ANNEE 2017 THESE : 2017 – TOU 3 – 4110

COMMUNAUTES DE RONGEURS ET RISQUE DE LEPTOSPIROSE SELON UN GRADIENT DE DEFORESTATION AU CAMBODGE

THESE
pour obtenir le grade de
DOCTEUR VETERINAIRE

DIPLOME D'ETAT

présentée et soutenue publiquement
devant l'Université Paul-Sabatier de Toulouse

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REMERCIEMENTS:

A Monsieur le Professeur Gérard Campistron

Professeur à l'Université Paul-Sabatier de TOULOUSE

Qui nous a fait l'honneur d'accepter la présidence du jury de thèse.

Hommages respectueux.

A Monsieur le Docteur Guillaume Le Loc'h

Maitre de conférences de l'Ecole Nationale Vétérinaire de Toulouse

Médecine zoologique et santé de la faune sauvage

Pour son support et sa confiance,

Sincères remerciements.

A Monsieur le Professeur Gilles Meyer

Professeur de l'Ecole Nationale Vétérinaire de Toulouse

Pathologie des ruminants

Qui nous fait l'honneur de participer à notre jury de thèse,

Veuillez accepter mes plus sincères remerciements.

A la fondation **France Vétérinaire International**,
Pour la bourse qu'ils m'ont attribué et qui m'a permis d'étudier le master Inter'Risk un an en Thaïlande.

A l'**Université Fédérale de Toulouse**,
Pour attribution de cette bourse qui aide les étudiants souhaitant accroitre leur compétence par la réalisation de projets à l'international. Sincère remerciement à Malaury Boissier pour sa flexibilité et gentillesse.

To **Kasetsart University** and the **Veterinary Faculty**,
For hosting this international master degree. I couldn't have learnt better how to manage and estimate health risks in South East Asia if I stayed in France. This master deeply influences my thoughts and beliefs. I am also very grateful for the Education Faculty and the women football club for their kindness and involvement towards me.

To the **master Inter'Risk teachers**,
For their involvement, the great quality of their teaching, their advice and support given all along the year.

Au Docteur Mathieu Pruvot,

Project Lead - Wildlife Health

Epidémiologiste au Wildlife Conservation Society Cambodia

Pour son encadrement durant ce stage, pour la pertinence de ses conseils, et pour son investissement tout au long de ma thèse.

To the WCS Team, Field work in the deep deforested areas in Cambodia is not easy tasks. It was only made possible thanks to the help and tenacity of a number of people. I am greatly thankful to every person from the WCS who participated in the field work. I am especially thankful to the team I've been working with: Sokha Chea, our coordinator and Srey Em Sours, Onthida Choeun, Sreyleap Torng, Sunsisonila Kang, Udom Hun, Sophornn Ton, Sithun Nuon and the villagers that provided their help.

To all the technicians of the virology unit of Institut Pasteur du Cambodge, I am grateful for the assistance given by Vibol HUL during my stay and for his previous work on the project with the PCR and on the sequences analyses. My special thanks are also extended to Jill-Léa Ramassamy who did an enormous job during the first year of the project.

Aux enseignants de l'Ecole Nationale Vétérinaire de Toulouse pour cette magnifique formation, et enfin à la **promo barbe**.

Qu'ils trouvent ici le témoignage de ma gratitude.

The present thesis is the result of an internship done with Wildlife Conservation Society and was part of the One Health LACANET program, funded by the European Commission.

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ABBREVIATIONS

c: probability of recapture (sign used during MARK analyses)

COI: Cytochrome c Oxydase I

CT: Cycle-to-threshold

ELC: Economic Land Concession

GPS: Global Positioning System

HCI: Higher limit of the 95% Confidence Interval

LCI: Lower limit of the 95% Confidence Interval

$\hat{N}$: Estimated abundance (estimated thanks to MARK software)

OECD: Organisation for Economic Co-operation and Development

p: probability of first capture (sign used during MARK analyses)

PCR: Polymerase Chain Reaction

RT-PCR: Real-Time Polymerase Chain Reaction

SEA: South-East Asia

SECR: Spatially Explicit Capture-Recapture

VTM: Viral Transport Media

WHO: World Health Organization

PART 1 – RÉSUMÉ

PART 1 – RÉSUMÉ

Dans le cadre de ma cinquième année vétérinaire, j'ai suivi le master d'évaluation et de gestion du risque en santé à l'interface entre homme, animal et environnement, appelé Inter'Risk. Ainsi, mon stage de master et cette thèse s'inscrivent dans le projet de recherche Lacanet soutenu par le CIRAD ainsi que l'association non gouvernementale appelée Wildlife Conservation Society.

L'étude des occurrences des maladies infectieuses émergentes entre 1940 et 2004 montrent que 70% d'elles sont des zoonoses et qu'entre les années 1990 et aujourd'hui une part grandissante de la transmission de ces zoonoses a pour origine la faune sauvage (Jones et al., 2008). L'Asie du Sud Est est une région singulière car elle se situe au carrefour des différents points chauds (hotspot) étant à la fois une région très riches en espèces notamment menacées (Schipper et al., 2008) et un lieu important d'émergence des maladies infectieuses (Jones et al., 2008). Morand et al. (2014) ont montré que le nombre d'espèces en danger est corrélé au risque d'émergence des maladies infectieuses. D'autres auteurs présentent le changement d'utilisation des terres, l'un des impacts majeurs de l'homme sur sa biosphère, comme un des mécanismes à l'origine de l'émergence de certaines maladies infectieuses.

Ainsi, les facteurs causant l'émergence des maladies infectieuses sont souvent discutés (Loh et al., 2015; Patz et al., 2004). Les grandes organisations internationales comme l'Organisation Mondiale de la Santé ou l'OIE s'accordent à dire qu'une vision intégrée de la santé est nécessaire pour faire face à ce risque accru d'émergence. De fait, depuis le début des années 2000, l'étude conjointe de la santé animale, humaine et celle de l'environnement s'est progressivement développée sous le concept de « One Health » qui permet une approche de la santé dans sa globalité.

Le Cambodge est un pays marqué par le régime des Khmer rouges, à l'origine d'un conflit extrêmement violent qui a débuté en 1975, et le pays n'a retrouvé une stabilité qu'une 1993 (Cambodia Tribunal Monitor, 2009). Aujourd'hui, le Cambodge est en plein développement avec un PIB annuel de 7% depuis 2011 (OECD, 2017),

notamment grâce au commerce de l'habillement et aux investissements étrangers. Malgré cette croissance économique persistante, la corruption est encore très présente comme l'attestent l'index de gini, marqueur des inégalités, qui était ainsi croissant entre 2004 et 2007 et le fait que l'inviolabilité du droit de la propriété ne soit toujours pas acquis. Ainsi, le gouvernement cambodgien a attribué des terres, parfois privées, à des concessions de terres (LICADHO, 2015).

De surcroît, le Cambodge présente l'un des plus fort taux de déforestation annuel d'Asie du Sud Est, celui étant d'environ1.57% entre les années 1990 à 2010 (source officielle de la FAO) De nombreux auteurs prédisent par ailleurs que la déforestation provoquerait une émergence des cas de leptospirose (Patz et al., 2004). Ainsi, la population rurale cambodgienne se situe à la confluence de l'insécurité des droits humains et d'une déforestation intense présentant un risque accru d'émergence des zoonoses. Cela nous a poussés à explorer les mécanismes, eux-mêmes, qui sous-tendent l'émergence de la leptospirose au cours de la déforestation.

La leptospirose est une maladie infectieuse dont la bactérie responsable est *Leptospira spp*. La bactérie se transmet par l'urine d'un animal infecté et persiste dans l'environnement. Les rongeurs sont considérés comme des réservoirs de la bactérie. En effet, la bactérie colonise les tubules proximaux des reins et continue de se multiplier pendant plusieurs années sans que les rongeurs ne présentent de symptômes (Levett, 2001).

Au cours de cette étude, nous avons fait l'hypothèse que le processus de déforestation augmente la circulation de *Leptospira spp.* entre les rongeurs. Ainsi, le but de cette étude est d'identifier les mécanismes menant à l'émergence de la leptospirose en partant d'une forêt intacte à une zone agriculturale. Nous avons fait les hypothèses suivantes :

(1) Une forêt avec un abattage intense constitue une zone de transition entre la forêt intacte et la zone d'agriculture de par une végétation intermédiaire. Ainsi, les différentes espèces de rongeurs se chevauchent et ont des contacts plus fréquents. Cette zone est alors considérée comme une zone de *spillover*, permettant la transmission du pathogène d'un individu naïf à un individu infecté.

(2) La zone cultivée, en tant qu'écosystème simplifié et manipulé par l'homme, est considérée comme moins résiliente aux changements entre saisons qu'une forêt intacte. En conséquence, les variations des dynamiques de population seront

différentes. Des variations importantes des populations de rongeurs sont attendues dans la zone cultivée, que l'on considère comme une zone d'amplification de la maladie.

(3) Dans le cas d'une transmission directe de la leptospirose par des espèces de rongeurs spécialistes, la déforestation diminuera le risque d'émergence de la leptospirose.

Pour tester ces hypothèses, nous avons étudié la dynamique des populations de rongeurs avec pour objectif d'estimer les abondances saisonnières pour chaque espèce de rongeurs et pour chaque niveau de déforestation. L'estimation de cette abondance a été mise en relation avec la probabilité de capture et de recapture. L'estimation de la détectabilité permet de distinguer une variation d'abondance réelle d'une variation liée à une différence probabilité de capture.

Dans le but d'étudier les mécanismes d'émergence de la leptospirose au cours de la déforestation, il est important de considérer le processus de déforestation comme un continuum dans le temps plutôt que le passage d'un écosystème de type forêt à un écosystème cultivé (Bradley, 2004). Pour ce faire, le processus de déforestation est étudié grâce à un design d'étude appelé *space for time substitution* (Bradley, 2004). Ce design considère que des zones avec des gradients de déforestation croissant, choisies en fonction de leur proximité géographique, reflète le processus temporel de la déforestation en lui-même. Il s'agit d'une substitution du temps pour l'espace. Ainsi, le design *space for time substitution*, s'affranchit d'un suivi longitudinal long et couteux mais assure le reflet temporel du processus de déforestation. Trois zones, présentant un gradient croissant de déforestation ont donc été définies : une zone de forêt intacte qui ne subit qu'un abattage sélectif d'arbre (il n'existe plus de forêt vierge au Cambodge), une zone de forêt perturbée qui subit un abattage non sélectif d'arbres et une modification du paysage au rythme le plus rapide et enfin une zone cultivée de moins de deux ans depuis la déforestation complète. Ces trois zones sont choisies géographiquement proches et reflètent le processus de déforestation à des temps différents. L'étude a été répétée dans cinq sites différents pendant la saison des pluies et la saison sèche dans les provinces de Mondulkiri et de Kampong Thom au Cambodge. Les rongeurs ont été capturés simultanément dans les trois zones d'un même site pendant huit nuits

consécutives. Les rongeurs capturés sont marqués par une boucle auriculaire, échantillonnés puis relâchés au niveau du piège. Les espèces ont été déterminées par analyses moléculaires (*barcoding*) (Bordes et al., 2015) et vérifiées par les données morphologiques récoltées. Les données de capture et recapture ont permis de créer des histoires de capture au cours des huit nuits consécutives pour chaque individu. Ces données appelées données de capture-marquage-recapture sont utilisées pour la modélisation de l'abondance et des probabilités de capture et recapture sous le logiciel MARK. Le statut infectieux vis-à-vis de la leptospirose a été déterminé par RT-PCR en ciblant les gènes *rrs* et *lipl32* (Smythe et al., 2002; Stoddard et al., 2009). Le gène *rrs* représente une séquence universellement portée par *Leptospira spp.* et que l'espèce soit pathogène ou intermédiaires, tandis que le gène *lipl32* représente une séquence uniquement présente chez les leptospires pathogènes.

Un total de 553 individus ont été capturés avec trois genres majoritairement capturés que sont *Maxomys.*, *Rattus* et *Mus*. Les résultats de la modélisation montrent que les estimations d'abondance de ces trois genres majoritaires varient entre les saisons et entre les différents niveaux de déforestation. En effet, les individus *Maxomys spp.* ne sont jamais capturés dans la zone cultivée tandis que les individus *Mus spp.* ne sont jamais capturés dans la zone de forêt intacte. Les individus *Rattus spp.* quant à eux, ont été capturés dans les trois niveaux de déforestation. Ce schéma de répartition est observé durant la saison sèche et la saison des pluies. De plus, le genre *Mus spp.* est celui qui présente la plus forte variation d'abondance entre les saisons dans la zone cultivée en passant de 63,52 individus [CI 95% : 38,56 ; 161,18] en saison sèche à 327,41 individus [CI 95% : 323,69 ; 331,29] en saison humide (abondance pour tous sites regroupés).

Les résultats de capture montrent également une proportion apparente des femelles *Mus spp.* différente entre les saisons dans la zone cultivée. En effet, la proportion de femelle de 0,71 en saison sèche est significativement différente de la proportion de femelle de 0,47 en saison humide dans la zone cultivée. Cependant, les analyses d'abondance corrigée contredisent cette différence de proportions entre femelles et mâles. Cela s'explique par une différente probabilité de capture en fonction du sexe de l'individu. En effet, les femelles présentent une probabilité de capture supérieure au mâle au cours de la saison sèche dans la zone cultivée, tandis

que la probabilité de capture est identique entre les mâles et les femelles pendant la saison humide.

Enfin, des estimations corrigées de la prévalence de leptospirose ont été calculées lorsque la taille de l'échantillon le permettait. Un indicateur du risque de l'émergence de *Leptospira spp.* a été calculé en combinant les abondances par genre et par site à la prévalence apparente de *Leptospira spp.* (la prévalence corrigée ne pouvant être estimée à cause d'une taille d'échantillon faible). Les résultats montrent notamment une augmentation du risque d'émergence de *Leptospira spp.* avec le niveau de déforestation pendant la saison humide. Ce risque n'augmente pas en saison sèche.

Ce dernier résultat est cependant à parfaire en utilisant des prévalences corrigées. En effet, la littérature scientifique nous informe que les mâles sont plus susceptibles d'être infectés par *Letpospira spp.* que les femelles (Ivanova et al., 2012). Ce fait est à mettre en relation avec la plus forte « visibilité » des femelles au cours de la saison sèche qui masque la prévalence réelle au sein de la communauté des rongeurs. Ainsi, la prévalence apparente de la leptospirose est sous-estimée au cours de la saison sèche dans la zone cultivée, ce qui entraine également une sous-estimation du risque d'émergence. De plus, la plupart des études préalables ne prenant pas en compte la probabilité de capture des femelles, leur prévalence est probablement également sous-estimée. La faible taille de l'échantillon de cette étude ne nous a pas permis de calculer la prévalence corrigée. Cependant, les résultats de la deuxième année de ce projet devraient fournir des nouvelles données pour compléter ce travail.

De par le design particulier de cette étude, il est possible de mettre en avant les conséquences du processus de déforestation lui-même. Nous avons montré une modification de la communauté de rongeur au cours de la déforestation avec une abondance plus importante pendant la saison humide que la saison sèche. Cette étude offre également un aperçu de l'importance de prendre en compte des probabilités de détection avant de tirer des conclusions sur la prévalence et l'écologie d'une maladie.

PART 2 – LITTERATURE REVIEW

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1. Health: links between wildlife, land use change and human outbreaks

1.1. Using One Health approach to tackle disease emergence

One Health is an emerging way of thinking, studying and solving challenging health threats, which aims to consider human, animal and environmental health linkages. Health has often been ill-defined and mostly human focused, by defining health as the absence of diseases. However, it can also be thought with a broader point of view which defined health as the presence of a well-being. Stephen et al. (2014) maintained the idea that 'One health' projects were mainly focused on diseases from an animal-human perspective, often leaving environment a step behind. They suggested that we should adopt an integrative definition of health that we can link with the concept of 'resilience' often used by ecologists. Resilience is the capacity to cope and recover from stressors or changes. They insisted on the fact that we could benefit from a socio-ecological approach to health and consider the reciprocal care of health and the environment for human well-being and this thought should be the base for 'One Health' projects. Myers et al. (2013) also highlighted that existing research on human health impacts of ecosystem alterations focused on a single outcome of health. Since ecosystem degradation has multiple impacts on health, Myers et al. (2013) advised to study their contribution to health outcomes. For example, we can ask 'how much is malaria a consequence of deforestation' and thus consider the net health effects. This would have more benefit for public health and conservation.

With this in mind, disease ecology, understudied at the moment but already a dynamic area of research could play an important role to understand mechanisms of diseases emergence and reduce number of cases. As illustrated by Karesh et al. (2012) in the figure 1 bellow showing the dynamic of zoonosis emergence from wildlife to domestic animals and humans. Early detections surveillance systems among wildlife population at the interface with human and animals could be a key component to improve public health. These challenges could be addressed using a One Health approach.

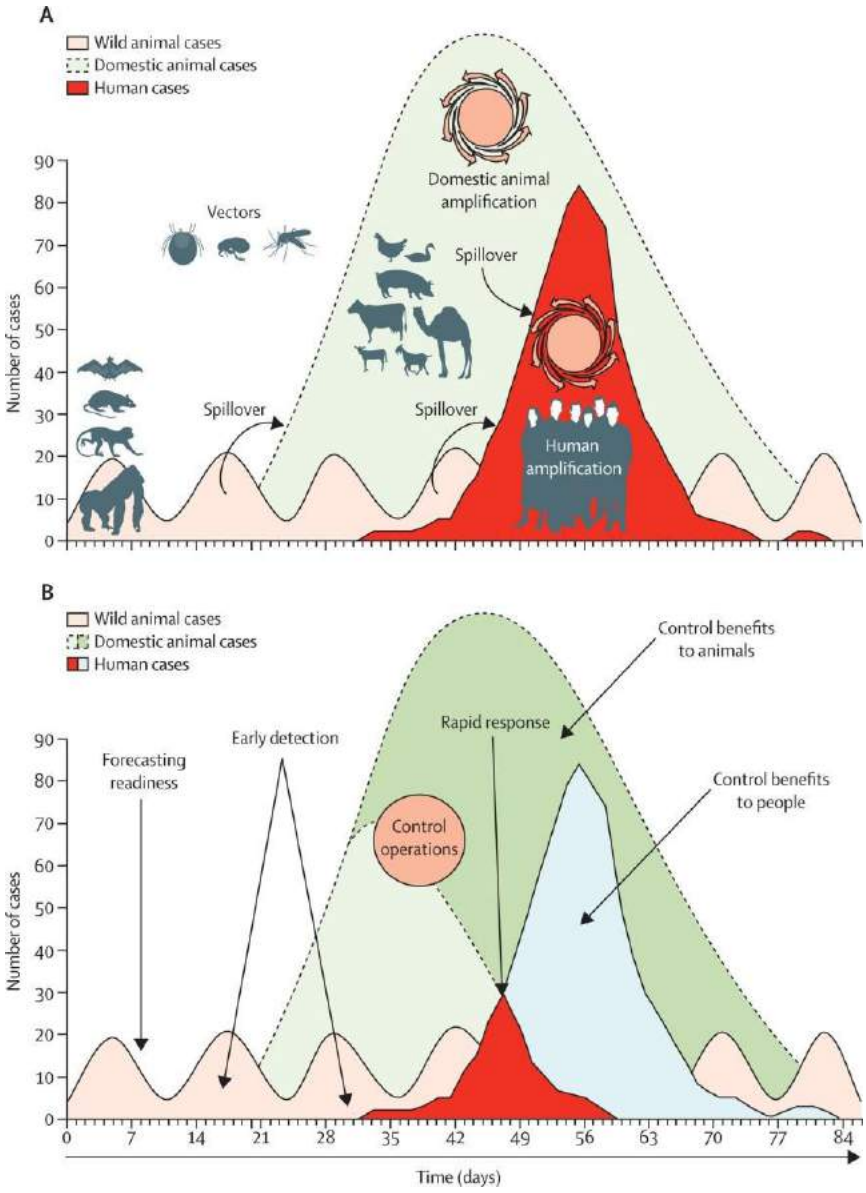

Figure 1 - *Ecology of zoonoses: natural and unnatural histories (Karesh et. al., 2012).*

A. *Transmission of infection and amplification in people (bright red) occurs after a pathogen from wild animals (pink) moves into livestock to cause an outbreak (light green) that amplifies the capacity for pathogen transmission to people.*

B. *Early detection and control efforts reduce disease incidence in people (light blue) and animals (dark green). Spillover arrows shows cross-species transmission.*

1.2. Links between wildlife, biodiversity and health

1.2.1. Ecosystem services provided by the forest in Cambodia

Persson et al. (2010) wrote a report for the Stockholm Environment Institute about the ecosystem services supporting livelihoods in Cambodia. Forest resources

were reported to be used in all seven villages surveyed. Twenty five percent of the villagers identified forest resources as their second most important source of income, as it is also observed in other tropical countries (Colfer et al., 2006). Timber, bamboo, rattan are the main forest products collected. Added to this, food such as snails, frogs, eels as well as edible plants or leaves are collected for household consumption and medicinal care. These goods collected by numerous households contribute to an important part of the household income as well as the protein supply, as revealed by interviews in this study.

Persson et al.'s report (2010) highlights that some villagers experienced a decline in availability due to the interdiction to collect forest products from new economic land concessions leading to the need to pay to collect these resources while it was free of charge before.

When people's food, health care, economic systems have always been intertwined with the forest, its loss have negative implications not only for their socio-economic status, but also for their mental health and well-being. These populations also become prone to infectious diseases because of an unpredictable sanitary situation and different exposure pathways to infectious diseases (Colfer et al., 2006).

Soil stabilization, erosion control, sustaining air quality, climate regulation, carbon sequestration are some examples among a long list of forest ecosystems services.

1.2.2. Land-use change and the (re)emergence of zoonosis

Two-thirds of known human infectious pathogens have emerged from animals, with the majority of recently emerging pathogens originating in wildlife (Taylor et al. 2001; Jones et al. 2008). Among others, the occurrence of chagas disease, yellow fever and leishmaniasis have been linked to the change in land use in tropical regions. This has been explained by the particularly intense changes faced by primary forests that opened to extractive industries (Karesh et al., 2012). These lands are also emerging disease hotspots because of their richness in wildlife biodiversity and thus probably richness in pathogens never seen by human populations. At the same time, contacts between human populations and unmodified ecosystems are increasing. Loh et al., (2015) identified zoonotic diseases attributed to land-use change and attributed a likelihood for each transmission pathways. Thus, zoonotic

diseases attributed to land-use change were more likely to be transmitted via the vector-borne pathway (52.5%), followed by direct animal contact (23.8%), the airborne pathway (19.8%), and a smaller proportion from the contaminated environment and oral transmission pathways (2%).

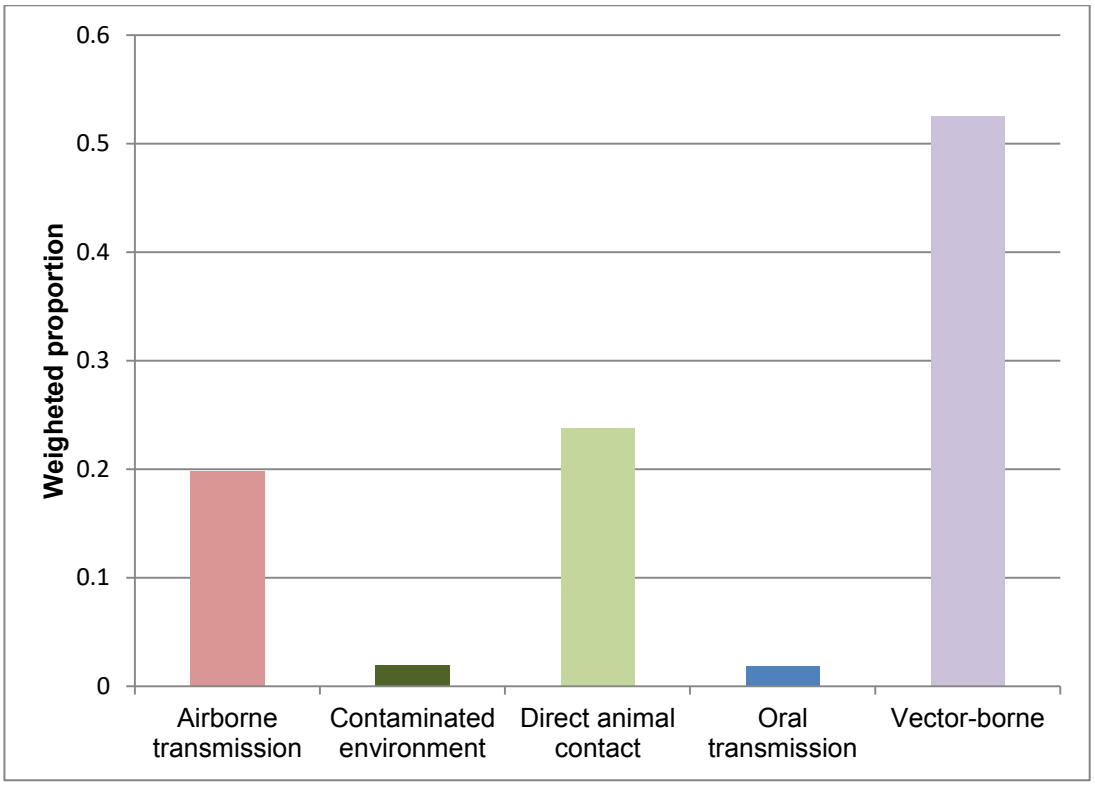

Figure 2 - *Land-use change as one of the primary driver of disease emergence: scaled number of zoonotic disease emerging infectious diseases events per transmission route categorized by the primary driver of disease emergence for each pathogen (Loh et al., 2015).*

Three main mechanisms leading to the emergence of humans pathogens after the clearing of forests has been suggested by Wilcox & Ellis, (2006): the exposure of immunologically naïve population to pathogen present in forests, an increase in the abundance of dispersal of pathogens influencing hosts abundance and distribution and finally, the alteration of ecohydrological functions which facilitate the survival and transport of waterborne pathogens.

Wilcox and Ellis (2006) said in their article called *forests and emerging infectious diseases of humans*: “disease emergence is a transient phenomenon in a human population, and in its most severe form is typically a consequence of rapid social and environmental change or instability”.

1.2.3. Impact of land-use change on wildlife and diseases

In SEA, the importance of rodent-borne diseases in regards to emergence of zoonotic diseases is very high (Morand et. al., 2015a). Moreover, (Serge Morand et al., 2014) showed that rather than the richness of birds and mammals, it is the number of threatened mammals and bird species that is positively correlated with outbreaks. Biodiversity is thus a source of pathogens, but the loss of biodiversity or its regulation seems to be associated with an increase in the number of zoonotic outbreaks.

Land-use change had considerably impacted the biodiversity (Sodhi et al., 2004; Wilcove et al., 2013). The reemergence of leptospirosis is recognized to be linked with deforestation (Patz et al., 2004).Myers et al., (2013) proposed a schematic of the complex relationships between altered environmental conditions and human health (appendix part 2 -1.2.3).

Galetti et al., (2015) studied the change in abundance and diet of rodents following the extinction of a dominant terrestrial mammal in a neotropical rainforests. Their results support the hypothesis that the local extinction of a dominant ungulate has an effect on the abundance and diversity of small mammals in species-rich communities. Two of the three rodent species were found with an increased abundance in defaunated forests (*Akodon montensis* and *Oligoryzomys nigripes*). This finding has important consequences in terms of human-health since these 2 species are important hosts of Hantavirus. Thus, Galetti et al.'study (2015) highlights that defaunated non-fragmented forests contribute to an increase in the population of Hantavirus hosts and ultimately-trigger the emergence and spread of lethal diseases in human populations.

2. Land-use change in Cambodia: trajectories and socio- economic context

Miettinen et al. (2011) identified that the main change trajectories leading to deforestation in South-East Asia, between 2000 - 2010, is due to the transition from forest to plantations. Stibig et al., (2014) ranked the main forest change processes in SEA between 1990 - 2010 and identified that the first cause of forest loss is the conversion to cash crop plantations (coffee, tea, sugarcane, oil palm). The second

cause identified being logging and thirdly, fast-growing forest plantations as rubber plantations. The latter trajectory was mainly occurring in Cambodia.

Moreover, official data on 200 Economic Land Concessions (ELCs) analyzed by Forest Trend (2015) indicate that their main purpose of deforestation was for rubber plantations (about 1.1 million hectares of concessions areas), sugar culture (150,000 ha) and pulp/paper (100,000 ha). ELCs are estimated to cover 12% of the country (LICADHO, 2015), see 2.1. These preliminary results support the conclusion made by Stibig et al. (2014) that rubber is the major driver of deforestation performed by ELCs in Cambodia.

2.1. Deforestation rate estimation in SEA and Cambodia:

Deforestation rate in SEA is recognized to be one of the highest in the world (Deforestation Dataset University of Maryland, 2014). FAO estimated in 1995 that SEA harbor 15% of the world's tropical forest (Stibig et al., 2014). Estimated rates of deforestation vary from one to another study. Miettinen et al. (2011) estimated an overall annual deforestation rate of 1.0% in continental and insular SEA between 2000 and 2010. This rate is lower than the estimation provided by FAO during the 1990's, which estimated a 1.5% - 1.7% deforestation rate. However, Stibig et al. (2014) indicate an annual deforestation rate of 0.67 for the 1990's and 0.59 for the 2000's. There are huge disparities between SEA's countries. Sumatra being the highest with an annual deforestation rate of 2.7% between 2000 – 2010 (Miettinen et al., 2011).

To focus on Cambodia, official data collected by FAO indicate a decrease of the forest land area from 12,944,000 ha in 1990 to 9,457,000 ha in 2015, with an annual deforestation rate of 1.57% from 1990 to 2010 (figure 3).

The national forest cover change assessment conducted in 2006 by the forest administration (the key government agency in the forestry sector in Cambodia) concluded that forest cover declined from 61% of the total land area in 2002 to 59% in 2006, which is equivalent to an annual rate of deforestation from 2002-2006 of 0.5% of the total land area (The forestry administration, 2010). According to a report of 2015 (Forest Trend, 2015), Cambodia is losing forest at a rate of 804 square mile a year, that's to say 1.15%.

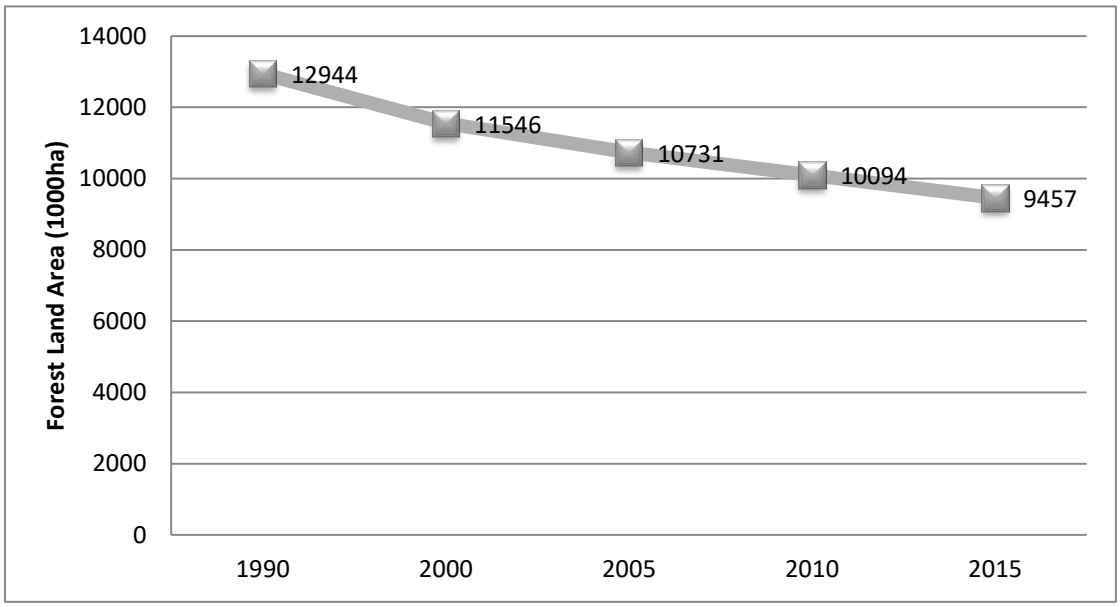

Figure 3 - *Forest land area (in 10000 ha) in Cambodia from 1990 to 2015 – exported data collected on FAO website (official data only)*

2.2. How economic development and land concessions led to deforestation in Cambodia

2.2.1. Insecurity of land title in Cambodia

After a three decades history of violent conflicts and traumatic Khmer Rouge regime, Cambodia has reached a political stability since 1993, when the newly elected government came to power (Cambodia Tribunal Monitor, 2009). This has led to an improvement of development indicators ("World Bank Cambodia Data," 2017), of the enrollment rate in primary education and of maternal health (World Bank, 2006). Cambodia is now considered as an emerging country, with a rapid and solid economic performance of a constant 7% annual GDP since 2011, which rank it in the top 3 of highest GDP of ASEAN 10 (OECD, 2017).

However, growth has been largely driven by the garment, tourism and construction sectors, located in the urban areas, while agricultural growth has been rather modest, with more than 90% of Cambodia's poverty in rural areas (*World Bank, 2006*). Thus, the primary drivers of growth have only few linkages with the majority of the population, leading to an urban growth bias (Rudi et al., 2014).

While, international indicators depict a decrease in the poverty trends, the inequality indicators are not doing so (appendix part 2 - 2.2.1.The World Bank," 2017). Indeed, the Gini coefficient (the most commonly used measure of inequality)

has risen from 0.38 in 2004 to 0.41 in 2007 before to decrease. Moreover, the Economist Intelligence Unit, (2009), based on its Political Instability Index[1], identified Cambodia as one of the most vulnerable nations to socio-political unrest due to social inequality and economic distress. Cambodia's corruption index lies at 2.1 out of 10, ranking it 156th position out of 176 countries; and for the second year Cambodia is the most corrupt South East Asian country on their list ("Transparency International," 2017).

The strategy of Cambodian government has been to promote investment influx in order to favor economic growth, regardless on human rights (Amnesty International, 2008; Rudi et al., 2014). With 56% of the country's ELCs granted to foreign companies, ELCs are estimated to cover 2,1 million hectares, ie. approximately 12% of the country (LICADHO, 2015).

The opaque process by which land titles are granted is based on selective and arbitrary law enforcement for those with connections to the powerful and weak institutions (De Lopez, 2002; LICADHO, 2015). Some forced evictions cases are not a last resort decisions and land title not a guarantee, depriving Cambodian from their human rights ("LICADHO," 2015).

Land grabs have been made through violence, in some cases involving Cambodian authorities (LICADHO, 2015; Amnesty International, 2008), and inadequateness of relocation sites (Land and house rights work group, 2009) created a more economic vulnerable population, that has also an impact on their mental health and well-being (Colfer et al., 2006).

Thus, the Kingdom of Cambodia is a post-conflict developing society characterized by weak democratic institutions, large inequality, in spite of consistent economic growth. The absence of security of tenure, in the context of endemic corruption, and a rapid influx of foreign investment and economic development, has led to a land rights crisis in Cambodia.

[1] This index includes 3 economic distress index and 12 vulnerability indicators which are: inequality; state history; corruption; ethnic fragmentation; trust in institutions; status of minorities; history of political instability; proclivity to labor unrest; level of social provision; a country's neighborhood; regime type (full democracy, authoritarian, etc); and the interaction of regime type with political factionalism.

2.2.2. The role played by economic land concessions in the deforestation

From 1997 to 2002, deforestation in Cambodia was mainly associated with smallholder agricultural encroachment along the boundaries between extensive forest and non-forest landscapes (Amariei, 2004). This form of deforestation appears relatively limited today as large scale agri-industrial plantations have rapidly encroached on forest lands since mid-2004.

Thus, ongoing deforestation in Cambodia is mainly explained by the large land concessions accorded to agricultural companies. Nearly 14% (nearly 12% according to LICADHO) of the country has been allocated to these corporations. Moreover, according to Engvall et al. (2007), due to the absence of constraints for investors, many of them have focused on harvesting existing forest resources and then left empty lands once trees were cut.

Using Nasa satellite images of forest fires and carbon emissions, Forest Trend localized ongoing deforestation. These records showed that ELCs are targeting the oldest and most valuable forests (many of them on national forest lands) for logging. This information is consistent with the maps published by LICADHO that made observations in the country (appendix part 2 - 2.2.2.)

Thus, lands are acquired by powerful people in connection with a corrupted government. The loss of forest cover observed in Cambodia is consistent with land use and land cover change patterns associated with demographic growth and economic development in most countries.

The combined effects of this land-use change have severe impacts on the livelihood of villagers facing insecure income and land title, as well as threats upon an exploited forest ecosystems.

3. Leptospirosis to study the impact of deforestation in Cambodia

Leptospirosis is an infection caused by bacteria of genus *Leptospira* that includes 9 pathogenic species and at least 5 intermediate species (with approximately 20 species and more than 300 serovars) (Bharti et al., 2003). Half of the pathogenic serovars belongs to species *L. interrogans* or *L. borgpetersenii*. Symptoms vary widely, making distinction between malaria, viral hepatitis, yellow fever, dengue and

viral meningitis very complicated and leading to misdiagnosis. In most cases, leptospirosis leads to a febrile illness. Asymptomatic or subclinical infection is believed to be common in endemic regions (Levett, 2001).

3.1. Leptospirosis overview in SEA:

Classified by WHO as a neglected zoonotic disease, a subset of neglected tropical diseases, leptospirosis is however not included in its top 17 priorities. At the same time, literature reports that leptospirosis burden is very likely underestimated in low-income tropical countries, and may therefore be comparable or even higher to other important neglected tropical diseases (visceral leishmaniasis, severe dengue and cysticercosis for example).

Several studies support the fact that leptospirosis is an important and emerging NTD which should be more taken into consideration (Costa et al., 2015; Mwachui et al., 2015; Picardeau et al., 2015). Moreover, as highlighted by Ewald et al. (2002), we should focus on diseases already globally distributed and prevalent, representing consequently a major threat for public health instead of focusing on famous and excessive media exposure that benefits some acute infectious diseases.

Indeed, leptospirosis is one of the world's most widespread zoonotic infectious diseases. Thailand, Cambodia, Laos, Vietnam are considered endemic areas for this disease.

3.1.1. High burden in Cambodia but under-reporting of cases:

WHO estimates the prevalence in tropical countries at 10 cases per 100 000 people, and can soar to over 100 cases per 100 000 people in case of epidemic (WHO | Leptospirosis Burden Epidemiology Reference Group, 2017). Costa et al., (2015) estimated the global burden of leptospirosis at over one million severe human cases per year, and approximately 60,000 deaths per year. However these numbers are likely underestimated due to limitations of surveillance systems in low income countries (Picardeau et al., 2015).

SEA is a region where incidence is high, and more and more countries report leptospirosis outbreaks (Cosson et al., 2014). Current trends of leptospirosis outbreaks, especially in endemic areas, indicate that geographic spread and

epidemics will increase in the future (WHO SEA Regional Office).Thailand, for example, which has a relatively good health system, reports several thousand cases of leptospirosis each year, while Cambodia reports very few. This discrepancy could be due to under-reporting. Indeed, the largest study in Cambodia on human leptospirosis tested N=612 hospitalized-patients with an infectious syndrome (among them 10% were previously tested negative to dengue from the dengue surveillance network). This study revealed that 14.4 % were tested positive for an acute *Leptospira* infection (detected by PCR targeting *rrs* gene and *lfb1* for confirmation) and 29.9% were positive by at least one biologic marker (IgM or PCR) (Berlioz-Arthaud et al., 2010).

In order to get free from the selection bias of hospitalized patients, a community based study has been conducted by Hem et al. (2012) in Kampong Cham, the most populated province in Cambodia. They aimed to estimate the risk of being infected by *Leptospira* among children and young adults (< 20 years old) with fever. A total of 8295 samples were first tested for the most common cause of fever in Cambodia (Dengue, Japanese encephalitis virus, Chikungunya virus, Influenza, Respiratory Syncitial Virus and Human Metapneumovirus). Positives samples were removed and a random selection of the 7162 remaining negatives samples was done. Among the 2358 samples tested for anti-leptospirosis IgM, 26.7% were found positives. Modeling analyses lead to an overall semestrial probability of having fever caused by leptospirosis of 1.03% (95%CI: 0.95%-1.22%) among all children and young adult under 20 years old with fever (Hem et al., 2012).

Thus, Cambodia is an endemic country of leptospirosis with a high burden of infections but with high under-reporting of cases.

3.1.2. Epidemiology of leptospirosis in SEA

The source of infection in humans is usually urine of an infected animal, the contamination being mainly for indirect contact. The main portal of entry is through abrasions or cuts after prolonged immersion in water. Although rats, mice and other rodents are believed to be reservoir, a wide range of other mammals (dogs, deer, rabbits, cattle, buffaloes, sheep, and pigs) also carry and transmit Leptospira.

This wide range of animals which can serve as an infection source explains the wide geographically distribution of this zoonotic disease. Moreover, some infected species, as rodents for example, can remain asymptomatic and shed infectious organisms in urine for their entire lifetime. Indeed, Leptospira colonize persistently the proximal renal tubules (Levett, 2001). Wildlife might play an important role in the transmission pathway (Mwachui et al., 2015) but the mechanisms are still unclear.

Most Leptospira are resistant in the environment with a longer survival in warm and humid conditions (Andre-Fontaine et al., 2015). Thus, we observe a seasonality of the disease with a peak incidence during rainy seasons in tropical countries; which are usually developing countries with greater contacts with livestock, domestic pets and wild animals (Levett, 2001).

The review from Bharti et al. (2003) underlined that isolated populations of mammals may have an important role in the maintenance of unusual serovars, and that a single species may carry different serovars in geographically distinct populations.

Clinical disease in wild animals appears to be less severe than the one described in subsequently infected humans. Although numerous pathogenic serogroups of Leptospira exist, not all exhibit the same virulence in each animal species.

- Leptospirosis transmission risk factors

In their review, Mwachui et al., (2015) aimed to assess the environmental and behavioral determinants of leptospirosis transmission, classified risk factors into the following categories: i) water related (eg. flooded areas), ii) agriculture area (eg. rice production), iii) landscape factors (eg. forest cover), iv) socio-economic status (specific home construction materials as a proxy), v) sanitation (eg. type of and proximity to sewage system), vi) behavioural (eg. walking barefoot), vii) animals.

Occupational exposure such as rice farming and other agricultural activities is significant, as well as the exposure of the general population during activities of daily living.

Floods and heavy rain were associated with leptospirosis in almost all studies investigating these risk factors (n = 17). This is consistent with the increasing number of outbreaks reported during flooding events and it can be considered as one of the

main risk factors in tropical countries (Lau et al., 2010). In addition they hypothesize that due to global warming, extreme weather events will occur with increasing frequency and intensity worldwide. Thus, the risk of flooding events is expected to increase leading to an expected increasing leptospira transmission risk.

Living in rural areas was associated with increased risk of leptospirosis infection in studies comparing rural and urban residents. This result was unrelated to geographic study location, which means the risk is higher in rural areas for developed countries as well as resource poor countries.

Thus, leptospirosis risk has also to be considered globally in the perspective of climate change.

3.2. Prevalence of leptospira among rodents in SEA:

The mean prevalence of leptospira among rodents in SEA varies from one study to another: from 4.4% (Della Rossa et al., 2016) to 7.1% (among 901 total rodents sampled) (Cosson et al., 2014) and even 12% (with 580 rodents sampled) (Ivanova et al., 2012). Morand et al. (2015), combined rodents' leptospirosis studies conducted in Thailand and estimated an overall prevalence of 8.1% among rodents. Details per species are shown in the following table 1 – A. associated with the main information about the study design (table 1 – B.).

3.3. Risk factors of rodents infections:

3.3.1. Flooding season

A clear seasonality pattern, with higher prevalence for Leptospira infections of rodent species during the flooding season has been showed by Ivanova et al., (2012). They showed that the wet season is favorable for transmission of Leptospira in rodents, particularly in rain-fed fields.

3.3.2. Host species

3.3.2.1. Rodent infection and habitat

Cosson et al. (2014) conducted a study in seven localities in SEA (three in Thailand, two in Cambodia, two in Lao PDR) within four different habitats types (forested areas, non-floodable and floodable lands, human dwellings). Their results showed a large variation of the mean prevalence in rodents across localities and habitats, but not between rodent species. *Leptospira* prevalence was very low in human dwellings (2%) and when removed, *Leptospira* prevalence was similar between floodable areas, forests and non-floodable agricultural fields.

However, another study showed that species living in forests and in non-flooded habitats, such as *Berylmys berdmorei* and *Niviventer fulvescens*, have similar level of infection to species inhabiting rice fields (i.e. with low slope values) (Ivanova et al., 2012).

These two studies suggest that not only rice fields but forests, secondary forests, and their interface with agricultural fields are also areas of potential risk for leptospirosis infection in humans. Thus it challenges the idea that leptospires mainly circulate in wetlands.

3.3.2.2. Rodent infection and species

The level of detection of leptospira in the different species presents considerable differences (Ivanova et al., 2012 ; Herbreteau et al., 2012 ; Cosson et al., 2014 ; Loan et al., 2015). *Bandicota spp.* and *Rattus spp.* are reported to be important hosts of leptospires of human health importance. Moreover, high prevalence was observed in rarely investigated species such as *Niviventer fulvescens*, whereas on the contrary, *Mus spp.* appeared to be not infected (Ivanova et al., 2012).

It has been suggested that the observed differences in prevalence may reflect differences in population densities, rather than intrinsic differences in susceptibility among species (Cosson et al., 2014). Differences in sample size, species distribution, as well as in laboratory methods for determining prevalence complicate comparisons across studies.

3.3.3. Individual characteristics explaining the prevalence

Ivanova et al., (2012) confirmed that prevalence of infection increases with age, a result consistent with a chronic and unlethal disease for rodents as previously mentioned. Males were significantly more likely to be infected than females (Cosson et al., 2014). Moreover, Loan et al. (2015) identified rat size (those in the fourth quantile of body size) as having an increased risk of testing positive (OR = 3.74).

Table 1 - *Comparison of leptospirosis prevalence among rodents of 5 studies conducted in SEA. A. Leptospirosis prevalence per species – results are a percentage (total number of sampled animal into brackets) B. Main study design characteristics (next page).*

A

Species	**Herbreteau et al., 2012**	**Della Rossa et al., 2016**	**Loan et al., 2015**	**Cosson et al., 2014**	**Ivanova et al., 2012**
Bandicota indica	10.1 (1006)	10.7 (65)	10.8 (65)	3.7 (27)	66.7 (3)
Bandicota savilei	2.6 (464)	-	-	1.92 (52)	21.3 (80)
Berylmys berdmorei	0 (6)	10 (10)	-	15.38 (13)	33.3 (12)
Berylmys bowersi	-	100 (1)	-	0 (1)	-
Leopoldamys edwardsi	-	-	-	0 (3)	0 (2)
Maxomys surifer	-	-	-	6.98 (43)	8.7 (104)
Mus caroli	0 (6)	0 (7)	-	5.98 (88)	0 (1)
Mus cervicolor	0 (12)	0 (7)	-	9.23 (65)	-
Mus cookii	-	0 (27)	-	18.82 (85)	-
Muss spp.	0 (4)	0 (9)	-	0 (14)	-
Niviventer fulvescens	-	-	-	-	21.4 (14)
Rattus andamanensis	-	-	-	0 (4)	-
Rattus argentiventer	5.9 (102)	-	4.8 (104)	13.51 (37)	29.2 (48)
Rattus exulans	3.9 (1242)	0 (63)	0 (16)	0.45 (220)	3.9 (155)
Rattus losea	7.0 (86)	-	-	12.77 (47)	-
Rattus nitidus	-	-	-	0 (6)	-
Rattus norvegicus	20.8 (860)	-	6.9 (29)	0 (10)	0 (27)
Rattus tanezumi	5.7 (1858)	2.8 (36)	3.3 (61)	9.68 (186)	11.2 (134)
Total	**8.1 (5646)**	**4.4 (225)**	**5.8 (275)**	**7.1 (901)**	**12 (580)**

B

	Herbreteau et al., 2012	Loan et al., 2015	Della Rossa et al., 2016	Cosson et al., 2014	Ivanova et al., 2012
Samples location	Compiled surveys of microparasites in rodents trapped - Thailand	Vietnam, Mekong Delta	Northern Thaïland	7 areas among Thailand, Lao PDR, Cambodia	Cambodia (2 provinces)
Types of habitats studied		Markets (5), Farms (20), Edge of rice fields (6), Tropical forest - Natural Park (with large numbers of canals) (4)	4 habitats: forest, non-flooded and flooded lands, humans settlements		
Study period		Dry season, rainy season only for market samples		2009-2010	Dry and rainy season
Laboratory analyses used		RT - PCR	RT - PCR	RT-PCR targetting *lipL32* gene	RT-PCR Mérien et al protocol (1992) identified saprophytic leptospires

4. Future research recommendations

A working group on land-use change and disease emergence published an article on policy recommendations as regards further research on landscape

fragmentation and infectious disease (Patz et al., 2004). They advised the acquisition of key data on pathogen load of wildlife, as well as the relative abundance of organisms (vectors, pathogens, hosts). These key information would unable the understanding of fragmentation's consequences and disease ecology.

Moreover, given the high heterogeneity of risk factors identified by the recent review from Mwachui et al. (2015), general recommendations for designing effective healthcare interventions are difficult to address. More knowledge is needed. Indeed, they highlighted the fact that the role of rodents was surprisingly understudied in SEA (2 studies out of their 64 selected studies). Even if we know that the underlying rodent population dynamic feeds environmental contamination, they advised that future epidemiological studies should address ecological, climatic and rodent demographic components for a more detailed understanding of habitat role. Authors also suggested that future attempts to develop leptospirosis transmission models should primarily address environmental water related exposures as a main driver for transmission (Mwachui et al., 2015).

As well, very few knowledge is available as regards epidemiology of leptospira among wild communities of rodents, pathogen and host dynamic. Also, whether environmental conditions determine *Leptospira* species distribution in nature remains largely unexplored (Cosson et al., 2014).

4.1. The concept of chronotone to study land-use change

Disease transmission is a dynamic and complex process which can be explained by a multitude of factors including the structure and organization of social and ecological systems but also the public health system (Scoones, 2017).

What happens during the transition period between two states at the equilibrium? The reorganization phase separating these two states is illustrated by the conceptual model below.

The concept of ecotone has been a very much useful tool to study mechanisms happening at the interface between two ecosystems. However, it becomes limited once we want to integrate the chronology sequence happening between two steady-states of ecosystems. Thus, the concept of chronotone, as introduced by Bradley

(2004), can be useful to understand an ecological process occurring in a short time period.

> *"As the ecotone is the boundary area between two ecosystems or habitat types in space, so the chronotone is the boundary in time between two types of land use or habitat. (…)The chronotone is therefore defined as the period of relatively rapid transformation separating the two long-term types of land use."* (Bradley, 2004)

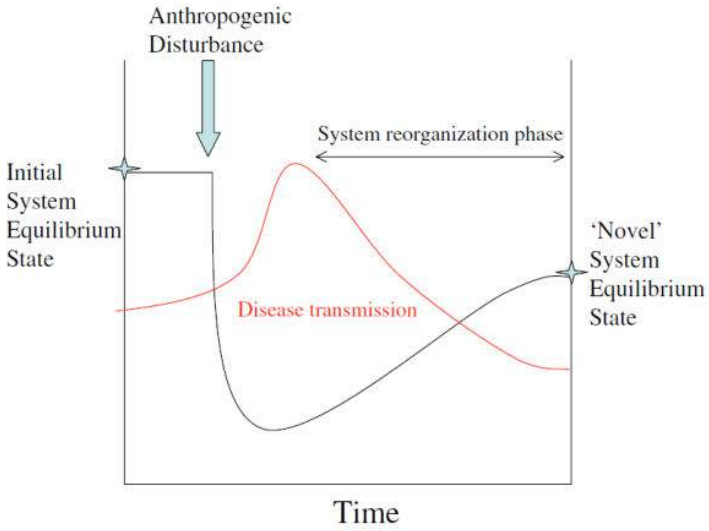

Figure 4 - *Conceptual model of the dynamic process of disease transmission - Gottdenker 2014*

When a forest is to be cleared for cultivation, we can expect a diverse set of changes in which we are interested to know the diseases dynamics as regards pathogens, hosts. Epidemiologically, these set of changes carry risks peculiar to itself that are essential to understand in order to implement some specific and relevant prevention measures.

When we use the concept of chronotone for multiple sites, one of the main assumptions is that all zones were at the same state when sampling was started and that they followed the same pattern of change.

These last years, a lot of studies focused on the consequences of deforestation using comparison between distinct areas but the process of deforestation in itself is still understudied (Brearley et al., 2013) and the use of chronotone concept might be usefully applied to this problematic.

PART 3 – HYPOTHESIS

PART 3 – HYPOTHESIS

The biodiversity of leptospires in the environment is affected by geography, climate, biotic interactions, and anthropogenic activities. Environmental conditions strongly affect the transmission of Leptospira by modifying the population biology, behaviour, or community ecology of spirochetes and their hosts.

While we have seen the links and complexity between land-use change and its impacts on human health, the mechanisms leading to the emergence of infectious diseases during the process of change itself are still unclear. Even though some studies are stating that biodiversity loss and habitat changes may be the very drivers of disease emergence (Serge Morand et al., 2014), only a limited amount of studies suggest mechanisms (Wilcox et al., 2006).

Our hypothesis is that during the process of deforestation, the circulation of *Leptospira spp.* between rodent species increases. Thus, our aim was to identify the mechanisms leading to the emergence of leptospirosis from intact forest to cleared forest. To do so, we made the following hypothesis:

(1) The forest altered by logging can be considered as an area of increased contacts between rodent species. This transition zone between intact forest and agriculture is composed of intermediate vegetation where different species that usually do not come into contact can overlap. Thus, we considered this area with increased contacts between species as a “spillover zone”, allowing transmission between infected and naïve animals.

(2) The cultivated area as a simplified and manipulated ecosystem can be considered less resilient than the intact forest to seasonal changes. As a consequence, variations in the population dynamics are expected to vary between intact forest and cultivated lands. Higher variations of the population are expected in the cultivated land which constitutes a target zone for amplification.

(3) Finally, in the case of a direct transmission with a specialist rodent, deforestation will lead to a decreasing risk of emergence. Indeed, their living environment will be destroyed and the host will either migrate or die.

More precisely, we focused on the population dynamics with the following objectives:

(1) Estimate the difference of abundance among seasons for each rodent species for each different zone (levels of deforestation) and identify factors explaining this variation (sex, age, infectious status).

(2) Assess the capture and recapture probability for each species for each zone during the wet and dry season. This will permit to distinguish whether the variation among rodent populations are explained by a different detectability or a different population dynamic.

Variations of the rodent species distribution and their population dynamics affect the pathogens they carry and thus would allow an assessment of the possible risk of human leptospirosis.

PART 4 – MATERIALS & METHODS

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1. Zone selection for a chronosequence design

In order to study the ongoing changes linked to deforestation, we used the concept of chronotone: the transition in time between two types of land-use or ecosystems (Bradley, 2004). To follow up the deforestation transition over time, we surveyed three zones at the same time and close geographically, with an increasing level of deforestation. We considered that these three zones were representative of the modifications observed during the process of deforestation giving access to a time sequence along this process (space for time or chonosequence design). By doing so, we were able to avoid a longitudinal follow-up.

The three increasing levels of deforestation were defined as follows:

(i) **Intact forest:** evergreen or semi evergreen forest from protected area or community forest with a selective tree logging (zone 1). The most valuable trees are cut in the first place.

(ii) **Disturbed forest:** tree logging and landscape modifications are happening at the quickest rate (zone 2).

(iii) **Agricultural land:** zone recently planted, less than two years since complete clearing, (zone 3).

2. Study sites and rodent trapping

We studied five different sites starting from June 2015 to April 2016. Rodents were trapped in Mondulkiri province (Keo Siema district) for sites S1, S2 and S4, and Kampong Thom province (San Dan district) for the sites S3 and S5 (figure 5).

Each site was visited during the rainy and the dry season. Sites' order was randomized so that the time interval between seasons was varying from 156 days to 287 days (table 2).

Rodents were trapped using locally made non-lethal Havahart traps (figure 6) separated by 20m intervals and placed at least 100m from the habitat edge in each zone

Table 2 - *Sampling time period for each sites and the time interval between seasons*

Sites	Rainy season 2015	Dry season 2016	Time interval (days)
Site 1	17th - 25th June	24th March - 01st April	281
Site 2	08th - 16th July	20th - 28th April	287
Site 3	30st July - 07th August	10th - 18th Feb.	195
Site 4	19th - 27th August	22nd - 30th Jan.	156
Site 5	10th - 18th Sept.	04th - 12nd March	176

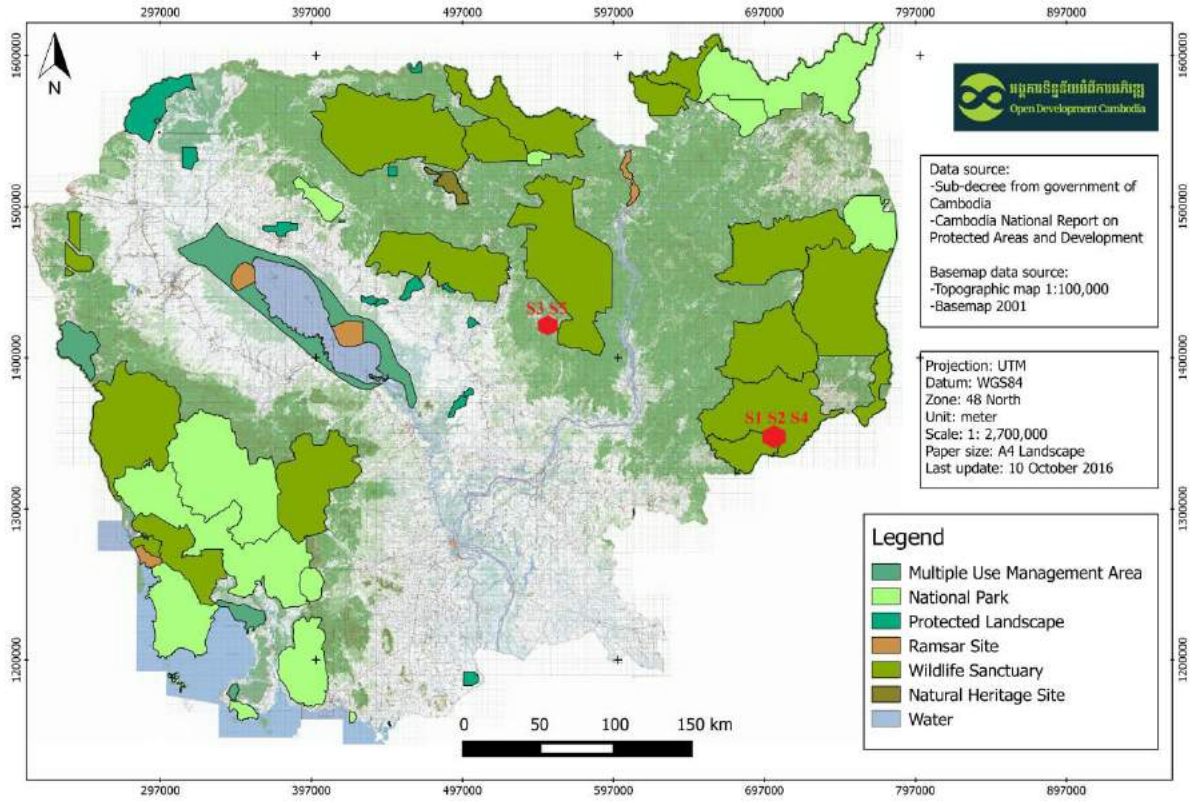

Figure 5 - Locations of sites sampled in red superimposed with the protected areas in Cambodia, map used from Open Development Cambodia.

Figure 6 - *Locally made non-lethal Havahart traps placed in the cultivated area*

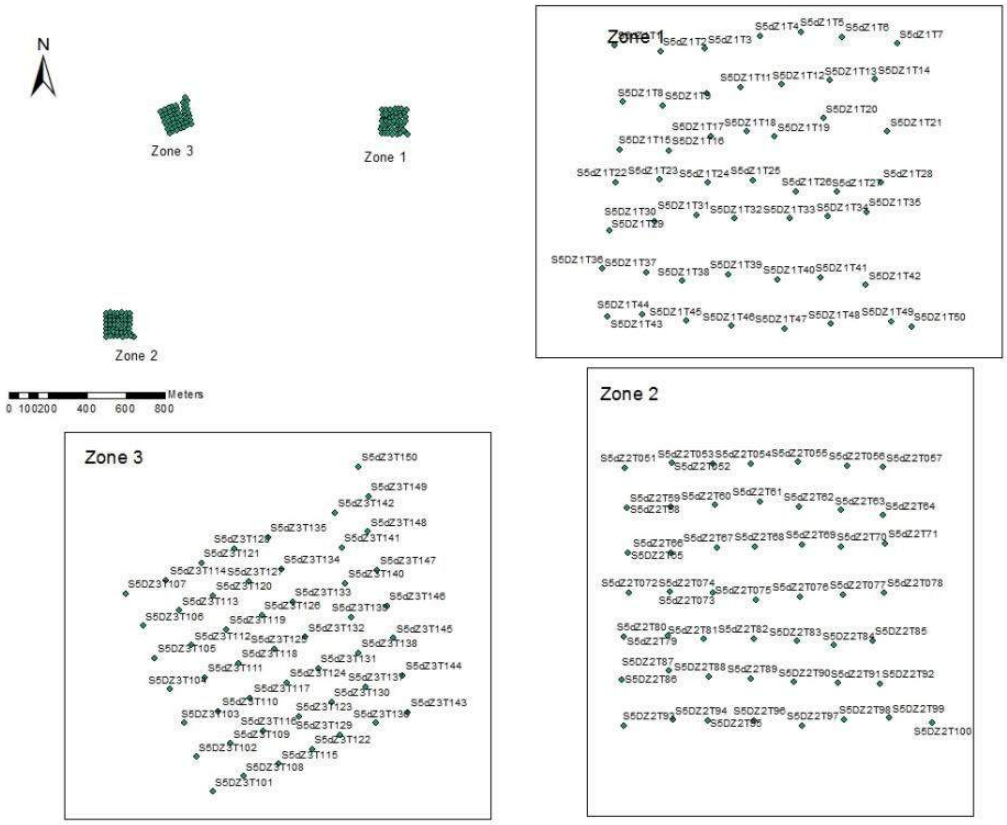

Figure 7 - *Example of a trapping grid with the three zones represented*

Each zone was 200m away from each other. At each locality, 5 lines of 10 traps, with a total of 150 traps for the 3 different habitats, were placed during 8 nights. (When the length of the area couldn't permit to place 10 traps, we placed 7 lines of 7 traps with 8 traps on the last line) (figure 7).

The sampling effort corresponded to a total of 1200 trap nights per site. In both seasons, trap lines were located in the same area using a global positioning system (GPS) receiver. Geographical coordinates of each trap line were systematically recorded by GPS.

2.1. Capture-Mark-Recapture design

All animals captured were identified using a unique ear tag number before being released at the same captured trap location. Each trap was loaded every evening with bait made of sweet potatoes covered with peanut butter. Following recapture successes were recorded providing an encounter history for each animal during the eight nights of capture occasions. Teams' shifts were set up when loading with bait

the different sites in order to reduce manipulators bias. Animals recaptured without a tag (removed accidently after release) were identified once again using a new tag number and two encounter histories were created for these individuals. This is equivalent to a total of 10 animals, 7 originating from S5.

3. Rodent manipulation

Captured rodents were anesthetized using isofluran inhalation until muscular relaxation was obtained (around 15 seconds were needed). We then proceeded to species identification, measurements of body parts and samples collection.

3.1. Rodent measurements and identification

After tagging, rodents' body lengths were measured (left ear, left foot, head & body, tail, skull, weight, anal genital distance) and main morphological characteristics were recorded (sex, age, species, sexual development state) (appendix part 4 - 3.1.). Finally, a picture of each rodent was taken.

3.2. Rodent samples

Skin, urine or uro-genital swab and feces or rectal swab were collected. Skin samples were preserved in 95% ethanol solution, while others samples were preserved in RNA and Viral Transport Media (VTM). All samples were stored in nitrogen solution before being transferred in a -80°C freezer.

4. Laboratory analyses

4.1. Leptospira species and genetic diversity

Urine and uro-genital swabs were used to identify rodent carriers of *Leptospira spp.*. Rectal swabs and feces were also used since contamination from urine happened in some cases. DNA was extracted using the Qiagen RNeasy® Mini Kit (Qiagen S.A.S., France). We performed two different polymerase chain reactions.

Real-time polymerase chain reaction (RT-PCR) targeting the *lipL32* gene was performed. *lipL32* gene is considered to be a virulence factor that encodes for an

outer membrane lipoprotein. This gene is not present in nonpathogenic species (Haake et al., 2000), allowing the detection of Leptospira species that are pathogenic to human.

A second RT-PCR amplified the *rrs* gene, universally present in *Leptospira* and thus detected in both pathogenic and intermediate *Leptospira* species allowing us to carry out a broader screening in rodents.

Were considered individuals' positive for *Leptospira* infection, sample which were positive for *lipL32* PCR and/or suspect or positive for the *rrs* gene.

4.1.1. Human pathogenic *Leptospira* infection status

RT-PCR using a TaqMan *lipL32* assay was performed in order to identify the human pathogenic strains. As previously described by Stoddard et al. (2009) we used the following primers: forward (5′-AAG CAT TAC CGC TTG TGG TG-3′), reverse (5′-GAA CTC CCA TTT CAG CGA TT-3′) and probe lipL32-189P (FAM5′-AA AGC CAG GAC AAG CGC CG-3′BHQ1). The amplification was performed on a BioRad Thermal Cycler CFX96. A Ct<40 (Ct: cycle to threshold) for the *lipL32* amplicons was considered positive for *Leptospira*.

4.1.2. *Leptospira* detection among rodents

This real-time PCR assay, previously described by Smythe et al. (2002), amplified the *rrs* (16S) gene. The primer set of Lepto-F (5'-CCC GCG TCC GAT TAG-3') and Lepto-R (5'-TCC ATT GTG GCC GRA CAC-3') were used for amplification with an expected size of 87pb and detected by the probe Lepto-probe (5'-6-FAM-CTC ACC AAG GCG ACG ATC GGT AGC-BHQ1-3'). Real-time amplification was performed using the BioRad Thermal Cycler CFX96. Positive samples were defined as having Ct value below 35.

4.2. Rodent species identification

4.2.1. Choice criteria for final species decision

Rodents species were first identified using morphological criteria in the field. In parallel, molecular techniques were used on all rodents sampled. The final decision

to attribute the species was taken according to agreement between the barcoding outcome (a molecular identification method) and the previous morphological identification, and data were always cross-checked with pictures.

In case of disagreement between the barcoding outcome and the picture, we chose:

(i) The barcoding identification if measurements of the animal were coherent with the barcoding result.

(ii) The genus identified from the picture and/or the morphological identification if measurements of the animal were not coherent with the barcoding result.

Finally, in case of impossible result from barcoding analyses, we used the animal's head and body length measurements cross-checked with pictures to decide for the genus.

4.2.2. Morphological rodents identification

We based our morphological identification in the field using a decision tree (appendix part 4 - 4.2.2.) created thanks to CERoPath field guide (Chaval et al., 2011) and A field Guide to the Mammals of South East Asia by Francis et al., (2008). The decision tree was validated by CERoPath researchers' team (CERoPath standing for Community Ecology of Rodents - Pathogens and habitat changes in Southeast Asia).

4.2.3. Barcoding: molecular technique for species identification:

DNA was extracted from skin tissue using the Qiagen DNeasy® Blood & Tissue Kit according to the manufacturer's instructions. The primer set of BatL5310 (5'-CCTACTCRGCCATTTTACCTATG-3') and R6036R (5'-ACTTCTGGGTGTCCAAAGAATCA-3') were used to amplify a 750 base pair fragment of the Cytochrome c oxydase I (COI) gene, as previously used in the CERoPath project (http://www.ceropath.org/). PCR products were visualized by gel electrophoresis and amplicons were sent for sequencing to Macrogen (Seoul, South Korea). Sequences were trimmed and assembled using CLC Genomics Workbench 3.6.1 (2013) software. Either the consensus (when obtained) or both sequences

(reverse and forward) were submitted for BLASTn search to obtain the species on the NCBI website (National Center for Biotechnology Information) and CERoPath website (http://www.ceropath.org/).

5. Statistical analyses – capture-mark-recapture modeling

5.1. Capture – Mark – Recapture data used to estimate the detectability

Contrary to the study of human diseases, sampling wildlife populations is rarely a census. Thus, in most wild populations, sampling and inference are strongly impacted by incomplete observations of the system state (Cooch et. al., 2012). Jennelle et al. (2007) underscored that when detection probability of diseased individuals varies over time, and not the detection probability of healthy individuals, we will observe a varying apparent prevalence over time, whereas true prevalence is time invariant. When studying wildlife diseases, we have to account for the observation bias since it is possible that disease status (diseased or not), gender or age influence our observation. An illustration on the apparent prevalence and encounter probabilities is given in appendix part 4 – 5.1.A.

Here, we considered estimation methods that explicitly account for differences in detection probability, using data from multiple encounters of known individuals. This class of model is referred as Capture-Mark-Recapture (CMR).

The data collected according to CMR approaches for one individual can be summarized as a series of ones and zeros, animals being recaptured (written 1) or not (written 0) during a series of capture sessions, named encounter histories (appendix part 4 – 5.1.B. for more details on capture histories input).

In order to estimate the abundance (ie. the population size), we used closed capture models. We focused on the three main genus captured (*Maxomys spp.*, *Mus spp.*, *Rattus spp.*) during the rainy and dry season 2015 – 2016 respectively. In a disease context, the use of a closed abundance estimators is useful to calculate the prevalence over the whole population and not only the visible or captured population. The repetition of capture occasions (eight nights of capture) in each zone and site enables to account for false absence.

Seven animals were captured twice the same day and adjusted to a single capture per day to be able to enter the data in the format required and to run the models. Six out of these seven animals were from site 5 and zone 1. Escaped animals (total of 15) and recaptured animals with a misread tag (total of 12) were excluded since identification or following identification were impossible (table 4).

Finally, encounter histories were created for all individuals for whom the genus was identified (table 3).

5.2. Assumptions of closed capture models

The rodent population is assumed to be closed: no changes in population size during the 8 nights time period. This assumption of closure is geographic (no movement on or off the study area) and demographic (no births and deaths).

A second assumption considered the absence of false positive errors: a species will never be detected at a site it does not occupy, while it will be detected with a given probability at sites where it is present. The key assumption of this model is that there is no unexplained site heterogeneity or if it exists it has been recorded by covariates.

5.3. Encounter histories, covariates and models selection using MARK software

5.3.1. About Mark analyses

Capture – Mark – Recapture data were analyzed using the software MARK. This software was used according to steps described in the guide “Program MARK A Gentle Introduction” by Evan G. Cooch & Gary C. White (17th edition).

The general approach to estimate the abundance and the probability of first capture *p* in closed populations is based on the Lincoln-Petersen estimator with the assumption that all individuals (marked or not) are equally catchable (that’s to say a random mixing of marked and unmarked individuals after the first sample - equation (1)).

$$(1) \quad \frac{m_2}{n_2} = \frac{n_1}{N}$$

N: the size of the population ; n_1, n_2: the number of individuals encountered and marked during the first, second occasion respectively ; m_2: the number of encountered animals during the second occasion that were previously encountered (called later recapture).

This leads to: (2) $\widehat{N} = \frac{n_1}{\hat{p}} = \frac{n_1 n_2}{m_2}$

Models are parameterized in terms of two different encounter parameters:

(i) *p* – the probability of first capture (i.e., the probability that an animal in the population will be captured and marked for the very first time),

(ii) *c* – the probability of recapture (conditional on having been captured at least once before). The *c* parameter is generally used to model for behavioral effects following initial capture.

The model parameters are estimated using a fitting algorithm based on the maximum likelihood[2]. We used a conditional likelihood approach to estimate abundance (described by Huggins, 1989), where we 'condition' the likelihood on individuals being encountered (so the encounter histories of individuals that were never caught doesn't appear).

With the condition likelihood approach, the estimated abundance $\hat{N}$ is not a parameter of the likelihood expression, but a derived parameter. This choice was taken to be able to include individual covariates in the model (eg. sex, age). Indeed, for animals that were never capture no covariates values are available. Consequently, we can't use a model including individuals never captured if we want to include individual covariates in the model. Thus, when individual covariates are used, a Horvitz-Thompson estimator is used to estimate $\hat{N}$.

[2] The maximum likelihood estimation method is firstly based on the calculation of the probability distribution of the observed data as a function of the parameters. We then transform it at a likelihood function, that's to say a function of the parameters conditional on the data. Finally, we find the values of the parameters that maximize this function. We answer the question: given the underlying model, for what values of the parameters are these data most likely? These are the maximum likelihood estimators.

$$(3) \quad \widehat{N} = \sum_{i=1}^{M_{t+1}} \frac{1}{1 - [1 - \hat{p}_1(x_i)][1 - \hat{p}_2(x_i)] \dots [1 - \hat{p}_t(x_i)]}$$

M_{t+1} the number of unique individuals caught at least once.

$\hat{p}_t$: first capture estimate at the capture occasion t

5.3.2. Models tested and model selection

For reasons of identifiability of closed population parameters, all p(t) and c(t) (ie. all first capture and recapture probabilities set for each capture occasion) cannot be estimated independently, and need to be constrained in the model. This means we have to set a constraint to specify *p* as a function of *c* or as a function of time (ie. capture occasions).

To do so, we choose different plausible models based on field observations and biological plausibility. We successively tested all the following models with all the combination of relevant covariates:

(i) M0: *p(.) = c(.)*, the first capture probability *p* and the recapture probability *c* are equal and constant over sampling occasions ;

(ii) Mb: *p(.), c(.), p* and *c* are different but stay constant over the sampling occasions. This model is equivalent to test for a behavioral effect, that's to say we constraint two different probabilities: a rodent being captured for the first time and a rodent being recaptured (could reveal for example a learning process that traps are not harmful and provide food).

(iii) Mt: *p(t) = c(t), p* and *c* are equal and vary for each sampling occasions ;

(iv) Mtb: *p(t) = c(t) + z,* a combination of the effect of time and behavioral effect ;

(v) M t + t²: *p* and *c* are equal and vary as a quadratic function of time (that's to say, it reflects an increasing probability of capture with time reaching a peak before to decrease. It is for example a window of time needed for rodent habituation before to enter the trap).

The best model was selected based on the lowest AICc. The use of AICc selects at the same time the most significant model as well as the one which best fits the data. Not convergent models (ie. model giving either unrealistic $\hat{N}$ results or

parameters without estimation because of a very large confidence interval) were removed even if they had the lowest AICc.

Finally, selection of models with a Δ AICc ≤ 4 were kept (MacKenzie, 2006) to calculate a $\hat{N}$ from the average of the models selected. Indeed all models with AIC difference of less than 2 have a substantial level of empirical support, 4 through 7 have substantially less support (MacKenzie, 2006).

5.3.3. Encounter histories and covariates

Capture histories were created for each of the 3 main genus captured (*Maxomys spp*., *Mus spp.* and *Rattus spp*.). Rodents were grouped by genus to provide a sufficient sample size within each genus, and to allow the inclusion of individuals that were only identified at the genus level (in case of inconclusive barcoding and morphological identification). These histories were created for each zone of each site when the sample size was > 3 animals and models described in the preceding section were tested. An example of the data format used for MARK analyses is given in the appendix part 4 – 5.1.B.

We hypothesized that sex, age (baby, juvenile, adult) and the leptospirosis infectious status of individuals had an influence on the capture and recapture probabilities. These three individual covariates were tested systematically. When the different covariate values were not well represented in the data (for example, 1 juvenile out of 22 *Maxomys spp.* captured in zone 1 during the dry season), the related covariate was not included in the model.

When the age information was missing, it was added using indicators of sexual maturity recorded for each animal. A female with an open vagina was considered as an adult, a juvenile otherwise; a male with testicule partially or fully descended was considered as an adult, a juvenile otherwise. No babies, easily recognizable given their small size, were involved among the missing age data.

5.3.4. Summary of the statistic procedure followed

We aimed to get a corrected abundance of the three main genuses captured while taking into account capture probabilities and possible covariates.

The abundance estimate was performed by zone by season for *Maxomys spp.*, *Rattus spp.* and *Mus spp.* and by zone by site by season for *Mus spp.* (bigger sample size).

We gathered data from the five sites by genuses and season. We tested systematically same models and used site as a covariate when the sample sized permitted it. Since all sites were not sampled at the same time period (table 2), we considered them as environmental covariates in order to account for sites heterogeneity. Moreover, by doing so, we free ourselves from the environmental differences between sampling period.

Thus, we ended up with an estimated abundance ($\hat{N}$) from the average of the best models selected (according to the explanation given in part 4 – 5.3.2.). This model average estimated abundance is calculated by genus, for each zone of each season.

We then focused on *Mus spp.* to investigate the sex influence on the capture probability. Our analyses were focused on the cultivated area – zone 3 for reasons of sample size.

Finally, fisher tests were used to assess whether there was significant difference in the sex proportion or the prevalence of Leptospira between season and zones. Statistical significance was set for $P< 0.05$.

PART 5 – RESULTS

PART 5 – RESULTS

1. Rodent community composition and structure

1.1. Rodent community dominated by three genuses

A total of 553 animals were captured from the five sites, with a marked difference between rainy and dry season with 435 and 118 animals captured, respectively (table 3).

Species couldn't be determined for 37 individuals' due to the absence of data recorded and/or unavailability of samples. Rodent genotyping was successfully determined for 494 individuals using molecular technics (barcoding analyses) and identified thirteen different rodent species. 22 individuals' genus was determined using pictures and measurements.

Table 3 - *Individuals distribution by zone and season for the main rodent genuses with the total number of individuals from other rodent species and individuals from unidentified species*

Genus	Rainy season 2015				Dry season 2016				TOTAL
	Z1	Z2	Z3	Total	Z1	Z2	Z3	Total	
Maxomys spp.	34	15	0	49	16	9	0	25	**74**
Rattus spp.	4	43	26	73	4	14	1	19	**92**
Mus spp.	0	39	232	271	0	31	31	62	**333**
Total / Zone / Season	**38**	**97**	**258**	**393**	**20**	**54**	**32**	**106**	**499**
Total other rodent species	1	5	1	7	4	5	1	10	17
Individuals from unidentified species	7	4	24	35	0	0	2	2	37

Maxomys spp. was never captured in the cultivated area (zone 3) and was mostly abundant in the forest area. *Mus spp.* was never captured in the forested area (zone1) and was mostly abundant in the cultivated area. *Rattus spp.* was the only genus captured in the 3 zones during the wet and the dry season, with a marked abundance in the disturbed forest (zone 2).

Of all captured rodents individuals with an identified species, *Mus spp.* constituted the highest number of captured animals (67.8% and 53.4% during the rainy and dry

season, respectively), *Rattus spp.* the second most captured (18.3% and 16.4% during the rainy and dry season respectively), followed by *Maxomys spp.* (12.3% and 21.6% during the rainy and dry season respectively). These three genuses accounted for 98.3% and 91.4% of the overall rodent community captured and successfully identified during the rainy and dry season respectively.

Each of the five other rodent genuses were represented by no more than 17 individuals. The detailed numbers of captured individuals by season, site, zone and species are presented in appendix part 5 – 1.1.. Given these species distribution, we focused our modeling analyses on the three main rodents genuses captured that's to say *Maxomys spp.*, *Mus spp.*, *Rattus spp.*.

1.2. Apparent *Mus* spp. sex proportion

We observed a distinct sex proportion difference between seasons when we focused on *Mus spp.* During the dry season, females' *Mus spp.* sex proportion was higher than during the rainy season (figure 8). Sex proportion was significantly different between seasons (Fisher test, P=0.0007) and was mainly supported by zone 3 (Fisher test, P=0.001).

Thus, female were significantly more captured than male during the dry season rather than the rainy season in the cultivated area (zone 3).

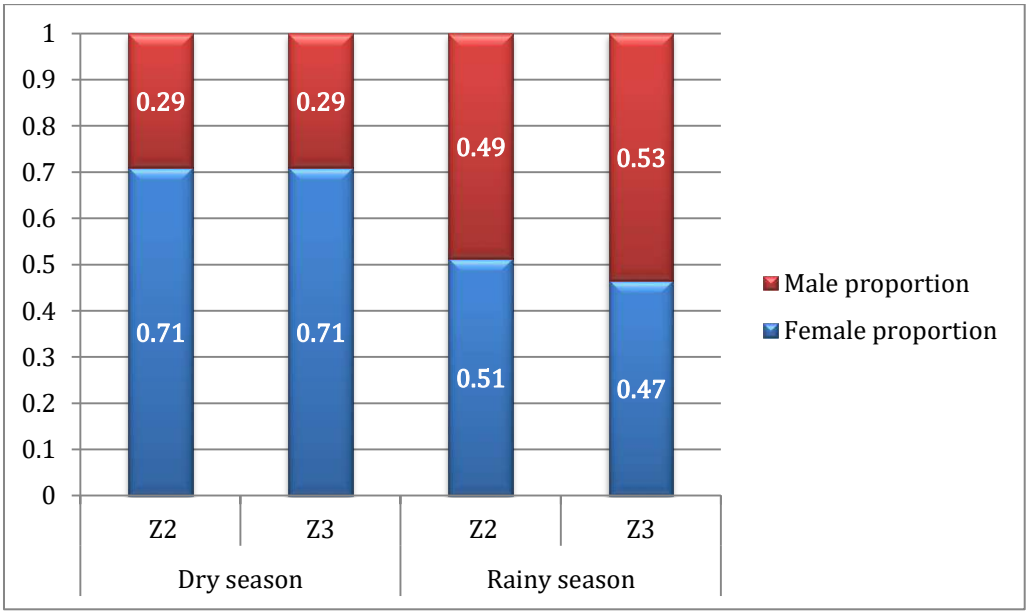

Figure 8 - *Mus spp. apparent sex proportion by zone by season*

2. Abundance estimation

In order to perform the abundance estimation under MARK, 36 animals captured were removed from the analyses because of either a missed identification of a recaptured animal (misreading of the tag number) or the animal escaped before being tagged. Consequently, no encounter histories could be associated for these 36 animals and could not be included to model *p* and *c* (detail given by zone/site/season and identification problem associated in table 4).

During the rainy season, most of animals removed came from the cultivated area - zone 3. Moreover, 25 rodents were removed from zone 3 out 32 removed during the rainy season. Note also that most of captured rodents during the rainy season came from zone 3 (table 3).

Table 4 - *Number of animal removed according to the identification problem by zone/ site/season.*

	Rainy season 2015								Dry season 2016			TOTAL
	Site 1		Site 2		Site 3	Site 5			Site 3		Site 5	
	Z2	Z3	Z2	Z3	Z3	Z1	Z2	Z3	Z2	Z3	Z1	
Escaped animals	1	2	0	2	1	1	1	6	0	1	0	**15**
Tag number misread	0	0	1	3	9	1	2	2	2	0	1	**21**
Total / Zone / Site / Season	**1**	**2**	**1**	**5**	**10**	**2**	**3**	**8**	**2**	**1**	**1**	**36**

2.1. N̂ when all sites analyzed collectively

The model average N̂ (abundance estimate) when all sites are analyzed collectively leads to an estimation of the rodents species abundance while considering all the same zones (for example all zones 1 - forested areas) of the different sites are equivalent to one same area.

Moreover, site is used as a covariate to take into account the site heterogeneity. Six season/zone/species combinations out of 14 successfully included sites as a covariate in the final best model (fifth column, table 6). All the 5 sites were included in the data and successfully used as a covariate for 5 out of 14 combinations (table 6).

Model average abundance estimates (N̂) by genus by zone by season are presented in table 5 with the unique number of animals captured (M_{t+1}) (see also

figure 9). The associated best model fitting the data for each season/zone/species combinations are presented in table 6. Details of the model used to generate the average N̂ are presented in appendix part 5 - 2.1.

Mus spp. N̂ were 50% higher than the unique number of animals captured (M_{t+1}), whereas the difference was smaller for the other two genuses.

Table 5 - *Estimated abundance (N̂) of model average for Maxomys spp., Rattus spp. and Mus spp. by zone, by season.;*

** one Maxomys spp. captured in site 2;*

*** one Rattus spp. captured in all the 5 sites;*

**** one Mus spp. captured in site 4.*

M_{t+1}: number of unique individuals caught at least once.

HCI: Higher limit of the 95% Confidence Interval

LCI: Lower limit of the 95% Confidence Interval.

Season	Zone	Sites excluded	Genus	N̂ of models average	LCI	UCI	M_{t+1}
Rainy	Z1		*Maxomys spp.*	39.02	35.37	78.18	35
		S1 S2 S4	*Rattus spp.*	4.25	4.01	13.48	4
	Z2	S2*	*Maxomys spp.*	23.78	19.59	31.80	15
		S4	*Rattus spp.*	50.89	48.86	53.63	43
		S4 S5	*Mus spp.*	143.47	103.98	206.96	39
	Z3		*Rattus spp.*	36.15	33.69	39.52	27
			Mus spp.	327.41	323.69	331.29	235
Dry	Z1		*Maxomys spp.*	25.39	23.11	32.41	22
		S2 S4	*Rattus spp.*	4.50	4.09	6.69	4
	Z2		*Maxomys spp.*	11.50	10.21	14.18	9
		S1	*Rattus spp.*	23.76	20.54	28.58	14
		S4 S5	*Mus spp.*	65.15	47.38	102.19	31
	Z3	**	*Rattus spp.*	--	--	--	--
		S4***	*Mus spp.*	63.52	38.56	161.18	30

Table 6 - *Best model that fitted the data by genus, by zone, by season. M0: p(.) = c(.), Mtb: p(t)=c(t)+z, Mt+t²: p and c are a quadratic function of time. Covariates abbreviations: Si: sites, Se: sex, A: age.*

** refers to models for which the sample size was too small to include sites as a covariate;*

*** one Maxomys spp. captured in site 2;*

**** one Rattus spp. captured in all the 5 sites;*

***** one Mus spp. captured in site 4.*

Season	Zone	Sites excluded	Genus	Best model with covariates
Rainy	Z1		*Maxomys spp.*	{Mtb+Si+Se}
		S1 S2 S4	*Rattus spp.*	{Mt+t^2}*
	Z2	S2**	*Maxomys spp.*	{M0+Si}
		S4	*Rattus spp.*	{Mt+t^2+Si}
		S4 S5	*Mus spp.*	{Mt+t^2+Si+Se+A}
	Z3		*Rattus spp.*	{Mt+t^2}*
			Mus spp.	{Mt+Si}
Dry	Z1		*Maxomys spp.*	{Mt+t^2+Si}
		S2 S4	*Rattus spp.*	{M0}*
	Z2		*Maxomys spp.*	{M0}*
		S1	*Rattus spp.*	{M0}*
		S4 S5	*Mus spp.*	{Mt+t^2+Se}*
	Z3	***	*Rattus spp.*	--
		S4****	*Mus spp.*	{Mt+t^2+Si+Se}

2.1.1. Best model

The Mt+t² model (capture and recapture probabilities follow a quadratic function of time), was the best model in half of the season/zone/species combinations. The model M0 (constant probability of capture and recapture during successive occasions) was the second model most frequently fitting the data of the season/zone/species combinations (table 6).

2.1.2. Significant covariates

Among all the covariates tested, sex was the covariate tested with a significant effect on the capture probability estimates. Most specifically, sex had a significant effect on *Mus spp.* capture probabilities during the dry season in zone 2 and 3. The

capture data for *Mus spp*. in zone 3 during the rainy season is best explained by the model {Mt+Si}, however the model {Mt+Si+Se} was within a Δ AICc of 2.0028 (appendix part 5 – 2.1.) which suggest a fair level of support for the model including sex as covariate.

The age had a significant effect only for *Mus spp.* in zone 2 during the rainy season (table 6). Age had a fair level of support in zone 3 during the rainy season (with the model {Mt+Si+A} and a Δ AICc of 1.629) as well as in zone 2 during the dry season (with the model {Mt+t²+Se+A} and a Δ AICc of 4.093) (appendix part 5 – 2.1.).

2.1.3. Population dynamic and variation between zones and season

The model average abundance estimate is represented by zone by season for *Maxomys spp*., *Rattus spp*. and *Mus spp.* in figure 9 (visual summary of table 5).

Corrected abundance calculation confirms the population structure and dynamic previously explained in part 5 - 1.1. with an increased abundance for each genuses during the rainy season compared with the dry season.

Mus spp. population increases the most between seasons with a 5.2 fold increase during the rainy season in the cultivated area (increase from 63.52 [95%CI 38.56 ; 161.18] during the dry season to 327.41 [95%CI 323.69 ; 331.29] during the rainy season). On the contrary, *Maxomys spp.* and *Rattus spp.* follow the same pattern between seasons: *Rattus spp.* is mainly abundant in the disturbed forest and *Maxomys spp.* in the forested area.

Disturbed forest is the only area with an overlap of the three main rodent genuses (dominated by *Mus spp.*, followed by *Rattus spp.* and *Maxomys spp.*).

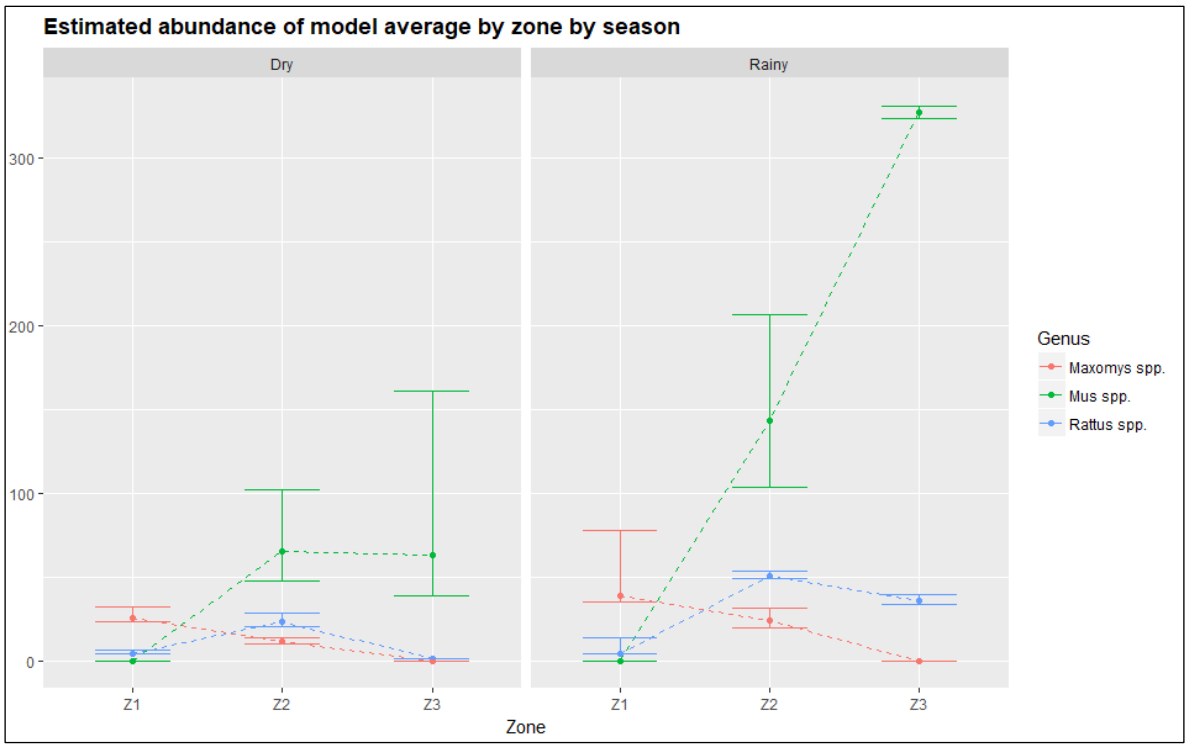

Figure 9 - *N̂ of a model average and their confidence interval for the three main genus captured along a deforestation gradient (zone 1 = intact forest, zone 2 = disturbed forest with intense tree logging, zone 3 = cultivated area).*

2.2. Abundance estimation for *Mus spp.* in zone 3

Mus spp. N̂ by site and season in zone 3 (cultivated area) was estimated when the sample size permitted it (table 7).

During the dry season, in the cultivated area - zone 3, the sum of *Mus spp.*'s estimated abundance (sum of N̂ = 54.83) (table 7) for all sites is consistent with the confidence interval of the model average N̂ including all sites (95% CI: 38.56 – 161.18) (table 5). However, the sum calculated for the rainy season (sum of N̂ = 372.80) (table 7) is higher than the highest confidence interval of the model average N̂ for all sites gathered (95% CI: 323.69 – 331.29) (table 5).

When sites were analyzed separately, sex covariate was found to be significant only for site 2 during the dry season, and age for site 1 during the rainy season. Moreover, no clear trend on the best model fitting the data was observed (ie. the constraint upon *p* and *c* best fitting the data) (table 8). The constraint *p(t)* = *c(t)* is

the only one found twice out of the five sites to be the best to fit the data during the rainy season (other constraints being *p(.) = c(.)* (model {M0}), p(.)=c(.)+z (model {Mb}), *p* and *c* following a quadratic function of time (model {Mt+t²}) table 8). Mt model was well supported when all sites were analyzed together (table 6).

Table 7 - *Mus spp. N̂ (estimated abundance) of models average in zone 3 (cultural lands) by site by season with the best model fitting the data. HCI: Higher limit of the 95% Confidence Interval and LCI: Lower limit of the 95% Confidence Interval.*

Season	Site	N̂ of models average	LCI	UCI	M_{t+1}
Rainy	S1	**82.11**	59.62	131.79	41
	S2	**51.76**	49.52	54.49	39
	S3	**58.70**	56.86	60.93	48
	S4	**76.01**	47.54	169.11	35
	S5	**104.22**	98.86	110.65	72
Dry	S1	--	--	--	2
	S2	**18.58**	11.42	61.78	10
	S3	**27.69**	14.16	199.86	13
	S4	--	--	--	1
	S5	**8.56**	6.97	12.81	6

Table 8 - *Best model that fitted the data for Mus spp. in zone 3 (cultural land) by season. M0: p(.) = c(.), Mb: p(.)=c(.)+z, Mt: p(t)=c(t), Mt+t²: p and c are a quadratic function of time. Covariates abbreviations: Se: sex, A: age.*

Season	Site	Best model
Rainy	S1	{M t + t² +Se}
	S2	{M0}
	S3	{Mt}
	S4	{Mb}
	S5	{Mt}
Dry	S1	--
	S2	{Mb+Se}
	S3	{Mt}
	S4	--
	S5	{M t+ t²}

3. *Mus spp.* capture probability and sex proportion

Mark-recapture modeling was used to differentiate a true variation in the sex proportion between seasons from a bias caused by the variation of the capture probabilities. Capture probabilities were calculated probabilities by sex, zone, site and season for *Mus spp.* (figure 10) using the best models identified in table 6. (Note that to be able to compare capture probabilities between zone and season, we added sex as a covariate for zone 2 during the dry season even if it wasn't included in the best model (table 6). Likewise, we removed the site's and age's covariates from the model in zone 2 during the rainy season to be able to compare with the dry season that didn't include these covariates. When sites are removed from the model (line labeled "0" in figure 10), it is equivalent to consider all zone 3 gathered in a same area without taking into account their heterogeneity. No male individual was captured in zone 3, site 1 during the dry season, thus, no capture probability is estimated.

3.1. Capture probabilities according to sex

Females and males' *Mus spp.* capture probabilities by season are shown in figure 8 .Capture probabilities followed approximately the same pattern through successive occasions: increasing progressively until the fourth or fifth occasion before to decrease or stay stable. Moreover, capture probabilities during the dry season have the same order of magnitude as the wet season (figure 10). It testifies that the abundance difference between seasons (figure 9) is linked to a population abundance variation rather than a capture probability variation.

In the cultivated area (zone 3), during the dry season, females' *Mus spp.* present a higher capture probability than males. This difference is not significant during the wet season (figure 10-B).

In the disturbed forest (zone 2), capture probabilities are significantly influenced by sex during both seasons. However, the capture probability difference between male and female is higher during the dry season than it is during the rainy season.

There is almost no overlap between the two capture probabilities according to sex during the dry season while it is not the case during the rainy season (figure 10-A).

Different capture probabilities between male and female are more significant during the dry season than the rainy season in zone 2 and 3.

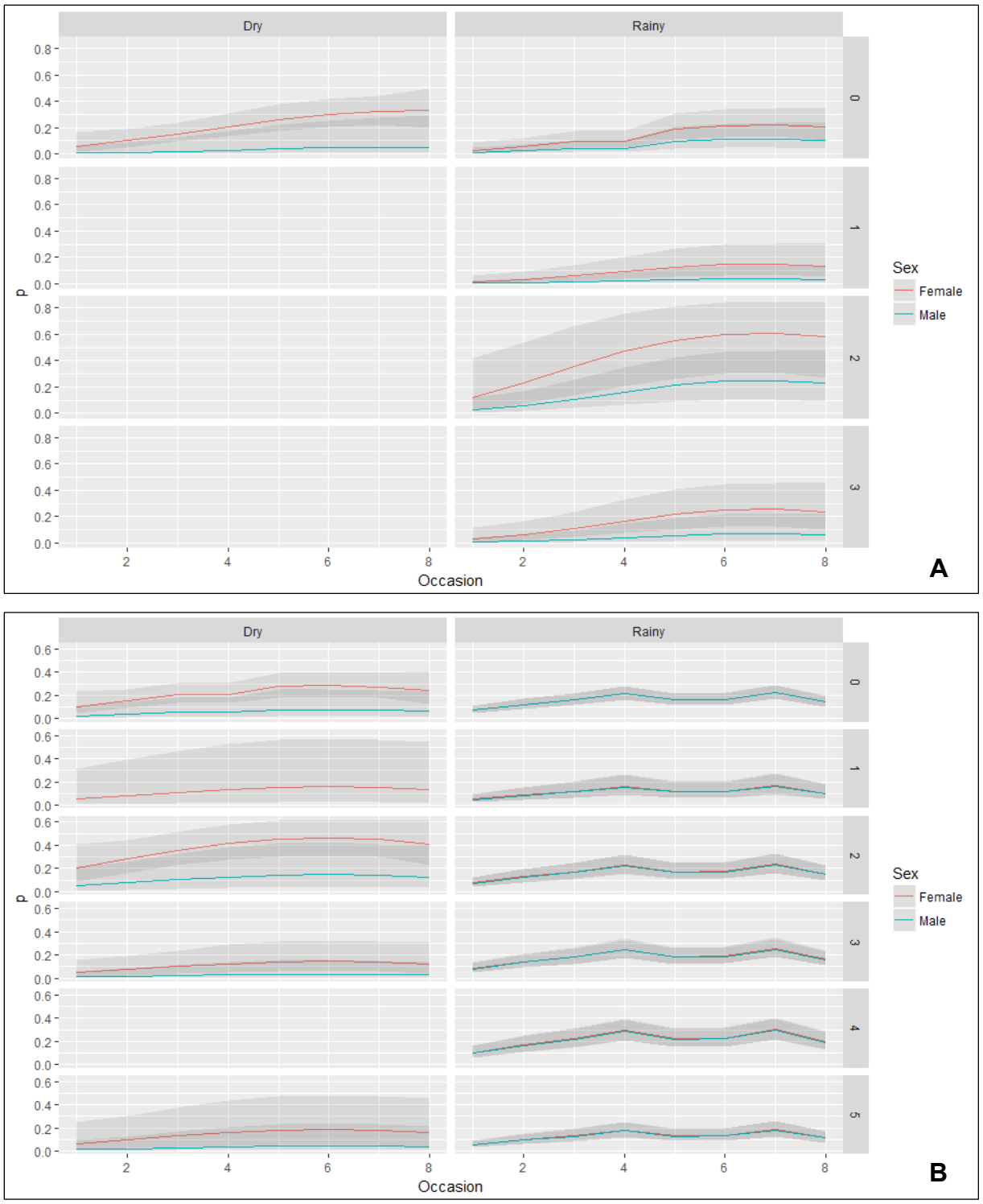

Figure 10 - *Capture probability and their confidence interval represented by sex, site and season in the disturbed forest - zone 2 (**A**) and the cultivated area - zone 3 (**B**).*

Sites are numbered from 1 to 5 (lines). The line labeled 0 shows o the capture probabilities when capture histories from all sites are gathered but sites were not included as a covariate. It is equivalent to consider all sites as one identical site.

3.2. Links between the capture probability and the sex proportion

We previously identified a significant sex proportion difference between seasons found in the cultivated land (zone 3) for *Mus spp*.'s apparent abundance (part 5 – 1.2.).

In order to get the corrected sex proportion, we estimated the *Mus spp.* abundance by sex using MARK modeling. Thus, to estimate the sex-specific abundance, we used the best model previously identified to best fit the data: {Mt+ Si} for the rainy season and {Mt+t²+ Si} for the dry season (table 6). We then used sex as a group instead of a covariate (data were separated in two groups according to their sex). By doing so, we were able to estimate the corrected abundance by group (sex). The sample size variation explains the difference in the total estimated abundance when we compare the results from table 5.

Table 9 - *Uncorrected and estimated abundance of Mus spp. in zone 3 by sex, season using {Mt+Si} for the rainy season and {Mt+t²+Si} for the dry season with the corrected and apparent sex proportion.*

Season	Sex	Estimated Abundance	SE	LCI	UCI	Apparent abundance	Corrected sex proportion	Apparent sex proportion
Rainy	Male	173.22	12.63	154.11	204.88	126	0.53	0.53
	Female	153.28	11.97	135.42	183.69	110	0.47	0.47
Dry	Male	23.83	13.06	13.06	74.51	9	0.45	0.29
	Female	29.49	5.98	23.44	50.49	21	0.55	0.71

Female and male are almost equitably distributed during the rainy and dry season.

As noted previously, *Mus spp.* is more abundant during the rainy season than the dry season. Also, female's capture probability of *Mus spp.* is higher during the dry season. Then, we "see" more females because their capture probability is higher and not because of an higher abundance than males.

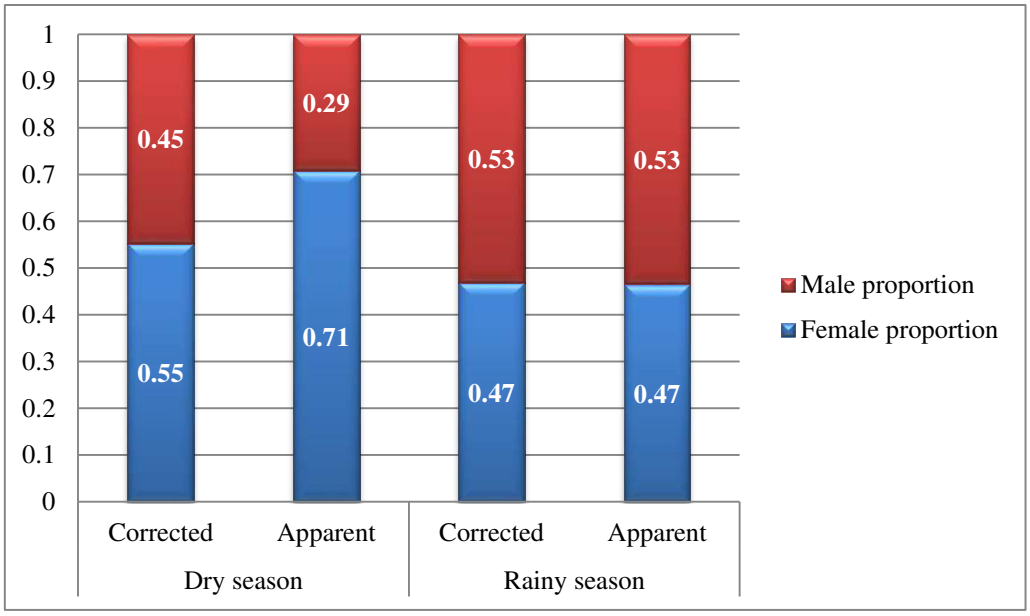

Figure 11 - *Mus spp. corrected and apparent sex proportion in zone 3 between seasons*

4. Leptospira infection and risk estimation

In order to estimate the risk of *Leptospira* emergence along a deforestation gradient, we put into perspective the infectious status of the three main rodent genuses captured with the genuses dynamic and more globally the rodent community dynamics.

4.1. *Maxomys spp.*, *Rattus spp.* and *Mus spp.* apparent *Leptospira* prevalence

Table 10 presents the apparent prevalence associated with the total number of tested rodents by zone and season. Seventy-two animals could not be tested for *Leptospira* infection during the rainy season and two during the dry season, as no samples were available. Three samples were positives using the pathogenic specific PCR (targeting *lipL32* gene) and negative with the broad range of *Leptospira spp.* PCR (using *rrs* gene). This is explained by a difference of conserved sequence between species.

At least one individual from the three genuses were found positive to *Leptospira* along the deforestation gradient for each season. The highest apparent

prevalence is located in the disturbed forest during the dry season (table 10, figure 10).

Table 10 – *Apparent prevalence in percentage of leptospirosis by zone and season for each of the main genus with the total number of positive individual and the total number of tested individual. Zone 1 = Intact forest, Zone 2 = Disturbed forest, Zone 3 = Agricultural land*

Genus	Leptospirosis infectious status	Rainy season				Dry season				TOTAL
		Z1	Z2	Z3	All	Z1	Z2	Z3	All	
Maxomys spp.	Number of positive	1	1	0	2	0	2	0	2	4
	Number of tested	22	14	0	36	16	9	0	25	61
	Apparent prevalence (%)	**4.55**	**7.14**	**-**	**5.56**	**0**	**22.2**	**-**	**8**	**3.28**
Mus spp.	Number of positive	0	6	33	39	0	0	1	1	40
	Number of tested	0	38	185	223	0	31	28	59	282
	Apparent prevalence (%)	**-**	**15.8**	**17.8**	**17.5**	**-**	**0**	**3.6**	**1.7**	**14.2**
Rattus spp.	Number of positive	0	6	1	7	1	4	0	5	12
	Number of tested	2	37	23	62	4	14	1	19	81
	Apparent prevalence (%)	**0**	**16.2**	**4.3**	**11.3**	**25**	**28.6**	**0**	**26.3**	**14.8**

Based on uncorrected prevalence (table 10), seasons were found to significantly affect individual infection: *Mus spp.* were more likely to be infected during the rainy season (fisher test: odds ratio = 12.2, p=0.0006), and was mainly supported by zone 2 (p= 0.02917), while it was at the limit of significance for zone 3 (p= 0.05602) for *Mus spp*.. Prevalence among male *Mus spp.* was significantly higher than female all seasons and zones gathered (fisher test: odds ratio = 2.149813, p = 0.039).

4.2. *Mus spp.* corrected *Leptospira* prevalence

Estimation of the corrected prevalence under MARK was calculated using *Leptospira* infected status as a group. We then fitted the corresponding best model identified in table 7 to get the final abundance of positive and negative individuals. From this corrected abundance according to the infectious status, we calculate a corrected prevalence. However, a single *Mus spp.*'s individual resulted positive

during the dry season, which prevents abundance's estimation for the positives *Mus spp.* individuals to *Leptospira spp.* (table 10).

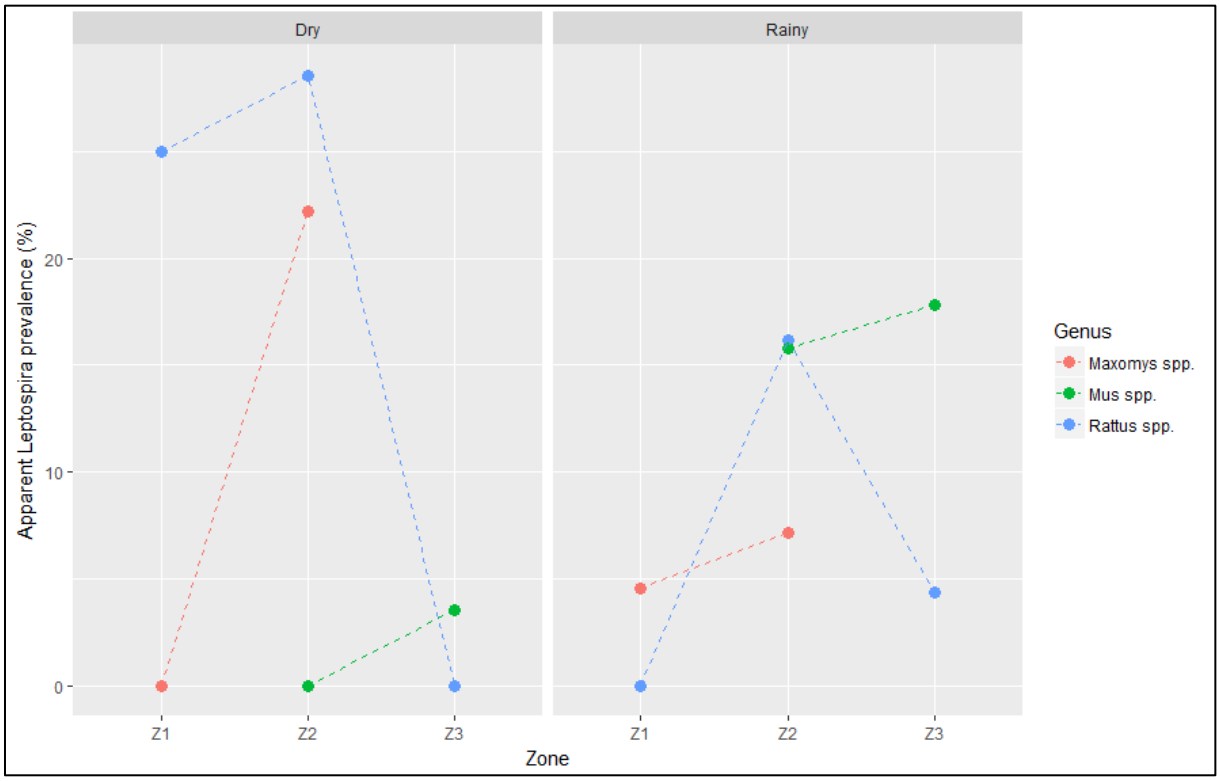

Figure 12 - *Apparent Leptospira prevalence by zone by season for the three most abundant genuses*

Table 11 – *Corrected and uncorrected prevalence of Mus spp. in zone 3 by season using {Mt+Si} model for the rainy season and {Mt+t²+Si} model for the dry season with the corrected and apparent prevalence (%). During the dry season, the estimated abundance of the number of positive couldn't be calculated due to a sample size of 1.*

Season	Leptospirosis infectious status	Estimated Abundance	SE	LCI	UCI	Corrected prevalence (%)	Apparent prevalence (%)
Rainy	Number of positive	43.477	5.563	36.945	60.825	16.8	17.8
	Number of negative	214.004	15.925	189.782	253.757		
Dry	Number of positive	-	-	-	-	-	3.6
	Number of negative	46.789	12.157	33.896	87.880		

4.3. Risk indicator for the emergence of *Leptospira spp.* along a deforestation gradient

A risk indicator for the emergence of *Leptospira* along a deforestation gradient is calculated by multiplying the apparent prevalence/genus/zone (table 10) by the estimated abundance/genus/zone (table 5) during the dry and the rainy season and then add up for the three main rodent genuses captured (figure 13).

$$Risk\ Indicator = \sum_{Genuses} (Abundance\ estimate\ _{per\ zone,\ per\ season}) \times (Apparent\ \ prevalence\ _{per\ zone,\ per\ season})$$

The highest risk of emergence is hosted by the cultivated area during the rainy season, and the disturbed forest during the dry season. Thus, during the rainy season, *Leptospira* risk among rodents is increasing along a deforestation gradient.

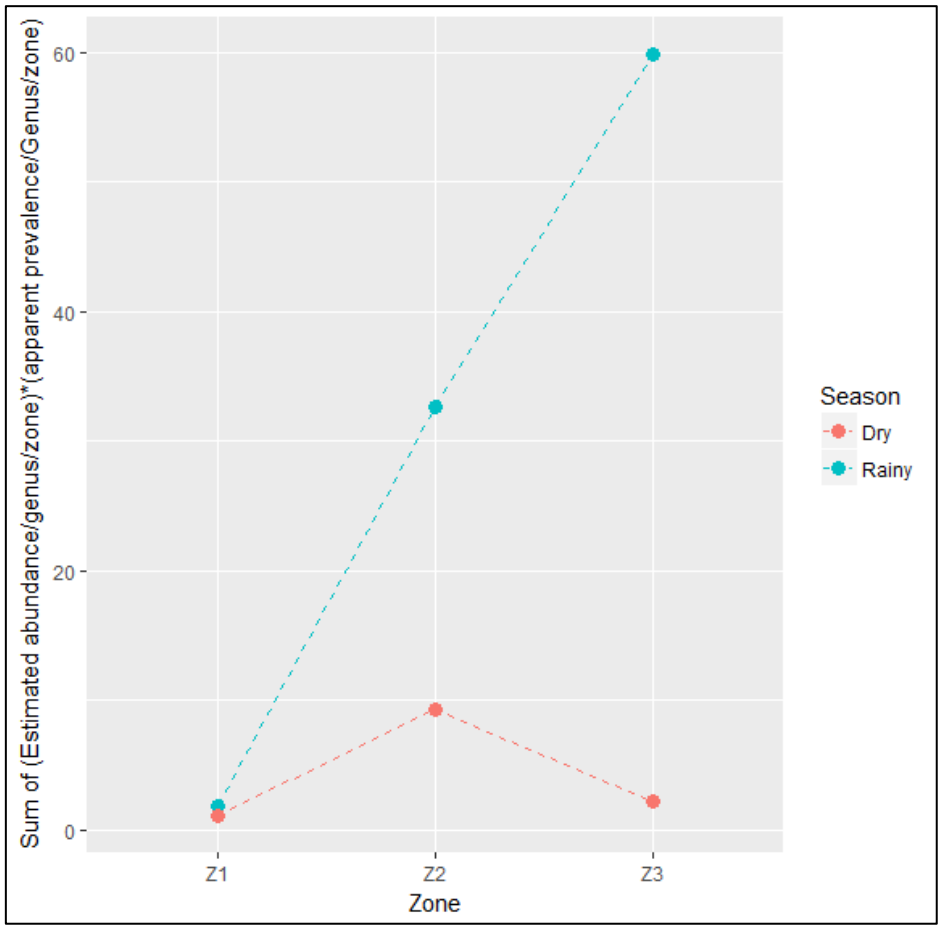

Figure 13 - *Risk of Leptospira spp. along a deforestation gradient by zone by season*

PART 6 – DISCUSSION

PART 6 – DISCUSSION

1. Ecological drivers of *Leptospira* infection in *Mus spp.*

1.1. Higher female Mus spp. capture probability than male during the dry season in the cultivated area and links with species behaviours

We were able to evidence a significant sex proportion difference for *Mus spp*. between seasons in the cultivated area. However, the corrected sex proportions do not confirm this apparent difference. At the same time, we identified higher female capture probabilities than male during the dry season in the cultivated area (zone 3). Thus, the apparent sex proportion is influenced by capture probability sex-dependent and is not linked to a different sex proportion.

Moreover, during the rainy season, we do not observe a different capture probability according to sex which testify for a season influence on the female visibility.

No literature has been found regarding the behavioral ecology to explain this variation of the population dynamics of South East Asian for *Mus spp.*. However, it is likely that the difference in *Mus spp.* sex proportion during the dry season is due to its reproductive cycle and the search for food. It is known that reproduction, predation and food availability are the three main drivers of behavior in other species. Thus, it is possible that with the dry weather, male are burrowing and female are foraging. During the dry season of the second year, we noticed a high proportion of pregnant female in zone 3 in comparison with other species and other zones. We hypothesized that the capture probability of pregnant female would be higher than male due to the impossibility to compromise on their nutritional inputs. However, this couldn't be investigated for the first year and could be the next research direction for this study.

We identified a significant difference of prevalence between seasons for *Mus spp*. as previously highlighted by Ivanova et al., (2012). *Leptospira* infections depict a seasonal effect and are also influenced by the population dynamic. Previous studies estimating prevalence have likely underestimated the prevalence among *Mus spp.* during the dry season. Thus, we highlight the importance to include the detection probability to estimate the prevalence. Future wildlife diseases studies should also be

attentive to account for the detection probability since it gives an indication on the population dynamic (Cooch et al., 2012).

1.2. *Leptospira* prevalence underestimated during the dry season in the cultivated area

Our result showing a significantly higher prevalence of *Leptospira* infections in male *Mus spp.* than female was consistent with previous studies (Cosson et al., 2014). The corrected sex proportion was lower than the apparent one (figure 9). Thus the apparent *Leptospira* prevalence is likely to be biased low in the agricultural area during the dry season. Indeed, the estimation of a corrected prevalence is key information to obtain in order to prove the population dynamic and the link with *Leptospira* infections. However, we were not able to calculate this corrected prevalence since a single positive animal was identified (table 10, table 11). Data collected from the second year survey will update this finding.

Male were found to have higher *Leptospira* prevalence. This finding may be explained by territorial and aggressive behaviors that may be more frequent in males than females. They also present a higher androgens concentrations that is linked to a reduced efficiency immune system and is associated with a higher infection rate (Cosson et al., 2014), which could influence susceptibility.

2. Rodent community dynamic and risk of *Leptospira*

2.1. Age distribution

The age distribution and links with infectious status was not investigated during this work. We expect adult hosts to be significantly more likely to be infected than juveniles (Ivanova et al., 2012). However, the infection is believed to occur during the youth of the individuals (Levett, 2001). Regardless, the age distribution will also be important indicators of the population dynamics of these different rodent species.

2.2. Habitat preference

The different rodent genuses investigated were shown to display clear habitat preferences, particularly with *Maxomys spp.* in forest habitat and with *Mus spp.* in non-flooded field. Even if we didn't calculated any habitat preferences index, our findings correlate with those from S. Morand et al., 2015a (appendix part 1 – 3.3.2.). From our results, *Rattus spp.* is the only genus to be found in the three habitats (figure 7), in favor of the low habitat specificity previously proven for this genus (Ivanova et al., 2012; S. Morand et al., 2015a).

2.3. Possible mechanisms of *Leptospira* emergence during deforestation

Figure 13 combines results from abundance and prevalence to come to an overall *Leptospira* risk indication along a deforestation gradient. These results have to be compared to human contacts expected along the deforestation gradient. We can reasonably consider that human-rodents contacts increase along a deforestation gradient since land-use by human increases (we can expect few contacts in forested area compared with cultivated land).

At the same time, we observed an overlap of the different rodents' genuses in the deforested area – zone 2 – which favors a potential spillover from infected to naive animals of different genuses.

Finally, the cultivated land (zone 3), mainly represented by *Mus spp.*, hosts a huge abundance variation between seasons (figure 9). Indeed, Mus spp.'s abundance increases enormously during the rainy season which favors multiplication of the bacteria. Cultivated area is both an area of favorable multiplication of this pathogen and an area of increased human-rodents contacts, leading to an overall risk of emergence of *Leptospira* increasing with deforestation.

Moreover, the biased low *Mus spp*'s *Leptospira* prevalence lead to a risk estimation also biased low during the dry season in the cultivated area. Indeed, we couldn't get a corrected prevalence due to the limited sample size of the positives individuals (table 10). The second year results could increase our sample size and refine our conclusion. To do so, we could use a new risk indicator calculated as follow:

$$Risk\ Indicator = \sum_{Genuses} \begin{matrix} (Prevalence\ estimate\ _{per\ zone,\ \ per\ season}) \\ \times (Abundance\ estimate\ _{per\ zone,\ \ per\ season}) \\ \times (Density\ estimate\ _{per\ zone,\ \ per\ season}) \end{matrix}$$

Cosson et al., (2014) challenged the idea that leptospirosis was mainly driven through water and highlighted the presence of two epidemiological cycles, one in humid habitats and another one in dry habitats. This study suggests that direct transmission could explain the circulation of leptospires in dry habitats. Density-dependent transmission usually displays strong relationships with contacts rates. Isolation of habitats may in fact increase contact rates and subsequent transmission and prevalence, possibly due to clumping of resources and individuals. A recent review of wildlife diseases by Tompkins et. al., (2011) identified that a major challenge with contact dynamics and disease transmission lies in distinguishing the contacts that are potentially important to transmission from those that are not. A detailed understanding of host social and population dynamics is essential to understand host-pathogen dynamics of direct transmission (Brearley et al., 2013).

3. Methodological considerations

3.1. Space-for-time study design

Longitudinal design studies are ideally suited to study temporal processes such as deforestation, even if the required length of follow up often makes this design impractical and too costly. In contrast, the fast rate of deforestation in Cambodia, and its unpredictable nature were major impediments in the planning and implementation of longitudinal studies.

A chronosequence design was used as an alternative to longitudinal studies, substituting space for time. A critical assumption of chronosequence designs requires that each zone in the deforestation sequence only differs by the stage (time) along the process and follow the same pattern. This assumption implies that abiotic and biotic conditions remained constant over the time span of the deforestation process. It also implies that all zones had the same pattern of change.

In our study, the three zones (intact forest; disturbed forest; recently cleared forest or cultivated area) were matched in close proximity in the same geographical location. Recently cleared fields were always less than one-year old since the last intact forest stage. The fast rate of deforestation and the simultaneous sampling of all zones of a site ensured limited changes of biotic and abiotic factors, other than those

related to the deforestation process. We avoided regrowth and recovery of the original vegetation structure in the logged forest.

Zone 1 - “intact forest” often had ongoing selective logging. It was difficult to find untouched forest areas. Thus, we were not able to cover the entire chronotone of deforestation, starting from the pristine forest, since these no longer exist in most regions of Cambodia.

3.2. Leptospira infection

In our study, we decided to include all suspect samples performed by the broad PCR (using *rrs* gene). These samples were classified as suspect because they presented a not clearly sigmoidal curve. Thus, we had to test them again for confirmation. Given the small number of positives animals, this decision might have an influence on the total prevalence estimated.

Moreover, the two RT-PCR do not detect the same sequence (pathogenic for *lipL*32 and a conserved sequence with *rrs* gene). We pooled all positives and suspect individuals to get an indication of *Leptospira spp.* circulation among rodents (and not of the pathogenicity circulation).

Studies in SEA used different PCR protocols and we defined our Ct value lower than previous studies using the same PCR method (Thaipadunpanit et al., 2011) ; all this limited our ability to compare leptospirosis prevalence in South East Asia (table 1).

3.3. The putative species *Rattus sp.* R3

The individuals of *Rattus sp.* R3, a putative “species”, identified by barcoding were capture in all three zones during both the rainy and dry seasons. However, the taxonomic status of these rodents is unclear and has not yet been explored. It seemed several species could be included in this clade. The evolutionary history of the Asian black rat is complex with an incongruence of phylogenetic analysis based on the mitochondrial DNA or nuclear DNA (Blasdell et al., 2015; Pagès et al., 2013). Thus, according to criteria used for classification, *Rattus sp.* R3 is alternatively closely related to *Rattus tanezumi* or *Rattus sakeratensis*.

4. Limitations of the statistical analyses performed

4.1. Goodness of fit and assumptions

The first step in modeling remains to test for the goodness of fit of the model chosen. This first step aimed to test whether closed models and their associated assumptions described correctly the data. We were not able to perform a test for the goodness of fit since it is not available under MARK for closed models. It is a work in progress in the scientific community.

However, we can reasonably consider that the closure assumption was met given that we studied rodent population during eight consecutive days. This short period of time is a biological reasonable window to meet our assumption of closure (no immigration or birth and no emigration or death). Mortalities induced after the manipulation of rodents, which we were not able to assess, could be a reason to reject this assumption.

4.1.1. Data deleted and consequences on *p* and *c*

Thus, to run the analyses under MARK, we deleted a total of 36 individuals (table 4). The removal of escaped animals leads to an underestimation of the capture and recapture probabilities for zones involved since we are not able to count them as a first capture. We might probably count them as a first capture during subsequent occasions while it should have been counted a second capture.

High misidentification of recaptured animals leads to an underestimation of the recapture probability, *c*. As an example, during the rainy season in zone 3, 14 recaptured animals couldn't be identified; it is 5.3% of all animals captured in this zone all sites joined. While missing the identification of individuals, we also missed information on the species. Since only 27 unique animals of *Rattus spp*. were captured, a small difference on the number of recapture can influence its recapture probability and as a consequence the estimated abundance of the species. It has indeed more consequences to miss recapture from an already small population than a bigger one (table 4).

Finally, given the deleted data, we expect our abundance estimates to be biased low when sampling situations present low encounter probabilities ($p \leq 0.2$) and a low

number of samples ($M_{t+1} \leq 5$) as it is the case for *Rattus spp.* in zone 1 during the rainy and dry seasons ($M_{t+1} = 4$) (table 5).

4.1.2. Small sample size prevent abundance estimation

We have to be aware of the links between the sample size, the number of parameters included in the model and the resulting confidence interval of the estimated abundance.

In our analyses, abundance can only be estimated with MARK when the sample size is big enough. This was not the case for all sites, mainly because the numbers of parameters were too high compared with the number of data points. This might be one of the reasons explaining the model {M0} (*p* and *c* constant over occasions and thus model {M0} presents the lowest number of parameters) is the second best model fitting the data (Table 6). It is then a possibility that in some cases of low sample size, the data couldn't support more parameters than the model {M0}. Moreover, it is questionable whether the model with a quadratic function of time ($Mt+t^2$) was the best model to fit the data since it was a simpler model than the one including time as regards the number of parameters.

Equally, when sex was used as a group, it reduces consequently our sample size available in two groups to estimate *p* and *c*. It explains the difference of the estimated abundance when we compare the same species of the same zone while considering the sex as a group or not (table 7, table 9).

We made the general observation that behavioral models {Mb} is complicated to interpret with small sample size. For example, the agricultural area of site 1, we had six *Mus spp*. individuals recapture once and two were recaptured three times for a total of 41 unique individuals captured. Given this low number of recaptured individuals, a behavioral model considers that the normal behavior is to not come back to the trap and estimates a low recapture probability. The final estimated abundance will be lower than the model $\{Mt+t^2\}$. The behavioral effect would have a biological meaning when the sample size is big enough since with low sample size we faced a high difference between individuals as regards the number of recapture.

5. Bias in the detectability

5.1. Individual heterogeneity

Individual heterogeneity is a common source of bias, typically causing capture-mark-recapture estimates of population abundance to be biased low. The best way to reduce bias is to get *p* (the first capture probability) as high as possible while we design and implement the study. When *p* is high there is little room for variation and little chance that an individual is not detected. Several studies demonstrated that different models of the form of individual heterogeneity can lead to very different estimates of abundance and fit the data equally well (Cooch et al., 2012). The magnitude of the differences in abundance estimates is related to *p*; when *p* is small the differences can be large.

Species detectability is the product of several mechanisms, including species and habitat characteristics, abundance, surveyor skills or detection method, survey effort and survey conditions. Therefore, it is recommended that predictors of detectability include not only site-specific covariates, but also survey specific covariates (e.g. weather conditions or observer identity) (Guillera-Arroita, 2017). In this study, we repeated exactly the same process (same detection method, survey effort) for each zones of each sites, and worked with shifted teams to reduce the observer bias.

The best way to take into account individual heterogeneity is to measure all probable covariates that could have an influence on the capture and recapture probabilities, which have been done under this study.

5.2. Food availability and environmental covariates

We faced a high heterogeneity regarding the number of capture between sites. This observation can be explained because trapping sessions of the five sites occurred at different time during each season (table 2) and can therefore, induce potential selection bias. The heterogeneity between sites might be due to a difference of habitat and also of food availability as the resource decrease with the increasing duration of the dry season. The balance of benefit-risk for rodents to get trapped vs getting food might influence the capture probability. Thus food availability is believed to influence the recapture probabilities of rodents between seasons and between sites and could be the object of future work.

Microclimatic variation has the potential to affect colonization by small mammal's (Whitehead, Goosem, & Preece, 2014). Microclimate data have been measured during this study. The microclimatic variations for each zone between seasons could explain the abundance variation for each species. Microclimate variations are strongly linked with the habitat complexity and the canopy cover. Future analyses could focus on the link between the abundance variations of species between seasons along the deforestation gradient. Particularly, we could imagine the resilience to cope with the climate variation decreases with the deforestation gradient and this decrease would be linked with higher population variations between season.

6. Research perspectives

6.1. The importance of the environment in the wildlife epidemiological cycle

Some leptospires present a long survival in the environment (Levett, 2001), and leptospirosis outbreaks are linked to flooding events in SEA, making of water a key transmission pathways. However, Della Rossa et al., (2016) made the distinction between human infection and rodent infection since the water factor, as depicted as distance to river, seems to have a greater influence on human than on rodent infection. Their results challenge the role of rodents as carriers or reservoirs of *Leptospira spp*.. The study done by Cosson et al., (2014) suggest that direct transmission could explain the circulation of leptospires in dry habitats. Moreover, they found that *Leptospira* prevalence was similar between floodable and non-floodable areas. This result also challenged the widely accepted belief that leptospires mainly circulate in wetlands. Thus, whether environmental conditions (outside the host) determine *Leptospira* species distribution in nature remains largely unexplored.

Moreover, a precise estimation of the *Leptospira* resistance in the environment is not available at the moment. Andre-Fontaine et. al, (2015) estimated the survival and persistent virulence of pathogenic strains of *Leptospira spp.,* serovar Icterohaemorrhagiae, under laboratory circumstances. They found that despite unfavorable storage conditions such as cold, nutrient-poor acidic waters, the survival

and virulence of pathogenic *Leptospira spp*. was fully preserved over at least 20 months. Study of *Leptospira* resistance in the environment and more specifically in risky areas would be helpful to assess the risk of emergence and understand its mechanisms.

6.2. Future research using modeling disease in wildlife

Include sites as a random effect could be done to be able to consider a level of heterogeneity between sites, and then estimates the effect of covariates as sex free from sites heterogeneity.

Further analyses could be performed using robust design models. These models allow the estimation of emigration and immigration of a super-population and thus decompose general parameters such as the apparent survival probability and the apparent encounter probability. Indeed, in this study we estimated the abundance with the calculation of *p*, the apparent encounter probability, that we can decompose into two more parameters based on the formula: $p = (1 - \gamma) \times p^*$. Using a robust design models we can estimate these two parameters: $(1-\gamma)$, the probability that conditional on being alive, and in the super-population, the individual is available to be encountered and p^*, the probability that an individual is encounter. This robust design model would include encounter histories interlinking the two seasons.

Moreover, even if disease ecology is a sector receiving an increasing interest in the scientific communities (Myers et al., 2013), a lot more research is needed as regards ecology of rodents in SEA (Cosson et al., 2014). More knowledge on the behavior and population dynamic would increase our understanding of *Leptospira* epidemiology. The use of models in the understanding and management of disease in wildlife populations has been limited, relative to their already large use in the study of human disease (Cooch et al., 2012). Modeling disease in wildlife is a promising research sector that we should deal with in depth.

PART 7 – CONCLUSION

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Population growth, deforestation and forest fragmentation, poverty and economic growth, health and emerging infectious disease are all intimately interconnected and encourage us to focus on these challenges with a One Health approach. While we can see the links and complexity between land-use change and its impacts on human health, it is still a challenging new way to deal with health.

In the context of this study, we focused on the rodent population dynamic and links with the emergence of leptospirosis among rodent communities on a deforestation gradient in Cambodia.

We used mark-recapture data modeling. By doing so, we could account for the detectability probabilities (depending on the genuses, zone, season, infectious status) and adjust the observed data to the corrected population dynamic. Thus, we showed that *Leptospira* infection presented a seasonal pattern with an increasing prevalence during the rainy season. Moreover, we showed that male *Mus spp*. were likely to be under captured, and since they are more likely to be infected, previous prevalence reported have underestimated the real *Leptospira* prevalence during the dry season. Little is known on the population dynamic and its consequences on disease ecology. This study offered a glimpse of the impact of detection probability when studying wildlife diseases.

ECOLE NATIONALE VETERINAIRE TOULOUSE

UNIVERSITÉ TOULOUSE III PAUL SABATIER

AGREMENT SCIENTIFIQUE

En vue de l'obtention du permis d'imprimer de la thèse de doctorat vétérinaire

Je soussigné, **Guillaume LE LOC'H**, Enseignant-chercheur, de l'Ecole Nationale Vétérinaire de Toulouse, directeur de thèse, certifie avoir examiné la thèse de **Caroline FILLEUX** intitulée **«Communautés de rongeurs et risque de leptospirose selon un gradient de déforestation au Cambodge»** et que cette dernière peut être imprimée en vue de sa soutenance.

Fait à Toulouse, le 15 novembre 2017
Docteur Guillaume LE LOC'H
Maître de Conférences
de l'Ecole Nationale Vétérinaire de Toulouse

Vu :
La Directrice de l'Ecole Nationale Vétérinaire de Toulouse
Isabelle CHMITELIN

ECOLE NATIONALE VETERINAIRE DE TOULOUSE

Pour la Directrice et par délégation,
le Directeur de l'enseignement
et de la vie étudiante
Hubert BRUGERE

Vu :
Le Président du jury :
Professeur Gérard CAMPISTRON

Vu et autorisation de l'impression :
Président de l'Université
Paul Sabatier
Monsieur Jean-Pierre VINEL

Le Président de l'Université Paul Sabatier
par délégation,
La Vice-Présidente de la CFVU
Régine ANDRE-OBRECHT

Mlle Caroline FILLEUX
a été admis(e) sur concours en : 2012
a obtenu son diplôme d'études fondamentales vétérinaires le : 23/06/2016
a validé son année d'approfondissement le : 13/07/2017
n'a plus aucun stage, ni enseignement optionnel à valider.

Université de Toulouse

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APPENDICES

APPENDIX PART 1 : LACANET project objectives

The LACANET One Health Surveillance and Laboratory Network project (also referred to as “LACANET”) is an EU-funded project which brings together partners in the human health, wildlife health and animal health sectors to create capacity to survey, diagnose and understand the drivers of disease at human-animal-environmental interfaces.

The overall objective is to develop a bi-national Lao PDR-Cambodia One Health Surveillance and Laboratory Network that will enable both countries to:

Build capacity for surveillance and field investigation for zoonotic diseases:

For this to happen, we are training district, provincial and national wildlife and livestock health authorities in both Lao PDR and Cambodia to jointly conduct surveillance for zoonotic disease pathogens in vectors, wildlife and livestock populations using various sampling techniques. We are also developing capacity to implement diagnostic testing for national priority diseases at the human-animal-environment interface between both human and veterinary diagnostic laboratories, using whenever possible similar techniques and standard operating procedures.

Improve laboratory capacity to detect zoonotic diseases

Laboratory experiments and analysis represent a significant part of the LACANET project, since we need to analyze all samples taken from the field. The Cambodian National Veterinary Research Institute (NaVRI) and the Lao PDR National Animal Health Laboratory (NAHL) regularly receive animal samples from suspected disease outbreaks from various Lao and Cambodian provinces for testing.

Improving Lao and Cambodian laboratory capacity therefore appears as being critical. Therefore, the Institut Pasteur du Cambodge (IPC) and the Lao-Oxford-

Mahosot Hospital Wellcome Trust Research Unit (LOMWRU) are providing laboratory training to NaVRI and NAHL respectively from year 1 to year 4.

Improve national and regional cross-sectoral collaborations by establishing a One Health surveillance and laboratory network

Much of our efforts are designed to initiate lasting connections between One Health practitioners (field biologists and veterinarians, laboratory diagnosticians and medical microbiologists) within and between Lao PDR and Cambodia to promote knowledge transfer through exchanges, workshops and trainings, to encourage timely information sharing for effective and coordinated responses to zoonotic outbreaks. We are also hosting workshops on disease epidemiology and diagnostic techniques, across both animal and human sectors, and meetings to discuss One Health coordination as well as the economic and sociological aspects of these pathogens.

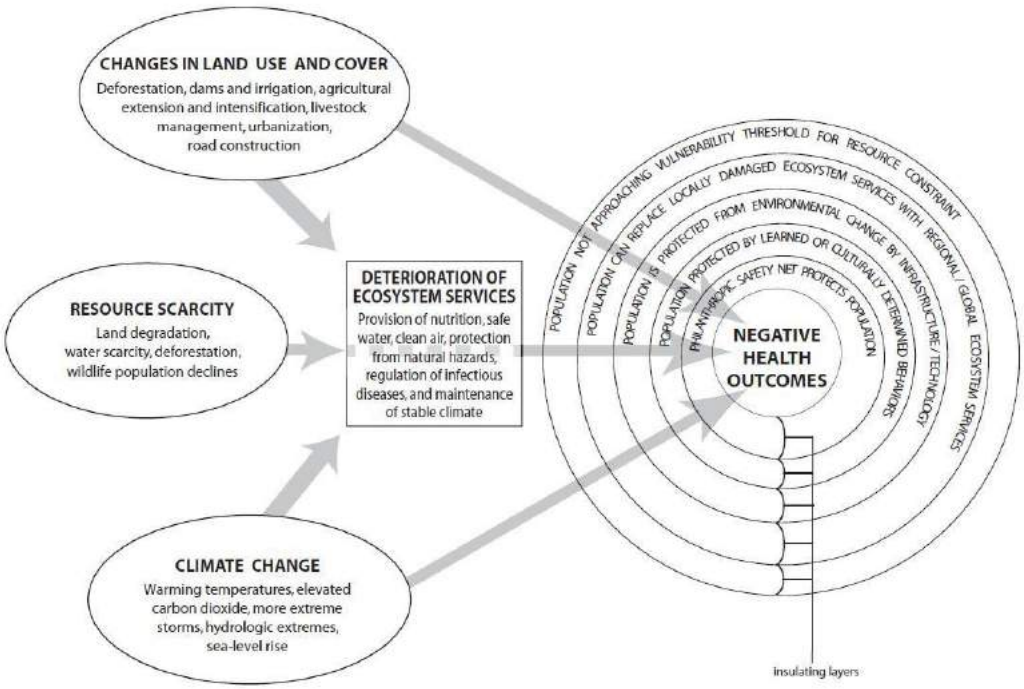
Conduct strategic research on two important drivers of disease emergence – Wildlife trade and land-use change:

We are investigating the role that land use change plays in disease dynamics by conducting surveillance for diseases with domestic and wild animal reservoirs, including Japanese encephalitis, leptospirosis and rickettsial diseases (as model disease systems) in vectors along a land use gradient, from pristine forest to industrial landscape.

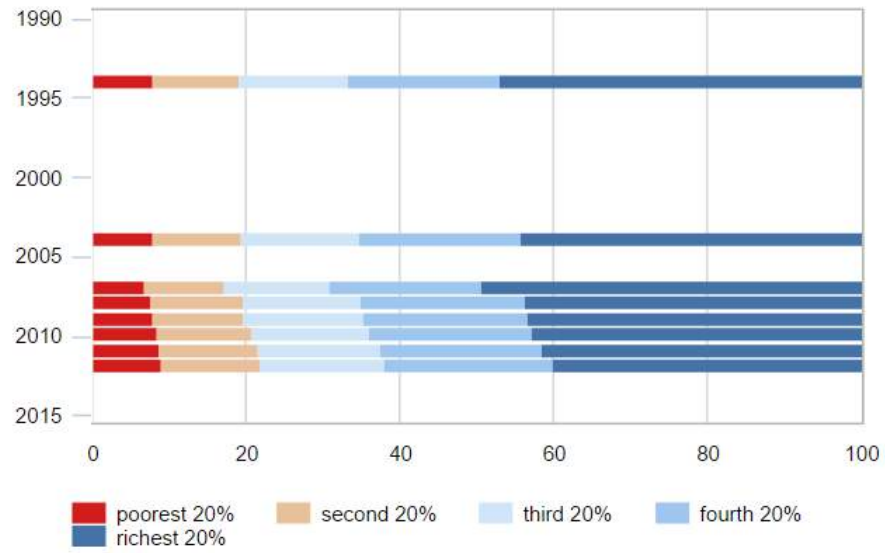
We are also examining the role wildlife trade plays in disease emergence, including diseases such as rabies, anthrax, leptospirosis, typhus and trichinellosis, by conducting surveillance at high risk human-wildlife interfaces in wildlife market.

APPENDIX PART 2 – 1.2.3: A schematic of the complex relationships between altered environmental conditions and human health (Myers et al., 2013)

Drivers of global environmental change (e.g., land-use change, resource scarcity, or climate change) can directly pose health risks or impair ecosystem services that subsequently influence health. Population level vulnerability, however, will be modified by multiple layers of social or infrastructure barriers that can buffer or eliminate risks associated with these exposures. Together, all components must be considered to achieve realistic assessments of population vulnerability.

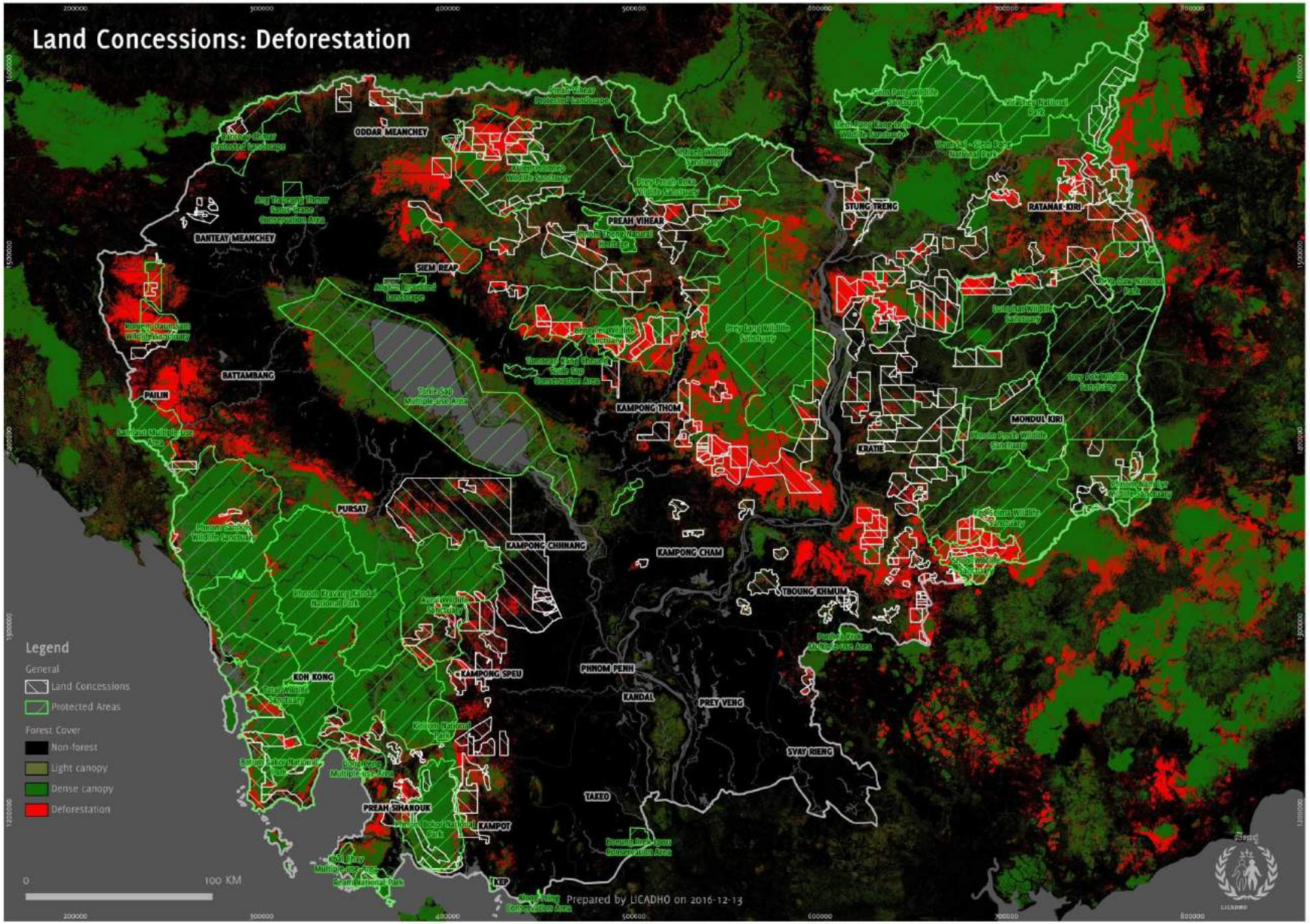


APPENDIX PART 2 – 2.2.1: "Poverty & Equity Data - Cambodia - The World Bank," (2017) Country inequality trend: distribution of income or consumption by quintile.

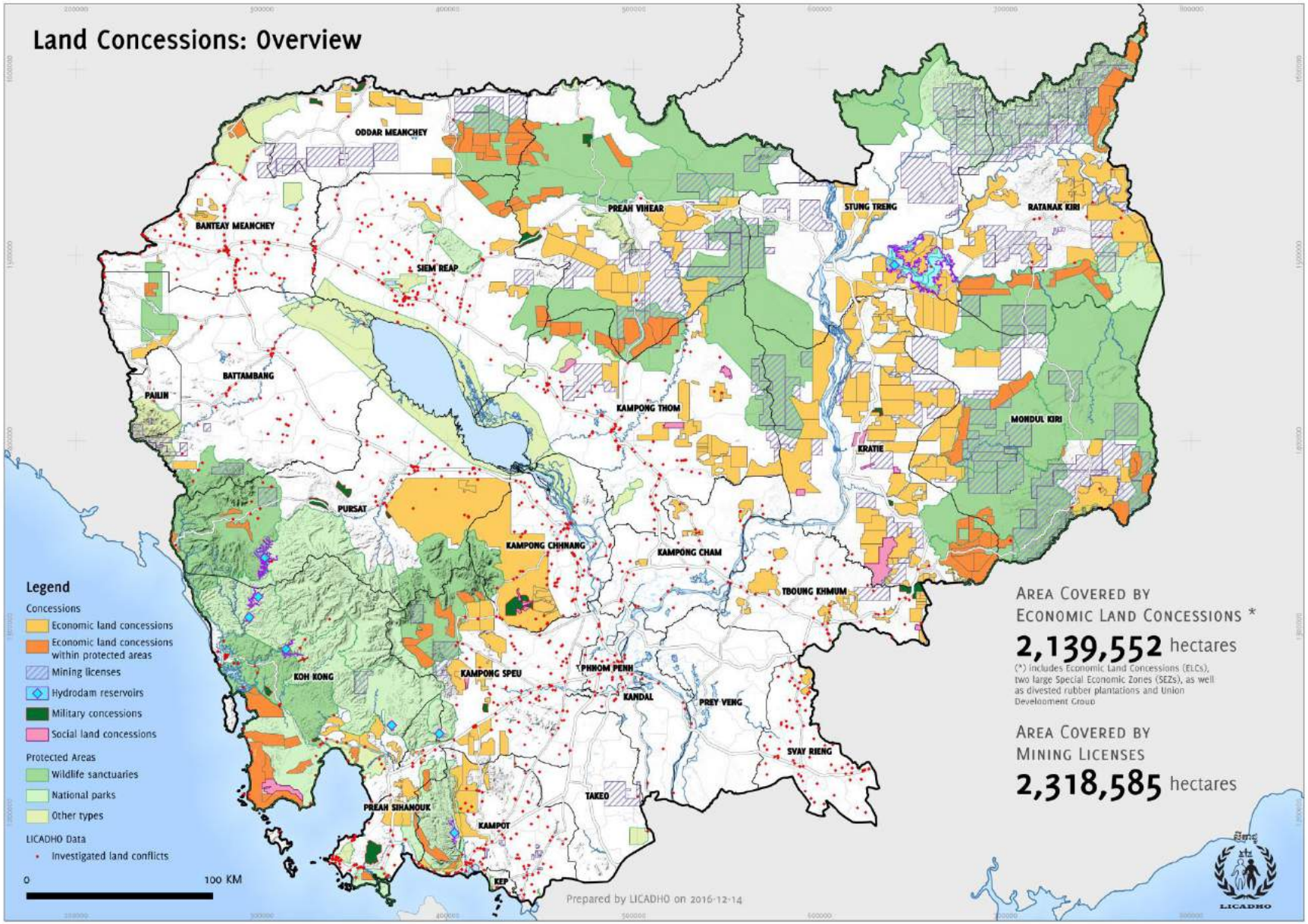


Source: Poverty & Equity Databank and PovcalNet

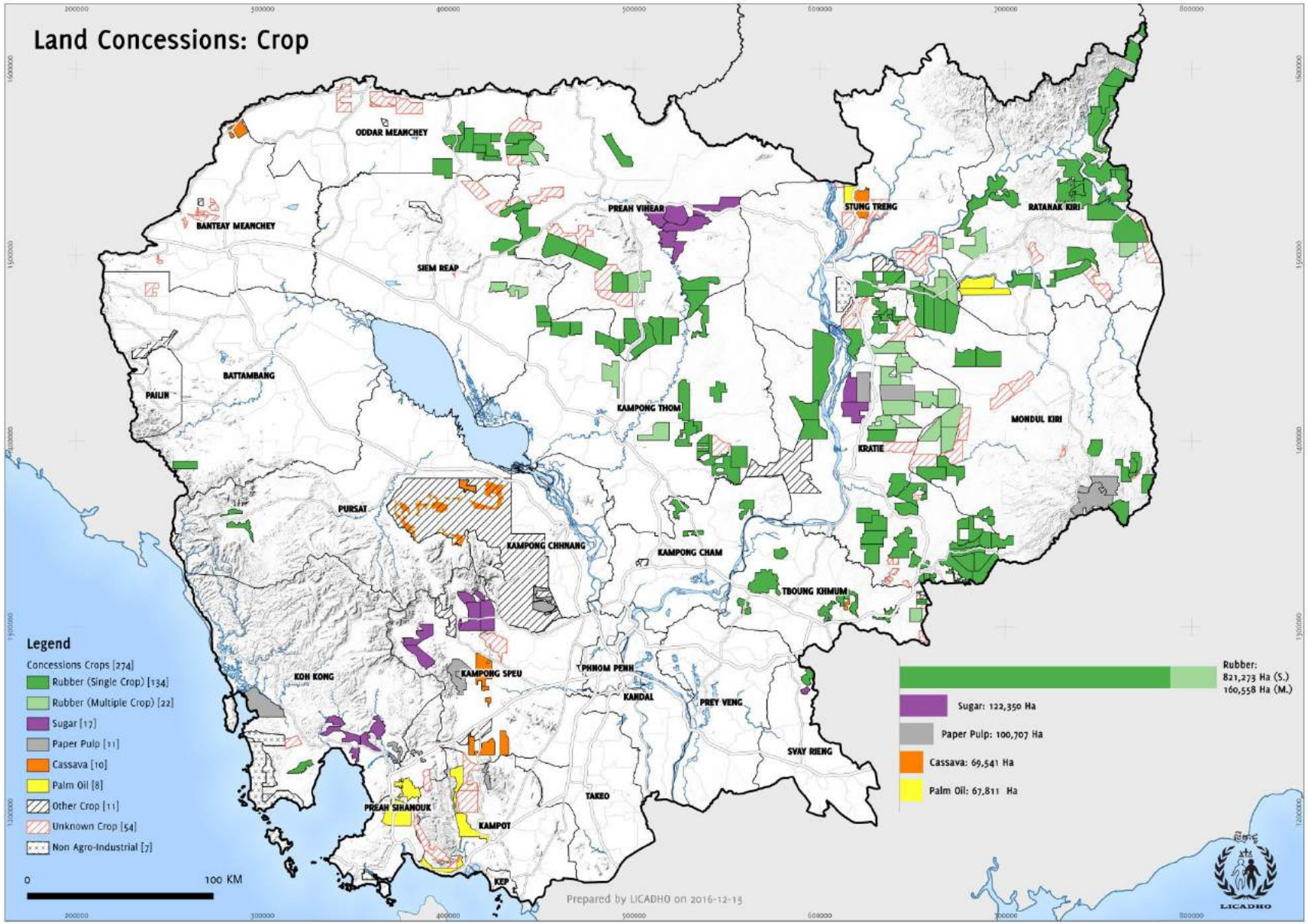
APPENDIX PART 2 - 2.2.2 A: Maps of deforestation and land concessions in Cambodia from LICADHO



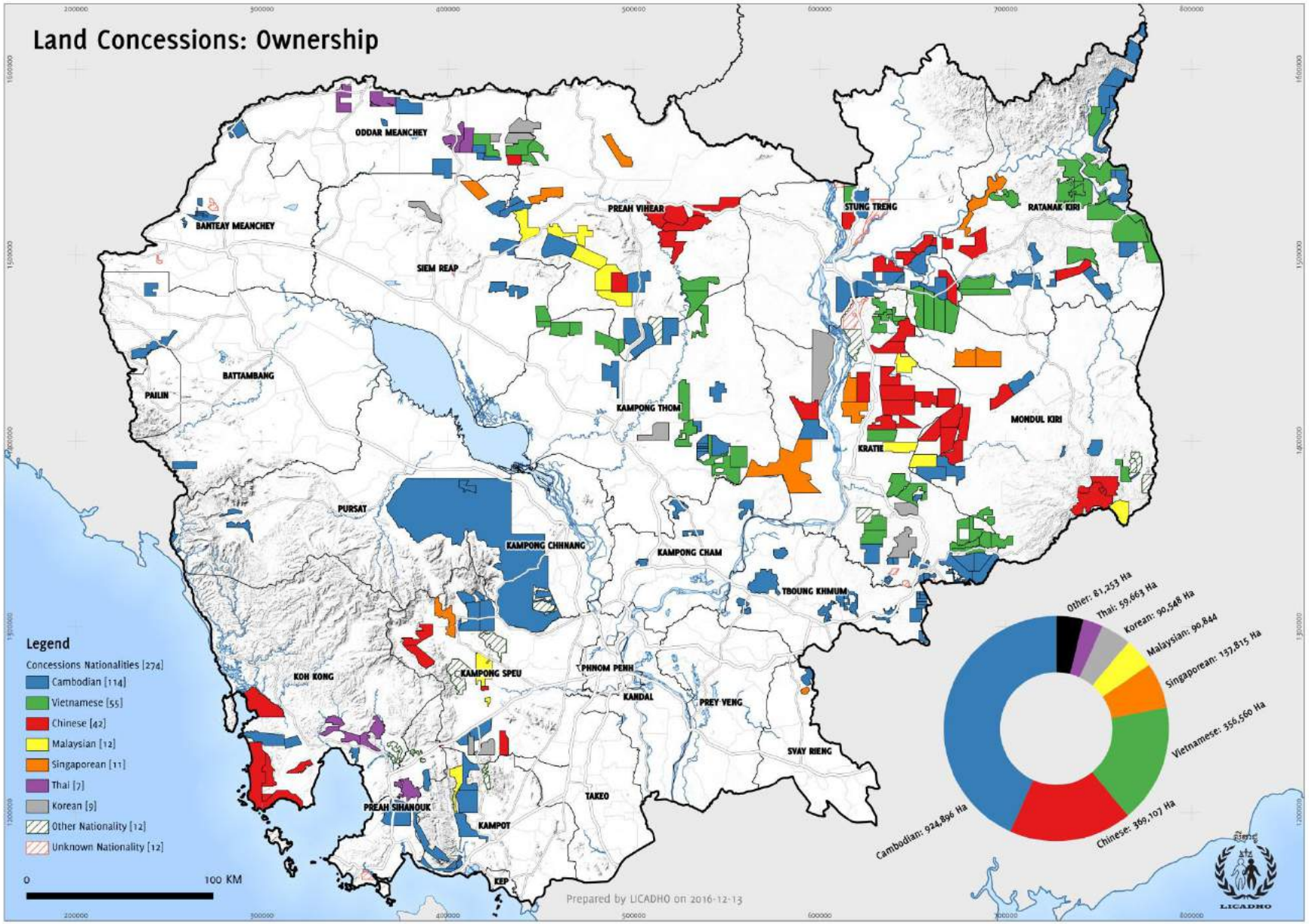
APPENDIX PART 2 - 2.2.2 B: Maps of land concessions areas repartition around protected areas in Cambodia from LICADHO



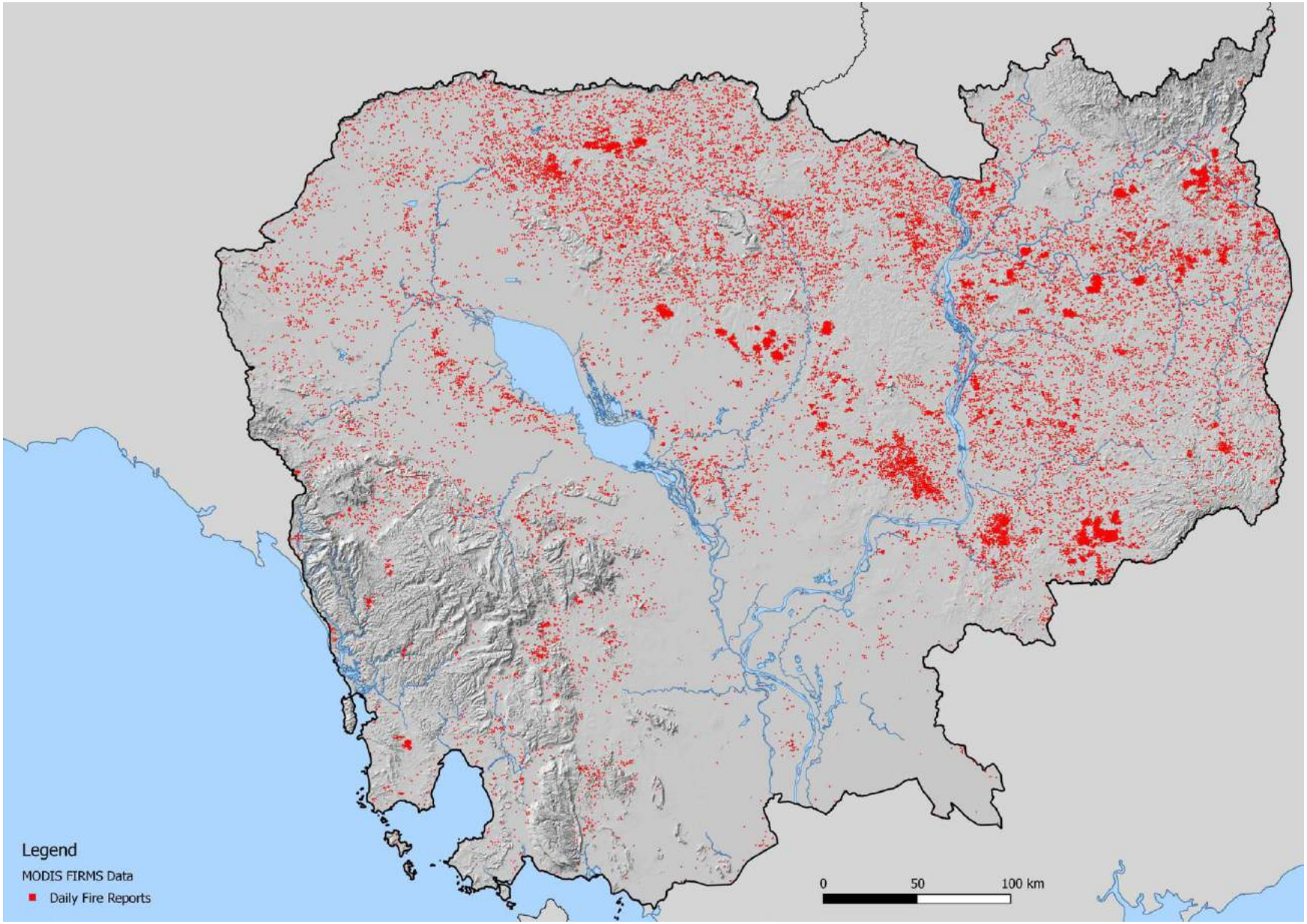
APPENDIX PART 2 - 2.2.2 C: Maps of land concessions crops in Cambodia from LICADHO



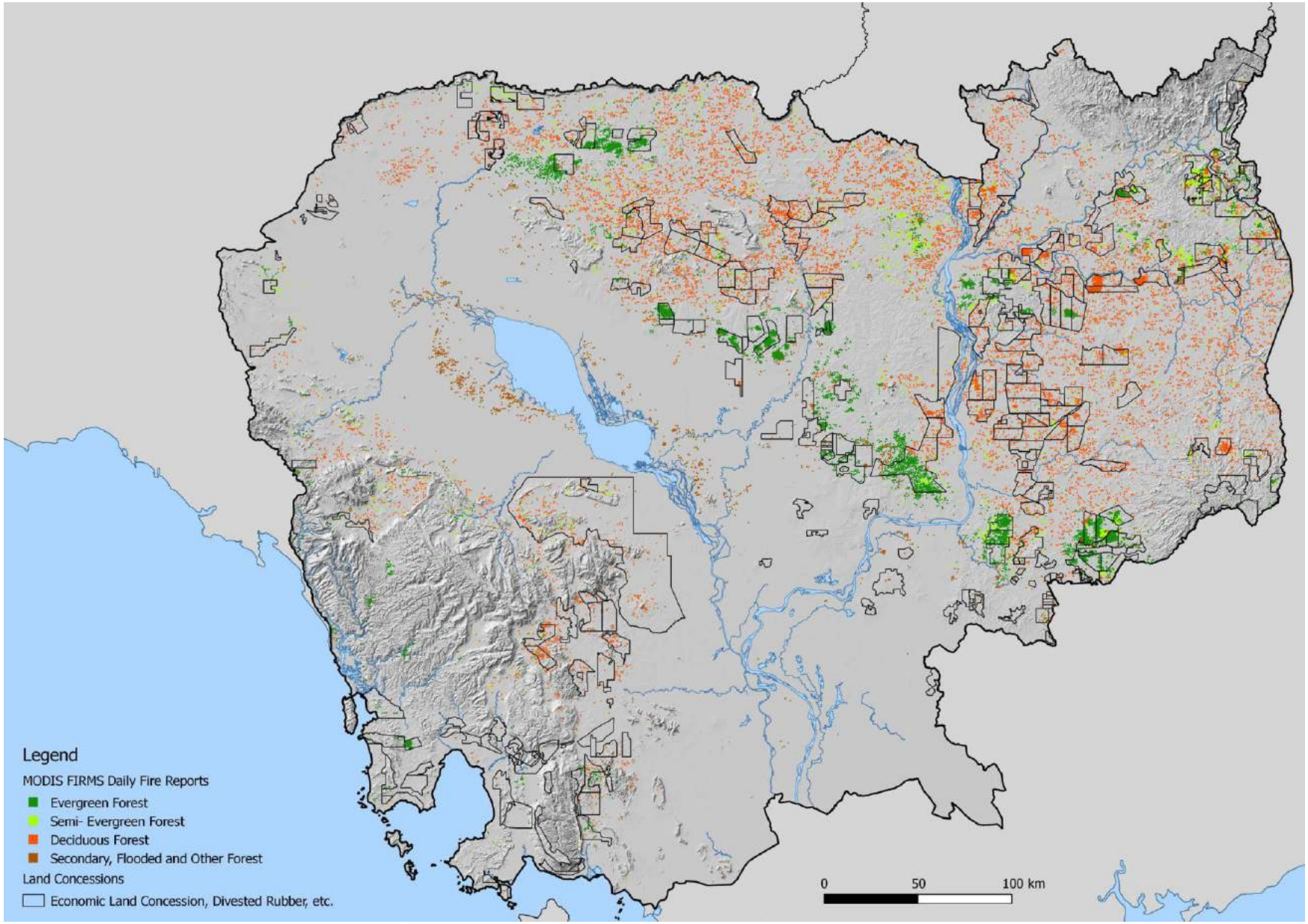
APPENDIX PART 2 - 2.2.2 D: Maps of land concessions ownership in Cambodia from LICADHO



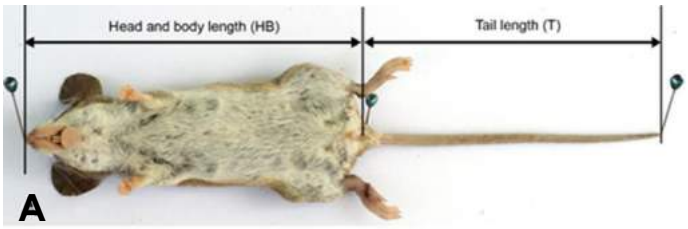
APPENDIX PART 2 – 2.2.2 D: Active Fire Reports October 2012 – March 2013 from Forest Trend (2015)



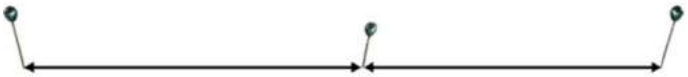
APPENDIX PART 2 – 2.2.2 E: Fire Distribution in Relation to Forest Formations and Land Concessions



APPENDIX PART 4 – 3.1: Animal measurements and identification

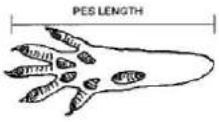


A



B

C



D

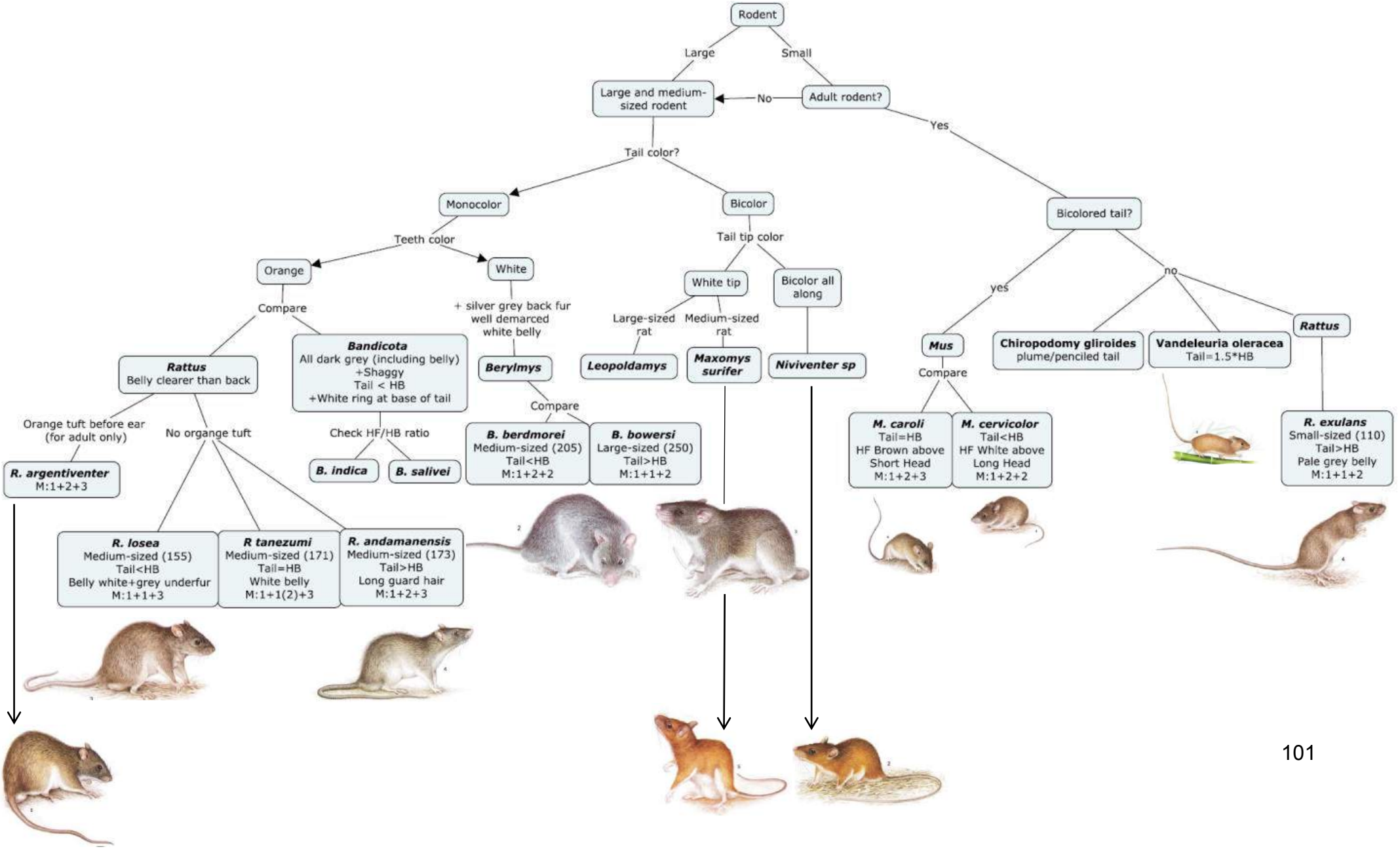
A. The HB length was measured from the tip of the nose to the middle of the anus.
B. The tail length was measured from the middle of the anus to the tip of the tail.
C. Ear length was measured from the bottom of the ear to the furthest point along the rim.
D. Foot length was measured from the base of the heel to the end of the toe pad on the longest toe.

GENERAL			MALE		FEMALE	
Sex	**Age**	**Species**	**Testicule score**	**Testicule length**	**Vagina**	**Teats score**
▪ Male ▪ Female	▪ Baby ▪ Juvenile ▪ Adult	See decision tree	▪ Non descended ▪ Partially descended ▪ Fully descended		▪ Close vagina ▪ Open vagina	▪ Indistinct ▪ Raised ▪ Lactating

MEASUREMENTS								
Left hind-foot	Left ear length	Anal genital distance	Head and body length	Skull length	Tail length	Total weight (bag + animal)	Bag weight	Number of injuries

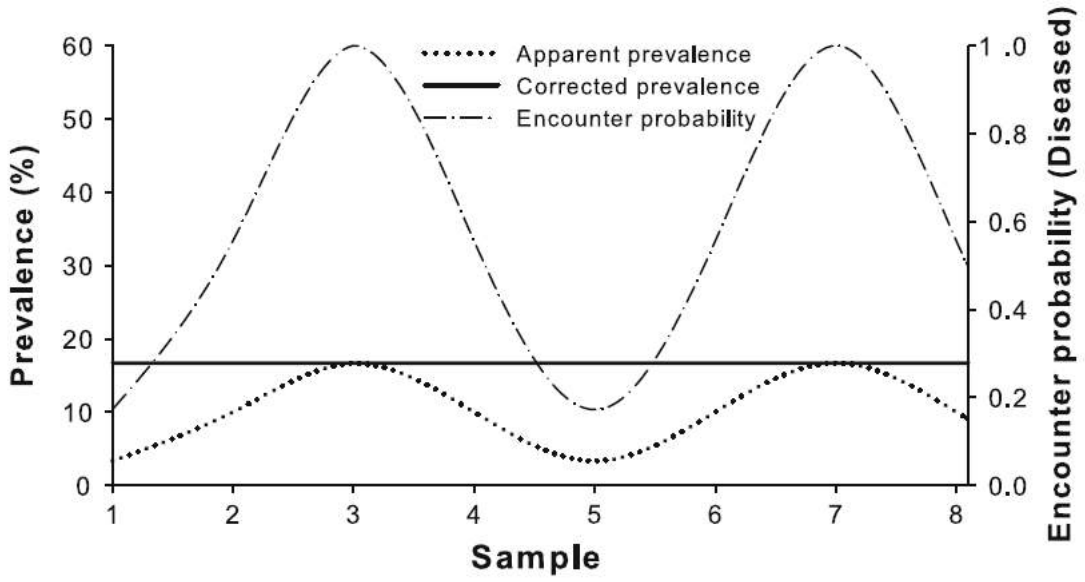
GENERAL				IDENTIFICATION			
Season	**Site**	**Zone**	**Trap number**	**Capture class**	**Tag number**	**Fate**	**Final animal ID**
Rainy	S1 to S5	Z1 to Z3	T1 to T150	New capture		Released	Site-Zone-Trap-Tag number e.g. S1Z3T45-00345
Dry				Recapture		Dead	
						Escaped	

APPENDIX PART 4 – 4.2.2.: Decision tree to guide rodent species identification and illustration from Francis 2008



APPENDIX PART 4 – 5.1.A.: Illustration from Cooch et al., (2012)

This graph shows how cyclic patterns of apparent (observed) prevalence could be an artifact of cyclic patterns in detection probabilities. In this case, only the detection probability of diseased individuals varies over time, while the detection probability of healthy animals (with respect to the condition under study) is time invariant (i.e., phealthy = 1.0). In this example, apparent prevalence varies temporally, whereas true prevalence is constant over time. This illustration is adapted from Jennelle et al., (2007)



APPENDIX PART 4 – 5.1.B: Encounter histories: input for mark analyses

The identification of the animal is between "/* */". The 8 following numbers indicate the encounter history of the animals S1dZ1T2-00505 and S5dZ1T49-00475 successively.

The two last numbers are in order, a column indicating the frequency (1 if released alive or -1 if dead which means no recapture probabilities have to be calculated for this animal), and a last column coding for the covariate (here the sex, 1 coding for male and 0 female). The appropriate number of columns was added according to the number of covariates. The abbreviations used for covariates in the following tables are "Se" standing for sex, "A" for age and "Si" for sites.

/*S1dZ1T2-00505*/00010001 1 1;	Alive male, first captured at the fourth occasions, not seen during the three next occasions and recaptured at the last occasion
/*S5dZ1T49-00475*/00001000 -1 0;	Female that died in the trap at its first encounter at the fifth occasion

APPENDIX PART 4 – 5.2.: Matrix created for modeling using MARK software

Design matrix used for modeling probability of first capture (p) and recapture probabilities (c) with**:**

A: M0: p(.) = c(.) ;

B: Mb: p(.), c(.) ;

C: Mt+t² ;

D: Mt: p(t)=c(t).

A

Design Matrix Specification: Huggi...

Design Matrix Specification (B = Beta)

B1: p Intercept	B2:	B3:	Parm	B4:	B5:
1	S2	S3	1:p	S4	S5
1	S2	S3	2:p	S4	S5
1	S2	S3	3:p	S4	S5
1	S2	S3	4:p	S4	S5
1	S2	S3	5:p	S4	S5
1	S2	S3	6:p	S4	S5
1	S2	S3	7:p	S4	S5
1	S2	S3	8:p	S4	S5
1	S2	S3	9:c	S4	S5
1	S2	S3	10:c	S4	S5
1	S2	S3	11:c	S4	S5
1	S2	S3	12:c	S4	S5
1	S2	S3	13:c	S4	S5
1	S2	S3	14:c	S4	S5
1	S2	S3	15:c	S4	S5

B

Design Matrix Specification: Huggins' p and c {Mb+Si}

Design Matrix

B1: p Intercept	B2: c Behavior	B3:	Parm	B4:	B5:	B6:
1	0	S2	1:p	S3	S4	S5
1	0	S2	2:p	S3	S4	S5
1	0	S2	3:p	S3	S4	S5
1	0	S2	4:p	S3	S4	S5
1	0	S2	5:p	S3	S4	S5
1	0	S2	6:p	S3	S4	S5
1	0	S2	7:p	S3	S4	S5
1	0	S2	8:p	S3	S4	S5
1	1	S2	9:c	S3	S4	S5
1	1	S2	10:c	S3	S4	S5
1	1	S2	11:c	S3	S4	S5
1	1	S2	12:c	S3	S4	S5
1	1	S2	13:c	S3	S4	S5
1	1	S2	14:c	S3	S4	S5
1	1	S2	15:c	S3	S4	S5

C

Design Matrix Specification: Huggi...

Design Matrix Specification (B = Beta)

B1: p Intercept	B2: t	B3: t^2	Parm	B4: S2	B5: S3	B6: S4	B7: S5
1	1	1	1:p	S2	S3	S4	S5
1	2	4	2:p	S2	S3	S4	S5
1	3	9	3:p	S2	S3	S4	S5
1	4	16	4:p	S2	S3	S4	S5
1	5	25	5:p	S2	S3	S4	S5
1	6	36	6:p	S2	S3	S4	S5
1	7	49	7:p	S2	S3	S4	S5
1	8	64	8:p	S2	S3	S4	S5
1	2	4	9:c	S2	S3	S4	S5
1	3	9	10:c	S2	S3	S4	S5
1	4	16	11:c	S2	S3	S4	S5
1	5	25	12:c	S2	S3	S4	S5
1	6	36	13:c	S2	S3	S4	S5
1	7	49	14:c	S2	S3	S4	S5
1	8	64	15:c	S2	S3	S4	S5

D

Design Matrix Specification: Huggins' p and c (M t+ Si)

Design Matrix Specification (B = Beta)

B1: p Intercept	B2: p Occasion 1	B3: p Occasion 2	B4: p Occasion 3	B5: p Occasion 4	B6: p Occasion 5	Parm	B7: p Occasion 6	B8: p Occasion 7	B9:	B10:	B11:	B12:
1	1	0	0	0	0	1:p	0	0	S2	S3	S4	S5
1	0	1	0	0	0	2:p	0	0	S2	S3	S4	S5
1	0	0	1	0	0	3:p	0	0	S2	S3	S4	S5
1	0	0	0	1	0	4:p	0	0	S2	S3	S4	S5
1	0	0	0	0	1	5:p	0	0	S2	S3	S4	S5
1	0	0	0	0	0	6:p	1	0	S2	S3	S4	S5
1	0	0	0	0	0	7:p	0	1	S2	S3	S4	S5
1	0	0	0	0	0	8:p	0	0	S2	S3	S4	S5
1	0	1	0	0	0	9:c	0	0	S2	S3	S4	S5
1	0	0	1	0	0	10:c	0	0	S2	S3	S4	S5
1	0	0	0	1	0	11:c	0	0	S2	S3	S4	S5
1	0	0	0	0	1	12:c	0	0	S2	S3	S4	S5
1	0	0	0	0	0	13:c	1	0	S2	S3	S4	S5
1	0	0	0	0	0	14:c	0	1	S2	S3	S4	S5
1	0	0	0	0	0	15:c	0	0	S2	S3	S4	S5

APPENDICE PART 5 – 1.1. : Total number of capture individuals from the three main genus captured by zone by site by season and the species identity. Zone 1 = Intact forest, Zone 2 = disturbed forest, Zone 3 = Agricultural land. Zeros are replaced by dashes for easy reading.

Genus	Rainy Season 2015															
	S1			S2			S3			S4			S5			Rainy
	Z1	Z2	Z3	Z1	Z2	Z3	Z1	Z2	Z3	Z1	Z2	Z3	Z1	Z2	Z3	Total
Mus spp.	-	20	41	-	6	39	-	13	48	-	-	35	-	-	69	**271**
Rattus spp.	1	11	4	-	12	5	1	11	13	-	-	3	2	9	1	**73**
Maxomys spp.	3	5	-	-	1	-	4	2	-	9	3	-	18	4	-	**49**
Total	**4**	**36**	**45**	**0**	**19**	**44**	**5**	**26**	**61**	**9**	**3**	**38**	**20**	**13**	**70**	**393**
Individuals with an unidentified species	-	-	-	-	-	5	-	1	2	-	-	2	7	3	15	**35**
Total other rodent species	-	-	-	1	1	-	-	1	-	-	-	-	-	3	1	**7**

Genus	Dry Season 2016															
	S1d			S2d			S3d			S4d			S5d			Dry
	Z1	Z2	Z3	Z1	Z2	Z3	Z1	Z2	Z3	Z1	Z2	Z3	Z1	Z2	Z3	Total
Mus spp.	-	3	2	-	4	10	-	24	12	-	-	1	-	-	6	**62**
Rattus spp.	1	-	-	-	2	1	1	3	-	-	2	-	2	7	-	**19**
Maxomys spp.	1	3	-	4	1	-	3	-	-	2	1	-	6	4	-	**25**
Total	**2**	**6**	**2**	**4**	**7**	**11**	**4**	**27**	**12**	**2**	**3**	**1**	**8**	**11**	**6**	**106**
Individuals with an unidentified species	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	**2**
Total other rodent species	1	1	-	2	-	-	1	2	1	-	-	-	-	2	-	**10**

APPENDIX PART 5 – 3.: Count of all captured individuals by species for each zone, site and season. Zone 1 = Intact forest, Zone 2 = disturbed forest, Zone 3 = Agricultural land. Zeros are replaced by dashes for easy reading.

Species	Rainy Season 2015															Rainy Total
	Site 1			Site 2			Site 3			Site 4			Site 5			
	Z1	Z2	Z3	Z1	Z2	Z3	Z1	Z2	Z3	Z1	Z2	Z3	Z1	Z2	Z3	
Berylmys berdmorei	-	-	-	1	-	-	-	1	-	-	-	-	-	-	-	**2**
Chiropodomys gliroides	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0**
Leopoldamys sabanus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0**
Maxomys surifer	3	5	-	-	1	-	4	2	-	9	3	-	18	4	-	**49**
Mus caroli	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	**5**
Mus cervicolor	-	13	37	-	6	39	-	13	48	-	-	35	-	-	64	**255**
Mus spp.	-	7	4	-	-	-	-	-	-	-	-	-	-	-	-	**11**
Niviventer fulvescens	-	-	-	-	1	-	-	-	-	-	-	-	-	3	1	**5**
Rattus andamanenis	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**1**
Rattus exulans	-	-	-	-	-	3	-	-	1	-	-	-	-	-	-	**4**
Rattus losea	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	**1**
Rattus sp. R3	-	11	3	-	12	2	1	11	11	-	-	3	2	9	1	**66**
Rattus spp.	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	**1**
Vandeleuria oleracea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0**
Total	**4**	**36**	**45**	**1**	**20**	**44**	**5**	**27**	**61**	**9**	**3**	**38**	**20**	**16**	**71**	**400**
Tupaia belangeri	-	-	-	-	-	-	-	-	-	-	-	-	2	5	-	**7**
Individuals with an unidentified species	-	-	-	-	-	5	-	1	2	-	-	2	7	3	15	**35**

Species	Dry Season 2015															Dry Total
	Site 1			Site 2			Site 3			Site 4			Site 5			
	Z1	Z2	Z3	Z1	Z2	Z3	Z1	Z2	Z3	Z1	Z2	Z3	Z1	Z2	Z3	
Berylmys berdmorei	-	-	-	2	-	-	-	-	-	-	-	-	-	1	-	**3**
Chiropodomys gliroides	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	**1**
Leopoldamys sabanus	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**1**
Maxomys surifer	1	3	-	4	1	-	3	-	-	2	1	-	6	4	-	**25**
Mus caroli	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0**
Mus cervicolor	-	3	2	-	4	10	-	24	12	-	-	1	-	-	6	**62**
Mus spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0**
Niviventer fulvescens	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	**2**
Rattus andamanenis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0**
Rattus exulans	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0**
Rattus losea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0**
Rattus sp. R3	1	-	-	-	2	1	1	2	-	-	2	-	2	7	-	**18**
Rattus spp.	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	**1**
Vandeleuria oleracea	-	-	-	-	-	-	-	2	1	-	-	-	-	-	-	**3**
Total	**3**	**7**	**2**	**6**	**7**	**11**	**5**	**29**	**13**	**2**	**3**	**1**	**8**	**13**	**6**	**116**
Tupaia belangeri	-	1	-	-	-	-	2	1	-	-	-	-	-	5	-	**9**
Individuals with an unidentified species	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	**2**

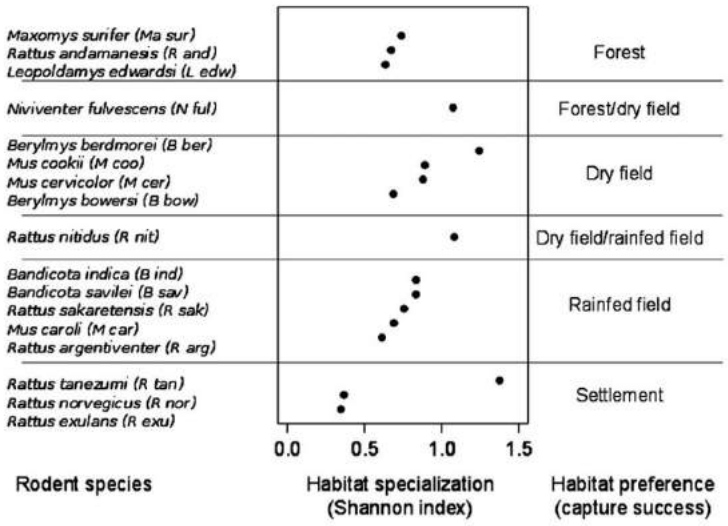
APPENDIX PART 5 - 2.1.: List of models used to calculate the average estimated abundance for *Maxomys spp., Rattus spp.,* and *Mus spp.* by zone by season along with the AICc, number of parameters included and the deviance of the model.

Season	Zone	Genus	Model	AICc	Delta AICc	AICc Weights	Model Likelihood	Number of parameters	Deviance	Estimated abundance	SE N̂	LCI	UCI
Rainy	Z1	*Maxomys spp.*	{Mtb+Si+Se}	332.0264	0	0.74206	1	13	304.6	39.2	13.6	35.2	125.1
			{Mtb+Si+Se+A}	334.1398	2.1134	0.25794	0.3476	14	304.5	38.6	10.5	35.2	102.9
		Rattus spp.	{Mt+t^2}	39.202	0	0.79558	1	3	27.6	4.1	0.3	4.0	6.2
			{M0}	43.0123	3.8103	0.11838	0.1488	1	36.1	4.2	0.5	4.0	7.0
			{Mb}	43.6507	4.4487	0.08603	0.1081	2	34.5	5.8	5.0	4.1	36.1
	Z2	*Maxomys spp.*	{M0+Si}	125.2307	0	0.39683	1	4	116.9	23.3	8.3	16.6	57.6
			{M0+Si+Se}	125.4081	0.1774	0.36314	0.9151	5	114.9	24.4	8.7	17.0	59.1
			{M t+t^2+Si}	127.5093	2.2786	0.127	0.32	6	114.8	23.1	8.2	16.6	57.0
			{M t+t^2+Si+Se}	127.7424	2.5117	0.11303	0.2848	7	112.7	24.2	8.6	17.0	58.5
		Rattus spp.	{Mt+t^2+Si}	361.7422	0	0.7359	1	6	349.5	50.9	4.7	45.7	66.2
			{Mt+t^2+Si+Se}	363.7917	2.0495	0.2641	0.3589	7	349.5	50.9	4.7	45.7	66.3
		Mus spp.	{Mt+t^2+Si+Se+A}	244.6956	0	0.66006	1	7	230.3	148.1	85.0	66.9	487.1
			{Mt+t^2+Si+Se+A+L}	246.7462	2.0506	0.23676	0.3587	8	230.3	147.0	83.8	66.6	478.8
			{Mt+t^2+Si+A}	248.4073	3.7117	0.10318	0.1563	6	236.1	105.8	44.5	58.1	261.1
	Z3	*Rattus spp.*	{M t+t^2+Si}	191.0796	0	0.37118	1	6	178.7	48.0	12.9	33.9	90.5
			{M t+t^2+Si+A}	192.613	1.5334	0.17243	0.4645	7	178.1	49.6	14.2	34.3	97.1
			{M t+t^2+Si+Se}	192.8596	1.78	0.15243	0.4107	7	178.3	48.7	13.4	34.1	93.3
			{Mb+Si}	193.6314	2.5518	0.10362	0.2792	5	183.3	115.9	121.8	38.8	693.5
			{M t+t^2+Si+Se+A}	194.4594	3.3798	0.0685	0.1845	8	177.7	49.7	14.2	34.4	96.9
			{Mb+Si+Se}	195.2348	4.1552	0.04648	0.1252	6	182.8	122.3	130.4	39.7	740.4
			{Mb+Si+A}	195.3802	4.3006	0.04322	0.1164	6	183.0	113.8	111.2	39.6	625.8
			{M0+Si}	195.4308	4.3512	0.04214	0.1135	4	187.2	48.9	13.3	34.3	92.6

			{M t+ Si}	1741.8618	0	0.34822	1	12	1717.7	326.8	19.0	296.5	372.0
			{M t+t^2+ Si}	1743.2854	1.4236	0.17089	0.4908	7	1729.2	327.7	19.1	297.2	373.3
			{M t+ Si+A}	1743.4908	1.629	0.15421	0.4429	13	1717.3	327.9	19.3	297.0	374.1
		Mus spp.	{M t+ Si+Se}	1743.8646	2.0028	0.12792	0.3674	13	1717.7	326.8	19.0	296.5	372.0
			{M t+t^2+ Si+A}	1744.9	3.0382	0.07623	0.2189	8	1728.8	328.8	19.5	297.7	375.3
			{M t+t^2+ Si+Se}	1745.2771	3.4153	0.06313	0.1813	8	1729.2	327.7	19.1	297.2	373.3
			{M t+ Si+Se+A}	1745.3991	3.5373	0.05939	0.1706	14	1717.2	328.0	19.4	297.1	374.3

Season	Zone	Genus	Model	AICc	Delta AICc	AICc Weights	Model Likelihood	Number of parameters	Deviance	Estimated abundance	SE Nhat	LCI	UCI
Dry	Z1	*Maxomys spp.*	{M t+t^2+Si}	217.0094	0	0.56621	1	7	202.3	24.3	2.2	22.5	33.2
			{M t+t^2+Si+Se}	219.0438	2.0344	0.20474	0.3616	8	202.2	24.3	2.2	22.5	33.2
			{Mb+Si}	219.5822	2.5728	0.15642	0.2763	6	207.1	31.3	9.5	23.8	70.5
			{M0+Si}	221.1167	4.1073	0.07263	0.1283	5	210.8	24.5	2.3	22.5	33.7
		Rattus spp.	{M0}	35.5628	0	0.64025	1	1	33.4	4.5	0.9	4.0	9.3
			{Mb}	37.8622	2.2994	0.20279	0.3167	2	33.4	4.6	1.6	4.0	14.0
			{Mt+t^2}	38.3746	2.8118	0.15696	0.2452	3	31.4	4.5	0.9	4.0	9.3
	Z2	*Maxomys spp.*	{M0}	69.6254	0	0.59866	1	1	67.6	11.5	2.5	9.5	21.8
			{M0+Se}	71.5519	1.9265	0.22848	0.3817	2	67.4	11.8	3.1	9.5	25.2
			{Mt+t^2}	72.1098	2.4844	0.17286	0.2887	3	65.7	11.2	2.3	9.4	21.0
		Rattus spp.	{M0}	97.7449	0	0.85195	1	1	95.7	23.8	7.0	16.8	48.2
			{Mt+t^2}	101.2448	3.4999	0.14805	0.1738	3	95.0	23.7	6.9	16.8	48.0
		Mus spp.	{Mt+t^2+Se}	231.4367	0	0.88559	1	4	223.3	65.1	36.1	37.3	217.3
			{Mt+t^2+Se+A}	235.5297	4.093	0.11441	0.1292	6	223.2	65.3	36.2	37.3	218.2
	Z3	*Mus spp.*	{Mt+t^2+Si+Se}	231.0738	0	0.91067	1	7	216.6	63.5	26.5	38.6	161.2

APPENDIX PART 5 – 3.3.2.: Rodents species ranked according to their habitat specialization (S. Morand, Jittapalapong, & Kosoy, 2015b)



Ranking of rodent species according to their habitat specialization (Shannon index) with main habitat preference (based on capture success) corresponding to the habitat (or the 2 habitats in which the highest number of captures was obtained.

FILLIEUX Caroline Anna Perrine

Title: Rodents community and leptospirosis risk on a deforestation gradient

The massive deforestation in Cambodia results in a drastic change in small- mammal community composition. This land use change process is believed to lead to a (re) emergence of zoonotic disease. The understanding of the disease ecology among rodents is thus an increasingly important subject to prevent possible outbreaks. This study aimed to determine the mechanisms driving the emergence of leptospirosis among rodent communities during the process of deforestation in Mondulkiri and Kampong Thom provinces, Cambodia. We focused on changes of rodent communities composition linked with their leptospirosis infectious status. Rodents trapping and mark-recapture techniques investigated rodents diversity, abundance and community composition from evergreen forest, disturbed forest, to cultivated land. Rodents community composition differed between habitats during the deforestation process. We identified that males Mus spp. had a lower capture probability <but a higher susceptibility to Leptospira infection, thus *Leptospira* apparent prevalence of Mus spp. is biased low during the dry season and is likely to be underestimated in previous studies estimating *Leptospira* prevalence among *Mus spp.* during the dry season.

Key words: Zoonosis, Leptospirosis, Rodents, Deforestation, Ecology, Epidemiology

Titre: Communautés de rongeurs et risque de leptospirose selon un gradient de déforestation

De la déforestation massive que subit le Cambodge découle des changements drastiques de la composition des communautés de petits mammifères. A travers ce processus de transformation des terres, la réémergence des maladies zoonotiques est attendue. Le but de cette étude est de déterminer les mécanismes qui entrainent l'émergence de la leptospirose au sein des communautés de rongeurs au cours du processus de déforestation dans les provinces de Mondulkiri et San Dan au Cambodge. Nous nous concentrons sur le lien entre les changements de composition des communautés de rongeurs et leur statut infectieux de leptospirose. Pour ce faire, des pièges de rongeurs et des techniques de capture-marquage-recapture ont été utilisés pour investiguer la diversité, l'abondance et la composition des communautés de rongeurs lors de la transformation d'un habitat de type forêt en une zone cultivée. Nos résultats montrent que la composition des communautés de rongeurs diffère au cours du gradient de déforestation. Les mâles *Mus spp*. présentent une probabilité de capture inférieure aux femelles mais une probabilité d'infection par les leptospires supérieure aux femelles. Ainsi, la prévalence apparente de *Leptospira* de *Mus spp*. est sous-estimée durant la saison sèche dans les zones cultivées.

Mots clés: Zoonose, Leptospirose, Rongeurs, Déforestation, Ecologie, Epidémiologie