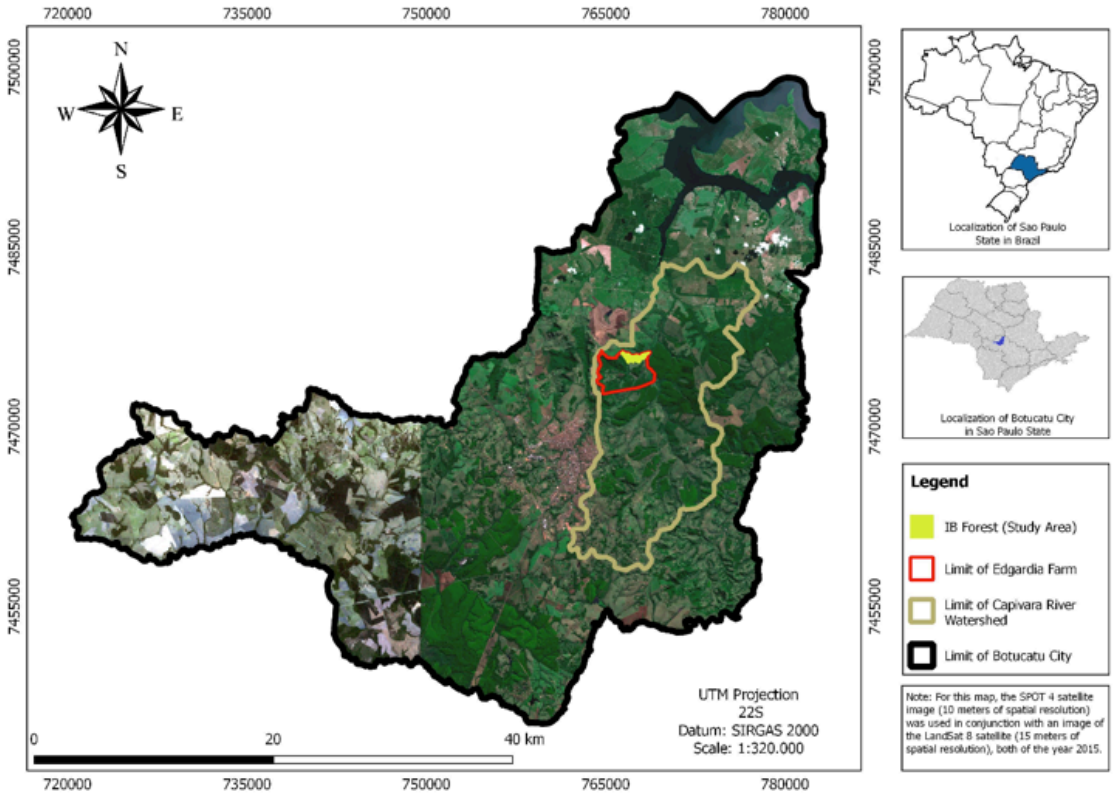

Figure 1. Limits of the municipality of Botucatu, state of São Paulo, highlighting the Capivara River basin and the Edgardia Experimental Farm.

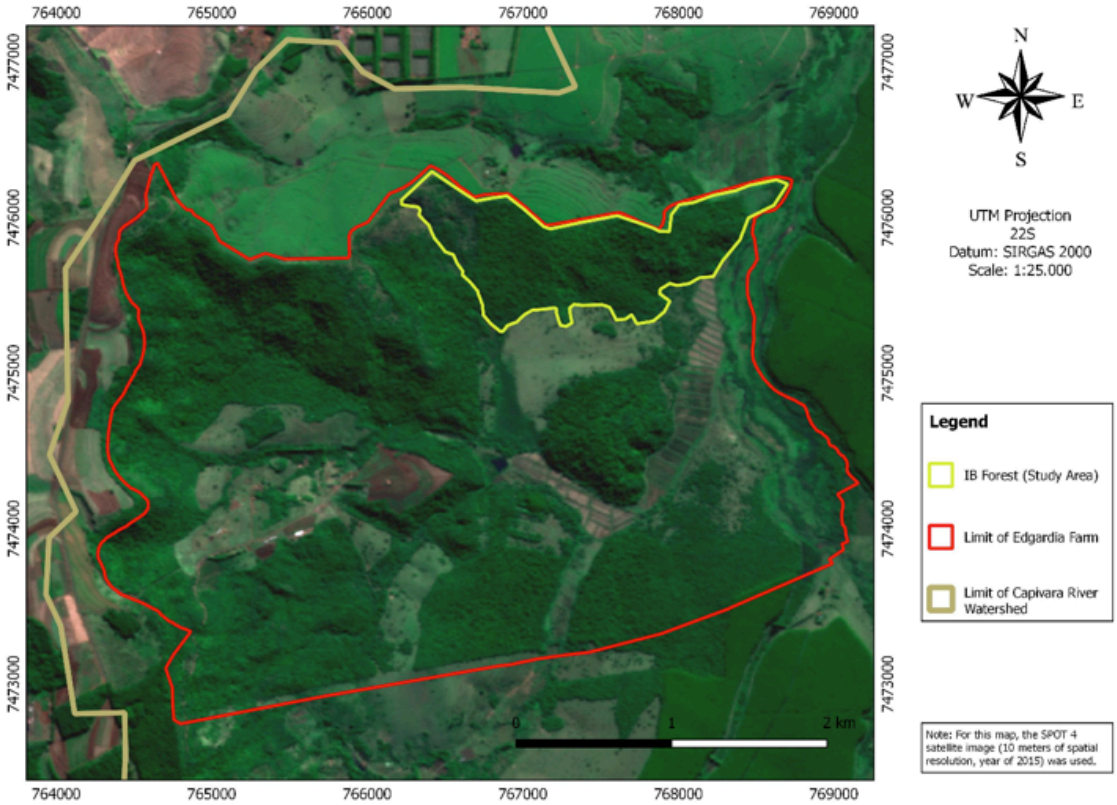

Figure 2. Limits of the Edgardia Experimental Farm and IB Forest (adapted from Google Earth 2018).

the same set were arranged so that they were at least 10m apart, resulting in a quadrangular shape (Fig. 3). Each plot was filled with fine, clear, and wet sand, with an average layer thickness of 3cm. They were delimited with PVC tubes and covered with an extended raffia bag, to assure that the sand mostly remained inside the plot area and that the sand humidity was maintained over time, respectively.

In order to optimize the recording of the mammals in our samples, we offered in each sand plot different kinds of baits to attract them, such as banana, bacon, avocado, sea salt, sardines, corn, pepperoni, and guava. These baits were chosen in relation to their attractiveness; additionally, the fruit selection was based on their lower risk of germination, greater resistance to decay, and portability (Pardini et al. 2003). The baits were separated in equal numbers and were also alternately distributed day after day (Image 1).

We checked sand plots during the dry and wet seasons as well as during transitional periods between them to access possible seasonal variations of mammals' footprints occurrence. We checked the plots every morning on seven consecutive days in April (wet-dry), July (dry), and October (dry-wet) of 2010, and in January (wet) of 2011. Our sample effort was of 252 plots per season.

For the mammals' footprints identification, we used Becker & Dalponte (1991) and Borges & Tomás (2004). To verify the accuracy of the scientific names of registered mammals, we used the nomenclature proposed by Vivo et al. (2011). We used the guide of Emmons & Feer (1997) to classify the recorded mammals as medium or large-sized. Moreover, we consulted two official lists of threatened fauna, the Red List of São Paulo State (MPSP 2014) and the Red List of Brazil (ICMBio 2016) to verify the species' state of extinction risk. We specified trophic categories of all registered taxa following the guides of Robinson & Redford (1986) and Fonseca et al. (1996).

Image 1. Plots of sand with various baits used. a - banana, b - sardine, c - corn with peanut butter, d - mixed (mixture of baits to attract any trophic category).

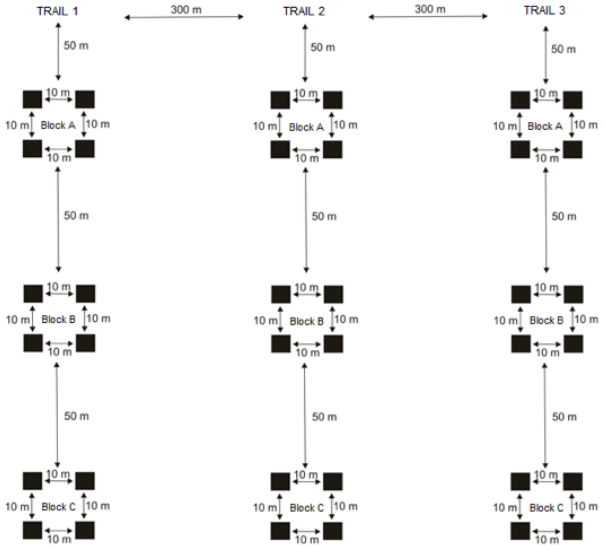

Figure 3. Organization of three tracks with three blocks of four plots (black squares) each and their respective distances in metres (arrows).

Data analysis

To verify if our sample effort was sufficient to record medium and large mammals, we performed the species accumulation curve. All registered mammal species were included to calculate this curve, which was adjusted by using the nonparametric estimator Mao Tau through Estimates Win 7.5 program (Colwell 2005). We used the rarefaction procedure performing 1,000 randomizations of samples (Colwell & Coddington 1994) to prevent the order in which the species were added along the accumulation curve in having any influence on the results. The cumulative curve tended to stabilize over time (Fig. 4).

RESULTS

We identified mammal species from different trophic categories in each set of sand plots (Table 1). We directly observed or detected any marks of the presence of other animals (lizards and birds) rarely. We identified 10 different mammal taxa — nine of them were detected considering our proposed method (footprinting in sand plots; Image 2), whereas just one was identified on occasion by direct visualization in the study area (Image 3). These 10 taxa were from five different orders: Artiodactyla, Carnivora, Didelphimorphia, Rodentia, and Xenarthra (Table 1).

Seven taxa were identified at the species level, including two different genera. The footprints most frequently recorded were of *Didelphis* sp., followed by *Dazyprocta azarae* (Lichtenstein, 1823) and *Leopardus pardalis* (Linnaeus, 1758), besides *Tamandua tetradactyla* (Linnaeus, 1758) and *Puma concolor* (Linnaeus, 1771), which were recorded only once (Table 2). From these identified taxa, *Leopardus pardalis* and *Puma concolor* are considered threatened species, while *Sapajus* cf. *nigritus* (Goldfuss, 1809) is considered a near threatened species in the São Palo State (MPSP 2014). Taking into account the national territory red list, *Puma concolor* is considered a vulnerable species (ICMBio 2016).

In relation to seasonal variations on the mammal footprints recordings, the dry season and the period of transition from dry to wet season have the highest frequency of occurrence of different taxa (eight taxa taking into account both seasons), followed by the wet season (five taxa) and the period of transition from wet to dry season (three taxa) (Table 2). Although *Dasypus novemcinctus* (Linnaeus, 1758) and *Eira barbara* (Linnaeus, 1758) were not the most frequently recorded species, as mentioned above, only these species were found in all the seasons (Table 2).

DISCUSSION

Here we demonstrate that at least 10 species of medium and large mammals occur in the IB Forest in Botucatu. Interestingly, among these recorded species, we found one threatened species, *Puma concolor*, and two others, *Leopardus pardalis* and *Sapajus* cf. *nigritus*, considered with some degree of threat at the state and

Table 1. Mammals identified in the study area with type of recording, food chain, and category of threat.

	Common name	Type of recording [1]	Trophic category [2]	Threatened species list [3]	
				SP	ICMBio
Artiodactyla Order					
Cervidae Family					
Mazama sp. (Rafinesque, 1817)	Small Brocket	P	FH	NT	NT
Carnivore Order					
Felidae Family					
Leopardus pardalis (Linnaeus, 1758)	Ocelot	P	CA	T	NT
Puma concolor (Linnaeus, 1771)	Puma	P	CA	T	VU
Mustelidae Family					
Eira barbara (Linnaeus, 1758)	Tayra	P	FO	NT	NT
Procyonidae Family					
Nasua nasua (Linnaeus, 1766)	South American Coati	P	FO	NT	NT
Didelphimorphia Order					
Didelphidae Family					
Didelphis sp. (Linnaeus, 1758)	Brazilian Common Opossum	P	FO	NT	NT
Primates Order					
Cebidae Family					
Sapajus cf. *nigritus* (Goldfuss, 1809)	Black-horned Capuchin	V	FO	AT	NT
Rodentia Order					
Dasyproctidae Family					
Dasyprocta azarae (Lichtenstein, 1823)	Azara's Agouti	P	FH	NT	NT
Xenarthra Order					
Dasypodidae Family					
Dasypus novemcinctus (Linnaeus, 1758)	Nine-banded Armadillo	P	IO	NT	NT
Myrmecophagidae Family					
Tamandua tetradactyla (Linnaeus, 1758)	Southern Tamandua	P	MY	NT	NT

[1] Registration Type: P (Footprints in the sand portion), V (direct view); [2] trophic categories according to Robinson and Redford (1986) and Fonseca et al. (1996): FO (frugivore-Omnivore), IO (Insectivorous-Omnivore), MY (Myrmecófago), CA (Carnivore) and FH (frugivore-herbivore); [3] Threat Category: NT (Not Threatened), VU (Vulnerable), AT (Almost Threatened) and T (Threatened), in the Reds Lists of the State of São Paulo (2014) and the Chico Mendes Institute for Biodiversity Conservation - Ministry of the Environment (2016).

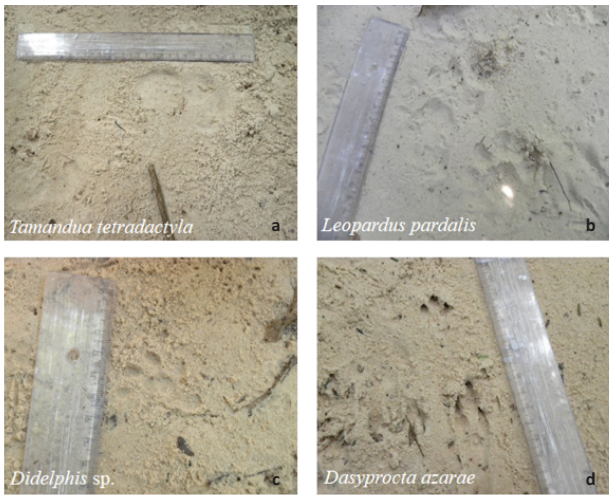

Image 2. Examples of footprints of mammals detected in the study area. a - *Tamandua tetradactyla*, b - *Leopardus pardalis*, c - *Didelphis* sp., d - *Dasyprocta azarae*.

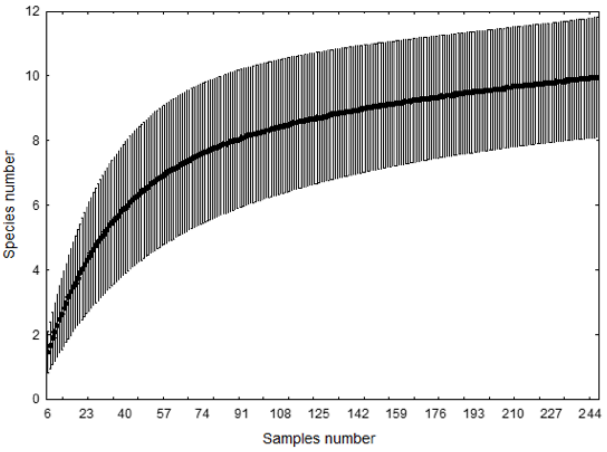

Figure 4. Average cumulative number of medium and large mammalian species with the increase of the sampling effort for sand plots in three tracks, using the Mao Tau estimator and confidence intervals (95%).

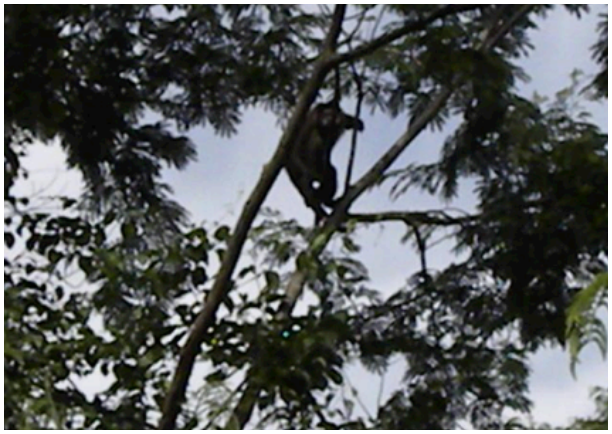

Image 3. Direct observation of Black-horned Capuchin *Sapajus* cf. *nigritus* in the study area.

national levels. Such degrees of threat, however, are not considered at the international level for *L. pardalis* and *P. concolor*, which are listed as Least Concern, but only for the endemic *S. nigritus*, which is internationally recognised as Near Threatened (IUCN 2018). Regardless of the international scenario, it is reasonable to emphasize the importance of the conservation of this area for such species that show a decreasing population trend. Moreover, some species were more frequently detected after a period of a year, mostly in the dry season than in the wet season. We discuss possible explanations for these findings below.

Considering that the cumulative curve of species tended to stabilize over time, we assume that our sampling effort by sand plots method was sufficient. The richness of species found in this study is similar with other studies conducted in the Atlantic Forest remnants of São Paulo State, being slightly higher (Dotta 2005) or equivalent (Gheler-Costa et al. 2002; Brocardo et al. 2012; Norris et al. 2012). Despite this, we found a lower richness of species than the majority of studies in the same state (Negrão & Valladares-Pádua 2006; Minami 2010; Siviero & Setz 2011; Alves et al. 2012; Magioli et al. 2014; Reale et al. 2014; Breviglieri et al. 2017). The higher richness in others' studies may have been favoured by increased sampling effort (more months or years of collection), a combination of sand plots with transects, much larger sample plots, or increased area of sampling (larger fragments).

Considering seasonal variations, more taxa were found in the dry season and dry-wet transition period, followed by the rainy season and wet-dry transition period. The fact that the wettest periods showed a low incidence of records may suggest less displacement of these mammals during rainy days, possibly because the search for water was reduced under this condition, though this needs further investigation. Another possibility here is that footprints may have been erased by the rains during the intervals of data recordings.

The taxon with the highest frequency of occurrence was *Didelphis* sp., small mammals that are among the most representative animals in fragmented and disturbed forests (Fonseca 1989). Moreover, this genus includes species that are generalists for habitat and diet resources, thus easily adjusting their populations to the environment (Fonseca & Robinson 1990). Although the most frequently detected footprints were from *Didelphis* sp,

Table 2. Occurrence of each taxon for seasons and total frequency of occurrence regardless of the season.

Taxon	Occurrence by season				Total frequency of occurence
	Wet-dry	Dry	Dry-wet	Wet	
Cebus cf. *nigritus* (Goldfuss, 1809)				X	1
Dasyprocta azarae (Lichtenstein, 1823)	X	X	X		11
Dasypus novemcinctus (Linnaeus, 1758)	X	X	X	X	8
Didelphis sp. (Linnaeus, 1758)		X	X	X	15
Eira barbara (Linnaeus, 1758)	X	X	X	X	7
Leopardus pardalis (Linnaeus, 1758)		X	X		11
Mazama sp. (Rafinesque, 1817)			X	X	3
Nasua nasua (Linnaeus, 1766)		X	X		7
Puma concolor (Linnaeus, 1771)				X	1
Tamandua tetradactyla (Linnaeus, 1758)		X			1
Total	3	7	7	6	65

X - presence of the species.

such footprints were not observed in all sampling periods. This may indicate that such animals could be transients in this fragment, in contrast with *Dasypus novemcinctus* and *Eira barbara* species that were recorded during all the seasons over the year (Table 2) and that use this fragment probably as a habitat. As we did not individualize our recorded footprints, however, we are not sure about how many individuals were sampled. Thus, these findings should be considered with caution.

On the other hand, the taxa less frequently recorded were *Tamandua tetradactyla* and *Puma concolor*. Taking into account that *T. tetradactyla* is an arboreal animal (Alves et al. 2012), this species is rarely recorded by its footprints; this may explain the reason why this species was recorded only once in our study. *Puma concolor* was also recorded just once, which is expected considering that these animals are top predators that require large areas for their maintenance, commonly using the fragments as a passage to expand their foraging area (Mazzolli 2010; Magioli et al. 2014). Even with only one recording, the presence of *P. concolor* in the evaluated area indicates the importance of the IB Forest in contributing for the species maintenance, since it is considered a threatened species in São Paulo State. Besides *P. concolor*, *Leopardus pardalis* and *Sapajus* cf. *nigritus* recorded in our study are also considered threatened or near threatened species, highlighting the IB Forest's importance and maintenance.

Furthermore, potential common preys of *Puma concolor* were also detected in our study as *Dasypus novemcinctus*, *Mazama* sp., and *Dasyprocta azarae.* This suggests that it is also important to conserve fragments as the one studied here not only focusing to protect possible threatened species such as in our case, but also considering the need to protect species that can be valuable to help maintain top predators such as *P. concolor*. In fact, prey animals are also one of the factors influencing carnivore population size and dynamics (a bottom-up process; Sandell 1989; Laundre & Hernández 2010). Moreover, the occurrence of potential natural common prey species of *P. concolor* in the studied area minimizes the risk of livestock predation, as already clearly demonstrated in the Atlantic Forest (Palmeira et al. 2015).

Even in larger forest fragments of the Brazilian Atlantic Forest, as in the corridor in southern Bahia with 50,000ha, the decline of mammal populations may be evident (Canale et al. 2012). This situation tends to be more aggravated for larger mammals, not only because they require larger areas to live, but as Galetti et al. (2017) reinforce, such species are more affected by hunting. Furthermore, the more recent economic crisis in Brazil may be contributing to defaunation in the largest Atlantic Forest remnants, since around 30% of all forest guards were dismissed from the protected parks in São Paulo State in the last few years (Galetti et al. 2017). In this scenario, considering threatened species that already have their population densities seriously compromised, protective actions should be more intense, including the reduction of fragmentation. Regarding this, Mansourian et al. (2017) recommend restoration across the landscape to connect habitat fragments for threatened species.

One may say that the richness of medium and large-sized mammals detected here can be a consequence of the baits used. We assume, however, that such kind of effect was minimized in our study because we changed the type of bait in each sampling unit daily, similar to other studies (Pires & Cademartori 2012; Reale et

al. 2014). Moreover, throughout the study, species of different trophic categories were recorded in each set of plots, thus reinforcing that such kind of bias was prevented. Furthermore, in our study, there were only isolated occurrences of animals that were not medium or large-sized mammals. Thus, such facts demonstrate that the baits used here were suitable for our focus.

CONCLUSION

Based on our findings, we suggest that the IB Forest incorporated in EEF is a refuge for wild mammals, including species with some extinction threat level in the state of São Paulo, such as *Leopardus pardalis*, *Puma concolor*, and *Sapajus* cf. *nigritus*, and also, in Brazil, *Puma concolor*. Since these mammals are subject to anthropogenic influences such as grazing, monocultures, and illegal hunting, we emphasize the importance of monitoring the occurrence of these mammals as well as of the maintenance of forest fragments in the region.

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Portuguese Abstract: O desmatamento e a fragmentação do habitat afetam intensamente mamíferos silvestres de maior porte, que requerem grandes áreas para estabelecer suas populações. Esses mamíferos podem ter importantes funções na estrutura e dinâmica das florestas tropicais, atuando como dispersores de sementes, reguladores de herbivoria e espécies guarda-chuva. Aqui caracterizamos a comunidade de mamíferos silvestres de médio e grande porte em um fragmento de Floresta Estacional Semidecidual, denominado "Floresta do IB" (Instituto de Biociências), na Fazenda Experimental Edgardia, UNESP, Botucatu, SP. Para isso, por meio do estabelecimento de parcelas de areia em três trilhas como método principal, identificamos a ocorrência de nove espécies, além de uma espécie por visualização direta. Algumas delas estão em listas vermelhas: duas como ameaçadas (*Leopardus pardalis*, *Puma concolor*) e uma como quase ameaçada (*Sapajus* cf. *nigritus*) no Estado de São Paulo, sendo que a *Puma concolor* é também listada como vulnerável em nível nacional. Assim, enfatizamos a importância deste remanescente como refúgio da vida silvestre, o que torna necessário monitorar a ocorrência desses animais na área e conservar fragmentos similares na região.

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