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Effect of anthropogenic and natural factors on the population density of sportive lemur from northern Madagascar



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Le présent rapport constitue un exercice pédagogique qui n'engage en aucun cas la responsabilité du laboratoire d'accueil

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RESUME

La densité des populations et la distribution géographique des espèces de lémuriers nouvellement décrites sont peu connues alors que ces populations font face à plusieurs menaces et pressions pouvant conduire à leur disparition à l'état sauvage. Les recherches menées sur l'effectif des populations et les effets des actions humaines sur ces populations peuvent aider à établir des stratégies de conservation efficaces. Les forêts de la région nord de Madagascar font partie des habitats les plus touchés par la dégradation malgré la diversité biologique et le taux d'endémisme élevé qu'elles abritent. Les espèces du genre *Lepilemur* sont parmi les moins étudiées dans cette région bien qu'elles soient les plus touchées par les effets de l'homme. Cette étude consiste à estimer les effectifs des populations de ces espèces dans toute la région nord de Madagascar, évaluer les effets des actions anthropiques mais aussi naturelles sur ces espèces. Pour les estimations des densités, nous avons adopté la technique de *Line Transect Distance Sampling* et les données ont été analysées à l'aide de la méthode de *Conventional Distance Sampling* grâce au programme DISTANCE. Nous avons utilisé un test de corrélation linéaire pour évaluer l'effet des actions naturelles et anthropiques sur ces populations de lémuriers. Les résultats des estimations des densités montrent des grandes variations en fonction des différents sites d'études avec des densités assez satisfaisantes au niveau des sous-régions d'Ankarana, Andrafiarana et dans l'*Inter River System* Loky-Manambato (215 ind/km²; 135 ind/km² et 151 ind/km² respectivement). Les estimations de densités sont assez faibles dans les forêts situées entre les rivières des Manambery et Bemarivo. Nos études de corrélations ont suggéré des effets négatifs des coupes d'arbres, des fréquences des feux et des forêts de types humides sur la densité des populations de *Lepilemurs* de cette région. Nous suggérons que des mesures strictes de conservation soient prises dans ces zones protégées pour éviter la perte de ces habitats naturels des lémuriers. Nous suggérons aussi que des mesures particulières doivent être prises dans les forêts entre les rivières de Manambato et Bemarivo vu que ces habitats ne sont pas protégés. Pour cela, les solutions à préconiser sont la sensibilisation des communautés villageoises, implémenter des solutions alternatives telles que le développement de techniques de l'agriculture écologique, maintenir la connectivité au niveau des habitats par le reboisement nécessaire à la viabilité de ces espèces.

Mots clés : sportive lemur, destruction de l'habitat, Distance Sampling, abondance, région nord de Madagascar

ABSTRACT

Population density and distribution of the most recently described lemur species remain poorly known while many appear to face threats and pressures and to have small geographical ranges, making them vulnerable to extinction. Research on the population size and the effects of human actions on these populations can contribute to establish efficient conservation strategies. Forests of the northern region of Madagascar are among the most affected by habitat degradation despite their highly endemic diversity. The genus *Lepilemur* is among the least studied nocturnal species in this region. These lemur species are among the most affected by the habitat loss and the human actions. This study aims at estimating the population density of these species in the entire northern region of Madagascar, to evaluate the natural and human action effects on the population densities of different species of sportive lemur in this region. For density estimates, Line Transect Distance Sampling was adopted and data were analyzed using the Conventional Distance Sampling method implemented in the DISTANCE software. We evaluated the impact of anthropogenic and natural factors on the sportive lemur population density using a linear correlation test. The results of density estimates show large variations depending on the study sites with satisfactory values at the sub-regions of Ankarana, Andrafiarana and the Inter River System (IRS) of Loky-Manambato (215 ind/km², 135 ind/km² and 151 ind/km² respectively). The sportive lemurs from the IRS of Manambery-Bemarivo have a population size relatively low. Our correlation studies suggest negative effects of tree cutting, frequency of fires and the humid forest on the sportive lemur population density of this northern region of Madagascar. We suggest that conservation measures must be taken in account to prevent the loss of natural habitats for these species. We also suggest that particular measures must be taken in the forests between the Manambato and Bemarivo rivers since these habitats are not protected. This action measure should involve the local population and implement alternative solutions for the villagers such as organic farming; maintain the connectivity of the habitat that is necessary to the conservation.

Key words: Sportive lemurs, habitat disturbance, Distance Sampling, abundance, northern region of Madagascar

I. INTRODUCTION

The deforestation rate in Madagascar is now reaching alarming levels and induces massive loss of biological diversity (Herrera *et al.*, 2011; Schwitzer *et al.*, 2014). The implementation of conservative measures of natural habitats are thus necessary but they remain a major challenge in conservative biology (Martinuzzi *et al.*, 2014; Schwitzer *et al.*, 2013). To be efficient these measures require a good knowledge of the target species, especially regarding their distribution and their population sizes, the effect of anthropogenic perturbations on their populations and their responses to habitat changes. Population density and geographical range are often used as indicators of the population status over time (they are for instance the primary criteria of Red Lists by IUCN). Under the management of the Malagasy fauna, lemurs are an example particularly interesting due to their dependence on natural habitats and forests (Mittermeier *et al.*, 2010). Previous researches on lemur population status and threats have showed a negative effect of the habitat loss on the diversity of these species (Ganzhorn 1995; Schäffler and Kappeler 2014; Schwitzer *et al.* 2013), negative effect that eventually led to 5 lemur species to be among the 25 most endangered primates in the world (Mittermeier *et al.*, 2009).

In the recent decades, the number of newly discovered species of lemurs has been increased (Blair *et al.*, 2014; Blanco *et al.*, 2013; R. A. Mittermeier *et al.*, 2008). The number of lemur species has also largely increased through taxonomic revisions based on genetic studies over the past two decades (Blair *et al.*, 2014; Blanco *et al.*, 2013). 99 different species and 103 subspecies of lemur are currently described (Schwitzer *et al.*, 2013). Many efforts have been made by the Malagasy authorities to preserve their natural habitats (Ratsimbazafy *et al.*, 2013; Schwitzer *et al.*, 2013). Currently, 94% (103 species) of all lemur species are classified as “Sufficient Data” among which 24 species are critically endangered, 49 species are endangered and 20 species are vulnerable (Schwitzer *et al.* 2013).

Several studies deal with lemur density (Kun-Rodrigues *et al.*, 2014; Quéméré *et al.*, 2010; Salmons *et al.*, 2013; Schäffler & Kappeler, 2014). However, the information available on lemur population densities mostly concern the diurnal species with large body that mostly inhabit protected areas (Ankarafantsika, Berenty, Vohibola III, Ranomafana, Marojeje etc., Table 3). For the nocturnal species, studies on population sizes are rare and 90% of the published studies were conducted in the north-west (Rakotondravony and Radespiel, 2009; Ralison, 2006a, 2006b; Randrianambinina, *et al.*, 2010), in the east (Lehman, *et al.*, 2005;

Lehman, 2007) and in the southern parts of the island (Ganzhorn and Kappeler, 1996; Hladik *et al.*, 1998; Rabeson *et al.*, 2006). These studies suggested large differences of the population densities between species and between study sites (Ganzhorn, 1997). Although the conservation status of the majority of lemurs are known (Schwitzer *et al.*, 2013), there are still several areas of Madagascar where no study about nocturnal species has been conducted. This is specially the case of the northern region, where little information is available regarding the density and the population size of these nocturnal species. The genus *Lepilemur* is among the least studied in this area (Meyler *et al.*, 2012). Until now, neither its distribution range, nor its population density have been described while it is known as the lemur genus the most threatened by habitat loss and hunting (Randrianambinina *et al.* 2010).

Four studies including density estimates have been carried out on *Lepilemur* in this northern region of Madagascar. Hawkins *et al.* in 1990 estimated densities ranging from 60 to 560 ind/km² in the Ankarana forest for the ankarana sportive lemur (*L.ankaranensis*) which was considered as *L.septentrionalis* at that time (Hawkins *et al.*, 1990). For the same species in this area, Ganzhorn (1992) estimated an average density value of 163 ind/km². Yet between 1992 and 2011, no study has been conducted to estimate the actual population size of sportive lemurs in the northern regions of Madagascar. Some censuses were conducted in 2005 (Rasoloharijaona *et al.*, 2005) and allowed estimating the encounter rates of the animals, but no density were estimated. In 2012, Meyler *et al.* conducted a study on the population density in two forest fragments in the Loky-Manambato IRS for the daraina sportive lemur (*L.milanoii*) and estimated densities ranging from 105 to 195 ind/km² (Meyler *et al.*, 2012). Ranaivoarisoa *et al.* (2013) conducted a study on the northern sportive lemur (*L.septentrionalis*) in the sub-region of the extreme north-east that suggested a limited population size (only 19 individuals, Ranaivoarisoa *et al.*, 2013). This last species is classified in the Red List of Critically Endangered (Ranaivoarisoa *et al.*, 2013).

The northern forests of Madagascar are particularly impacted by anthropogenic activities and logging rate. Forest cover is being reduced by slash-and-burn agriculture, and illegal logging transforming pristine habitat to secondary forest formations, scrub, and savanna (Banks *et al.*, 2007; Rakotondravony, 2006; Ranaivoarisoa *et al.*, 2013). Due to the increase of the number of sportive lemur taxa (10 to 26 species) in the recent years (Mittermeier *et al.*, 2010), species are becoming increasingly threatened because of their small distributions. Therefore, studies of the distribution and the population densities of these

nocturnal lemurs and their responses to habitat changes are needed to establish an efficient conservation strategy.

The aim of our study is to estimate the density of the sportive lemur genus (*Lepilemur*) in the northern region of Madagascar. This study is the first that estimate densities using a large number of observations on lemurs in Madagascar and the first to include several sites in the north for *Lepilemur* species. It is the first that density estimates for these species on the most visited forest of Madagascar including all the areas between the Manambato and Bemarivo rivers in the north-east sites. The objective is to study the size of each population of sportive lemur for each forest fragment, discuss the results in relation to existing data in all *Lepilemur* species and try to explain the causes of these population variations in the surveyed sites.

II. MATERIALS AND METHODS

My work is a part of the project carried out by the Population and Conservation Genetics Group (PCG), in the Instituto de Gulbankian de Ciência (IGC) between 2010 and 2013 under the collaboration signed by the IGC and the *Département de Biologie Animale et Ecologie* of the University of Mahajanga, Madagascar. The main objective of this project is the conservation of lemurs on the northwest, north and northeast of Madagascar. During my five-month internship at the IGC and I have been in charge of the analysis of available data in order to estimate *Lepilemur* density and to assess the effects of different factors (natural and anthropogenic factors) on their population density in the north and northeast of Madagascar.

1. Study Sites

This study was conducted throughout the northern region of Madagascar Between 2010 and 2013. Several sub-regions and Inter-River-systems (IRS) were visited. These sub-regions are:

the extreme north-east (1), the sub-region of Ankarana(2), the Analamerana-Andrafiarana(3) or the *massif d'Andrafiarana*, the sub-region of Andavakoera(4), the IRS of Loky-Manambato(5) and finally the sub-region between the Manambato and Bemarivo rivers which divided into three IRS:

the Manambato-Manambery (6), the Manambery-Fanambana (7) and Fanambana-Bemarivo (8) (Figure 1).

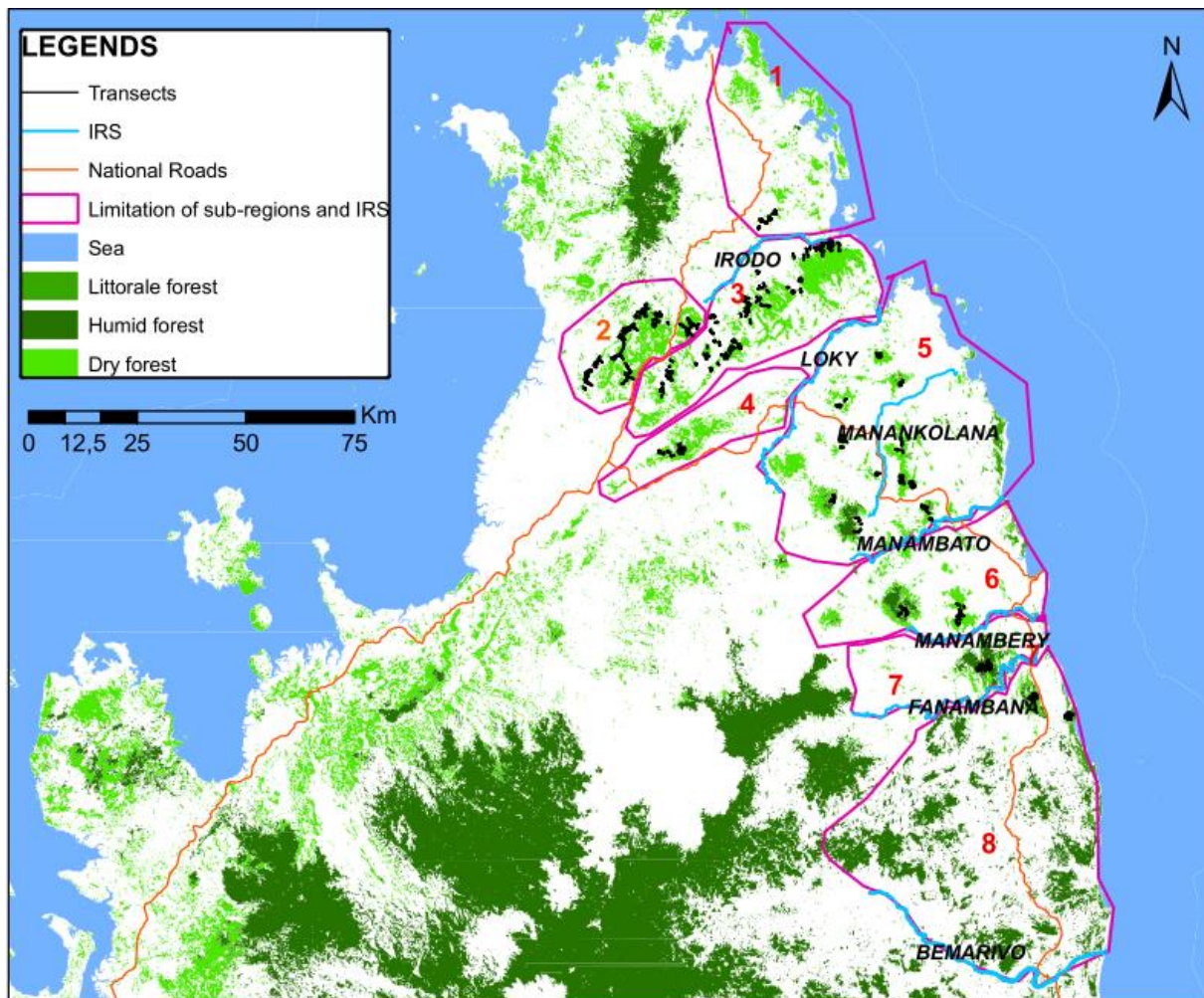


Figure 1: Map of the sites studied to estimate the population densities of lepilemur in north and northeast of Madagascar.

1: Sub-region of the extreme north-east, 2: Ankarana; 3: Massif d'Andrafiamena, 4 Andavakoera, 5-Loky Manambato, 6: Manambato-Manambery, 7: Manambery-Fanambana, 8: Fanambana-Bemarivo

This part of the island is characterized by dry forests types including the IRS of Loky-Manambato and the Andrafiamena, Andavakoera and Ankarana sub-regions, with some rain and transition forests (Meyers & Wright, 1993; Meyers, 1993). In the east, the climate is tropical and humid with heavy rain on some forests such as the Analalava forests.

2. Field study

A. Density

To estimate the densities of the populations, we conducted nocturnal lines transect nased on distance sampling survey type (Buckland *et al.*, 2001; Meyler *et al.* 2012; Salmona

et al. 2013) commonly used for forest dwelling primates (Buckland *et al.*, 2010) and particularly well suited for sportive lemur (Meyler *et al.*, 2012). We established transects lines and mostly used existing trails when available. To facilitate the walk during the nocturnal observations, each transect and trail was marked by flags every 20 meters and was surveyed two to four times by a two-member teams walking slowly (0.5km/h) between 6 PM and 9:30 PM with headlamps. Transects/paths are measured with a tape measure or by GPS. When an animal or group of animals was seen, the following information were recorded: time of observation, the GPS point, the height where the animal was seen on the tree, the number of individuals and an estimated perpendicular distance (pd) between the animal to the line transect. This last information is crucial for the calculation of the density and therefore is carefully collected. Species are recognized by their morphology, their body sizes (between *Microcebus*, *Cheirogaleus* and *Phaner*) or directly by their postures and locomotion among families *Lepilemuridae*, *Cheirogaleidae*, and *Lemuridae*. A headlamp with low intensity allows easily detecting the animal by eye reflection. Another high intensity lamp is used to determine the family of the animal and often to the genus level.

B. Habitat Characterization

For each surveyed forest, information about the state of the habitat were noted. These information were collected regularly along transects but also unsystematically during the prospection and the catch of individuals outside transects. Along transects, the information are collected at an interval of 20m from the starting point of the transect, which averaged a total of 50 collection points for a transect of 1000m. The type of habitat (humid and dry forest), the type of forest (primary or secondary), the abundance of large, medium and small trees, the height of the canopy, the presence of permanent and temporary rivers, and all signs of threats for the animals and the forests are noted within a radius of 10m around the observer. All these information are needed to characterize the habitat but also to discuss the abundances of encountered animals.

3. Data analysis

A. Population density

The population density (D) is defined as the number of individuals encountered during the observations per unit of area according to the following formula: $D = N/S$.

N is the number of individuals counted and S the area of sampling. The area is calculated by the formula: $S = 2ESW \times L$, with L the total length of transect and ESW the effective strip width. The density (D) is obtained as following:

$$D = \frac{N_t}{2 \times ESW \times L_t}$$

N_t (total number of observations) is obtained by counts of the total individuals seen in the field and L_t (total length of transects) calculated using the ArcGIS software. While N_t and L_t are easily obtained, the difficulty of density estimation with Line Transect Distance Sampling remains in the estimation of ESW which depends on the probability of detecting an animal at a given distance. Several methods are developed to estimate the effective width ESW: such as the method of Müller (Müller *et al.*, 2000); the Kelker method (Struhsaker, 1981); the method of King currently known as the method of Mean of Perpendicular Distances (MPD) (Kun-Rodrigues *et al.*, 2014) etc.

In our analyses, we estimated the population density using the software DISTANCE 6.0 (Thomas *et al.*, 2010) based on distance sampling data. We used the method of Conventional Distance Sampling (CDS) (Buckland *et al.*, 2001; Thomas *et al.*, 2010) assuming that the detection probability on the transect line equals 1. DISTANCE fits a detection function to the histogram of observed distances and correct rough densities for undetected animals as well as for failed identification at greater distance from the transect. It permits to model the distribution of observing an animal as its distance from the transect increases. This method is widely used to estimate lemurs density (Axel and Maurer, 2011; Kelley *et al.*, 2007; Kun-Rodrigues *et al.*, 2014; Meyler *et al.*, 2012a; Norscia, 2008; Quéméré *et al.*, 2010; Salmons *et al.*, 2013; Schäffler & Kappeler, 2014).

To calculate ESW, this approach considers four models that can be fitted to the dataset (Uniform, Hazard rate, Half normal and Negative exponential detection functions). The choice of the detection function is performed using the Akaike Information Criterion (AIC) with a truncation of 5% of the data as recommended by Buckland (Buckland *et al.*, 2001). This method requires a minimum number of observations per study site estimated at 40 to be properly applied (Buckland *et al.*, 1993). The model "Hazard rate" was chosen because of its smallest AIC values in the forests where the number of observations is greater than 40. In the sites where the number of observations is less than 40, the density was estimated using ESW across the sub-regions.

B. Impact of environment on densities

The frequency of occurrence of habitat disturbances, of tree cutting, the presence of zebus, and the presence of trails but also the natural factors such as the humid forest, the presence of permanent and temporary rivers are obtained from formula:

$$Fi(\%) = \frac{Ni}{N} \times 100$$

Fi = Frequency of the considered variable, Ni= Number of observation for the considered factor, N = Number of presence and absence of the concerned factor.

To study the correlation between population densities and the habitat, we estimated the density for each transect using ESW for each sub-region assuming that the transects from the same habitats (region) must have the same visibility (Kun- Rodrigues *et al.*, 2014).

The relationship between the fragment size, the level of anthropogenic disturbances and the natural factors was tested with a linear correlation using the STATISTICA 12 software.

Dry and humid forest were taken in account, using forest layers from the Madagascar Vegetation Mapping Project (Moat & Smith, 2007) available at http://www.kew.org/gis/projects/mad_veg/datasets.html and the size of each forest fragment and region was calculated using the Arc Gis software.

We estimated the population size by multiplying the considered density with the total surface of each corresponding forest fragment.

III. RESULTS

1. Nocturnal observations

We surveyed 305 transects and trails for a total of 560km with 1949 sportive lemurs observed (Table 1).

The number of sportive lemurs in a given site ranged from 3 to 157. In three sites (Salafiana, Ambohitrandrina and Bezavona), we did not find any sportive lemur.

2. Population densities

Table 1: Density estimates of lepilemur by site, forest and sub-region in the northern part of Madagascar in 2010-2013.

Sites/Forest	#obs	Effort (m)	Ind/km	ESW (m)	Density (ind/km ²)				Population size		
					D	CI95		Aire (km ²)	A	CI95	
						Min	Max			Min	Max
Loky-Manambato	609	181,865	3.3	10.7	151	118	192	335	50,448	39,540	64,367
Ambilondambo	4	2,237	1.8	10.7	63	8	479	7	438	56	3,353
Ampodrabé_Daraina	91	7,374	12.3	10.1	590	125	2789	16	9,448	2,000	44,627
Antsahabe	26	16,145	1.6	10.7	72	24	200	9	650	219	1,804
Antsaharaingy	9	6,604	1.4	10.7	64	3	847	13	826	39	11,010
Bekaraoka_2010	56	18,950	3.0	10.7	126	57	276				
Bekaraoka_2011	215	47,644	4.5	12.1	184	147	229	54	9,910	7,927	12,388
Benanofy	21	16,978	1.2	10.7	51	12	271	7	355	87	1,899
Binara	26	6,347	4.1	10.7	203	23	1277	40	8,103	908	51,088
Bobankora	53	25,201	2.1	11.6	90	55	148	14	1,265	773	2,068
Solaniampilana_2010	43	20,509	2.1	4.5	213	105	429				
Solaniampilana_2011	65	13,876	4.7	12.2	186	110	314	10	1,862	1,103	3,142
Manambato-Manambery	74	17,683	4.2	11.5	167	108	257	169	#	#	#
Analafiana	74	13,507	5.5	11.2	224	153	330	37	8,304	5,656	12,192
Salafiana	0	4,176	0.0	0.0	0			60			
Manambery-Fanambana	3	13,624	0.2	11.5	10	1.1	15.8	93	379	98	1,467
Bezavona-Ankirendrina*	3	13,624	0.2	11.5	10	1.1	15.8	80	326	84	1,262
Ankirendrina	3	8,487	0.4	11.5	15	2	26				
Bezavona	0	5,137	0.0	0.0	0						
Fanambana-Bemarivo	18	19,543	0.9	11.1	33	9	126	603	#	#	#
Ambohitrandrina	0	9,075	0.0	0.0	0			32			
Analalava	18	10,468	1.7	11.1	69	20	238	36	2,475	716	8,551
Massif d'Andrafiama	444	155,781	2.9	10.2	135	108	168	327	44,059	35,316	54,970
Antsoy*	155	59,112	2.6	11.4	112.3	77.9	161.9	44	4,942	3,428	7,125

Ambohibory	35	7,493	4.7	10.2	230	45	516				
Ampasimaty	28	7,367	3.8	10.2	180	131	758				
Ankavanana	17	10,292	1.7	10.2	81	26	201				
Andrafiambany	29	11,205	2.6	10.2	127	49	256				
Andranotsimaty	21	9,894	2.1	10.2	90	32	146				
Betsiaka	25	12,860	1.9	10.2	96	56	195				
Antserasera	28	7,801	3.6	10.2	151	74	299	36	5,452	2,671	10,754
Anjahankely-Ampantsogno	95	32,382	2.9	9.6	151,2	98,3	232,7	45	6,805	4,422	10,470
Ampantsogno	22	12,396	1.8	10.2	87	36	150				
Anjahankely	73	19,985	3.7	9.3	193	115	323				
Analabe*	162	56,487	2.9	9.6	145,6	92,6	228,9	121	17,619	11,209	27,693
Menagisy	22	4,723	4.7	10.2	229	87	6177				
Ampondrabe-Analamenara	43	19,578	2.2	15.5	69	46	104				
Anteninaomby	101	32,186	3.1	9.1	162	78	335				
Ankarana	736	133,956	5.5	12.2	215	173	267	37	8,014	6,457	9,946
Ankarana*	736	133,956	5.5	12.2	215,0	173,2	266	22	4,729	3,810	5,869
Ambondromifehy	72	12,128	5.9	12.5	231	158	338				
Analamahitsy	97	15,448	6.3	10.0	313	185	531				
Andrafiabe_americans	72	15,118	4.8	15.6	142	47	426				
Andrafiabe_jongovy_jiaby	37	9,301	4.0	12.2	154	74	894				
Andrafiabe_tenan'ankarana	43	5,291	8.1	9.6	415	177	974				
Mahamasina_anilotra	72	11,476	6.3	12.2	235	132	418				
Mahamasina_prince	109	22,024	4.9	12.8	181	90	364				
Marotaolana	29	8,435	3.4	12.2	158	57	435				
Marovato_marovato	48	18,259	2.6	11.7	101	49	209				
Marovato_ankotra	157	16,477	9.5	12.5	374	248	563				
Andavakoera	34	25,999	1.3	6.2	87	36	212	92	7,987	3,276	19,466
Andavakoera	34	25,999	1.3	6.2	87	36	212	11	965	395	2,351
Extrême-Nord-Est	31	11,702	2.6	6.8	194	105	359	144	#	#	#
Ankarongana	31	11,702	2.6	6.8	194	105	359	6	1,164	629	2,152

obs = number of observations; Ind / km= individuals per kilometer= encounter rate; ESW = effective strip width; D = density; CI = Confidence Interval; A = Abundance; Min=Minimum; Max=Maximum. The gray lines represent the regions and IRS; *: Forests encompassing several sites; **: The ESW of the region between the Manambato and Bemarivo rivers; # = no extrapolation in the region; the ESW are in bold at the sites whose have a number of observation over 40

The population density in the sub-regions, IRS and sites showed considerable variations with average values varying between 15 ind/km² in Ankirendrina and 590 ind/km² in Ampondrabe at ste level and between 10 ind/km² in the Manambery-Fanambana and 214 ind/km² in Ankarana at sub-region and IRS level.

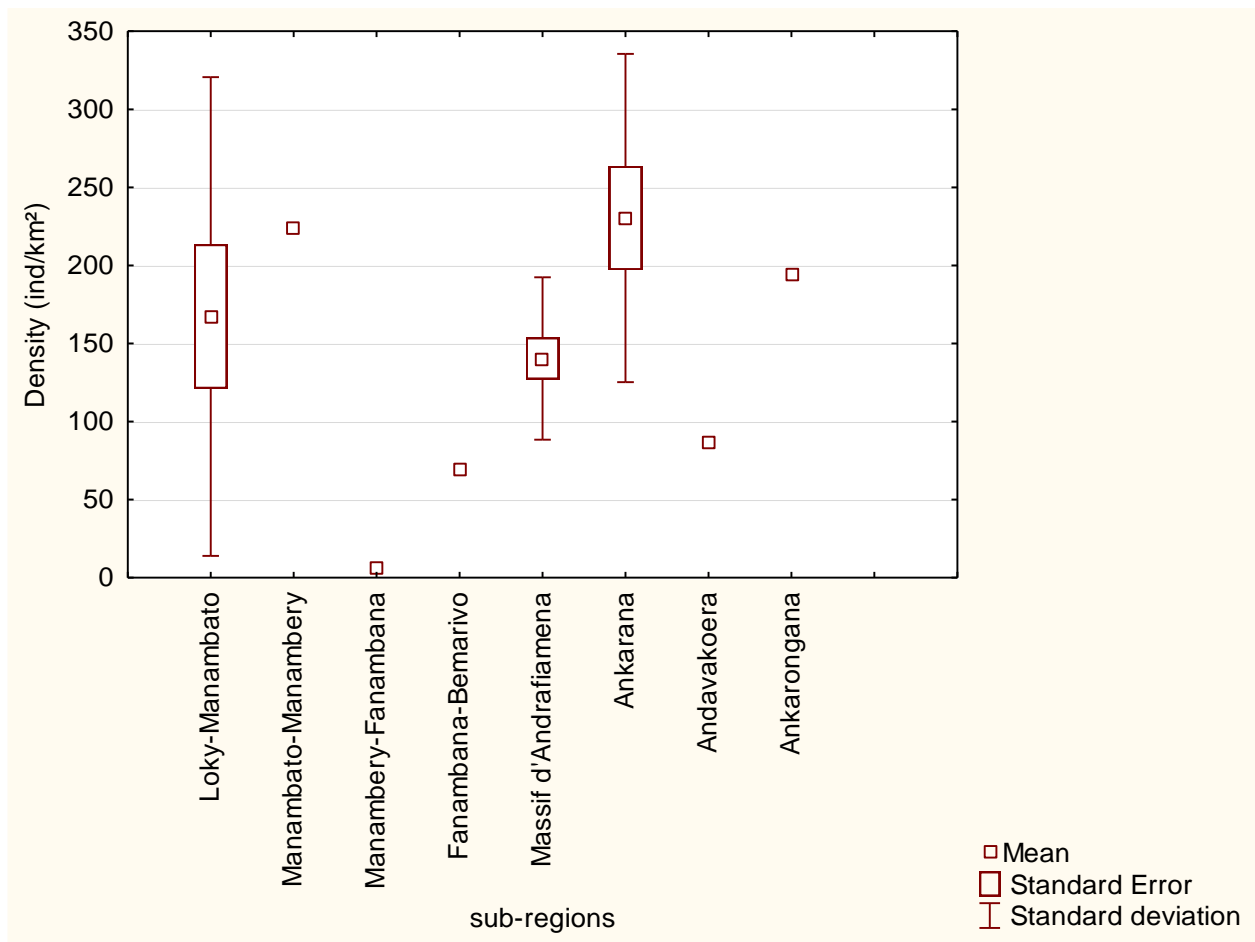


Figure 2: Variation of the density per region

Across the sub-regions and the IRS, the highest density is found in the Ankarana. The most important variations of densities are reported in the IRS of Loky-Manambato. The IRS of Manambato-Manambery, Manambery-Fanambana and Fanambana-Bemarivo and the sub-region of Andavakoera are represented each by one site (one value of density).

3. Correlation between forest fragment sizes and population density.

During these correlation studies, 21 of 23 forests were surveyed. In the two forests (Ambohitsitoroina and Antsaharaingy), we did not collected any information about density and habitat characterizations..

The fragment forest sizes varied from 6 to 80 km² in the forest of Ankarongana and Bezavona-Ankirendrina respectively.

Our correlation analyses revealed no significant correlation between forest fragment size and population density (N =19, r= -0.09, p = 0.69).

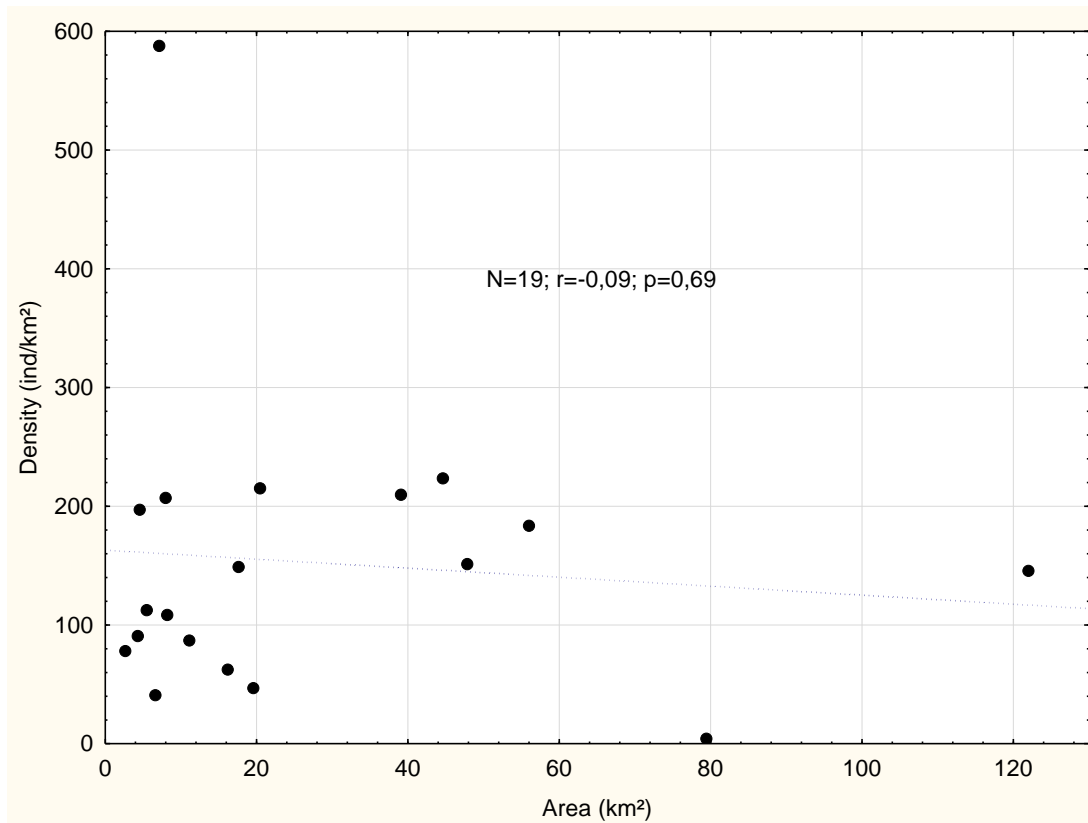


Figure 3: Correlation between population density and fragment forest size

The forest where the density are equal to zero are excluded from the analyze.

4. Correlation between anthropogenic and natural factors on the population density

In this study of habitat, a total of 184 transects were surveyed with 10063 points of collects.

Table2: Correlation between anthropogenic factors on the population density

Sites		T.cutting	Fire	Zebus	PT	ALT	PLT	PMT	PST	PPR	PTR	HF
All sites	N	183	183	184	183	180	179	180	183	183	183	179
	p	0.00	0.04*	0.84	0.18	0.74	0.69	0.75	0.51	0.71	0.75	0.00
	r	-0.24	-0.14	-0.01	-0.09	0.03	0.03	0.02	-0.05	0.02	-0.02	-0.28
Loky-Manambato	N	32	32	32	32	32	32	30	32	32	32	32
	p	0.43	0.3	0.03*	0.22	0.05*	0.63	0.70	0.57	0.47	0.08	0.59
	r	-0.10	-0.18	0.36	-0.22	-0.33	-0.08	0.07	-0.10	-0.10	-0.30	-0.10
Manambato-Manambery	N	13	13	13	13	13	13	13	13	13	13	13
	p	0.46	0.18	0.44	0.85	0.02	0.501	0.61		0.17	0.81	0.04*
	r	0.22	-0.39	0.23	-0.05	-0.64	-0.21	-0.15		-0.40	0.08	0.5
Manambery-Fanambana	N	14	14	14	14	14	14	14	14	14	14	14
	P	0.17	0.69	0.60	0.13	0.29	0.39	0.60	0.61			0.58

	r	-0.40	0.11	-0.15	-0.42	-0.31	0.24	0.15	0.14			0.15
Fanambana-Bemarivo	N	----	----	----	----	----	----	----	----			----
	p	----	----	----	----	----	----	----	----			----
	r	----	----	----	----	----	----	----	----			----
Andavakoera	N	9	9	9	9	9	9	9	9	9	9	9
	p	0.62	0.11	0.60	0.22	0.12	0.69		0.40	0.14		
	r	0.18	0.55	0.20	-0.44	0.54	0.15		0.20	-0.50		
Massif d'Andrafiomena	N	46	46	46	46	45	42	44	44	45	46	46
	P	0.18	0.90	0.64	0.59	0.37	0.69	0.71	0.90	0.57	0.32	0.81
	r	-0.20	-0.01	0.06	-0.08	0.13	0.06	0.05	-0.01	-0.10	-0.10	-0.03
Ankarana	N	49	49	49	49	49	48	49	47	49	49	49
	p	0.05	0.84	0.37	0.47	0.83	0.30	0.89	0.53	0.22	0.84	
	r	-0.30	-0.02	-0.12	-0.10	-0.03	0.10	-0.02	0.09	0.17	0.02	

p- value = 0.05 , p - value = 0.01 Bonferonni between (trees-cutting, presence of fire , presence of zebus and presence of trails) and p-value-Bonferonni= 0.03 between (altitude and humid forest) ; N: Number of transects ; r: correlation coefficient ; p : level of significance ; PLT : presence of large trees , PMT : Presence of Meddle Trees; PPA: Presence of Small Trees; PPR: presence of permanent rivers, PTR: presence of temporary rivers ; HF: Rainforests ; PT: Presence of trails; ALT : Altitude ; *: Correlation no-significant according to the Bonferonni correction; no correlation study in the IRS Fanmbana-Bemarivo because of insufficient data.

In this study, we found a negative correlation between tree cutting and the population density. We encountered significantly more sportive lemur when trees cutting frequency was lower. It also suggests a negative correlation between the presence of fire and the density but this correlation is not significant after Bonferonni correction. Regarding the other anthropogenic factors, no correlation was significant. On the other hand, according to our study, the density of sportive lemurs is negatively related to the humid forest types. Across the region, the density of sportive lemurs is negatively correlated to the altitude in the IRS of Loky-Manambato and Manambato-manambery (p=0.05 and 0.02 respectively). As for other factors, no correlation is emphasized. However, although there is no significant correlation between these factors, negative trends were observed between the population densities and the frequencies of light, trees cutting, and the presence of trails in the vast majority of regions (IRS, table 2) .

IV. DISCUSSION

1. Density Estimates from Line Transect

Our results presented in Table 1 suggest densities of 215 ind/km²; 151 ind/km²; 135 ind/km²; 87 ind/km²; and 10 ind/km² for the sub-regions of Ankarana, the IRS Loky-Manambato the sub-region of Massif d'Andrafiarana, Andavakoera, and the IRS Manambery-Fanambana respectively. In all the IRS and sub-regions but in the IRS Manambery-Fanambana (10ind/km²), these values seem usual in terms of density estimation for sportive lemur. Meyler, in 2012 (Meyler *et al.*, 2012) suggested values of density ranging from 105 to 195 ind/Km² in the IRS Loky-Manambato for the Daraina sportive lemur (*L.milanoii*). The discrepancies between our results and this previous study on *L. milanoii* (Meyler *et al.*, 2012) in the IRS Loky-Manambato may mostly be due to the effect of the sample size and the number of surveyed sites (99 observations at two sites in the study of Meyler et al in 2012 against 609 observations at 9 sites in our study). Our density estimation is closed to the population density reported by Ganzhorn and Kappeler (1996, 163 ind/km²) in the Montagne d'ambre forest for the northern sportive lemur (*L. septentrionalis*) (Ganzhorn and Kappeler, 1996). The population density we estimate is however law than the one of the Ankarana sportive lemur (*L. ankaranensis*) ranging from 30 to 564 ind/km² in the Ankarana and Analamerana forests (Hawkins *et al.*, 1990). These differences may be due to the effect of the analysis method. To calculate the ESW during their studies, Hawkins *et al.* in 1990 used the method of Mean of Perpendicular Distance (MPD). This method generally overestimates the population density (Kun-Rodrigues *et al.*, 2014; Meyler *et al.*, 2012).

Table 3 : Review of published population density of sportive lemurs in the different regions of Madagascar

Year	Species	Sites	Status of the site	Regions	Area(km ²)	D(ind/km ²)	Methods	References
1987	<i>L.ankaranensis</i>	Nord	Protected	North		30-560	MPD	(Hawkins <i>et al.</i> , 1990)
2003-2004	(<i>L. microdon</i>) <i>L.betsileo</i>	Vohibola III	Protected	East	20,34	9,9	Kelker	(Lehman <i>et al.</i> , 2005) (Müller <i>et al.</i> , 2000)
1995	<i>L. edwardsi</i>	Anjamena	Protected	North_West		110	Müller	(Mohamed-Thani <i>et al.</i> , 2013)
2012	<i>L. edwardsi</i>	Mariarano	Classed forest	North_West	15,8	200-220	CDS et Müller	(Ganzhorn, 1992)
	<i>L. edwardsi</i>	Ampijoroa	Protected	North_West		57	MPD	
2005	<i>L. leucopus</i>	6 sites in SW Madagascar	no Protected	South_West		18-239	MPD	(Ralison, 2006a)
1970	<i>L. leucopus</i>	Berenty	Protected	South_Est		810	MPD	Hladik et al, 1971

1997	<i>L. leucopus</i>	Berenty	Protected	South-east		790	MDP	Hladik et al, 1998
2010	<i>L. microdon</i>	Analamazaotra	Protected	North-east	67	13	MDP	(Ganzhorn 1992)
	<i>L. milanoii</i>	Daraina	Protected					
2004	<i>L. mustelinus</i>	RS Marotandrano	Protected	West		38-75	MDP	Ralison et al 2006b
	<i>L. mustelinus</i> (?)	Marotandrano Special Reserve	Protected	North-east	42	38-75	Kelker	Ralison 2006b
1990	<i>L. ruficaudatus</i>	western Madagascar	Protected	West	134	0-136	UM-GIS	Smith et al. 1997
1996	(<i>L. mustelinus</i>)	Marojejy	Protected	North-east		0-20	Min Convex Polygone	(Sterling & McFadden, 2000)
	<i>L. seali</i>	National Park		West		250	MDP	Hladik et al, 1980
1987	<i>L. ruficaudatus</i>	Kirindy	Protected	South-east		144	----	Ganzhorn and Kapeler 1996
	<i>L. ruficaudatus</i>	Morondava		South-east		357	MDP	Ganzhorn, 1992
2003-2005	<i>L. ruficaudatus</i>	Kirindy	Protected	South-west		87-159	MDP	Hilgartner 2006
	<i>L. mustelinus</i>	Vohibola III	Protected	East		9-12.5	Kelker	Lehmann 2007
1990	<i>L. ruficaudatus</i>	Morondava		South-West		357	MDP	Ganzhorn, 1992
2010	<i>L. ruficaudatus</i>	western Madagascar				0-136	UM-GIS	Smith et al. 1997
	<i>L. sahamalazensis</i>	Ankarafa	Protected	North-west		7-23		
2009-2011	<i>L. seali</i>	Makira Forest Block (12 sites)		North-east	3761,56	30		Silier et al 2013
2005-2006	<i>L. septentrionalis</i>	Ankarana	Protected	North Central-south-east		163	----	Rasolofoson et al. 2007
2000	<i>L. sp.</i>	Kalambatritra	Protected		282,5	72	Kelker	Ganzhorn, 1992
2005	<i>L. sp.</i>	Beakora				6		Irwin et al. 2001
2005	(<i>L. ruficaudatus</i>) <i>L. sp.</i>	Andranomanitsy Forest	No Protected	North-west		416	MDP	Rabeson et al. 2006 (Ralison, 2007)

Comparing to other species of sportive lemur from other regions, we found that our results of density estimation seem to be similar to the densities estimated and published in the literature : for example, 110 ind/km² for *L. edwardsi* (Müller et al , 2000.) 87-159 ind/km² for *L. ruficaudatus* of the West (Hilgartner, 2006); 136 ind/km² for *L. ruficaudatus* of the Western region (Smith et al., 1997), 239 ind/km² for *L. leucopus* from the southwest (Ralison, 2006a), 144 ind/km² for *L. ruficaudatus* from Kirindy (Ganzhorn and Kappeler , 1996). Our estimations are low compared to the values suggested by Hladik et al (1971) and (1998) (790 ind/km² and 810 ind/km² respectively) for *L. leucopus* in the Berenty forest, and the values obtained by Ralison (Ralison, 2006b, 416ind/km²) in the Andranomanintsy forest, northwest for *Lepilemur spp.* Confused to *L. ruficaudatus* at this period. Ganzhorn in 1992 suggested an average estimation of 357 ind/km² for this last species, which seems too high compared to our average values per sub-region and per IRS. It is very difficult to know which factors may be responsible for these large discrepancies since that they are related to

different species living in different habitats. However, we emphasize that in all these studies, the authors used the method (MPD) to estimate the ESW, and as we noted above, this method appears to lead to density overestimates. Other studies have suggested low values of density for sportive lemur from different part of Madagascar. For example: 6 ind/km² for *Lepilemur* sp. from Beakora in the south east (Rabeson *et al.*, 2006); 9.9 ind/km² for *L. betsileo* from Vohibola III forest confused to *L. microdon* at this period (Lehman *et al.*, 2005), 30 ind/km² for *L.seali* in the Makira forest, north east (Rasolofoson *et al.*, 2007). However, ecological factors, such as the type of the forest, the quality of the habitat and the methodology may account for these differences in those estimation of population densities (Ganzhorn, 1992; Lehman *et al.*, 2006).

2. Methodological limitations

In the field, all data were collected during the dry season, during which food resources are limited for the animals. No data collection was made during the rainy season although this factor can have influences on the estimation of the density. Other constraints concerning our line transects were encountered. These transects had varying lengths and are not necessarily straight. On several occasions, we need to avoid obstacles such as *tsingy*(calcarous formations typical of Madagascar) and large valleys, that make the transects not straight and with unwanted lengths.

For the analyzes, we found the CDS method to be the most appropriate for population density estimation because it models the detection functions of the animal and the decreasing detectability with increasing distance from the line which allow to correct the undetected animals. Yet, relatively recent improvement of Distance sampling methodology allows including covariates on the shape of the detection function (multiple covariate distance sampling, MCDS; (Marques *et al.*, 2007) such as season that may affect the scale of the detection function. We therefore suggest a revision of the methodology adopted, to try to correct the results with other calculation methods that consider these parameters.

3. Studies of correlations

A. Effect of forest size on the population density

While some studies have reported a negative effect of forest size on lemurs density (Seiler *et al.*, 2013; Sussman *et al.*, 2006), our analyzes suggest that sportive lemurs from the northern region of Madagascar have neutral responses to the size of the area. (Selier *et al.* 2013) suggested that the Sahamalaza sportive lemur (*L.sahamalazensis*) has a lower density

in the large forest. Sussman *et al.* in 2006 suggested also low densities in the forests with large sizes for the ringtailed lemur (*Lemur catta*) from the Berainty private reserve (Sussman *et al.*, 2006). Yet, this study was conducted in the southern region of the island with arid conditions. Moreover these extremely harsh conditions are coupled with a massive logging. These challenging conditions may have an impact on the reproduction of these populations that in turn may be responsible for the low population size. Neutral effects of the sizes of forests densities have also been shown in some studies on non-lemur primates: the brown spider monkeys (*Ateles hybridus*) and the red howler monkeys (*Alouatta seniculus*) (Rimbach *et al.*, 2013).

A. Effects of Natural and anthropogenic factors on the population density

The density of the genus *Lepilemur* from the Northern region of Madagascar seems to be related to the frequencies of tree cutting and fire. Lower population densities have been reported in lowlands with high frequency of tree cutting pressure. The high density of these populations may be associated with the absence of pressures (tree cutting and high frequency of fire) and high density of the large trees. Randrianambinina *et al.* in 2010 suggested that the *L. grewcockorum* population has never been encountered in degraded areas (Randrianambinina *et al.*, 2010).

It has previously been shown that sportive lemurs have more difficulties coping with habitat changes such as an increasing degree of habitat fragmentation and habitat degradation (Ganzhorn *et al.*, 2000; Olivieri *et al.*, 2005). Regarding to the other factors, such as the presence of zebu , the presence or absence of trails, the abundance of large trees and shrubs, the presence of permanent and temporary rivers and the types of forests, the study suggests no effect of those factors on the density of this species.

Usually the presence of trails and the absence of large and medium trees are considered to have a negative effect on the population size of sportive lemur (Hawkins *et al.* 1990, Lehman, 2007, Seiler *et al.* 2013), but our results suggest no correlation between these factors and the population density of sportive lemur. These animals require forests with good conditions and mature trees (Ganzhorn, 1988; Randrianambinina *et al.*, 2010; Schwitzer *et al.*, 2013). Although the frequency of tree cutting and fire has negative effects on the sportive lemur density, with all our data combined, these negative correlations were not, however, significant on any region (Table 2). This could be due to the limited sample sizes at these spatial scales.

a) IRS of Loky–Manambato

Although these forests are included in the system of protected areas of Madagascar, these habitats are subjected to heavy exploitation such as tree cutting or fire, which have a negative impact on the population density of lemurs (Meyler *et al.*, 2012; Quéméré *et al.*, 2010; Vargas *et al.*, 2002). However, these pressures seem to be moderate compared to other protected areas such as the sub-region Ankarana and Andrafiamena areas.

The population density estimated in this sub-region leads to high population size estimation for the Daraina Sportive lemur (Table 1). The regional estimation of 60,000 (Table 1) is a good news compared for example to the population size of 18,000 estimated for the Golden crowned sifaka (*Propithecus tattersalli*) by Quéméré *et al.* (2010) which almost shares the same distribution range with the Daraina sportive lemur. This area contains the forest with the highest value of density in the entire northern region of Madagascar (590 ind/km² for the daraina sportive lemur).

b) Sub-regions Ankarana, Andrafiamena and Andavakoera

These regions contain three types of protected areas: The Ankarana National Park, the Analamerana special reserve, and the protected areas managed by the NGO Fanamby of Andrafiamena and Andavakoera. Despite this protection, these forests are among the most exploited of the northern region of Madagascar. These are also the forests where we recorded most traces of hunting. Despite these high rates of pressures, the population densities are relatively high. That represents a good news for the population because of their large geographical range (Table 1).

c) Sub-region of the extreme north-east

This area is a non-protected forest. According to our results, this is the most exploited forest of our study (see figure: index). Due to the none-protection and its strategic position near the towns of Anivorano and Antsiranana, several pressures exist especially tree cutting, fire of culture and other forms of exploitation such as hunting and the presence of zebu in the forest. In this sub-region, we were unable to assess the effects of forest degradation on the population density because we did not have sufficient transects to conduct such study. However, Ranaivoarisoa and collaborators in 2013 showed that the northern sportive lemur faces imminent danger of extinction, more than any other lemur. Habitat loss and hunting continue to be the principal threats to the long-term survival of this species with a very small population size (Ranaivoarisoa *et al.* 2013). Our studies suggest a high density of this species

in this sub-region; this is probably due to the fact that we visited only one small forest fragment (Ankarongana, 5 km²) with a low transects effort (11.7km).

d) Regions between the rivers of Manambato and Bemarivo in the North East

This sub-region hosts a sportive lemur species whose taxonomical status is still unknown. Neither their ecological and biological characteristics nor their population size and distribution range have been described yet. Our results suggest the lowest densities in the forests of these IRS. We were not able to extrapolate densities at the IRS Manambato-Manambery and Fanambana-Bemarivo because we did not have data of density on some of their forests (Table 1). Only the Manambery-Fanambana IRS had a density value (10 ind/km²) with a population sizes ranging from 80 to 1000 individuals. Although the negative correlations suggested between the rate of degradation and fire are not significant in these regions probably because of low sample size, these factors probably have a major effect on the abundance of lemurs (Banks *et al.*, 2007; Dunham *et al.*, 2008; Ganzhorn, 1995). As this area is not protected, these actions can be combined with the effects of hunting that contribute to the decline in the number of individuals (Ganzhorn et al 2000, Olivieri et al, 2005). Ganzhorn and Kappeler (1996) showed that the trees cutting has no negative effect on the population density of large lemurs such as *Propithecus vereauxi* and *L.rufucaudatus* in the Berinty protected area where the degradation is limited, and where the trees cutting are selective (Ganzhorn and Kappeler, 1996). Our study suggests no correlation between the presence of zebu and the density while the presence of zebu in the forest is a sign of regular human presence, which may increase hunting pressure (Olivieri *et al.*, 2005).

Ganzhorn in 1997 showed that hutting did not have a significant effect on lemur populations in some protected forests such as the *Montagne d'Ambre* forest. However, it is important to mention that in the vast majority of our study sites, this hunting was really active. This species (sportive lemur) is among the most widely consumed of all lemurs (Lehman, 2007) and according to our observations. These animals are easy and defenseless prey for hunters that find their sleeping sites during the day (Randrianambinina *et al.*, 2010).

On the other hand, these low density values of the IRS may also be due to other factors such as the climatic conditions. Our analyses suggest a negative impact of rainforests on sportive lemur population density. In rainforests, the temperature is low. Knowing that the density of lemurs decreases with decreasing temperature (Lehman, 2014; Meyers and Wright, 1993; Meyers, 1993), this may be a factor that could explain these low densities. According to

Meyers (1993), Lemur density falls with the temperature decreasing and with higher altitude (Lehman, 2014; Meyers & Wright, 1993; Meyers, 1993).

Sportive lemurs were previously reported to sleep in tree holes during the dry season and in tangles of vines and leaves during the rainy season (Rasoloharijaona *et al.* 2003; 2008). As these species seemed to choose their sleeping sites and the microhabitat around them based on tree density, height of tree, and quality of the habitat, the negative correlation between rain forest and sportive lemur density could be explain by the holes filled with water, that can be a bad living conditions for these animals. These low population densities in the IRS Manambery-Bemarivo may also be explained by the edge effect that is affecting the population density. Lehman reported in (2007) that *L. mustelinus* exhibited a neutral edge response but is more abundant inside forest than in border. This edge effect on the lemur population density was also noted in other nocturnal species (*Avahi laniger*) responding neutrally to the edge effect but which nevertheless had the highest density values inside the forest than in edge (Lehman *et al.* 2006). Other lemur species (Eulemur, Cheirogaleus) have negative responses to edge (Lehman *et al.* 2006). This correlation is related to the change in the distribution of resources such as the availability of fruits (Lehman *et al.* 2006). In summary, sportive lemur may avoids edges due presumably to reduced fruit abundance in the degraded habitat, which may have negative effects on seed dispersal for fruit trees. However, each lemur species has its sensitivities and adaptations to the habitat effects (Ganzhorn 1988).

V. CONCLUSION

The population density of the sportive lemurs from the northern Madagascar is relatively high despite the significant degradation of their habitats. In the North-Eastern sub-region (IRS of Manambery-Bemarivo), the estimation of the population density is relatively low with some sites where sportive lemurs were not found. This uncontrolled sub-region faces to different anthropogenic pressures that eventually lead to a progressive fragmentation and degradation of the forest. This fragmentation is exacerbated by the practice slash-and-burn agriculture that induces net habitat loss. These practices may generate migration or a progressive extinction of the local isolated populations such as those occurring in this sub-region limited by large rivers that limit migration possibilities. It thus seems urgent that strict measures of control and effective protections of all protected area should be implemented to prevent a total loss of these habitats. In the case of the North-East sub-region (IRS of Manambery-Bemarivo), we suggest that particular measures should be taken by the conservation actors and local authorities as these habitats are still unprotected. This action measure should involve the local population and: implement alternative solutions for the villagers such as the ecologic agricultural technique; maintain the connectivity of the habitat that is necessary to the conservation.

The different responses observed in different species of sportive lemur for each sub-region and IRS do not allow us generalizing the factors causing the differences in population sizes. However, our study suggests a negative effect of trees cutting, frequency of fire, absence of large trees, humid forest and the altitude on population density. These factors must affect the quality of habitat, the availability of resources such as breeding sites and activity (foraging, reproduction...) of different species. Thus, changes in habitat (quality and structure) lead to the difference in density observed for each species. However, it is important to mention that the taxonomic status of the sportive lemur that occurs in the eastern sub-region “between the Manambato and the Bemarivo rivers” are still unknown. If undiscovered species inhabit these small isolated forests, they could be in critical danger of extinction due to their low population size and their restricted distribution.

Our study allows obtaining information on population sizes throughout the northern region of Madagascar. It also allows us highlighting how the population sizes vary according to different anthropogenic and their sensitivities towards changes in habitat. Results from our analyzes will contribute with the help of the data of presence/absence and distribution to

update the conservation status of sportive lemurs in this northern region according the UICN criterions and to design effective conservation plans. However, to understand which factor has an influence on sportive lemur population density in these regions, many issues remain to be clarified and it is important to continue research on sportive lemurs' ecology and habitat requirement. These studies should gather vegetation data, such as density of large and means trees, the DBH (diameter at breast height), the heights of the trees, and data on climate and rainfall factors, including the entire north-eastern part in order to generalize which factors cause the variations in densities in these regions. Larger sample sizes as well as data on food abundance and quality may allow a more detailed understanding of the conservation status of each population, understanding that is crucially needed to establish a better strategy to protect sportive lemur and all the northern forest in general.

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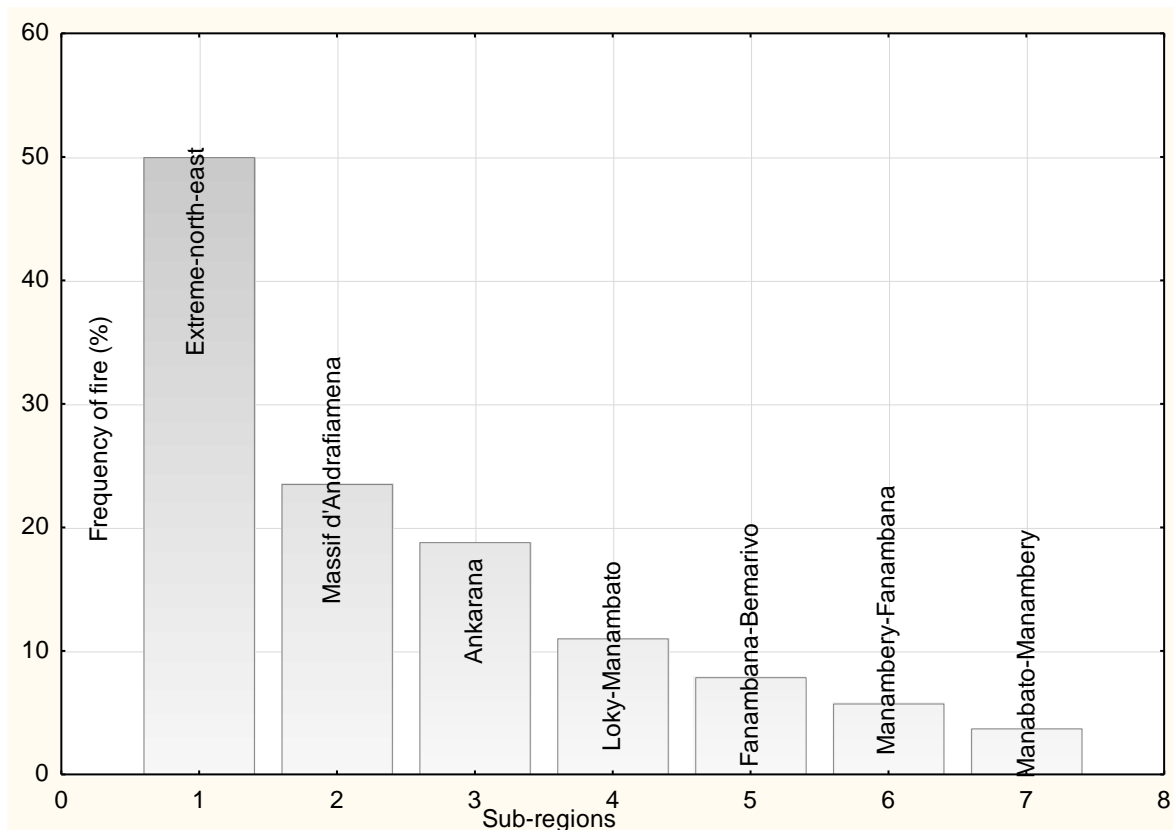
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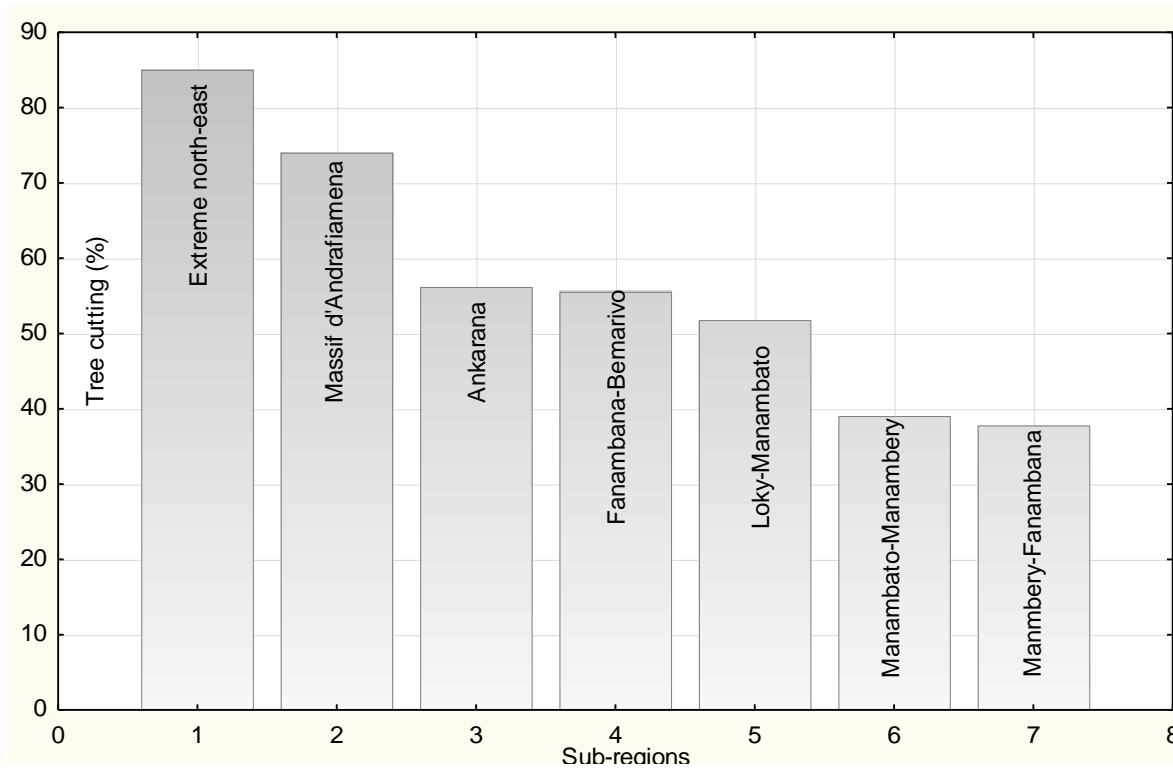
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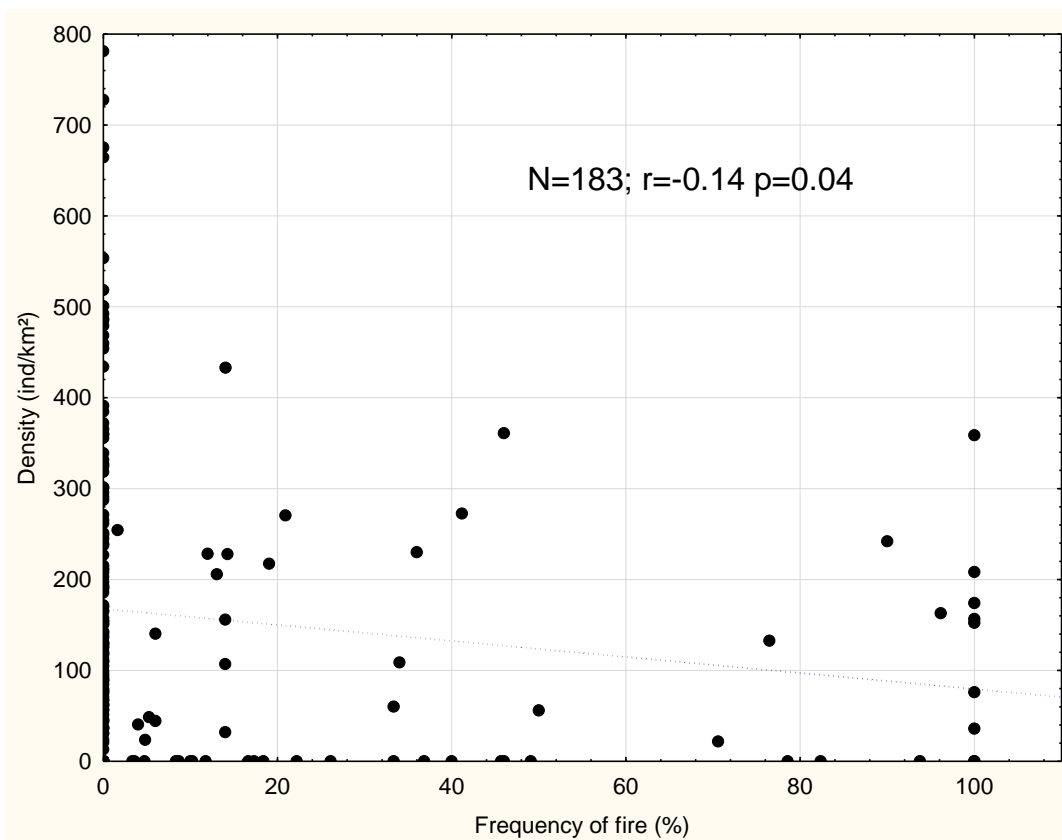
Annex



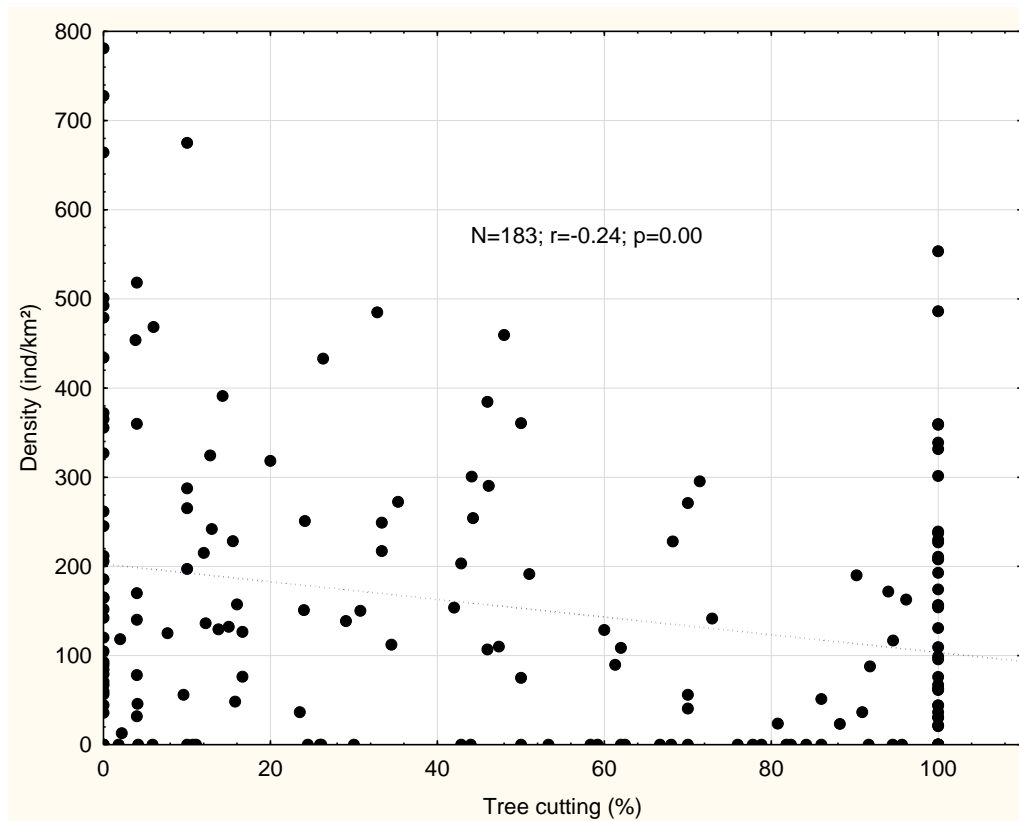
Annex 1: Frequency of fire per sub-region



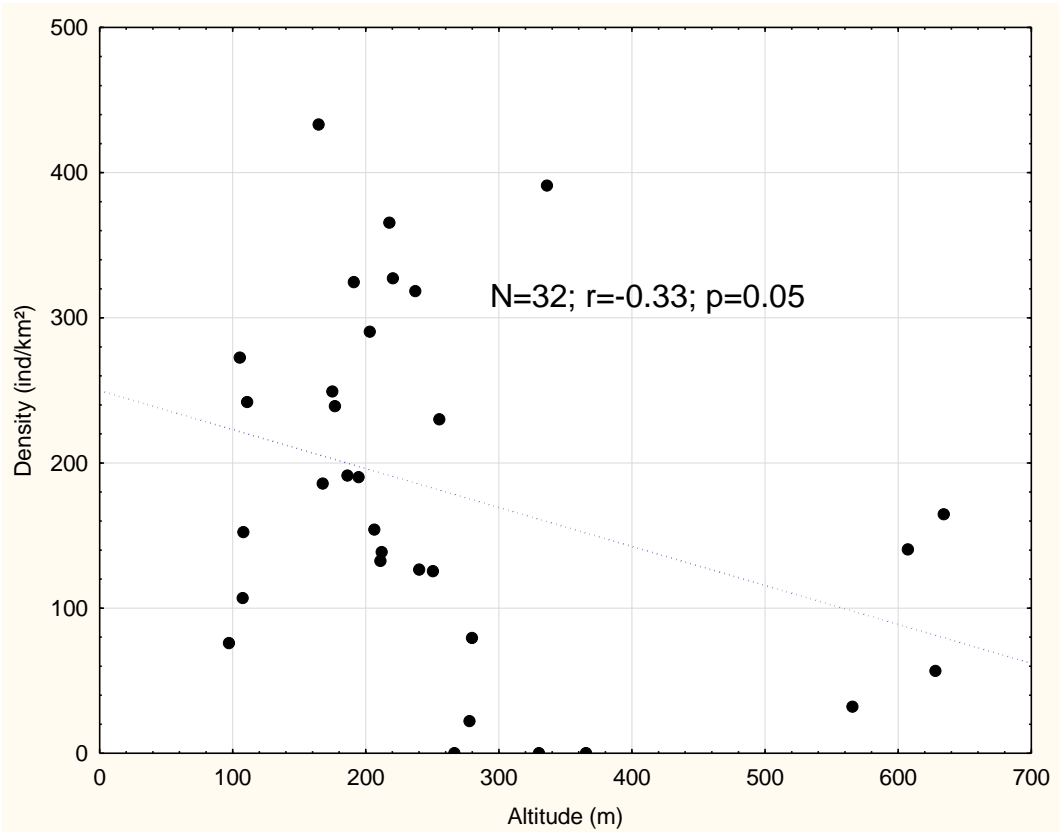
Annex 2: Frequency of tree cutting per sub-region



Annex 3: Correlation between population density and frequency of fire



Annex 4: Correlation between population density and trees cutting



Annex 5: Correlation between population density and Altitude in the Loky-Manambato IRS