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The Influence of Spatial Attributes on Fragment Occupancy and Population Structure in the Mexican Mantled Howler (*Alouatta palliata mexicana*)

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Abstract It is essential to document habitat occupancy patterns and population structure to facilitate the survival of primates in areas of anthropogenic disturbance. The overlapping of the Nearctic and Neotropical regions in the Olmec region of Mexico make this area particularly important as part of a natural biological corridor that harbors a high number of endemic species and connects the Atlantic and Pacific coastal plains. We surveyed *Alouatta palliata mexicana* (Mexican mantled howlers) in a 300-km^2 area to determine if fragment occupation and subpopulation structure were related to the spatial attributes of the fragments. We measured the fragment size and shape as well as the distances to the nearest road, human settlement, agricultural field, and nearest neighboring fragment. During 1 year (*ca*. 4500 fieldwork hours) we surveyed 48 fragments, 17 of which were occupied, and counted 198 howlers. Larger fragments that were farther from agricultural activities were more likely to be occupied. Subpopulation size and number of individuals in all age–sex classes increased in larger fragments that were closer to other fragments. We found more females and

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juveniles, as well as more females per male in fragments that were farther from roads and we found fewer immatures per female, females per male, and individuals per area in more irregular fragments. In addition, more males and immatures per female occurred in fragments that were farther from agricultural fields. The Olmec Region is located at the center of the geographic distribution of mantled howlers in Mexico, and could therefore play a fundamental role in maintaining the contact between different populations. However, our study suggests that mantled howlers are highly threatened by anthropogenic habitat disturbance in this area, mainly through the loss of their habitat and contact with humans.

Keywords Anthropogenic disturbance · Fragmentation · Habitat loss · Howlers

Introduction

The disturbance of natural habitats caused by human activities, such as agriculture, industry, and forestry, are leading to their loss, fragmentation, and transformation (Cowlishaw and Dunbar 2000). Human disturbances are the main drivers of the ongoing biodiversity crisis (Dirzo and Raven 2003), which is characterized mainly by ecosystem disruptions, e.g., boreal ecosystems (Mayor *et al.* 2012) and semiarid ecosystems (Ribeiro *et al.* 2015) and species loss, e.g., birds (Pimm *et al.* 2006) and mammals (Ceballos *et al.* 2015). In the face of this disturbance, documenting variation in species presence and population structure in response to anthropogenic habitat disturbance is necessary to aid predicting future persistence of individuals and to develop effective conservation and management strategies.

In disturbed habitats primate mortality increases as a consequence of human activities, such as hunting (Marshall *et al.* 2006), fires (Kinnaird and O'Brien 1998), and road kills (Kumara *et al.* 2000). In addition, individuals likely produce fewer offspring or have lower offspring survival, e.g., black howlers (*Alouatta pigra*: Dias *et al.* 2015) and simakobu (*Simias concolor*: Erb *et al.* 2012). Reduced fecundity and survival of individuals in disturbed habitats are probably proximately linked to poor nutrition, physiological stress, and disease (Chapman *et al.* 2015; Dias *et al.* 2015; Mbora *et al.* 2009). Occupancy patterns and population size in a variety of primate species are positively affected by forest fragment size (Arroyo-Rodríguez *et al.* 2013; da Silva *et al.* 2015; Ménard *et al.* 2014) and shape (Arroyo-Rodríguez *et al.* 2013; Dias *et al.* 2015), and are negatively affected by human forest use (da Silva *et al.* 2015; Ménard *et al.* 2014) and habitat isolation (da Silva *et al.* 2015; Sharma *et al.* 2014). These negative effects of habitat disturbance on primates ultimately result in population reduction, which may lead to changes in species distribution patterns and increased extinction probabilities (Chapman and Lambert 2000; De Vleeschouwer and Raboy 2013).

Howlers (*Alouatta* spp.) are large-bodied arboreal primates that live in nearly all types of tropical forests of the Americas (Kowalewski *et al.* 2015). They are particularly resilient to habitat disturbance, and are common throughout the Neotropics, even in areas where other mammals, including primates, have become locally extinct (Rangel-Negrín *et al.* 2014; Terborgh *et al.* 2001). Furthermore, of the 12 species currently recognized (Cortés-Ortiz *et al.* 2015), only four are considered Threatened (IUCN 2015). There is, however, abundant evidence that howlers are negatively affected by habitat disturbance (Arroyo-Rodríguez and Dias 2010), and at the subspecific level several taxa are Threatened (IUCN 2015). Such is the case of *A. palliata*, the mantled howler. This is one of the howler species with the widest latitudinal distributions, and it is currently categorized as Least Concern (IUCN 2015). However, four out of its five subspecies are categorized as Threatened (IUCN 2015). The Mexican mantled howler (*A. p. mexicana*) is present in southern Mexico and northwest Guatemala (Rodríguez-Luna *et al.* 2009) and is currently categorized as Critically Endangered (IUCN 2015). In Mexico, mantled howlers live in protected areas, such as Los Tuxtlas Biosphere Reserve and extensive nonprotected areas, such as the Selva Zoque (Fig. 1). Previous research has identified forest fragment size, shape, isolation, and proximity to human activities as the main predictors of the presence and abundance of mantled howlers in Mexico (Anzures-Dadda and Manson 2007; Arroyo-Rodríguez *et al.* 2008; Cristóbal-Azkarate *et al.* 2005; Estrada and Coates-Estrada 1996).

It is estimated that a majority of populations of mantled howlers live in highly disturbed areas (Rodríguez-Luna *et al.* 2009). One of these areas is the Olmec Region in southern Veracruz (Fig. 1). The original vegetation in this area was mainly evergreen and semievergreen rainforests, as well as hydrophytic vegetation in flooded areas near the Coatzacoalcos River (Ellis and Martínez-Bello 2010). From a zoogeographical perspective, this is a particularly important area, because here the Nearctic and Neotropical regions overlap, resulting in a high number of endemic species, and it is part of a natural biological corridor connecting the Atlantic and the coastal Pacific plains (Peterson *et al.* 1999). For mantled howlers, it connects the northernmost population of Los Tuxtlas, with populations of the Selva Zoque and the lowlands of Tabasco (Fig. 1). Currently, 41.3 % of the Olmec Region territory is dedicated to agricultural activities, and the oil industry has also disturbed large tracts of vegetation (Ellis and Martínez-Bello 2010).

To improve our knowledge about the responses of primates to habitat disturbance, we 1) surveyed the Olmec Region for the presence of mantled howlers and 2)

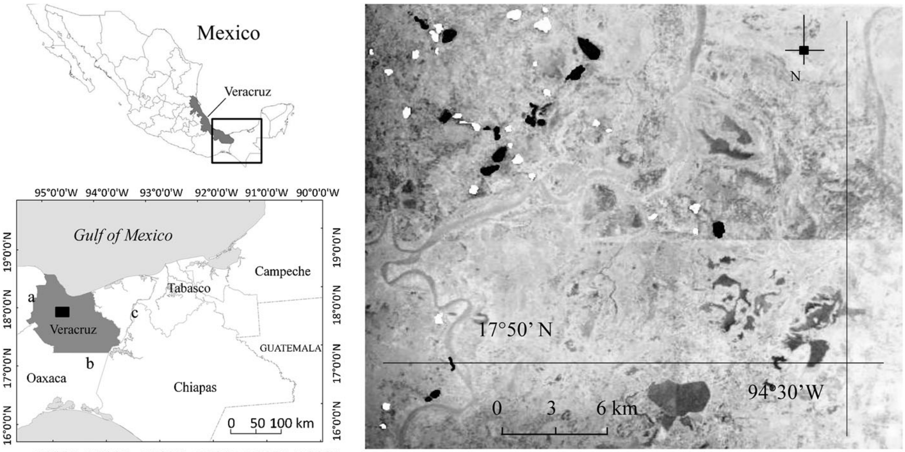

Fig. 1 Map of southern Veracruz, Mexico (*top left, shaded in grey*). The Olmec Region (*dark gray* area in the map at *bottom left*) connects the northernmost subpopulations of *Alouatta palliata mexicana* of Los Tuxtlas (**a**) with those at the Selva Zoque (**b**) and at the plains of Tabasco (**c**). The study area is represented as *solid black square* in the map at *bottom left* and figure at *right* (fragments with howlers in *black* and fragments without howlers in *white*).

determined if in this region forest fragment occupation and subpopulation structure of mantled howlers are related to the spatial attributes of the fragments. We predicted that the probability of fragment occupancy and the abundance of mantled howlers should increase for larger fragments with regular shape that were closer to other fragments and farther from human activities (settlements, roads, and agricultural fields).

Methods

Study Area

We conducted our study in the Olmec Region, Veracruz state (Mexico; Fig. 1). We concentrated our surveys in an area of *ca.* 300 km^2. We selected this area because there were informal reports of the presence of mantled howlers. The climate is warm and humid, with mean ambient temperature of 25.6 °C and annual rainfall of 2283.5 mm. Rainfall varies seasonally, with mean rainfall in the months of December to May (76.9 mm) representing only 25.3 % of rainfall in June–November (303.7 mm).

Survey and Census of Howler Subpopulations

We used orthophotos (1:10,000, resolution 1 m) of the study area taken between 2004 and 2006 and obtained from the National Institute of Statistics and Geography of Mexico (INEGI), to digitize vegetation fragments in the study area with ArcGIS 9.3.1 (ESRI Inc., Redlands, CA, USA). We defined a fragment as a patch of forest with size ≥0.5 ha that was not connected to other patches. This choice was based on previous studies that identified a 0.5 ha criterion as the minimum habitat requirement for fragment occupation by howlers (Arroyo-Rodríguez *et al.* 2008; Cristóbal-Azkarate *et al.* 2005). We corroborated the presence of fragments during field surveys, and confirmed that they were composed of natural vegetation rather than plantations.

Between June 2006 and May 2007 we surveyed all forest fragments within the 300-km^2 area described in the previous subsection for the presence of mantled howlers. We used three methods to survey howlers. First, we positioned in strategic spots before sunrise and waited for the howlers to howl. When howls were heard, we approached the emitter and started the survey. Second, we walked slowly (*ca.* 1.25 km/h) around and inside each fragment between 06:00 and 19:00 h, to detect visual cues (e.g., movement in the canopy, feces, consumed food items) or vocalizations of howlers. We performed walks inside the fragments along parallel north–south transects 40 m apart (visibility of monkeys in each side of trails was *ca.* 20 m) using a compass; we walked each trail back and forth twice each day. Total survey time using this method was thus a function of fragment area, as the number of trails per fragment varied between 1 and 15 and trail length varied between 23 and 418 m, with a mean (±SD) of 900.1 ± 501.7 m walked per fragment per day. Third, we interviewed local people about the presence of howlers. Whereas in small fragments (<5 ha) the first method was sufficient to detect individuals, in large fragments the second method was usually required to ensure that no individuals were missed during surveys. We resurveyed an area if we had found no howlers but informants told us they were present.

Once an individual was located, we determined sex through visual inspection of genitals. We classified as a juvenile any individual that moved independently from the mother, was roughly half her size, and was very active; as an infant any individual clinging to the mother's dorsal or ventral hair, totally dependent on her for travel, feeding, and that left the mother only for short periods of time; and as an adults any individual that was neither juvenile nor infant (National Research Council 1981). We also noted characteristics of each individual that could be used for identification, such as body size and proportions; scars; broken fingers; genital morphology and pigmentation; as well as blond hairs and skin pigmentation on the feet, hands, and tail. When censusing groups, we performed repetitive counts until consistent results on their size and composition were obtained. These criteria allowed us to identify each group; i.e., individuals and groups were not recorded more than once in each survey. We visited each forest fragment at least bimonthly, resulting in 388 days and *ca.* 500 h of fieldwork. Sampling effort varied as a function of fragment size, such that each small fragment (0.5–4.99 ha) was sampled for a mean (±SD) of 6.2 ± 0.5 days during the study, medium-sized fragments (5–10 ha) for 9.4 ± 0.6 days, and large fragments (>10 ha) for 14.8 ± 6.5 days.

Spatial Attributes of Fragments

During surveys we noticed that several fragments had a different spatial configuration from that recorded in orthophotos. We therefore conducted our measurements of fragment spatial attributes in situ with a global positioning system (GPS). We studied the following spatial attributes of the fragments: size, shape, proximity to human activities, and proximity to other fragments (i.e., isolation). To calculate size, we walked the perimeter of each fragment collecting waypoints with a GPS at 5-m intervals, which were then uploaded in ArcGis and used to calculate fragment perimeter and area. We calculated a shape index for each fragment (Forman and Godron 1986): $\mathrm{SI} = P/\sqrt{A\pi}$; where P is the perimeter (in meters) and A is the area (in square meters), respectively. This index varies between 1, for a circular shape, and 5, for a highly irregular shape. To calculate proximity to human activities, we located and positioned with a GPS the nearest road, human settlement, and agricultural field to each fragment. To estimate isolation we located and positioned the nearest forest fragment, and then we calculated the linear distance between each fragment and nearest road, human settlement, agricultural field, and forest fragment with ArcGis. We recorded the nearest waypoint to each of these landscape elements when determining fragment size.

Statistical Analyses

We surveyed 48 fragments and observed 198 mantled howlers in 17 fragments. Subpopulation size varied between 1 and 53 individuals, and a total of 24 groups were recorded [Electronic Supplementary Material (ESM) Table SI).

We used generalized linear models (GLMs) to model the influence of fragment spatial attributes on each of 10 dependent variables [occupancy, number of adult females, number of adult males, number of juveniles, number of infants, the ratio of immatures (i.e., juveniles and infants) to adult females, the ratio of immatures to adult females, the ratio of adult females to adult males (i.e., socionomic sex ratio; hereafter sex ratio), total subpopulation size, and subpopulation density (number of individuals/fragment size)]. Predictor variables were fragment size, fragment shape, distance to nearest road, distance to nearest human settlement, distance to nearest agricultural field, and distance to nearest fragment. We used the Akaike's information criterion (AIC) to select the most plausible model, that is, the combination of spatial attributes that best explained each dependent variable (Burnham and Anderson 2002). For subpopulation structure-dependent variables, we used second-order AIC values, i.e., AIC_c (Burnham and Anderson 2002) for model selection due to small sample size ($N = 17$ fragments occupied by mantled howlers). Because only a very small subset of populations included infants, unique parameter estimates could not be calculated for predictors of infant to female ratios, so this variable could not be analyzed further. We assessed model plausibility through AIC differences (Δ_i) and weights (w_i), and we selected the model with the lowest Δ_i for significance testing, i.e., the most plausible model (Burnham and Anderson 2002). In addition to the most plausible models, we considered models with $\Delta_i = 0$–2 to receive substantial empirical support (Burnham and Anderson 2002), and we also described them. To analyze fragment occupancy, we ran a GLM in which the presence/absence of mantled howlers in fragments was a dichotomous response variable (and used a binomial distribution with a log link-function in modeling) and fragment size, shape index, distance to nearest road, distance to nearest human settlement, distance to nearest agricultural field, and distance to nearest forest fragment were the predictor variables. To determine the relationship between fragment spatial attributes and subpopulation structure of fragments that were occupied by howlers we ran GLMs for each response variable, i.e., number of adult females, number of adult males, number of juveniles, number of infants, the ratio of immatures to adult females, sex ratio, total subpopulation size, and subpopulation density. Independent variables were fragment size, shape index, distance to nearest road, distance to nearest human settlement, distance to nearest agricultural field, and distance to nearest fragment. We selected a Poisson distribution with a logit link-function to analyze the number of adult females, number of adult males, number of juveniles, number of infants, and total subpopulation size. We selected a normal distribution with an identity link-function to analyze the ratio of immatures to adult females, the ratio of infants to adult females, sex ratio, and subpopulation density. We assessed the distribution of continuous dependent variables (i.e., immature to female ratio, sex ratio, and subpopulation density) via QQ plots. We determined normality with a Kolmogorov–Smirnov test. To improve the adequacy of model fit, we transformed total subpopulation size to square roots. We calculated each predictor's variance inflation factors (VIF; Neter *et al.* 1990) in each of the most plausible models as the inverse of the coefficient of nondetermination ($1/(1 - R^2)$) for a regression of that predictor on all others. All predictors had $VIF \leq 1.5$, indicating no multicollinearity, and were therefore included in the GLMs described in the preceding text. To account for multiple testing of the hypothesis that subpopulation structure was influenced by fragment spatial attributes ($N = 8$ models), we calculated statistical significance controlling for the False Discovery Rate (FDR) (Benjamini and Hochberg 1995). Finally, to determine the importance of predictive variables in explaining variation in dependent variables, we calculated the effect size of each predictor using partial correlation coefficients and 95 % confidence intervals as described by Nakagawa and Cuthill (2007).

Ethical Note

This study was noninvasive and complied with the legal requirements of the Estados Unidos Mexicanos. Our research protocols were reviewed and approved by the Facultad de Biología de la Universidad Veracruzana, Xalapa (Mexico). The authors have no conflict of interest to declare.

Results

Fragment Occupancy

At least one mantled howler was present in 35.4 % of surveyed fragments (cf. supplemental data in ESM Table SI). The best model predicting the presence of howlers in fragments included fragment size, distance to nearest road, distance to nearest agricultural field, and distance to nearest fragment (Table I). Three additional models received substantial support, although they were at least 13 % less plausible than the best model. In the best model, distance to nearest agricultural field and fragment size had the largest effect sizes and had significant effects on occupancy, such that fragments that were farther from agricultural fields [occupied (mean±SE) = 133.3 ± 35.4 m; nonoccupied = 19.5 ± 65.6 m] and that were larger (occupied = 8.3 ± 2.1 ha; nonoccupied = 3.1 ± 3.5 ha) had higher probabilities of being occupied.

Influences of Fragment Spatial Attributes on Subpopulation Structure

Population size varied between 1 and 53 individuals (ESM Table SI). The majority of the 17 fragments where we found mantled howlers had a single group (53 %), followed by fragments with more than one group (23.5 %) and fragments including only males (23.5 %), either solitary or in pairs. Heterosexual populations usually included more females (mean = 5.8 individuals, range = 1–21) than males (mean = 4.8, range = 1–17), and more adults (mean = 10.6, range = 2–38) than immatures (mean = 4.2, range = 0–15). Sex ratio varied between zero (i.e., populations containing only males) and three females per male. In addition to the four populations that comprised only males, there were two fragments in which we never observed immatures, and four in which no infants were observed. Therefore 35.3 % of the fragments occupied by howlers did not include immatures. The immature to female ratio ranged between 0 and 1, whereas the infant to female ratio ranged between 0 and 0.38. Population density ranged between 0.2 and 8.9 individuals/ha.

Fragment size (14 models), distance to nearest fragment (12), and shape index (11) were the predictors included more frequently in models with substantial empirical support, followed by distance to nearest agricultural field (7), distance to nearest road (5), and distance to nearest human settlement (2) (Table I). For the number of females, males, juveniles, and infants in subpopulations best models were significantly more plausible than other models based on their Δ_i and w_i values. However, for immatures to female ratio, sex ratio, total individuals in the subpopulation, and subpopulation density several models were equally plausible.

Table I Results of model selection using the Akaike information criterion implemented with generalized linear models investigating the influence of spatial attributes (predictors) on fragment occupancy and subpopulation structure of Mexican mantled howlers in the Olmec region (Mexico) surveyed between June 2006 and May 2007

Model	Predictors					Δ_i [a]	w_i
	1	2	3	4	5		
Occupancy	FRS	DNR	DNA	DNF		0.00	0.22
	FRS	DNA	DNF			1.69	0.09
	FRS	SI	DNR	DNA	DNF	1.98	0.08
	FRS	DNR	DNH	DNA	DNF	1.99	0.08
Females	FRS	DNR	DNF			0.00	0.45
Males	FRS	DNA	DNF			0.00	0.23
	FRS	DNA				1.71	0.10
	FRS	DNF				1.81	0.09
	FRS	SI	DNA	DNF		1.84	0.09
	FRS	DNR	DNF			0.00	0.51
Juveniles	FRS	DNF				0.00	0.32
Infants	SI	DNF				0.00	0.13
Immatures/females	SI	DNA				0.02	0.12
	SI	DNR				1.21	0.07
	SI	DNH				1.41	0.06
	SI	DNA	DNF			1.93	0.05
	SI	DNR	DNF			0.00	0.13
Sex ratio	FRS	SI	DNR			1.61	0.06
	FRS	DNF				0.00	0.12
Total individuals	FRS	DNA				0.63	0.09
	FRS					0.80	0.08
	SI					1.58	0.05
	FRS	DNA	DNF			1.89	0.05
	FRS	DNH				1.99	0.04
	SI					0.00	0.14
Subpopulation density	FRS					1.15	0.08
	SI	DNF				1.22	0.08

FRS fragment size, *DNR* distance to nearest road, *DNA* distance to nearest agricultural field, *DNF* distance to nearest fragment, *SI* shape index, *DNH* distance to nearest human settlement

[a] Δ_i are Akaike differences; w_i are Akaike weights. Only models with substantial empirical support, i.e., $\Delta_i < 2$, are represented

In the best models (Table II), the number of females, males, juveniles, infants, and total individuals was influenced mainly by fragment size (i.e., predictor with the largest effect size), which had positive effects on those variables. Distance to nearest fragment was also included in the best models of the same variables, and in the sex ratio model. Distance to nearest fragment had negative effects on all models when it was included,

Table II Results of generalized linear models investigating the most plausible models for the influence of spatial attributes (predictors) on fragment occupancy and subpopulation structure of Mexican mantled howlers in the Olmec region (Mexico) surveyed between June 2006 and May 2007

Model	Predictor	B [a]	*r* (ES)	95 % CI		*P*	Benjamini–Hochberg *P*
				LL	UL		
Ocurrence	FRS	−0.278	0.33	0.08	0.57	0.028	–
	DNR	−0.003	0.25	0.25	0.25	0.072	–
	DNA	−0.015	0.41	0.40	0.42	0.005	–
	DNF	0.004	0.24	0.24	0.25	0.076	–
Females	FRS	0.103	0.82	0.79	0.86	0.000	0.000^{*}
	DNR	0.001	0.69	0.69	0.69	0.001	0.003^{*}
	DNF	−0.010	0.80	0.79	0.80	0.000	0.000^{*}
Males	FRS	0.080	0.80	0.77	0.83	0.000	0.000^{*}
	DNA	−0.003	0.55	0.55	0.55	0.022	0.033^{*}
	DNF	−0.002	0.40	0.39	0.40	0.087	0.097^{*}
Juveniles	FRS	0.110	0.76	0.71	0.81	0.000	0.000^{*}
	DNR	0.002	0.65	0.64	0.65	0.004	0.008^{*}
	DNF	−0.013	0.76	0.75	0.76	0.000	0.000^{*}
Infants	FRS	0.160	0.74	0.66	0.81	0.000	0.000^{*}
	DNF	−0.011	0.69	0.68	0.69	0.001	0.002^{*}
Immatures/females	SI	−0.407	0.56	0.24	0.89	0.015	0.026^{*}
	DNA	0.003	0.33	0.33	0.33	0.116	$0.116^{\text{n.s.}}$
Sex ratio	SI	−0.247	0.55	0.34	0.76	0.022	0.033^{*}
	DNR	0.001	0.63	0.63	0.63	0.005	0.010^{*}
	DNF	−0.003	0.46	0.46	0.46	0.057	0.068^{*}
Total individuals	FRS	0.035	0.52	0.49	0.55	0.027	0.037^{*}
	DNF	−0.002	0.34	0.34	0.34	0.109	$0.115^{\text{n.s.}}$
Subpopulation density	SI	−0.387	0.44	0.04	0.83	0.056	0.068^{*}

FRS fragment size, *DNR* distance to nearest road, *DNA* distance to nearest agricultural field, *DNF* distance to nearest fragment, *SI* shape index

[a] B are the unstandardized regression coefficients; *r* are effect sizes calculated with partial correlation coefficients; 95 % CI are the 95 % confidence intervals of the effect sizes; Benjamini–Hochberg *P* are significance values adjusted for the False Discovery Rate (* indicates a significant effect; n.s. indicates a nonsignificant effect)

and presented moderate to high effect sizes ($r > 0.6$) in four (number of females, juveniles, and infants and sex ratio). Distance to the nearest road had positive moderate ($r > 0.60$) effects on the number of females and juveniles per subpopulation, and on sex ratios. Shape index was the predictor with the largest effect size on the immatures to female ratio; was the only predictor included in the best subpopulation density model; and had a moderate effect on the sex ratio. Shape index had a negative effect in all models when it was included. Distance to nearest agricultural field had a moderate effect size ($r = 0.55$) on the number of males per subpopulation model and was also included in the immatures to female ratio model, although with a nonsignificant effect.

Overall, the best models indicate that subpopulation size and number of individuals in all age–sex classes increased in larger fragments that were closer to other fragments. More females and juveniles and a higher proportion of females per male occurred in fragments that were farther from roads. Fewer immatures per female, females per male, and individuals per area occurred in fragments with more irregular shapes. Finally, more males and immatures per female occurred in fragments that were farther from agricultural fields.

Discussion

Our results suggest that for Mexican howlers fragment occupancy patterns and subpopulation structure within the fragments surveyed in the Olmec Region are influenced by the spatial attributes of forest fragments. All studied predictors were included in a least one model, although some had stronger influences on occupancy and subpopulation structure than others, as assessed by their inclusion in models with substantial empirical support and effect sizes. Fragment size, distance to nearest fragment, and shape index were the best predictors, followed by distance to nearest road and distance to nearest agricultural field. Therefore, we provide evidence that mantled howlers are negatively affected by anthropogenic disturbance in this area, mainly due to forest loss, but also through the transformation of their habitat, as assessed by distance to agricultural fields and roads.

As predicted by theoretical models (MacArthur and Wilson 1967), habitat availability pervasively influences species distribution and abundance, which has been demonstrated by studies analyzing species–area relationships in primates (Cowlishaw 1999; Harcourt and Doherty 2005). Small habitats support fewer individuals, and small populations are more vulnerable to the effects of demographic and environmental stochasticity, genetic drift, inbreeding depression, and Allee effects (Fischer and Lindenmayer 2007). At the individual level, these threats may lead to a reduction in survival and reproduction, whereas at the population level, threats may lead to reductions in population size and genetic diversity (Fahrig 2003). Our results align with this evidence and are consistent with other studies on howlers in which fragment size predicts occupancy patterns and subpopulation structure (Arroyo-Rodríguez and Dias 2010). Our results are probably determined by a positive relationship between fragment size and food availability, and by negative relationships among fragment size and anthropogenic pressures, physiological stress, and parasite loads (Arroyo-Rodríguez and Dias 2010). We found indirect evidence that food availability may be affecting subpopulation structure. Variation in fragment shape affects vegetation structure and composition owing to its relationship with edge effects, as irregular fragments have a higher proportion of edge than those with regular shapes (Ranta *et al.* 1998). Because tree mortality and damage are higher in forest edges (Laurance *et al.* 1998), the negative effects of shape index on immature to female ratio and subpopulation density could reflect a decrease in food availability in fragments with irregular shapes.

The positive relationship between distance to agricultural activities and fragment occupancy suggests that, besides changes to forest cover measured through fragment size, other types of disturbance may impact howler subpopulations in the Olmec Region. Whereas several occupied fragments were surrounded by abandoned or fallow fields, the majority of nonoccupied fragments were adjacent to pasturelands or crop fields that were visited daily by people. Noise (e.g., tractors), harassment by domestic dogs, and presence of people inside fragments to collect firewood are factors with the potential to disturb howlers (Aguilar-Mello *et al.* 2013; Arroyo-Rodríguez *et al.* 2008; Rangel-Negrín *et al.* 2011). During our surveys we observed such disturbances more often in fragments closer to agricultural activities (Puig-Lagunes *unpubl. data*). Furthermore, although we did not find direct evidence of hunting, there were reports in the area of immature howlers being hunted for pet trading and it is possible that hunting is more likely to occur in fragments that are easily accessible.

Mantled howler monkeys probably face higher extinction risks in the Olmec Region than in other areas where this species has been studied in Mexico (Anzures-Dadda and Manson 2007; Arroyo-Rodríguez *et al.* 2008; Cristóbal-Azkarate *et al.* 2005; Estrada and Coates-Estrada 1996). In addition to being an unprotected area, the percentage of forest cover, the number of forest fragments and the mean area of fragments are smaller and mean distance to the nearest fragment is greater in our study site than in the majority of subpopulations of this subspecies studied in Mexico (Table III). In the Olmec Region subpopulation size was also lower than in the adjacent Los Tuxtlas area (Cristóbal-Azkarate *et al.* 2005), suggesting that mantled howlers may be more vulnerable in the former than in the latter to the risks inherent in small population size, as mentioned previously.

Although distance to nearest fragment was related to occupancy and several subpopulation structure variables, we did not find evidence that monkeys moved among forest fragments, either through direct observation or inferred by changes in subpopulation composition between surveys (Puig-Lagunes *unpubl. data*). At other sites, mantled howlers may cross pasturelands walking on the ground at distances up to 200 m and use remnant trees or live fences to travel between fragments (Anzures-Dadda and Manson 2007; Estrada and Coates-Estrada 1996), suggesting that

Table III Comparison of landscape and fragment attributes among studies of Mexican mantled howlers

Study/area	% forest cover	No. of fragments/ sampling area	Mean fragment size (ha)	Mean distance to nearest forest fragment (m)
This study/Olmec Region	0.8	0.16	5	253.6
Anzures-Dadda and Manson 2007/Chiapas	5.1	0.95	3.8[a]	129.7
Arroyo-Rodríguez *et al.* 2008[b]/Los Tuxtlas	13	0.9–1.8	8.7	168
Cristóbal-Azkarate *et al.* 2005/Los Tuxtlas	–[c]	–[c]	22.1	50 % of the fragments were <100 m apart
Estrada and Coates-Estrada 1996/Los Tuxtlas	25	0.25	199.1	1436.8

[a] These authors defined fragments as remnants of primary or secondary forest with an area ≥0.2 ha

[b] Data in this study refer to three adjacent landscapes. We present mean values for the three landscapes

[c] Data not available

movements between forest fragments may be more difficult in the landscape we studied. In addition to higher isolation of fragments in the Olmec Region, lack of dispersal could relate to the flooding of many areas during the wet season, which in principle represents a barrier to howler movements (cf. Gonzalez-Socoloske and Snarr 2010). Independent of its causes, a lack of dispersal opportunities increases extinction probabilities in fragmented landscapes due to genetic drift and increased endogamy (Fahrig 2003; Hanski 1999). Interestingly, the effect size of distance to nearest fragment was two times higher for females than for males, suggesting that females may be more sensitive to isolation than males. A similar pattern occurs in black and gold howlers (*Alouatta caraya*: Oklander *et al.* 2010), in which dispersal patterns vary between continuous and fragmented habitat.

During the initial stages of disturbance (either through habitat loss or fragmentation), individuals distribute randomly in the remaining habitat, and population declines may not be observed immediately. This delay between the occurrence of disturbances and their ecological costs, termed extinction debt (Tilman *et al.* 1994), has been observed in several primate species living in disturbed habitats (Cowlishaw 1999), including howlers (e.g., black and gold howlers: Kowalewski and Zunino 1999). Extinction debt could explain why a negative relationship between fragment size and subpopulation density occurs in several mantled howler populations (Cristóbal-Azkarate *et al.* 2005). Such a relationship was not observed in the present study, suggesting that subpopulations may be already declining in the Olmec Region. Supporting this possibility is the absence of juveniles and/or infants from many fragments and the low immature and infant to female ratio, which indicates a low reproductive output that could result from low fertility (Cristóbal-Azkarate *et al.* 2005; Terborgh *et al.* 2001) or fecundity (Dias *et al.* 2015) of females. Furthermore, on occasion, food availability is higher in small than in large habitats occupied by mantled howlers (Gómez-Espinosa *et al.* 2014), suggesting that carrying capacity varies as a function of the particular structure and composition of the vegetation in each habitat (Brugiere *et al.* 2002). For example, our study fragment number 25 (ESM Table SI) was occupied by a large group, although it had a small area (2 ha). The fragment had many large trees (tree height >20 m), several of which were *Ficus* sp., a preferred food for Mexican mantled howlers (Cristóbal-Azkarate and Arroyo-Rodríguez 2007). Our future research will focus on 1) sampling vegetation structure and composition in this area to further assess whether variation in howler subpopulation structure and density depends on local food availability; 2) documenting temporal variation in demography to determine if the patterns identified in the present study only reflect subpopulation fluctuations that could not be assessed because of the short duration of the censuses; and 3) collecting evidence on the impact of hunting on population structure.

In addition to its inherent biological value, the Olmec Region is located in the center of the geographic distribution of mantled howlers in Mexico, and therefore could play a fundamental role in maintaining the contact between different populations. It could, for instance, facilitate gene flow between populations in the north (Los Tuxtlas), where genetic diversity is higher (Argüello-Sánchez 2012), and in the south (Selva Zoque), where genetic diversity is lower (Dunn *et al.* 2014). For now, our study suggests that mantled howlers in the Olmec Region are highly threatened by anthropogenic habitat disturbance, mainly through the loss of their habitat and contact with humans. Therefore, urgent conservation and management actions are required to increase the probabilities of future persistence of Mexican mantled howlers in this area. These actions include the protection of existing forest remnants, the restoration of suitable habitat, and the increase of habitat connectivity through the establishment of corridors.

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