Effects of puma on the diversity and composition of Neotropical mammals

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Abstract: Prey seek to minimize predation risk by moving across the landscape in search of safer areas. Yet, these movements are associated with risks that depend on the intrinsic attributes of the species involved. We evaluated the effect of presence of an apex predator (*Puma concolor*) on the composition of a community of medium and large-bodied terrestrial mammal species in 23 forest fragments in the State of São Paulo, Brazil, based on overnight footprint surveys over 2 y. We tested if (1) presence of the puma decreases species richness (prey and mesopredator), (2) landscape features interact with puma effects, altering the composition and richness of prey, (3) darker nights strengthen the predation risk effects of puma and (4) this effect can vary among prey species. The puma reduced the richness of prey species by ∼45% and presence of mesopredator by 11%. Larger forest fragments and darker nights strengthened the effects of puma on the mammal community. Most prey species showed negative associations with the apex predator, while others were unaffected or showed a positive association. These results add new knowledge about the effects of predation risk and of the landscape characteristics on the composition of the mammal community and the behaviour of different species. Furthermore, our results indicate that medium and large mammals live in a landscape of fear in Neotropical forest remnants.

Key Words: carnivores, fear, landscape, mesopredator, predator, prey, risk, vertebrates

INTRODUCTION

Predators reduce the density of prey populations, thus they can determine the richness and composition of the species that coexist in ecological communities (Ripple & Beschta 2004, Ripple *et al.* 2014, Schmitz 2010). Apex predators can potentially limit prey populations via predation, as well as limit mesoconsumer populations via competition and/or intraguild predation (Allen *et al.* 2014, Schmitz 2010). Moreover, in the face of predation risk, prey can reduce rates of foraging and intensify their vigilance (i.e. Lima 1998, Ripple & Beschta 2004, 2006). Thus, the decision of prey in reducing the probability of encounters with predators can influence the spatial and temporal use of home ranges, resulting in a concept called the landscape of fear (Laundré *et al.* 2010). A crucial element in this concept is that prey identify variable levels of predation risk at a spatial scale and this often leads to a negative spatial relationship between prey and predator

in which prey avoid the most risky sites in the landscape (Schmidt & Kuijper 2015).

Several studies have shown that the relationship between vertebrate apex predators and prey trigger a landscape of fear (Laundré & Hernández 2003, Ripple & Beschta 2006, Valeix *et al.* 2009). Wirsing & Ripple (2011) found that in both aquatic and terrestrial environments the non-lethal effect of vertebrate apex predators causes similar antipredator responses by their respective prey, comparing the effect of wolf and shark species on herbivores. Therefore, we know that large vertebrates are able to perceive signals from their predators and that these prey are able to respond to predation risk with elaborate antipredator strategies (Laundré *et al.* 2010), but that the responses may depend on the identity or traits of these mammals (e.g. size or behaviour). For example, it is known that larger prey are less vulnerable to predation (Sinclair *et al.* 2003) and that some mammal species can use shelters to protect themselves from predators or aggressors (Desbiez & Kluyber 2013, Sunquist & Sunquist 1989).

We investigated whether the presence of *Puma concolor* and the interaction of this predator with

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the characteristics of the environment influences communities of medium and large-bodied terrestrial mammals in 23 Neotropical forest fragments (an overall area of 84 600 ha), based on overnight footprint surveys over 2 y. We assume that the effects of this apex predator are predominantly non-consumptive, since (1) the surveys were conducted in a period shorter than 24 h, and (2) large solitary felids (e.g. puma, tiger and jaguar) on average kill only one prey individual per week (Cavalcanti & Gese 2010, Clark *et al.* 2014, Miller *et al.* 2013); the interval between hunting increases proportionately with the size of felled prey (Cavalcanti & Gese 2010), mainly because felids revisit the carcasses of their prey regularly for several days (Bacon & Boyce 2010, Cavalcanti & Gese 2010). Therefore, we hypothesize that (1) the presence of puma decreases species richness (prey and mesopredator) via non-consumptive effects, and that (2) landscape features (e.g. fragment size, sugar cane developmental stage) interact with apex predator effects, thus causing variable effects on the composition and richness of prey and mesopredators. In addition, we hypothesize that (3) darker nights strengthen the predation risk effects of puma on prey community. Finally, we hypothesize that (4) this non-consumptive effect can vary among prey species depending on their size or behaviour.

MATERIALS AND METHODS

Study region and fragment selection

The present study was conducted in 23 semideciduous forest remnants (Floresta Estacional Semidecidual – FESD), which are distributed in two sites of the State of São Paulo, Brazil. Of these, 13 fragments were located in a 62 800-ha area surrounding the city of Araçatuba (20°56′–21°7′S, 51°59′–51°4′W). The remaining 10 were located in a 21 800-ha area surrounding the city of Presidente Prudente (22°25′–22°41′S, 51°47′–51°29′W). The remnants are surrounded by a matrix dominated by the cultivation of sugar cane and are isolated from each other by a variety of manmade features including roads, cities, sugar cane monocultures, ponds and dams. The fragments in Araçatuba are separated on average (± SD) by 6.25 ± 3.19 km in Presidente Prudente by 5.74 ± 2.36 km. The mean ± SD size of the Araçatuba and Presidente Prudente forest fragments is 167 ± 119 ha and 367 ± 205 ha, respectively. The climate of both sites is classified as Aw (tropical hot and humid), presenting two distinct seasons (Rolim *et al.* 2007): rainy (October–March), with an average monthly rainfall of 21.5 ± 4.13 mm, and dry (April–September), with an average of 2.27 ± 0.92 mm.

Community of mammals in the study area

Currently there are records for 45 species of terrestrial mid- and large-bodied mammal in the State of São Paulo (Vivo *et al.* 2011). Species characteristic of both cerrado biome (i.e. Brazilian savanna) and FESD (Atlantic forest) occur in both areas studied. The orders commonly recorded in the forest fragments studied are: Cingulata, Pilosa, Primates, Rodentia, Lagomorpha, Perissodactyla, Artiodactyla and Carnivora (Appendix 1). However, hunting in the region resulted in the local extinction of species, such as giant otter (*Pteronura brasilensis*), giant armadillo (*Priodontes maximus*) and jaguar (*Panthera onca*) (Cheida *et al.* 2006, Vivo *et al.* 2011). Thus, we consider *Puma concolor* as the only apex predator commonly recorded in the forest fragments studied.

Sample design

We evaluated the effect of the presence of puma on the composition of mid- and large-bodied terrestrial mammal communities in 23 randomly selected forest fragments, based on overnight footprint surveys obtained in plots previously inspected and demarcated.

In each forest fragment a rectangular 4000-m^2 plot (4 × 1000 m) was established using a GPS, between the forest remnant and sugar cane monoculture. All inspections were made on foot during the early morning (07h00–09h00) to avoid the loss of records throughout the day. We did not sample during rainy or windy days, and only fresh footprints (one night, less than 24 h) were considered in each sample. Each of these plots was inspected seven times, every 3 mo between January 2011 and January 2013, in search of footprints to confirm the presence/absence of puma and mid- and large-bodied terrestrial mammal species (prey and mesopredator).

Plots were established on a substrate that comprised mostly sandy soil, which provides an ideal environment for recording footprints of mid- and large-bodied terrestrial mammals (Becker & Dalponte 1991). This method facilitates obtaining records of species that are difficult to visualize or capture (e.g. felids) and species identity was confirmed using identification guides (Becker & Dalponte 1991, Borges & Tomás 2004). Furthermore, camera traps and direct observations helped to confirm mammal identity. We analysed only mid- and large-bodied terrestrial mammal species (i.e. including prey, mesopredadors and apex predators) that moved between the fragments and sugar cane monocultures.

We considered as mid-sized those species with a mass between 2 and 7 kg, and as large those exceeding 7 kg (Emmons & Feer 1997). We considered three response variables: (1) richness of prey, (2) presence/absence of mesopredators (i.e. *Cerdocyon thous* and *Leopardus*

pardalis) and (3) presence/absence of apex predator (i.e. *P. concolor*). The classification of the mammal species in these functional groups was based on four works (Eisenberg & Redford 1999, Emmons & Feer 1997, Reis *et al.* 2011, Wilson & Reeder 2005) that addressed the diet, taxonomy, geographic distribution and behaviour in the Neotropics. The landscape variables used to construct the models, which may potentially predict the species richness were: (1) the developmental stage of the sugar cane adjacent to the fragments; absence denotes bare soil to budding stage (up to ~18 cm) and presence denotes maturing sugar cane (2–3 m tall). We expected that, in the absence of sugar cane, animals would be more exposed during movements/foraging to predators than in the presence of sugar cane. (2) The fragment area as a factor that directly affects resource availability (i.e. home range, shelter or food). (3) We also consider the influence of moonlight (i.e. percentage of moonface illuminated – %MFI) obtained by Moontool program for Windows 2.0 (https://www.fourmilab.ch/moontoolw/moontool16.html) the night preceding each sample, as a factor that could possibly influence the animal activity (i.e. prey, mesopredators and apex predator). Thus, we consider that darkest nights were represented by MFI between 0–50% and brighter nights between 60–100%.

Statistical analyses

We used permutational multivariate analysis of variance (PERMANOVA, Anderson 2001), coded in the adonis function of the vegan package in R, with Bray-Curtis dissimilarity coefficient and 999 permutations, to compare species richness between sites and between large and small forest fragments. We used linear mixed-effect models coded in the lme function of the nlme package in R to evaluate the influence of the apex predator (i.e. *Puma concolor*) presence on the richness of prey species. Generalized Linear Mixed Models (GLMMs) coded in the glmer function of the lme4 package in R was used to evaluate the influence of the apex predator presence on the presence of mesopredators. Presence of the apex predator was a fixed factor, and seasonality (i.e. samples every 3 mo) was a random factor with random intercept. We used Akaike's information criterion (AIC), corrected for small sample sizes (AICc, Burnham & Anderson 2002) to evaluate and weight the importance of the variables. We considered those models with the lowest AICc values (ΔAICc < 2) as the more plausible ones. We also selected models with Akaike weights (wAICc) higher than 0.1 (i.e. > 10%) as plausible. The wAICc index allows estimates of the relative efficiency of a model, given a list of competing models attempting to explain the patterns in the data (Burnham & Anderson 2002, Zuur *et al.* 2009). We also computed the contribution of the explanatory variables

that appeared in the plausible models, by adding up the wAICc of all models in which these variables appear. This model selection procedure was run separately for prey richness, and for presence of each mesopredator species (i.e. *Leopardus pardalis* and *Cerdocyon thous*). This procedure was also used to test the effects of environmental features on the occurrence of the apex predator (*Puma concolor*). The selected predictor variables were: (1) fragment area, (2) the presence/absence of *Puma concolor*, (3) the developmental stage of sugar cane and (4) percentage of moonface illuminated (%MFI). In addition, for model simplification (i.e. to evaluate interaction terms), we performed a Likelihood Ratio Test (LRT) to compare the effect of additive and multiplicative models. This procedure allowed identification of the relevant interaction terms among the variables selected by AICc.

To check the probability of association, i.e. Pr ($>|z|$), between the presence/absence of *Puma concolor* and presence/absence of each prey species we used generalized linear models (GLM) with binomial error family and log-logit as link function. To establish the effect magnitude of apex predator on each prey species, we used Cramer's association coefficient phi (ϕ) (Cramér 1999). Although this coefficient only ranges from 0–1, we represent graphically the direction of the effects for each of the phi value using the z-value (i.e. positive and negative) estimated by the GLM analysis.

We explored the potential mechanisms mediating the richness of prey by using a piecewise structural equation model (SEM). To infer the relative effects on prey richness we used (1) the presence/absence of the puma, *L. pardalis* and *C. thous*, (2) area of the fragments (ha), (3) the development stage of sugar cane and (4) percentage of moonface illuminated (%MFI). Piecewise SEM combines information from multiple separate linear models into a single causal network (Shipley 2009). Because the individual models can incorporate random structure and non-normal distributions, piecewise SEM is a powerful and flexible alternative to traditional variance-covariance-based SEM. Moreover, piecewise SEM can be used to circumvent restrictions on sample size (Shipley 2000). This approach constructs the path model as a set of hierarchical linear mixed models, each of which was fitted using restricted maximum likelihood with the *nlme* package (version 31.1-117) in R, and the overall path model (the SEM) was fit using R package piecewiseSEM (Lefcheck 2015).

All analyses were run using the R software and language (http://www.r-project.org) and the significance level was set at $\alpha = 0.05$. We checked heterogeneity of variances, normality, outliers and multicollinearity by graphical inspection (e.g. qq-plots, Cook's d, Influence) and statistical tests (Variance Inflation Factor (VIF), Levene's test); data were transformed when

Table 1. Category, family, species and common names of species recorded in the 23 fragments during the seven sample campaigns. The functional groups classification (i.e. prey, mesopredator and apex predator) were based on books that addressed the diet, taxonomy, geographic distribution and behaviour in the Neotropics. It is noteworthy that all species are terrestrial and move between the forest fragment and the matrix constituted by the planting of sugar cane.

Functional groups	Family	Species	Common name
Prey	Dasypodidae	*Dasypus novemcinctus*	Nine-banded armadillo
		Euphractus sexcinctus	Six-banded armadillo
	Tapiridae	*Tapirus terrestris*	Tapir
	Cervidae	*Mazama americana*	Red brocket deer
		Mazama gouazoubira	Brown brocket deer
	Tayassuidae	*Pecari tajacu*	Collared peccary
		Tayassu pecari	White-lipped peccary
	Dasyproctydae	*Dasyprocta azarae*	Azara's agouti
	Leporidae	*Sylvilagus brasiliensis*	Forest rabbit
	Procyonidae	*Procyon cancrivorus*	Crab-eating raccoon
Apex predator	Felidae	*Puma concolor*	Cougar
Mesopredator	Canidae	*Cerdocyon thous*	Crab-eating fox
	Felidae	*Leopardus pardalis*	Ocelot

needed, but back-transformed for the construction of figures.

RESULTS

We recorded footprints from 13 mid- and large-bodied mammal species. Of these, 10 were classified as prey, two as mesopredators and one was the apex predator (Table 1). Species composition was similar between sites (PERMANOVA, $R^2 = 0.0221$, $P = 0.25$) and forest fragments of different sizes (PERMANOVA, $R^2 = 0.0191$, $P = 0.34$).

Predictive models showed that the puma presence, puma presence + sugar cane and puma presence + fragment area influenced the richness of prey species (Table 2). The same pattern can be observed if we consider the cumulative wAICc for the richness of prey species (Table 3). Additive and multiplicative models including these predictors (i.e. puma presence associated with the sugar cane or with the fragment area) did not differ significantly (LRT = 2.15, P = 0.142 and LRT = 1.19, P = 0.273, respectively), meaning that their interactions are little relevant to prey richness. Whereas the presence of *P. concolor* reduced on average 45% the richness of prey species, the magnitude of effect of sugar cane stage and size of fragments were low, representing a decrease of only 7% and 0.2%, respectively. When sugar cane plantation is present, the average ± SD species richness was 4.97 ± 1.99, whereas in the absence this value declined to 4.63 ± 2.17. In relation to the size of the forest fragments, in large fragments the average ± SD of species richness was 4.83 ± 2.09, whereas in small fragments this value was 4.82 ± 2.05. Indeed, SEM demonstrated that prey richness decreased only in the presence of

P. concolor ($\beta = -0.64$, standardized coefficient, Figure 1).

Predictive models showed that the variables %MFI, puma presence, fragment area and sugar cane influenced the occurrence of the mesopredator *L. pardalis* (Table 2), although with lower magnitude as also confirmed by SEM (Figure 1). The same pattern can be observed if we consider the cumulative wAICc for the occurrence of *L. pardalis* (Table 3). *Leopardus pardalis* was present in 69% of the samples in fragments in darkest nights (%MFI between 0–50) and 78% in brighter nights (%MFI 60–100). When the sugar cane was taller, *L. pardalis* was present in 75% of the samples, whereas in the low sugar cane this value declined to 72%. In relation to the size of the forest fragments, in large fragments the occurrence of *L. pardalis* was 76%, whereas in small fragments this value declined to 69%. Additive and multiplicative models including the predictors puma presence and %MFI, %MFI and fragment area or puma presence and fragment area did not differ (LRT = 0.442, P = 0.502; LRT = 0.42, P = 0.461 and LRT = 0.41, P = 0.513, respectively), meaning that their interactions were not relevant to the occurrence of *L. pardalis*.

In contrast, a multiplicative model including puma presence × %MFI better explained the presence of the mesopredator *C. thous* (variables selected by ΔAICc, Table 2) when compared with an additive model (LRT = 4.80, P = 0.028). This means that the occurrence of *P. concolor* on darker nights decreased the frequency of this mesopredator in 11%. These results were also supported by SEM model ($\beta = -0.17$, standardized coefficient, Figure 1). The same pattern can be observed if we consider the cumulative wAICc for the occurrence of *C. thous* (Table 3). The predictive model sugar cane did not influence the occurrence of *C. thous* (Table 2), a result

Table 2. Models structures that best explain the species richness of mid- and large-bodied terrestrial mammals (i.e. prey, mesopredators and apex predator) in 23 semideciduous forest remnants in two sites of the State of São Paulo, Brazil. The table shows model structure, degrees of freedom (df), Akaike's information criterion corrected for small sample size (AICc), differences in AICc (ΔAICc) relative to the lowest value and the Akaike's weights (wAICc). The models with the lowest AICc values (ΔAICc < 2) and with wAICc higher than 0.1 (i.e. > 10%) were considered the most plausible. %MFI represents percentage of moonface illuminated. + indicates an additive effect and: multiplicative effect.

Model structure	df	AICc	ΔAICc	wAICc
Species richness				
SR ~ Puma presence + Sugar cane	5	616.8	0.0	0.5212
SR ~ Puma presence	4	617.8	1.1	0.3076
SR ~ Puma presence + Fragment area	6	620.0	2.2	0.1695
SR ~ Puma presence + %MFI	6	636.3	11.5	<0.001
SR ~ *Cerdocyon thous*	4	694.4	77.7	<0.001
SR ~ Sugar cane	4	698.4	81.7	<0.001
SR ~ Fragment area	4	699.5	82.7	<0.001
SR ~ *Leopardus pardalis*	4	699.7	82.9	<0.001
SR ~ %MFI	4	702.0	85.3	<0.001
SR ~ %MFI + Fragment area	5	703.5	86.7	<0.001
Leopardus pardalis				
LP ~ %MFI	3	188.4	0.0	0.41
LP ~ Puma presence	3	189.1	0.7	0.29
LP ~ Fragment area	3	190.2	1.8	0.17
LP ~ Sugar cane	3	190.7	2.3	0.13
Cerdocyon thous				
CT ~ Puma presence: %MFI	5	189.5	0.0	0.54
CT ~ Puma presence + %MFI	4	192.3	2.7	0.14
CT ~ Puma presence	3	192.5	2.9	0.12
CT ~ Sugar cane	3	193.3	3.7	0.08
CT ~ %MFI	3	194.1	4.4	0.06
CT ~ Fragment area	3	194.8	5.1	0.04
Puma concolor				
PC ~ Fragment area + %MFI	5	235.7	0.0	0.754
PC ~ Fragment area	4	238.9	3.2	0.153
PC ~ %MFI	4	240.1	4.4	0.082
PC ~ Sugar cane	4	244.5	8.8	0.009

Table 3. Cumulative weight of evidence (wAICc) for each individual explanatory variable (i.e. species richness and occurrence of the *Leopardus pardalis*, *Cerdocyon thous* and *Puma concolor*), in 23 semideciduous forest remnants in two sites of the State of São Paulo, Brazil. These values are computed by summing the wAICc of all models in which these variables are represented in Table 2. %MFI indicates percentage of moonface illuminated.

Model structure	Cumulative weight of evidence (wAICc)			
	Species richness	*L. pardalis*	*C. thous*	*P. concolor*
Puma presence	0.9983	0.29	0.80	–
Fragment area	0.1695	0.17	–	0.907
Sugar cane	0.5212	0.13	–	–
%MFI	–	0.41	0.68	0.754

that is also supported by SEM ($\beta = 0.11$, standardized coefficient, Figure 1).

The occurrence of *P. concolor* was affected by the predictors fragment area + %MFI and fragment area (i.e. selected by ΔAICc < 2 and wAICc > 0.1, Table 2). The same pattern can be observed if we consider the cumulative wAICc for the occurrence this apex predator (Table 3). *Puma concolor* was present in 68% of the samples

in large fragments and only 31% in smaller fragments. Additionally, *P. concolor* was present in 64% of the samples in fragments in darkest nights and only 34% in brighter nights. SEM demonstrated that presence of *P. concolor* and its effect on species richness were mediated by a positive effect of the size of the forest fragment (ha) ($\beta = 0.23$, standardized coefficient, see Figure 1) and a negative effect of %MFI ($\beta = -0.32$, standardized coefficient, Figure 1).
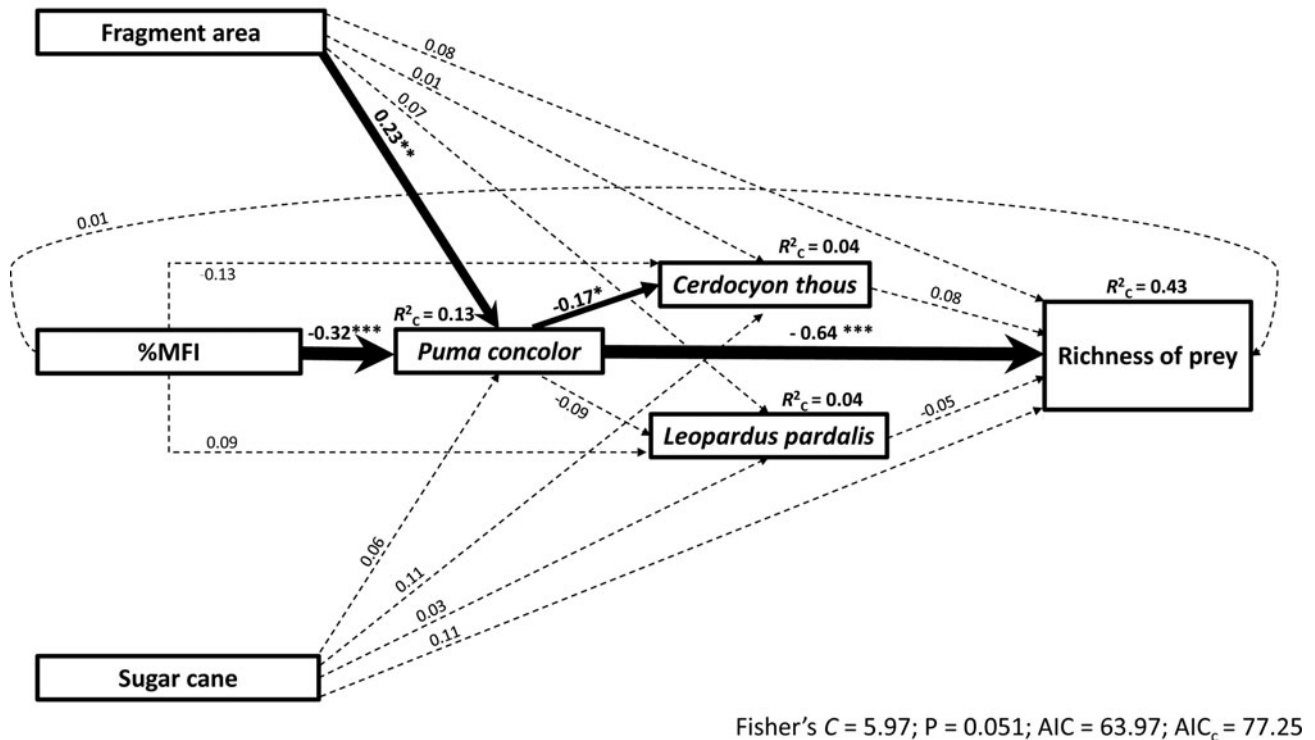
Figure 1. Results of structural equation modelling (SEM) showing the relative effects of fragment area (i.e. average $\pm$ SD; larger: $\sim$357 $\pm$ 145 ha and smaller: $\sim$54 $\pm$ 26.8 ha), the developmental stage of the sugar cane adjacent to the fragments (i.e. absence: denotes bare soil to budding stage up to $\sim$18 cm and presence: denotes maturing sugar cane above 2–3 m tall) and percentage of moonface illuminated (i.e. darkest nights: %MFI between 0–50 and brighter nights: %MFI between 60–100), on the presence of *Puma concolor* and consequently the presence of *Leopardus pardalis* and *Cerdocyon thous* (i.e. mesopredators) and species richness of prey. The dashed arrows represent no significant effects. The thickness of the arrows represents the magnitude of the standardized coefficient for each path. The values associated with arrows between variables represent standardized coefficients paths. The conditional R^2_c are presented above the boxes of the endogenous variables. *$P < 0.01$; **$P < 0.001$; ***$P < 0.0001$.

Through the GLM analysis and estimator of the coefficient of correlation Phi (φ), we detected that most of the prey species had a negative association with the presence of *P. concolor* (Figure 2). The intensity of the apex predator effect appears to be greater for prey species with a mass ranging between 20 and 25 kg, i.e. *Mazama* spp. (Figure 2). In contrast, *S. brasiliensis* (small prey) and *T. terrestris* (large prey) and mesopredators were not affected by *P. concolor* (Figure 2). Moreover, *D. azarae* showed a positive association with the presence of apex predator (Figure 2).

DISCUSSION

Our findings demonstrated that the richness of prey species and presence of the mesopredator *C. thous* (on darker nights) declined in the presence of *P. concolor*, corroborating our first hypothesis. Contrary to our second hypothesis, interactions of landscape features (e.g. sugar cane development stage or fragment area) with presence of apex predator were less relevant to the composition and

richness of prey. *Puma concolor* occupied larger fragments with higher frequencies, and were more active on darker nights, thus supporting our third hypothesis. In addition, we reported that prey have contrasting associations with the presence of *P. concolor*, i.e. while most showed negative associations with the apex predator, others were unaffected, and there was still another species that showed a positive association, corroborating our fourth hypothesis. These results add new knowledge about predation risk, antipredator behaviour and the landscape features that influence the community composition of mid- and large-bodied mammals in fragmented Neotropical regions. Furthermore, our results suggest a landscape of fear for mid- and large-bodied mammals in Neotropical forest remnants.

Several studies from temperate regions have shown that the presence of vertebrate apex predators inhibit the activity of their prey in response to predation risk (Laundré & Hernández 2003, Ripple & Beschta 2006, Valeix *et al.* 2009) and/or influence the structure and composition of mesopredator (or scavenger) communities (Allen *et al.* 2014, 2015; Crooks & Soulé 1999). Our results show
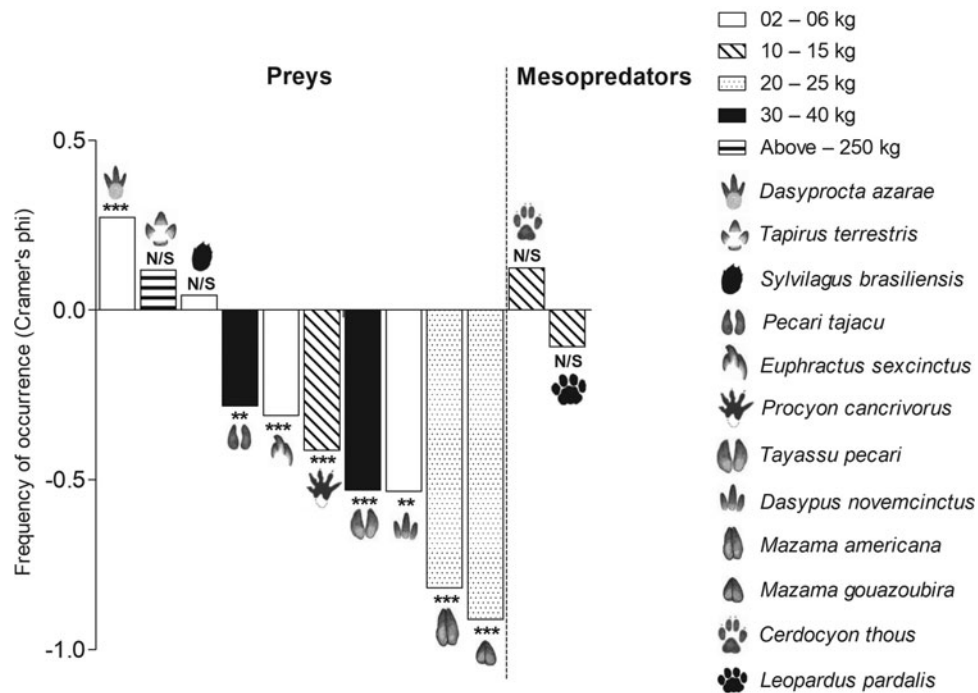
Figure 2. Binary association coefficient (Cramer's phi) between the presence of each prey and mesopredator (i.e. *Leopardus pardalis* and *Cerdocyon thous*) species with the presence of apex predator *Puma concolor*. Negative and positive values of Cramer's phi denote only the direction of the effect (i.e. z-value in the GLM analysis) on the occurrence of *Puma concolor* prey and mesopredators. **P <0.001; ***P <0.0001; n/s, no significant variation in the GLM analysis. Colouring shows different species weight classes (white = 2–6 kg, diagonal hatching = 10–15 kg, dotted = 20–25 kg, black = 30–40 kg and horizontal hatching = above 250 kg).

that this effect is also relevant in tropical environments. On the other hand, the landscape characteristics or their interactions with the presence of apex predators were less relevant to the richness of prey. It is known that habitat structure may play an important role during interactions between predators and prey (Schmidt & Kuijper 2015). In fragmented landscapes, prey can avoid areas or habitat patches with high density of predators or perceived risk (Lima & Dill 1990). However, when either the availability of high-quality habitats is low or when individuals are energetically stressed, prey may be forced to use patches in which predation risk is high (Lima & Dill 1990) or may be forced to increase their inter-patch movements (Johannesen *et al.* 2003). In addition, in fragmented landscapes *P. concolor* has advantages over its prey since it may quickly move from one habitat patch to another (Chiarello 1999), and choose best conditions for a successful hunt (e.g. hunting cover; Laundré & Hernández 2003, Sunquist & Sunquist 1989). As a consequence, prey do not know the whereabouts of the apex predator or the real predation risk at certain location (Brown *et al.* 1999), regardless of the landscape features, so they can increase vigilance rate (Lima 1998). To minimize vigilance by prey, *P. concolor* seems to rotate among large and small fragments; indeed,

we found this apex predator occupying large and small fragments. This rotation decreases vigilance enough so that predators have greater success in future attacks (Laundré & Loxterman 2007).

Our findings also indicate that *P. concolor* was active or present in forest fragments mainly on darker nights, regardless of fragment size. Generally, *P. concolor* does not alter its activity between different moon phases (Harmsen *et al.* 2010). However, under high rates of prey vigilance, *P. concolor* may seek to optimize the success of hunting by adopting different strategies. Thus, we assume that the hunting cover provided by forest remnants, regardless of size, can favour the success of predation, especially on darker nights, when many prey species are active (Rode-Margono & Nekaris 2014). Therefore, fragmentation might coerce prey to choose the remnant patches regardless of quality, thus negatively influencing prey foraging success. On the other hand, the prey can choose the most productive fragments regardless of the predation risk, and as a result, they may be facing death traps in the landscape of fear (as observed in the interaction between ungulates and apex predators in Poland; Schmidt & Kuijper 2015). Therefore, in the system studied here, the scarcity of potential sites to forage and the high risk of predation may explain the irrelevance

of landscape features and moon phase on the composition of the prey community.

The responses of prey and mesopredators to predation risk depend on the identity or traits of these mammals (e.g. size or behaviour). Our results indicate that Neotropical large prey (i.e. mass exceeding 7 kg) showed a negative association with the presence of *P. concolor* (except *T. terrestris*, which has mass of ~ 250 kg), suggesting that these species seek to minimize predation risk, occurring more in safer places within the landscape context, as reported for large herbivores from other zoogeographic regions (Laundré & Hernández 2003, Ripple & Beschta 2006, Valeix *et al.* 2009). However, the medium-sized prey (2–7 kg) showed different associations with *P. concolor* occurrence, revealing different adaptations to minimize the effects of predation risk within the landscape context. For example, these mammals can use natural cavities or burrows abandoned by armadillos (e.g. *Priodontes maximus*, Desbiez & Kluyber 2013) as refuges (i.e. the positive association of *D. azarae* in the presence of *P. concolor*), demonstrating the importance of these ecosystem engineers as providers of shelter from predators in Neotropical regions (Desbiez & Kluyber 2013, Sunquist & Sunquist 1989). Furthermore, *P. cancrivorous* can find refuges on trees (Emmons & Feer 1997). Thus, we would expect that both burrow builders (e.g. armadillos *D. novemcinctus* and *E. sexcinctus*) and species that adopt scansorial behaviour (*P. cancrivorous*) are sheltered and therefore would not be strongly affected by the presence of *P. concolor*. Future studies could investigate the effects mediated by mammalian predators in communities considering different strata of the landscape (e.g. underground, canopy).

Larger species (e.g. *T. terrestris* ~250 kg) were not affected by occurrence of *P. concolor*. Due to its size and weight, *T. terrestris* is preyed upon mainly by humans and occasionally by *Panthera onca* (Cheida *et al.* 2006). *Tapirus terrestris* can defend itself against attack by *P. onca* and hunting dogs by kicking them (Padilla & Dowler 1994). Thus, it is possible that *T. terrestris* does not perceive any predation risk due to its low vulnerability, as suggested by both empirical data and a meta-analysis which showed that large prey are less vulnerable to attack by predators than smaller prey (Breviglieri *et al.* 2013, Romero *et al.* 2011).

Regarding the response of mesopredators to the presence of an apex predator (*P. concolor*), whereas *C. thous* decreased, *L. pardalis* was not influenced by this predator. Although there is evidence that *P. concolor* feeds on *C. thous* (Silva-Pereira *et al.* 2011), we believe that the relationship between mesopredators and apex predators in the system studies is mostly competitive. *Leopardus pardalis* was not affected by *P. concolor*, likely because they do not share the same feeding items, i.e. *L. pardalis*

feeds on mainly small rodents and birds (Silva-Pereira *et al.* 2011). Thus, *L. pardalis* would not be competing directly with *P. concolor* for the same resources. On the other hand, *C. thous* decreased in the occurrence of *P. concolor* likely due to competitive displacement. *Cerdocyon thous* can consume carrion (Pedó *et al.* 2006), thus this species can exploit carcasses recently killed by *P. concolor*. However, the magnitude of the effects of the apex predator on this mesopredator was much smaller compared with herbivores (decrease 11% only). Therefore, we suggest that *C. thous* developed specific behavioural adaptions to exploit carrion and minimize competition with *P. concolor*, as also happens in the interaction between scavengers and apex predators (i.e. *Ursus americanus* and *P. concolor*) in California (Allen *et al.* 2014, 2015). The possible negative and positive effects of this interaction between Neotropical species of apex predator and scavengers could be further explored in future.

In conclusion, we report that the presence of *P. concolor* decreases species richness of mid- and large-bodied Neotropical mammals, but other variables had less importance. Generally the prey species change their pattern of occurrence influenced by the presence of *P. concolor* and seek sites that can minimize the effect of the risk, either via moving to areas with lower levels of predation risk or by using different strata of the landscape that limit the activity of their predators (e.g. underground, canopy). Thus, our results suggest that in Neotropical environments, the concept landscape of fear may be valid for different scales or strata (i.e. subsoil or canopy) when considering the different sizes, behaviours or dispersion capacity of the prey species that make up the community of mammals. For example, larger animals are not affected by the presence of *P. concolor* and, on the other hand, small prey seeks refuge in underground or on tree canopies. We suggest that future studies could address the effects of constant predation risk on each prey species in the community (i.e. reproduction and stress hormone). In addition, we demonstrate that the use of footprints may be a viable method for predator-prey studies thereby contributing to a better overall understanding of the landscape-of-fear theory.

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Appendix 1. Orders, families, species and common names of the mid- and large-bodied mammal recorded in 23 semideciduous forest remnants in two sites of the State of São Paulo, Brazil.

Orders	Family	Species	Common name
Cingulata	Dasypodidae	*Dasypus novemcinctus* Linnaeus, 1758	Nine-banded armadillo
		Euphractus sexcinctus (Linnaeus, 1758)	Six-banded armadillo
Perissodactyla	Tapiridae	*Tapirus terrestris* Linnaeus, 1758	Tapir
Artiodactyla	Cervidae	*Mazama americana* (Erxleben, 1777)	Red brocket deer
		Mazama gouazoubira (Fischer, 1814)	Brown brocket deer
	Tayassuidae	*Pecari tajacu* (Linnaeus, 1758)	Collared peccary
		Tayassu pecari (Link, 1795)	White-lipped peccary
Pilosa	Myrmecophagidae	*Myrmecophaga tridactyla* Linnaeus, 1758	Giant anteater
		Tamandua tetradactyla (Linnaeus, 1758)	Lesser anteater
Rodentia	Caviidae	*Cavia aperea* Erxleben, 1777	Guinea pig
		Hydrochoerus hydrochaeris (Linnaeus, 1766)	Capybara
	Dasyproctydae	*Dasyprocta azarae* Lichtenstein, 1823	Azara's agouti
Primates	Callitrichidae	*Callithrix jacchus* (Linnaeus, 1758)	White-tufted-ear marmoset
		Callithrix penicillata (É. Geoffroy, 1812)	Black-tufted marmoset
	Cebidae	*Sapajus nigritus* (Goldfuss, 1809)	Black-horned capuchin
	Atelidae	*Alouatta caraya* (Humboldt, 1812)	Black howler
		Alouatta guariba (Humboldt, 1812)	Brown howler monkey
Lagomorpha	Leporidae	*Sylvilagus brasiliensis* (Linnaeus, 1758)	Forest rabbit
Carnivora	Procyonidae	*Procyon cancrivorus* (G. [Baron] Cuvier, 1798)	Crab-eating raccoon
	Felidae	*Puma concolor* (Linnaeus, 1771)	Cougar
		Leopardus pardalis (Linnaeus, 1758)	Ocelot
		Herpailurus yagouaroundi (É. Geoffroy Saint-Hilaire, 1803)	Jaguarundi
	Canidae	*Cerdocyon thous* (Linnaeus, 1766)	Crab-eating fox
		Chrysocyon brachyurus (Illiger, 1815)	Maned wolf
	Mustelidae	*Eira barbara* (Linnaeus, 1758)	Tayra
		Galictis cuja (Molina, 1782)	Lesser grison
		Lontra longicaudis (Olfers, 1818)	Long-tailed otter
	Procyonidae	*Nasua nasua* (Linnaeus, 1766)	South American coati