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ECO-EPIDEMIOLOGIE DES ARENAVIRUS À L'INTERFACE RONGEUR-HUMAIN AU MOZAMBIQUE.

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DEDICATION

I dedicate this work to the memory of Dr. Dércio Rodrigues, our expert on rodents during the fieldwork. Unfortunately, a few months after the last field mission, his great dream and will to contribute on knowledge about rodent ecology in Mozambique was brutally interrupted. May his soul rest in peace.

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ABBREVIATIONS

CMR	Capture Mark Recapture
EIDs	Emerging infectious diseases
IDS	Indice de diversité de Simpson
KAP	Knowledge Attitudes Practices
LNP	Limpopo National Park
MIE	Maladies infectieuses émergentes
NWG	New World group mammarenaviruses
OSR	Operational sex ratio
OWG	Old World Group mammarenaviruses
PTS	Primary trapping session
SDI	Simpson's Diversity Index
STS	Secondary trapping session

ABSTRACT

Emerging infectious diseases (EIDs) have been a major global concern and more than 70% of new EIDs are zoonotic diseases that mostly originated from wildlife. EIDs caused by Lassa and Lujo mammarenaviruses are responsible for fatal cases of hemorrhagic fever in the West and southern Africa, respectively. The reservoir of the *Lujo virus* is yet unknown while the main reservoir of the *Lassa virus* is *Mastomys natalensis*, a rodent species widely distributed in sub-Saharan Africa. Other several mammarenaviruses yet regarded as non-pathogenic for humans were detected mainly from *M. natalensis* in southern Africa. Thus *M. natalensis* is one of the most important rodent species that carry zoonotic or potential zoonotic diseases. Moreover, the lives of rodents and people are often interwoven, increasing the interaction between rodents and humans as well as the risk of the emergence of zoonotic rodent-borne diseases.

This study aims to assess the circulation of mammarenaviruses and identify their drivers at the human/rodent interface in the Limpopo National Park and its buffer zone in Mozambique, to improve our knowledge of the potential risk of emerging rodent-borne zoonotic diseases. To reach our main objective we studied rodent ecology, then we detected and genotyped *Mammarenavirus* in *M. natalensis* trapped in irrigated croplands, rainfed cropland, mopane woodland forest, and villages. We also studied the drivers of the viral circulation in *M. natalensis* and finally we assessed human knowledge, attitudes, and practices toward rodents.

Our results indicated that the small-mammal community structure in our study area included seven rodent species and one *Elephant shrew*. *M. natalensis* was present in all four habitats sampled during the study. However, the irrigated agricultural constitute the main habitat for this rodent species. *M. natalensis* started breeding during the rainy season and its highest peak was observed in the middle of the dry season in June. *Mopeia virus* circulates in all habitats investigated and the proportion of positive samples 16,9% [IC 95%: 13,9-20,3] was the same across all habitats. However, the risk of mammarenaviruses transmission between rodents and humans varies over the habitats because of rodent population abundance differences and the type of human activities that constitute the risk factors for exposure to rodents. The irrigated croplands and villages (homes) are more likely to be the habitats with a high risk of pathogen transmission from rodents to humans. Anthropogenic factors jointly with climatic ones are importantly influencing the dynamic of the

rodent population and the human vulnerability to rodent-borne diseases in our study area. Here we identified the high-risk periods and the potential routes of mammarenaviruses transmissions to humans, despite the limitation of our sampling scheme. Our results have an implication for designing and implementing an action plan for the prevention and surveillance of rodent-borne diseases in humans in our study area.

Keywords: *Mammarenavirus*, *Mastomys natalensis*, epidemiology, disease ecology, one health.

1 RÉSUMÉ ÉTENDU EN FRANÇAIS

Eco-épidémiologie des mammarenavirus a l'interface rongeur-humain au Mozambique

1.1 ABSTRACT

Les maladies infectieuses émergentes (MIEs) constituent une préoccupation majeure au niveau mondial et plus de 70 % des nouvelles MIEs sont des zoonoses provenant principalement de la faune sauvage. Les MIEs causées par les mammarenaviruses Lassa et Lujo sont responsables de cas mortels de fièvre hémorragique en Afrique occidentale et australe, respectivement. Le réservoir du *Lujo virus* est encore inconnu tandis que le principal réservoir du *Lassa virus* est le rongeur *Mastomys natalensis* largement répandu en Afrique subsaharienne. Plusieurs autres mammarenaviruses considérés comme non pathogènes pour l'homme ont été isolés principalement chez *M. natalensis* en Afrique australe. Ainsi, *M. natalensis* est l'une des plus importantes espèces de rongeurs porteurs de zoonoses ou de zoonoses potentielles. De plus, les vies des rongeurs et des hommes sont souvent entremêlées, ce qui augmente l'interaction rongeur/homme ainsi que le risque d'émergence des maladies zoonotiques transmises par les rongeurs.

Cette étude vise à évaluer la circulation des mammarenaviruses et à identifier ses déterminants à l'interface homme/rongeur dans le parc national du Limpopo et sa zone tampon au Mozambique, afin d'améliorer notre connaissance du risque potentiel d'émergence de zoonoses transmises par les rongeurs. Pour atteindre notre objectif principal, nous avons étudié l'écologie des rongeurs, puis nous avons détecté et génotypé les *Mammarenavirus*, nous avons étudiés les facteurs influençant leur circulation chez *M. natalensis* et enfin nous avons évalué les connaissances, les attitudes et les pratiques humaines vis-à-vis des rongeurs.

Nos résultats indiquent que la structure de la communauté des petits mammifères dans notre zone d'étude comprend sept espèces de rongeurs et une musaraigne. *M. natalensis* était présent dans les quatre habitats contrastés échantillonnés pendant l'étude, cependant, les champs agricoles irrigués constituent l'habitat principal de cette espèce de rongeur. *M. natalensis* a commencé à se reproduire pendant la saison des pluies et un pic de population a été atteint au milieu de la saison sèche, en

Juin. Le *Mopeia virus* circule dans tous les habitats étudiés et la proportion d'échantillons positifs 16,9% [IC 95%: 13,9-20,3] était la même dans tous les habitats. Cependant, le risque de transmission des mammarenaviruses entre les rongeurs et les humains varie selon les habitats en raison des différences d'abondance des populations de rongeurs et du type d'activités humaines qui constituent les facteurs de risque d'exposition aux rongeurs. Les facteurs anthropogéniques, associés aux facteurs climatiques, influencent considérablement la dynamique de la population de rongeurs et la vulnérabilité de l'homme aux maladies transmises par les rongeurs. Nous avons détecté les périodes à haut risque et les voies potentielles de transmission des mammarenavirus à l'homme, malgré la limitation de notre plan d'échantillonnage. Nos résultats ont une implication dans la conception et la mise en œuvre d'un plan d'action pour la prévention et la surveillance des maladies humaines transmises par les rongeurs dans notre zone d'étude.

Mots-clés: *Mammarenavirus*, *Mastomys natalensis*, épidémiologie, écologie de la santé, une santé.

1.2 INTRODUCTION

Au cours des dernières décennies, les maladies infectieuses émergentes (MIE) ont constitué une préoccupation mondiale majeure en raison de leur impact sur la santé publique et l'économie. Environ 70% des nouvelles MIE sont des zoonoses et proviennent principalement de la faune sauvage. Parmi les animaux sauvages, les rongeurs hébergent au moins 60 maladies zoonotiques et sont donc considérés comme une source majeure de zoonoses. De plus, la vie des rongeurs et celle de l'homme sont souvent imbriquées. L'étude de l'interface homme/rongeur est donc particulièrement intéressante pour prévenir les épidémies d'MIE, notamment dans les pays où les ressources disponibles pour la santé publique sont limitées.

En Afrique sub-saharienne, l'une des espèces de rongeurs les plus importantes à véhiculer des zoonoses est *Mastomys natalensis*. Cette espèce de rongeur est largement répandue dans cette région où elle transmet le *Lassa virus* et le *Mammarenavirus*, responsables de milliers de cas de fièvres hémorragiques chaque année en Afrique de l'Ouest. Un deuxième *Mammarenavirus* zoonotique, le *Lujo virus*, est apparu lors d'une seule épidémie en Afrique du Sud en 2008 ; quatre des cinq patients humains infectés sont morts. Le réservoir du *Lujo virus* n'a pas encore été découvert mais plusieurs *Mammarenavirus*, considérés jusqu'à présent comme non pathogènes pour l'homme, ont été isolés principalement de *M. natalensis* en Afrique australe.

Plusieurs facteurs écologiques, anthropiques, génétiques et socio-économiques déterminent la circulation des agents pathogènes dans les réservoirs animaux ainsi que leur transmission à l'homme. En raison de la complexité de ces facteurs, il est nécessaire d'adopter des approches globales de la santé pour contrôler les MIE, comme le suggèrent les approches One Heath. En accord avec ces approches, des chercheurs dans les domaines de l'écologie, de l'épidémiologie et de la microbiologie sont nécessaires pour étudier les différents compartiments de l'interface et comprendre les différents mécanismes qui sous-tendent la circulation des agents pathogènes.

Malgré le risque potentiel d'urgence des *Mammarenavirus* en Afrique australe, peu d'études sur l'éco-épidémiologie du pathosystème rongeur-ont été menées dans la région. Pour combler cette lacune, nous avons réalisé cette étude exploratoire sur la circulation des *Mammarenavirus* et leurs

déterminants à l'interface homme/rongeur au Mozambique, afin d'améliorer notre connaissance du risque potentiel de zoonoses émergentes transmises par les rongeurs. À cette fin, nous avons étudié quatre aspects : tout d'abord, nous avons décrit la structure de la communauté des petits mammifères dans différents habitats et étudié la dynamique de la population de *M. natalensis*, puis nous avons détecté et caractérisé les *Mammarenavirus*, mené une étude éco-épidémiologique des *Mammarenavirus*, et enfin nous avons évalué les connaissances, les attitudes et les pratiques des humains vis-à-vis des rongeurs.

1.3 ZONE D'ÉTUDE ET PROTOCOLES D'ÉCHANTILLONNAGE

L'étude a été réalisée dans le district de Massingir au Parc National de Limpopo (LNP) dans la province de Gaza, au sud du Mozambique. Les rongeurs ont été piégés entre mars et novembre 2019 dans trois habitats différents (villages, champs de cultures et forêt de mopane) provenant de localités situées à l'intérieur du LNP (Bingo, Macavene et Mavodze) et de sa zone tampon (Chibotane, Machavule et Madingane). Le district de Massingir a une superficie de 5893 km2, 37 664 habitants, est dominé par des zones sèches de type semi-aride avec des températures annuelles moyennes de 30 °C et des précipitations de 600 mm. La zone d'étude fait partie de la zone de conservation transfrontalière du Grand Limpopo, adjacente au parc national Kruger (KNP) d'Afrique du Sud et au parc national Gonarezhou du Zimbabwe. La zone soutient une agriculture de subsistance dominée par des cultures pluviales sur des terres élevées et avec de petits systèmes d'irrigation près des rivières et du barrage de Massingir. Il existe une production animale extensive. Quatre localités sont situées à l'intérieur du LNP, sans barrière physique pour séparer la faune sauvage et l'homme. La communauté des mammifères sauvages comprend des animaux terrestres de grande, moyenne et petite taille, comme les éléphants, les buffles, les lions, les chiens sauvages, les cochons sauvages et les zèbres.

Le travail de terrain a été mis en œuvre pour couvrir les deux principales périodes de l'année (saison sèche et saison des pluies). Cinq sessions de piégeage primaire (PTS) ont été mises en œuvre en Mars (PTS1), Mai (PTS2), Juin (PTS3), Août (PTS4) et Novembre (PTS5). Deux types de sites ont été inclus au cours de cette étude: les sites de capture-marquage-recapture (CMR) et les sites « un coup ».

Les sites CMR étaient destinés à l'étude de la dynamique des populations de rongeurs et nous avons donc effectué une session de capture au cours de chaque PTS. Dans ces sites, seuls les rongeurs identifiés morphologiquement comme des *Mastomys* ont été marqués et relâchés pour une recapture potentielle. Deux sites ont été échantillonnés selon le protocole CMR : un site (cbf1) dans le champ de cultures irriguées et l'autre (mzf1) dans le champ de cultures pluviales. Des pièges à capture vivante Sherman de taille moyenne et petite (H.B. Sherman, Inc., Tallahassee, FL, USA) appâtés avec de la noix de coco grillée, de l'avoine et du beurre de cacahuète ont été déployés de manière intercalée, chaque piège moyen étant suivi du petit.

Les sites « un coup » ont été établis pour l'étude de la diversité des rongeurs et le dépistage du Mammarenavirus. Ils ont donc été échantillonnés en un ou deux PTS et tous les rongeurs capturés ont été sacrifiés. Les sites de prélèvement comprenaient des villages (n=5), des champs de culture (8) et des forêts de mopane (n=1). Dans les sites villageois, les rongeurs ont été piégés à l'intérieur des maisons et des greniers. Les captures dans les champs de culture comprenaient des sites irrigués (n=7) et pluviaux (n=1).

Initialement, les caractéristiques morphologiques ont été utilisées pour l'identification des espèces de rongeurs sur le terrain (Herbreteau et al., 2011). Comme les espèces de certains genres de rongeurs africains sont difficiles à reconnaître et que la taxonomie de nombreux groupes n'est pas encore bien résolue (Monadjem et al., 2015), nous avons effectué une identification génétique sur des rongeurs sélectionnés pour la confirmation des espèces. Nous avons appliqué l'approche de l'ADN-barcoding pour amplifier et séquencer (par la méthode Sanger) le gène mitochondrial complet du cytochrome b (CYTB ; 1140 pb) des individus sélectionnés.

Dans notre étude, l'effort de piégeage n'était pas homogène sur les différents sites et habitats et il a été déterminé par des considérations pratiques. Davantage de champs de cultures irriguées ont été échantillonnés car l'un de nos principaux objectifs était d'échantillonner des tissus de *M. natalensis* pour le dépistage des *Mammarenavirus*. Selon la littérature et les informations collectées localement, les champs de cultures irriguées étaient le type d'habitat où nous pouvions avoir des captures importantes de *M. natalensis*. En raison de la limitation des fonds, deux semaines ont été allouées pour chaque session de piégeage et elles n'ont pas été suffisantes pour

couvrir de nombreux sites dans les quatre habitats. Ainsi, les données obtenues à partir des sites «one shot » pourraient ne pas convenir à l'analyse de certains aspects de l'écologie des rongeurs.

Les espèces suivantes ont été génotypées : *Aethomys ineptus, Thallomys paedulcus, Rattus rattus et Rattus tanezumi*. Cependant, tous les individus de *Saccostomus campestris, Gerbilliscus leucogaster* et *M. natalensis* n'ont pas été génotypés. Nous n'avons pas génotypé *Saccostomus campestris* en raison de ressources limitées et aussi parce que nous étions confiants avec l'identification morphologique notamment basée sur sa queue courte (Mikula et al., 2016). Tous les *Gerbilliscus leucogaster* (n=5) génotypés (sur 10 captures pendant l'étude) ont été identifiés morphologiquement correctement, donc nous sommes également plus confiants que tous les individus ont été correctement identifiés.

Au total, 13,5% (138/1020) des *M. natalensis* capturés pendant toute l'étude ont été génotypés et deux d'entre eux ont été génétiquement identifiés comme étant *Aethomys ineptus*. Ainsi, nous pensons que l'erreur possible dans l'identification des espèces capturées pendant notre étude peut être la présence d'*Aethomys ineptus* identifié comme *M. natalensis*. Par conséquent, il est important d'attribuer un code-barres à tous les individus identifiés morphologiquement comme *M. natalensis* dans les champs de cultures pluviales et dans les forêts de mopane car ils font partie des habitats préférés d'*Aethomys ineptus*. Le nombre d'individus piégés et non génotypés dans les villages est gérable, ils peuvent donc tous être génotypés. Cependant, le nombre de *M. natalensis* non génotypés dans les champs de cultures irriguées est énorme, ainsi, nous suggérons de faire un code-barres pour tous les individus testés positifs au dépistage du *Mopeia virus* et dans le futur, tous les individus positifs au dépistage d'autres pathogènes devraient être génotypés.

1.4 STRUCTURE DE LA COMMUNAUTE DES PETITS MAMMIFERES, DANS LE PARC NATIONAL DE LIMPOPO ET SA ZONE TAMPON, MOZAMBIQUE

Nous avons décrit ici la structure de la communauté des petits mammifères et l'abondance des rongeurs dans différents habitats. La composition des espèces dans les différents habitats a été évaluée en utilisant la richesse en espèces qui est le nombre d'espèces piégées dans une communauté ou une unité géographique définie (Magurran, 2003; Begon et Townsend, 2021:574). Nous avons également utilisé l'indice de diversité de Simpson (IDS) pour évaluer la diversité entre les habitats (Simpson, 1949; Whittaker, 1972). Le nombre de captures par rapport à l'effort de piégeage (**nombre de captures / nuits de piégeage x100**) a été utilisé comme estimateur de l'abondance de la population (Granjon et al., 2005)

Avec 7290 nuits-pièges, un total de 1235 petits mammifères ont été piégés. La richesse en espèces de petits mammifères dans la zone d'étude comprenait sept espèces de rongeurs, à savoir *Aethomys ineptus, Gerbilliscus leucogaster, M. natalensis, Rattus rattus, Rattus tanezumi, Saccostomus campestris, Thallomys paedulcus*, et et un individu de *Macroscelide sp*. Dans l'ensemble, les champs de cultures irriguées avec deux espèces de rongeurs avaient une faible richesse en espèces (n=2) et l'indice de diversité de Simpson le plus bas (0,09). Les trois autres habitats avaient la même richesse en espèces (n=4), cependant, l'indice de diversité de Simpson indiquait que la forêt de mopane avec 0,68 avait une diversité d'espèces relativement plus élevée (**Table 1**). La diversité des espèces (indice de diversité de Simpson) différait significativement entre les quatre habitats (t=3,4; DF=3; p=0001), cependant, les différences n'étaient pas significatives entre les trois habitats ayant la même (n=4) richesse en espèces (t=6,0; DF=2; p=0,0).

La plus grande abondance de rongeurs a été enregistrée dans les champs de cultures irriguées 28.9% (n=961) et la plus faible dans la forêt de mopane 1.2% (n=13). *M. natalensis* a été piégé dans tous les habitats et représentait 14.0% (n=1020) des petits mammifères piégés et l'espèce de rongeur la plus abondante dans les champs de cultures irriguées 27.5% (n=917), les champs de cultures pluviales 2.6% (n=46), et la forêt de mopane 0.5% (n=6). *Rattus rattus* n'a été piégé que dans les villages et c'est l'espèce de rongeur la plus abondante dans cet habitat 11.5% (n=127).

Rattus tanezumi 0.2% (n=17) a été détecté dans un site du village. *Rattus tanezumi* n'a été détecté que dans un seul village (**Table 1**).

Table 1: Structure de la communauté des petits mammifères et abondance relative (RA) sur quatre habitats différents dans notre zone d'étude. n = le nombre d'individus piégés

Espèces	Nombre d'individus piégés et pourcentages dans chaque habitat				Global n (RA)
	Champs de cultures irriguées n (RA)	Champs de cultures non irriguées n (RA)	Forêt de mopane n (RA)	Village n (RA)	
Aethomys ineptus	/	2 (0.1)	/	/	2 (0.0)
Gerbilliscus leucogaster	/	10 (0.6)	1 (1.0)	/	11 (0.2)
Mastomys natalensis	917 (27.5)	46 (2.6)	6 (0.5)	51 (4.6)	1020 (14.0)
Rattus rattus	/	/	/	127 (11.5)	127 (1.7)
Rattus tanezumi	/	/	/	17 (1.5)	17 (0.2)
Saccostomus campestris	44 (1.3)	4 (0.2)	5 (0.5)	/	53 (0.7)
Thallomys paedulcus	/	/	/	4 (0.4)	4 (0.1)
Elephant shrew	/	/	1 (0.1)	/	1 (0.0)
Nombre total de captures	961 (28.9)	62 (3.5)	13 (1.2)	199 (18.1)	1235 (16.9)
Total des nuits-pièges	3331	1759	1100	1100	7290
Nombre de sites	8	2	1	5	
Richesse des espèces	2	4	4	4	
Indice de diversité de Simpson	0.09	0.43	0.67	0.45	

Nous avons conclu que les champs de cultures irriguées avaient une forte abondance de rongeurs mais une faible diversité d'espèces, tandis que la forêt de mopane avait une faible abondance de petits mammifères mais une grande diversité d'espèces. *M. natalensis* était présent dans tous les habitats et constituait l'espèce de rongeur la plus abondante dans les champs de culture et la forêt de mopane. *Rattus rattus* était la principale espèce de rongeurs dans les villages. Globalement, nos résultats sont compatibles avec l'idée que la diversité des petits mammifères est plus faible dans les agrosystèmes que dans les écosystèmes plus naturels. A notre connaissance, nous rapportons pour la première fois la présence de *Rattus tanezumi* au Mozambique.

1.5 DYNAMIQUE DE LA POPULATION DE *MASTOMYS NATALENSIS* DANS LES CHAMPS DE CULTURES IRRIGUEES

Les paramètres démographiques de *M. natalensis* ont été analysés pour évaluer la saison de reproduction de *M. natalensis* dans notre zone d'étude. Les résultats obtenus ici ont ensuite été utilisés pour étudier l'éco-épidémiologie des *Mammarenavirus*.

Comme mentionné plus haut, le nombre de captures par rapport à l'effort de piégeage (**nombre de captures / nuits de piégeage x100**) a été utilisé comme estimateur de l'abondance de la population (Granjon et al., 2005). La sex-ratio est la proportion de femelles sur l'ensemble de la population (Mlyashimbi et al., 2020) car le moment de la reproduction de *M. natalensis* dépend de la condition sexuelle des femelles (Leirs 1994 :65). Les femelles sont considérées comme sexuellement actives lorsque le vagin est ouvert et ou les mamelles sont proéminentes alors que les mâles sont considérés comme sexuellement actifs lorsque les testicules sont en position scrotale et/ou la vésicule séminale est développée (Leirs, 1994: 61; Herbreteau et al., 2011). Sur la base de nos classes de poids et des données sur la maturité sexuelle, nous avons fixé à 22 g la limite de poids pour distinguer les juvéniles des adultes dans notre étude.

Les sites « one shot » n'ont pas montré une tendance claire dans la variation des paramètres démographiques de *M. natalensis*. Nous pensons que cela pourrait être dû à des différences dans l'effort de piégeage entre les sites. Un nombre élevé de pièges augmente la possibilité pour les individus d'accéder à un piège vide, ce qui augmente la possibilité de capturer un plus grand nombre de rongeurs. D'autre part, un nombre plus faible de pièges limite le nombre de captures car même si la densité de rongeurs est plus élevée, moins de pièges sont disponibles.

Une autre explication qui peut avoir contribué à la tendance peu claire de la variation des paramètres démographiques de *M. natalensis* est le fait que certains sites ont été échantillonnés dans deux PTS. Dans ces sites, les individus capturés et retirés pendant le premier PTS, en plus de modifier la structure de la population, ont réduit le nombre d'individus capturables lors des captures suivantes. Pour les raisons décrites ci-dessus, l'évaluation de la dynamique de la population de *M. natalensis* présentée ici s'est concentrée sur les sites CMR.

Nos données ont montré une prépondérance des femelles, des tendances croissantes du recrutement des juvéniles et de l'abondance de la population de la fin de la saison des pluies vers le milieu de la saison sèche. Ces tendances sont compatibles avec une saison de reproduction en cours. Ainsi, nos données suggèrent que la reproduction de *M. natalensis* a commencé pendant la saison des pluies et que son pic a été atteint au milieu de la saison sèche (Juin/Juillet). Le changement dans la tendance des paramètres de reproduction mentionnés ci-dessus alors que nous approchons de la fin de la saison sèche suggère un déclin de la saison de reproduction. Le schéma de reproduction suggéré par nos données semble comparable à ceux décrits en Afrique du Sud et en Tanzanie où la principale saison de reproduction de *M. natalensis* commence pendant les pluies et s'étend jusqu'à la saison sèche avec des variations spatiales et annuelles dépendant des différences dans le schéma annuel des pluies Coetzee, 1975; (Leirs et al., 1993; Massawe et al., 2011; Mulungu et al., 2013). Le lien entre la reproduction de *M. natalensis* et les précipitations a été attribué à l'influence des pluies sur la disponibilité des ressources en qualité et en quantité pour les rongeurs.

1.6 DETECTION ET DIVERSITE GENETIQUE DU VIRUS *MOPEIA* CHEZ *MASTOMYS NATALENSIS* PROVENANT DE DIFFERENTS HABITATS DU PARC NATIONAL DE LIMPOPO, MOZAMBIQUE

Les tissus pulmonaires de *M. natalensis* ont été examinés afin d'évaluer la circulation des *Mammarenavirus* dans différents habitats. Deux paires d'amorces P1-LSF/R et P2-LSF/R (Li et al., 2015) ciblées sur des régions conservées du segment L des *Mammarenavirus* ont été utilisées pour amplifier une longueur de 611 pb. Puis nous avons réalisé l'analyse phylogénétique pour identifier les espèces de *Mammarenavirus* circulant dans l'étude. Le séquençage Sanger a été réalisé en utilisant la paire d'amorces P2-LSF/R chez LGC Genomics (Berlin, Allemagne). Les séquences d'acide nucléique générées dans cette étude ont été déposées dans la GenBank sous les numéros d'accession MZ512094 à MZ512143.

La proportion globale d'individus positifs pour la détection de l'ARN du *Mammarenavirus* était de 16,9% [IC 95%: 13,9-20,3] (**Table 2**). Les proportions de rongeurs positifs au *Mammarenavirus* ne différaient pas significativement entre les champs agricoles et les villages (χ^2 = 0,018; DF=1; p = 0,893) mais différaient significativement entre les localités (χ^2 = 12,512; DF=3; p = 0,0058). Le faible nombre d'échantillons testés dans les villages peut avoir contribué à ne pas détecter de

différences dans les proportions de rongeurs positifs entre les deux habitats. Les localités ont été échantillonnées à des périodes différentes, ce qui pourrait expliquer les différences dans la proportion d'échantillons positifs détectés entre les localités.

Table 2: Nombre de M. natalensis testés (N) et nombre d'échantillons positifs (n) dans différents habitats et localités du Parc National de Limpopo et de sa zone tampon

	Habitat							
	Champs agricoles		**Villages**		**Forêt de mopane**		**Total**	
Localité	n (N)	Proportion [CI, 95%]	n (N)	Proportion [CI, 95%]	n (N)	Proportion [CI, 95%]	n (N)	Proportion [CI, 95%]
Bingo	17 (78)	21.8 [14.1-32.2]	3 (10)	30.0 [10.8-60.3]	/	/	20 (88)	22.7 [15.2-32.5]
Chibotane	28 (144)	19.4 [13.8-26.7]	0 (19)	0.0 [0.0-16.8]	/	/	28 (163)	17.2 [12.2-23.7]
Macavene	/	/		/	1 (6)	16.7 [3.0-56.4]	1 (6)	16.7 [3.0-56.4]
Machavule	/	/	2 (2)	100.0 [34.2-100.0]		/	2 (2)	100.0 [34.2-100.0]
Madingane	1 (62)	1.6 [0.3-8.6]	1 (8)	12.5 [2.2-47.1]		/	2 (70)	2.9 [0.8-9.8]
Mavoze	36 (201)	17.9 [13.2-23.8]	1 (4)	25.0 [4.6-69.9]		/	37 (205)	18.0 [13.4-23.9]
Total	82 (485)	16.9 [13.8-20.5]	7 (43)	16.3 [8.1-30.0]	1 (6)	16.7 [3.0-56.4]	90 (534)	16.9 [13.9-20.3]

Cinq individus avaient des résultats RT-PCR douteux dans Bingo (n=1), Chibotane (n=2) et Mavoze (n=2)

L'analyse phylogénétique a indiqué que le *Mopeia virus* est le *Mammarenavirus* présent dans notre zone d'étude. Des recherches antérieures sur les *Mammarenavirus* au Mozambique ont également décrit la présence du *Mopeia virus* au Mozambique (Wulff et al., 1977). Cependant, les virus que nous avons détectés dans notre étude forment un clade spécifique de *Mopeia virus* différent de celui rapporté dans le centre du Mozambique par Wulff et al. (1977). De plus, le *Mopeia virus* étudié ici a formé huit sous-clusters, mais la topologie de l'arbre n'a pas été bien résolue car un fragment très court a été amplifié et séquencé.

Le *Mopeia virus* a également été détecté dans les pays voisins: Zimbabwe (Johnson et al., 1981), Tanzanie (Borremans et al., 2011) et Afrique du Sud (Grobbelaar et al., 2021). En effet, le *Mopeia virus* est encore considéré comme non pathogène pour l'homme. Cependant, sa large distribution dans la région de l'Afrique australe laisse penser qu'après le *Lujo virus* et ses cas humains mortels, le *Mopeia virus* mérite l'attention. Par conséquent, des recherches supplémentaires sont nécessaires

pour comprendre dans quelle mesure le *Mopeia virus* peut constituer une menace pour la santé publique.

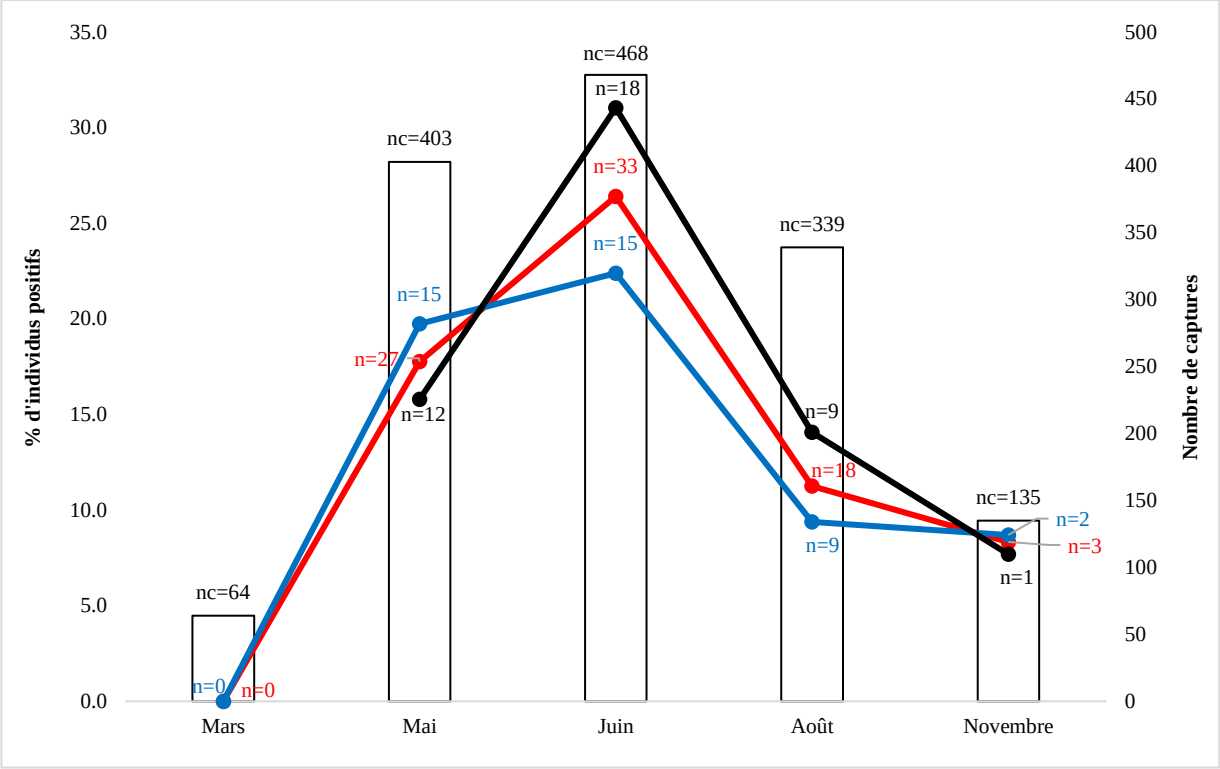
1.7 ECO-EPIDEMIOLOGIE DU VIRUS *MOPEIA* DANS LE PARC NATIONAL DU LIMPOPO ET SA ZONE TAMPON

La transmission des maladies peut être affectée par les interactions entre l'écologie de l'hôte et les facteurs abiotiques et biotiques (Bordes et al., 2015; Tong et al., 2008). Nous avons évalué ici l'effet des caractéristiques de l'hôte *M. natalensis* sur la transmission du *Mopeia virus*.

Nous avons effectué une analyse épidémiologique descriptive des résultats. Seules les données provenant des champs de cultures irriguées ont été prises en compte, car dans les autres habitats nous n'avions pas un nombre suffisant d'échantillons. Sur la base des résultats RT-PCR obtenus ci-dessus, la proportion d'échantillons positifs a été estimée pour chaque site, PTS, sexe, âge et condition de reproduction. L'âge et la condition reproductive ont été évalués comme nous l'avons décrit ci-dessus.

En regroupant les données par PTS, nous avons observé le pic de la proportion d'individus positifs en Juin. Elle a augmenté de Mars à Juin, puis a diminué jusqu'en Novembre (***Figure 1***). Ces différences d'échantillons positifs entre les PTS étaient significatives (χ^2=13,5; DF=3; p= 0,004).

La proportion globale d'échantillons positifs pour *M. natalensis* lors de la RT-PCR pour la détection de l'ARN du *Mopeia virus* était de 15,6% (n=41) pour les adultes et de 19,0% (n=40) pour les juvéniles, cependant, les différences de positivité du *Mopeia virus* entre les deux classes d'âge n'étaient pas significatives (χ^2=0,7; DF=1; p=0,4). Les différences d'échantillons positifs au *Mopeia virus* entre les sessions de piégeage étaient significatives pour les juvéniles (χ^2=6,7; DF=2; p=0,03) mais non significatives pour les adultes (χ^2=7,0; DF=3; p=0,07).



>22 g	1[0.0-79.4]	76 [12.3-30.0]	67 [14.1-33.7]	96 [5.0-16.9]	23 [2.4-26.8]
≤22 g		76 [9.3-25.6]	58 [20.6-43.8]	64 [7.6-24.6]	13 [1.4-33.3]
Global	1 [0.0-79.4]	152 [12.5-24.6]	125 [19.5-34.8]	160 [7.2-17.1]	36 [2.9-21.8]
Nuits pièges	636	836	628	749	482

Figure 1: Évolution du nombre de captures et des proportions d'échantillons positifs à la RT-PCR pour la détection de l'ARN du Mopeia virus chez les adultes (>22g) et les juvéniles (≤22g) au cours des sessions de piégeage dans les champs de cultures irriguées. Les barres affichent le nombre de captures (n) tandis que les lignes indiquent les proportions d'échantillons positifs. Le nombre total d'échantillons testés (N), l'IC95% et les nuits de piégeage sont affichés sous chaque session de piégeage tandis que le nombre d'échantillons positifs (n) est indiqué le long des graphiques.

La proportion d'échantillons positifs de *M. natalensis* lors de la RT-PCR pour la détection de l'ARN du *Mopeia virus* était de 20,7% (n=45) pour les femelles et de 14,1% (n=35) pour les mâles, cependant, cette différence n'était pas significative pour l'ensemble des données (χ^2=3,2 ; DF=1; p=0,07).

La différence de prévalence du *Mopeia virus* entre les individus sexuellement matures et immatures présentait le même schéma que celui observé entre les individus adultes et juvéniles. Cette similitude n'est pas surprenante puisqu'on s'attend à ce que les individus adultes soient sexuellement matures et que les juvéniles soient immatures.

Les pics suivants se sont produits au même moment en Juin : proportion globale d'échantillons positifs au *Mopeia virus*, proportion de juvéniles positifs et abondance de la population de *M. natalensis*. Nos données sur la dynamique de population indiquent que le pic des juvéniles et l'abondance de la population ont eu lieu conjointement en juin. Ainsi, la circulation du *Mopeia virus* était plus élevée pendant le pic de la saison de reproduction. La fluctuation des échantillons positifs au *Mopeia virus* au cours des périodes d'échantillonnage que nous avons décrites ici, suggère l'existence d'une période spécifique de risque plus élevé pour l'exposition de l'homme à une éventuelle infection par le *Mopeia virus* ou d'autres *Mammarenavirus* zoonotiques émergents.

1.8 LA VULNERABILITE AUX MALADIES ZOONOTIQUES EMERGENTES COMME CADRE ALTERNATIF POUR ABORDER L'INTERFACE HOMME-RONGEUR A LA LUMIERE DE L'HISTOIRE ENVIRONNEMENTALE, DANS LE PARC NATIONAL DE LIMPOPO, AU MOZAMBIQUE

Cette étude a examiné l'interface homme-rongeur dans une communauté rurale du Mozambique. Initialement conçue dans le cadre d'une recherche sur les *Mammarenavirus*, cette étude socio-anthropologique a exploré les dimensions culturelles et sociales de l'interface rongeur-homme, en considérant sa variabilité spatiale et temporelle. Elle contribue à la compréhension de la dynamique socio-écologique, dans laquelle de nouvelles transmissions virales peuvent se produire et la maladie se propager.

Une méthodologie basée sur la vulnérabilité a été utilisée pour évaluer l'exposition humaine, la sensibilité et la capacité d'adaptation à l'interaction rongeurs-humains. Les principaux résultats de cette étude sont: i) La valeur ajoutée de l'utilisation d'une méthodologie basée sur la vulnérabilité, par rapport à la méthodologie plus classique KAP (Connaissances, attitudes, pratiques), pour capturer l'expérience vécue et dynamique de cette interface; ii) Les transformations, observées localement au cours des dernières décennies, avec un nombre croissant de rongeurs invasifs au détriment des espèces indigènes; iii) Les moteurs socio-écologiques attribués à ces transformations : le changement climatique, les nouvelles infrastructures (par exemple la construction d'un barrage), les activités (par exemple, le développement de l'irrigation) et les politiques (réinstallation des populations); iv) l'évolution des interactions entre l'homme et les rongeurs, conséquence de l'évolution de la population de rongeurs et de l'exposition accrue des femmes aux

contacts avec les rongeurs; v) le fardeau multidimensionnel que représente localement l'interface homme-rongeur (par exemple, les pertes de récoltes, les biens endommagés) et les préoccupations limitées concernant les maladies liées aux rongeurs ; vi) enfin, malgré la perception d'un fardeau élevé associé aux nouveaux rongeurs invasifs, la capacité limitée à entreprendre des actions individuelles et collectives pour atténuer les dommages connexes.

Ces résultats montrent comment les changements socio-écologiques peuvent créer de nouvelles voies pour les virus émergents. Ils montrent également comment l'étude de la vulnérabilité (à travers ses différentes composantes) de la population potentiellement exposée peut informer sur les risques associés, plutôt que de se concentrer sur les lacunes dans les connaissances.

1.9 CONCLUSIONS GÉNÉRALES

Nos résultats indiquent que la communauté de petits mammifères de notre zone d'étude comprend au moins sept espèces de rongeurs et une musaraigne. *M. natalensis* était présent dans les quatre habitats échantillonnés pendant l'étude, cependant, les champs agricoles irrigués constituent l'habitat principal de cette espèce de rongeur. *M. natalensis* a commencé à se reproduire pendant la saison des pluies et son pic le plus élevé a été observé au milieu de la saison sèche en juin/juillet.

Le *Mopeia virus* circule dans tous les habitats étudiés, et la proportion d'échantillons positifs 16,9% [IC 95%: 13,9-20,3] était la même dans tous les habitats. Cependant, le faible nombre de captures de *M. natalensis* dans des habitats autres que les champs de cultures irriguées n'a peut-être pas permis de détecter des différences dans la proportion d'échantillons positifs entre les habitats. Les humains sont potentiellement exposés au risque d'infections par les mammarenavirus dans tous les habitats étudiés. Le risque de transmission des mammarenavirus entre les rongeurs et les humains peut être présent tout au long de l'année dans les maisons; les femmes et les jeunes filles constituent le groupe le plus à risque en raison de leurs tâches domestiques. Dans les champs cultivés, le risque est encore plus élevé pendant le pic de la saison de reproduction de *M. natalensis* au milieu de la saison sèche et, là encore, les femmes et les filles constituent le groupe à risque car ce sont elles qui effectuent les activités à risque dans les champs (désherbage et récolte) lorsque l'abondance de *M. natalensis* est plus élevée. Les filles sont également exposées aux rongeurs tout au long de

l'année dans la forêt lorsqu'elles cherchent du bois de chauffage pour cuisiner. Les hommes et les garçons sont les principaux groupes à risque dans les bois en raison de leurs activités de chasse et de pâturage du bétail tout au long de l'année. Cependant, une plus grande abondance de rongeurs dans les champs et les vacances scolaires peuvent attirer les garçons vers les activités de chasse.

1.10 RECOMMANDATIONS ET PERSPECTIVES

Les résultats de cette étude indiquent la circulation du *Mopeia virus* dans le parc national du Limpopo et sa zone tampon et décrivent le risque d'infections possibles de l'homme par des *Mammarenavirus*. Cependant, en raison des limitations de ressources et de temps, nos données ne sont pas suffisantes pour soutenir certaines conclusions de notre étude, il reste donc plusieurs aspects à clarifier.

Nous recommandons une étude à long terme d'au moins trois ans pour couvrir plus de répliques de sites et de saisons. Un minimum de trois ans est nécessaire pour permettre la collecte de beaucoup plus de données pour une meilleure évaluation des variations intra-annuelles de l'écologie des rongeurs et de la dynamique de transmission des *Mammarenavirus*. Les répétitions de sites provenant de champs de cultures pluviales et de zones boisées devraient être augmentées. L'échantillonnage doit couvrir toutes les saisons de l'année, aussi bien la saison des pluies que la saison sèche, et il doit être effectué au début, au milieu et à la fin de chaque saison.

Le niveau d'exposition aux rongeurs décrit dans cette étude suggère que les humains risquent de contracter des maladies transmises par les rongeurs. Par conséquent, une surveillance active pour détecter le *Mopeia virus* chez l'homme est recommandée dans notre zone d'étude. En tenant compte de la dynamique de transmission des *Mammarenavirus* chez *M. natalensis* décrite ci-dessus, le milieu de la saison sèche (juin/juillet) serait la période appropriée pour la surveillance. Une surveillance sérologique et virologique doit être réalisée chez l'homme pour détecter et caractériser d'éventuels *Mammarenavirus* circulant chez l'homme et les relier à ceux des rongeurs. Les femmes et les jeunes filles doivent être le groupe cible et, dans la mesure du possible, les hommes et les garçons doivent également être inclus.

Des campagnes de sensibilisation communautaires devraient être mises en œuvre pour combler le manque de connaissances sur les rongeurs et leurs maladies. En ce qui concerne plus particulièrement les mammarenaviruses, la prévention doit mettre l'accent sur les facteurs de risque (tels que l'hygiène, le stockage de la nourriture, la chasse, les pratiques agricoles et autres), y compris les périodes à haut risque. Les communautés ont exprimé que les méthodes qu'elles utilisent pour le contrôle des rongeurs dans les champs de culture et les maisons ne sont pas efficaces. De plus, au cours de l'étude, nous avons remarqué que les communautés étudiées ont un accès limité à la source d'approvisionnement en eau. Ainsi, le plaidoyer au niveau gouvernemental est essentiel pour répondre à la nécessité d'aider les communautés à améliorer les méthodes de contrôle des rongeurs, les sources d'approvisionnement en eau, et d'autres qui nécessitent l'intervention des autorités.

2 GENERAL INTRODUCTION

2.1 INTRODUCTION

Emerging infectious diseases (EIDs) have been a major global concern due to their impact on public health and the economy over the past decades with increasing records of emerging or reemerging pathogens (Mohan and Vinod, 2020; Suárez Rodríguez et al., 2022). Approximately 70% of newly EIDs are zoonotic diseases and mostly originated from wildlife (Jones et al., 2008; Gebreyes et al., 2014). Among the wildlife animals, rodents are the most diverse order of mammals and host at least 60 zoonotic diseases so some species of rodents are considered a major source of zoonosis and a source of potential new emerging infectious diseases (Luis et al., 2013; Han et al., 2015; Nieto-Rabiela et al., 2019; Dahmana et al., 2020). Moreover, the lives of rodents and people are often interwoven (Singleton, 2003), thus, the study of the human/rodent interface is of particular interest to prevent outbreaks of EIDs, especially in countries with limited resources available for public health (Bordes et al., 2016).

In sub-Saharan Africa, one of the most important rodent species that carry zoonotic diseases is *M. natalensis* (Arruda et al., 2021; Olayemi et al., 2018). This rodent species is widely distributed across this region (Monadjem et al., 2015) and is responsible for the transmission of the *Lassa virus* among several pathogens (Fichet-Calvet and Rogers, 2009; Akhmetzhanov et al., 2019). *Lassa virus* is a *Mammarenavirus* of the *Arenaviridae* family, responsible for thousands of cases of hemorrhagic fever each year in West Africa and therefore is a threat to public health (Balogun et al., 2021). The most recently discovered zoonotic *Mammarenavirus*, the *Lujo virus*, emerged in one outbreak in South Africa in 2008 and resulted in the death of four out of five infected human patients (Briese et al., 2009; Paweska et al., 2009). The reservoir of the *Lujo virus* is yet to be discovered (Simulundu et al., 2016; Grobbelaar et al., 2021), however, other mammarenaviruses so far described as non-pathogenic for humans have been isolated in rodents in southern Africa, including the *Mopeia virus* described for the first time worldwide in Mozambique in 1977 (Wulff et al., 1977). Here in the context of rodent borne diseases a reservoir is a rodent harboring disease causing organism and thus serves as potential sources of disease outbreaks (Meerburg et al., 2009).

Several ecological, anthropogenic, genetic, and socioeconomic factors drive the circulation of pathogens in animal reservoirs as well as transmission to humans and, due to the complexity of

these factors, controlling EIDs needs global approaches to health as suggested by the One Health and EcoHealth paradigms (Roger et al., 2016; Harrison et al., 2019). In line with these approachs, researchers from the of ecology, epidemiology, microbiology social sciences are needed to study the different compartments of the interface and understand the different mechanisms underlying the circulation of pathogens (Bordes et al., 2015; Plowright et al., 2016). The eco-epidemiology of the rodent-mammarenaviruses pathosystem has still not been extensively studied and ecological studies on the reservoir species combined with virological studies are needed for a better understanding of the drivers of circulation of these viruses in their natural hosts. In particular, host demographics and diversity are key in the circulation and emergence of zoonotic diseases (Fichet-Calvet et al., 2008, 2007; Lecompte et al., 2006; Redding et al., 2016; Taal Levi et al., 2016), thus their influence on mammarenaviruses circulation in rodents need to be assessed. Furthermore, a better understanding of the rodent/human interface, including human perception of and practices related to rodents, is needed to identify the exposure and group at risk as well as the processes of emergence of these zoonotic viruses in human populations (Spiegel et al., 2005; Stewart Ibarra et al., 2014; Lapinski et al., 2015).

With several mammarenaviruses known to circulate in southern Africa (Wulff et al., 1977; Ishii et al., 2011,2012; Grobbelaar et al., 2021) including a highly pathogenic one for humans (Paweska et al., 2009), limited studies were undertaken in the region. Below I expanded the literature review on rodent ecology, mammarenaviruses and their transmission between rodents and to humans, then I introduce objectives of the study.

2.2 LITERATURE REVIEW

2.2.1 Rodents

2.2.1.1 The order Rodentia

The order Rodentia is the most abundant and distributed worldwide (except in Antarctica and on some oceanic islands) order in the class Mammalia and is subdivided in 36 families, 513 genera and 2552 species (Burgin et al., 2018). About 463 rodent species are found in Africa (Monadjem et al., 2015).

Rodents are present in different ecosystems including natural, agricultural and urban (Monadjem et al., 2015).The habitat structure determines the rodent community structure and species richness depending on several factors among them availability of resources for shelter and food. (Avenant and Cavallini, 2007). Differences on small mammals species composition over different type of habitats have been reported by several studies (Odhiambo et al., 2006; Chidodo et al., 2020) and it has been suggested that natural habitats have a variety of resources and ecological niches to support high rodent diversity contrary to disturbed ecosystems where the lesser variety of resources will limit the diversity of residing species (Monadjem, 1999; Vera and Rocha, 2006; Agerie and Afework, 2015). Moreover, natural factors such as climate changes and human activities including urbanization and agriculture are responsible for the disturbance of ecosystems, thus affecting small mammals community structure (Massawe et al., 2005; Taylor et al., 2012; Ssuuna et al., 2020). Examples of impact of anthropogenic factors on biodiversity were reported in China, where the abundance of *Apodemus agrarius,* the natural reservoir of *Hantavirus,* was higher in lands for agricultural purposes (Yan et al., 2007). Similarly, a study in Tanzania suggested that the need for agricultural intensification to increase crop production may create conditions for colonization by *M. natalensis* (Makundi et al., 2007).

The factors mentioned above as drivers of species diversity have an impact on the dynamics of disease transmission between natural reservoir species as well as transmission to humans (Bordes et al., 2015). The mechanisms behind the effect of biodiversity on increasing or decreasing the transmission of zoonotic diseases (Ostfeld and Keesing, 2012) were detailed in the transmission ecology section (see §2.2.3).

2.2.1.2 Rodents and public health

Rodents are considered a major threat to public health as they host more than 60 pathogens (virus, bacteria and parasites) that can be transmitted to humans. (Meerburg et al., 2009; Rabiee et al., 2018; Tambo et al., 2018). A review study in sub-Saharan Africa recorded occurrence of approximately twenty pathogens transmitted from rodents to humans (Gratz, 1997), however, recent studies in rodents focus more on occurance of Leptospirosis, Toxoplasmosis, Plague and

mammarenaviruses (Belmain, 2006; Borremans et al., 2015; Grobbelaar et al., 2021). A study from Mozambique detected occurance of antibodies against Plague, Leptospirosis and Toxoplasmosis in both humans and rodents (Nala, 2006). In addition, more than ten pathogens hosted by rodent (e.g. causing Lyme disease, Crimean-Congo hemorrhagic fever, hemorrhagic fever due to Hantavirus, Rift valley fever and Cryptosporidiosis) yet not studied in rodents in Mozambique were recorded in humans (Salkeld et al., 2008; Augusto et al., 2009; Chau et al., 2017; Muianga et al., 2017; Messa et al., 2021; Rogier et al., 2022).

It is difficult to predict where and when emergence may come from (Howard and Fletcher, 2012). Attempted based in a forecasting tool developed for zoonotic outbreak investigation identified over 150 rodent species as potential sources of more than one pathogen beyond the ones so far known to carry (Han et al., 2015). This prediction highlighted the role of rodents as reservoirs of future zoonotic diseases. Despites all these records, rodent-borne diseases are neglected due to the limited capacity for surveillance conditioned by a weak and poorly resourced health system in many countries (Molyneux et al., 2018; Karpagam and Ganesh, 2020). Henceforth, understanding the processes that could contribute to the emergence/re-emergence of these diseases is necessary to limit their public health and economic consequences (Granjon and Duplantier, 2009). Thus, improved epidemiological surveillance of infectious diseases in species known to present the greatest risk is needed for earlier detection and to alert those responsible for formulating public health policy to put in place strategies for combating emerging diseases (Howard and Fletcher, 2012).

2.2.1.3 Mastomys (Thomas, 1915)

We focused on *Mastomys* because they are reservoir of several human pathogens including mammarenaviruses, the agents responsible for the targeted EIDs in our study.

Taxonomy, nomenclature and distribution

The genus *Mastomys* belongs to the family Muridae and it contains eight species namely *M. awashensis* Lavrenchenko, Linkhnova & Baskevich 1998, *M. coucha* (Smith, 1834), *M.*

erythroleucus (Temminck, 1855), *M. huberti* (Wroughton, 1909), *M. Kollmannspergeri* (Petter, 1957), *M. natalensis* (Smith, 1834), *M. shortridgei* (St. Leger. 1933) and *M. angolensis* (Bocage, 1890) (Granjon and Duplantier 2009; Monadjem et al., 2015; Nicolas et al., 2021; Hánová, 2021). This genus is the most common rodent is sub-Saharan Africa with some limited records in Morocco, northern Chad and northern Sudan (Granjon et al., 1997; Monadjem et al., 2015).

M. natalensis has a wide distribution over sub-Saharan Africa (**Figure 2**) while the other species have a limited geography (Monadjem et al., 2015). A study on distribution and diversity of *Mastomys* in Ethiopia reported occurrence of four species (*M. awashensis, M. natalensis, M. erythroleucus* and *M. kollmannspergeri*) mostly inhabiting natural biotopes (Martynov et al., 2020). In Senegal, *M. erythroleucus* was reported in different habitats while *M. huberti* tends to habit natural or cultivated humid areas and *M. natalensis* was restricted to inside villages (Duplantier et al., 1996). In Mali, *M. huberti* was reported from annually flooded shrub savanna (Granjon et al., 2005; Crespin et al., 2008) but it has been reported from different habitats in Western Africa mainly from lowland areas (IUCN, 2016). In Guinea sympatric occurrence of *M. natalensis* and *M. erythroleucus* was reported in rural villages and (Lecompte et al., 2006; Fichet-Calvet et al., 2007, 2008). The studies in Guinea where *M. natalensis* is intensively investigated as *Lassa virus* reservoir, reported its presence inside houses during the dry season when food resources are scarce outside but when food is available in the it disperse outside houses (Fichet-Calvet et al., 2007).

Four species of *Mastomys* were recorded in southern Africa, namely *M. natalensis, M. coucha, M. shortridgei and M. angolensis* (Venturi et al., 2004; Hánová et al., 2021). *M. natalensis* occurs all over Southern Africa region in a wide range of habitats including inside homes, agricultural , grassland and savannas (Leirs et al., 1996; Massawe et al., 2011; Colangelo et al., 2013; Monadjem et al., 2015). In our neighboring countries, the multimammate *M. natalensis* has been intensively studied in Tanzania and was more recorded in fallow land and cultivated than in houses where black rats and house mice are dominant (Leirs, 2006). This observation was previously reported in South Africa (Coetzee, 1975), where *Mastomys* was found co-inhabiting with other rodents species such as *Tatera, Rhabdomys, Lemniscomys, Aethomys, Thamnomys, Praomys* and *Mus*. Furthermore, the study of Coetzee (1975) suggested that the association of *Mastomys* with *Rattus*

rattus is rare and the replacement of the former by the last one was common in human habitats in towns and cities.

In Mozambique *M. natalensis* was reported in a wide range of habitats including inside houses, savanna and the cultivated (Wulff et al., 1977; Gliwicz, 1985; Leirs, 2006; Belmain, 2009). *M. natalensis* is targeted species in our study because of its wide occurrence in the country, besides being the main natural reservoir of several mammarenaviruses (Kenmoe et al., 2020; Grobbelaar et al., 2021).

The mitochondrial phylogenetic analysis split *M. natalensis* into six matrilineage phylogroups, namely A-I, A-II, A-III, B-IV, B-V, B-VI (**Figure 2**) (Colangelo et al., 2013; Hánová et al., 2023). Among these phylogroups previous studies reported parapatry occurrence of two lineages of *M. natalensis* (B-V and B-VI) in Mozambique where phylogroup B-VI occurs across the country while phylogroup B-V is geographically restricted to the northernmost part of the country (Colangelo et al., 2013; Gouy de Bellocq et al., 2020; Hánová et al., 2021).

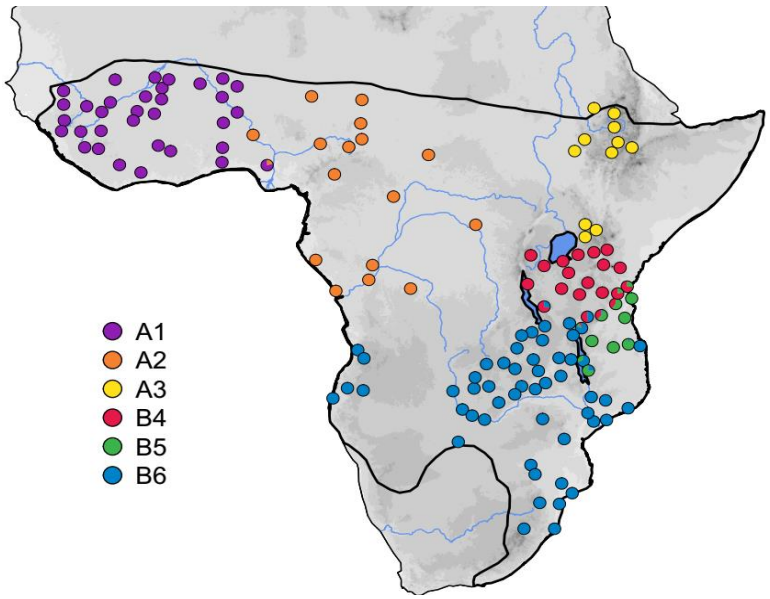


Figure 2: Distribution of matrilineage phylogroups of M. natalensis across sub–Saharan Africa. Source (Hánová et al., 2023)

General description of M. natalensis

The morphological characters are not sufficient to distinguish the members of the genus *Mastomys* particularly because of sibling species such as *M. natalensis* and *M. coucha* (Venturi et al., 2004) so genotyping is required (Granjon et al., 1997; Monadjem et al., 2015). Like other *Mastomys* are also designated multimammate because the females have between eight up to 12 pairs of nipples continuously distributed from the pectoral to the inguinal region (Leirs, 1994:10; Monadjem et al., 2015). The colour of the pelage varies according to the age of the animal. The dorsal pelage colour varies from grey to greyish-brown, brown or reddish buff and the ventral side is lighter to grey (Isaäcson, 1975; Monadjem et al., 2015). The body length is ±7.6-15.5 cm and the tail has approximately the same size (Leirs, 1994:10; Monadjem et al., 2015).

M. natalensis has nocturnal behaviour and reaches its activity peak just three hours after the onset of darkness (Coetzee, 1975). The multimammate *M. natalensis* is a generalist species feed on a wide range of food resources (grass stems, seeds, rhizomes and insects) depending on the season and the habitat (Coetzee, 1975; Monadjem and Perrin, 1998; Odhiambo et al., 2008; Mulungu et al., 2011). Studies in Tanzania suggested that the preference for grains may explain *M. natalensis* abundance in cultivated , especially grain plantations (Odhiambo et al., 2008; Mulungu et al., 2011).

M. natalensis is a very prolific species and due to their short gestation period and interval between litters of approximately three weeks for both parameters (Leirs 1994 :59; Coetzee, 1975). The **Table 3** gives a summary of other important reproductive parameters of *M. natalensis.*

Table 3: Reproduction parameters of M. natalensis

Parameters	Indicator	Reference
Age of sexual maturity	65-76 days	Leirs 1994 :59 ; Duplantier et al.1996
Light pregnant female	28 g	Granjon and Duplantier, 2009
Gestation period	21-22 days	Coetzee, 1975; Duplantier et al.1996; Granjon and Duplantier, 2009
Age at first litter	94.1± 19.19 days	Granjon and Duplantier, 2009
Interval between litters	21-26 days	Coetzee, 1975; Duplantier et al.1996; Leirs 1994:60
Litter size	10[6.5-12.1]	Coetzee, 1975; Duplantier et al.1996
	8.9[1-19]	Granjon and Duplantier, 2009
Lifetime	339 days	Coetzee, 1975

M. natalensis ecology

Small mammal populations fluctuate widely on an irregular basis (Delany, 1972; Cheeseman and Delany, 1979; David and Jarvis, 1985; Delany and Monro, 1986) and studies indicated that *M. natalensis* display both temporal and spatial population fluctuations (Monadjem and Perrin, 2003; Mulungu and Lopa, 2016).

Besides immigrations, reproduction is the main mechanism responsible for building up the population (Delany, 1972; Leirs, 1994: 59). As such, it has an impact on density and age structure (Makundi et al., 2007; Székely et al., 2014; Mlyashimbi et al., 2020).

M. natalensis population fluctuation has been linked to its seasonal reproduction which is most probably due to the rain influence on vegetation cover, quantity and quality of food resources availability (Leirs et al., 1989; Mulungu et al., 2013). The main breeding season for *M. natalensis* in South Africa starts at the end of the rains toward the dry season (Coetzee, 1965). In Tanzania the breeding in *M. natalensis* starts soon after the onset of rains and continues until the dry season (Leirs et al., 1989; Massawe et al., 2011; Mulungu et al., 2013).

The timing of *M. natalensis* breeding season depends on presence of sexually active individuals, mainly females since sexually active males may be present over a year and a single male can mate and impregnante several females (Leirs 1994: 65). A higher proportion of sexually active females were recorded towards of the end of rainy season in Tanzania (Leirs 1994:67; Odhiambo et al., 2006; Mulungu et al., 2013).

The outcome of the breeding season is recruitment of juveniles. A study in Tanzania reported two waves of juveniles' pulse. The first was seen in March and April and the second one was observed in July. The explanation given for these two waves was the existence of two rainy seasons. When the first rains (*vuli* rains) are abundant a short breeding period occurs early in the year. During the second and heavier rainy season (*mesika* rains) the main breeding season occurs (Leirs 1994: 84 and 119). Still in Tanzania more juveniles were observed in August and September and was linked to the main rainy breeding season (Mulungu et al., 2013) and juveniles' recruitment built up the population, thus increasing the population size. Peaks of rodent density were recorded in the middle of the dry season in South Africa (**Figure 3**) and Tanzania (**Figure 4**).

The above studies clearly indicated that presence of sexually active females, juveniles' recruitment and increase in population density depends on the reproduction pattern. Moreover, the onset and duration of the rainy season will drive the onset and duration of reproduction and when the host demographic will peak. In chapter three we assessed *M. natalensis* population dynamic and more details about its ecology were provided.

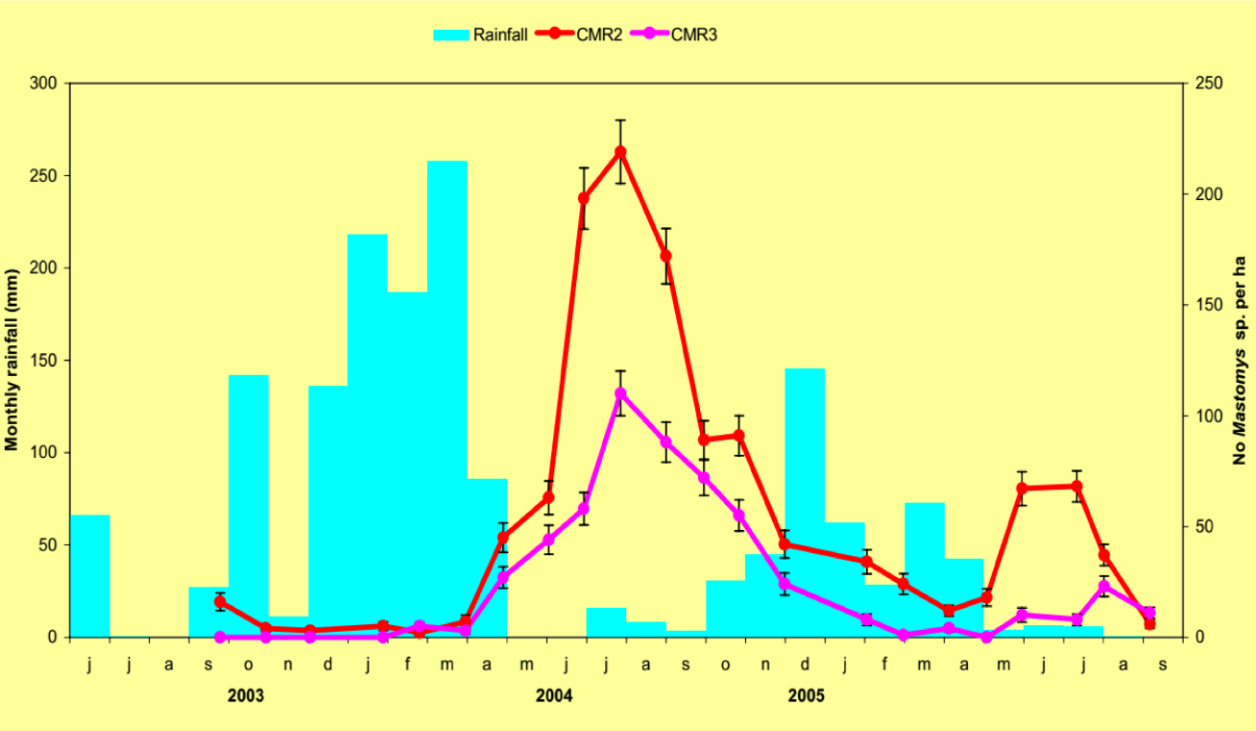


Figure 3: Evolution of M. natalensis population densities in two CMRs sites during the rainy and dry seasons between 2003 and 2005 in northeastern South Africa. Source: Leirs, 2006

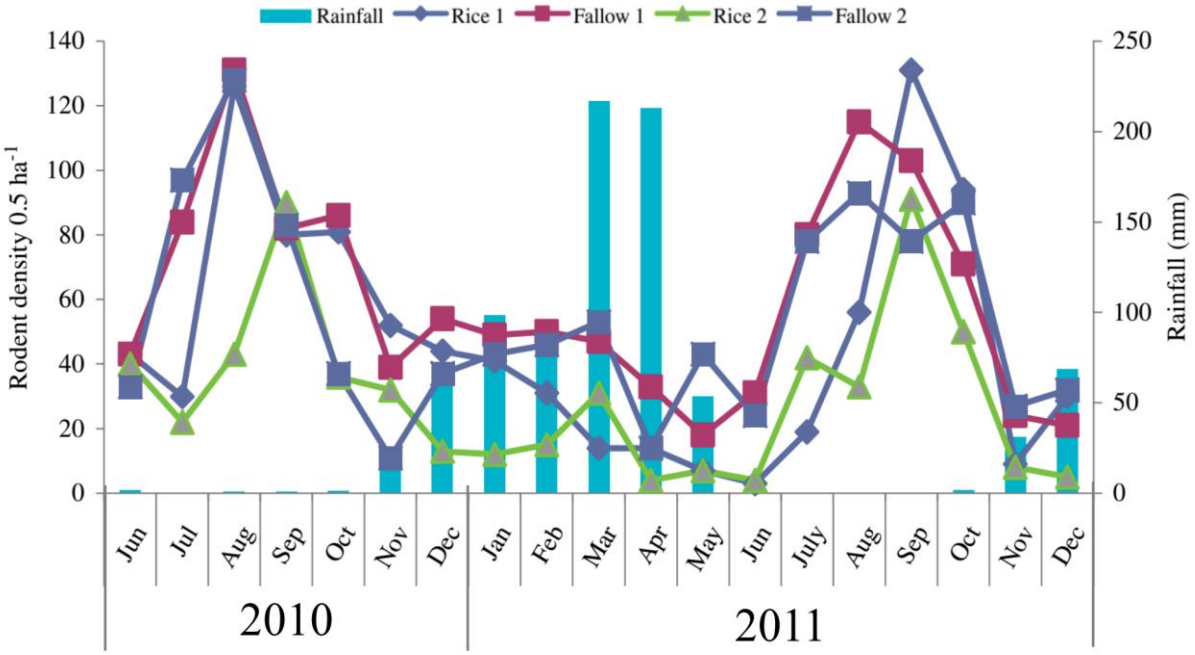


Figure 4: Evolution of M. natalensis population densities in rice and fallow land fields in the irrigated rice crop area during the rainy and dry seasons in 2010 and 2011 in eastern Tanzania. Source: Mulungu et al., 2013

2.2.2 The viruses of family Arenaviridae

2.2.2.1 Structure, proprieties and classification of Arenaviridae viruses

Mammarenavirus belongs to the *Arenaviridae* family. The members of this family are enveloped viruses with two or three single-strand RNA molecules. The genome consists of large (L) and small (S) RNA segments (***Figure 5***). The S segment encodes the viral nucleoprotein (NP) which is the most abundant structural protein and the glycoprotein precursors (GPC). The GPC (GP1 and GP2) is responsible for attachment and entry into the host cell where GP1 is associated with cellular receptors binding while GP2 mediates fusion of the viral and cellular membranes. The L segment encodes for the viral RNA-dependent RNA polymerase (L protein) at the 3' end and the zinc binding matrix protein Z at the 5' end. The protein Z drives budding of virions while the role of L protein is transcription to produce NP and L mRNAs. Sequences at the 3' end of the L and S segments are highly conserved (Payne, 2017; Burrell et al., 2017; Radoshitzky et al., 2019).

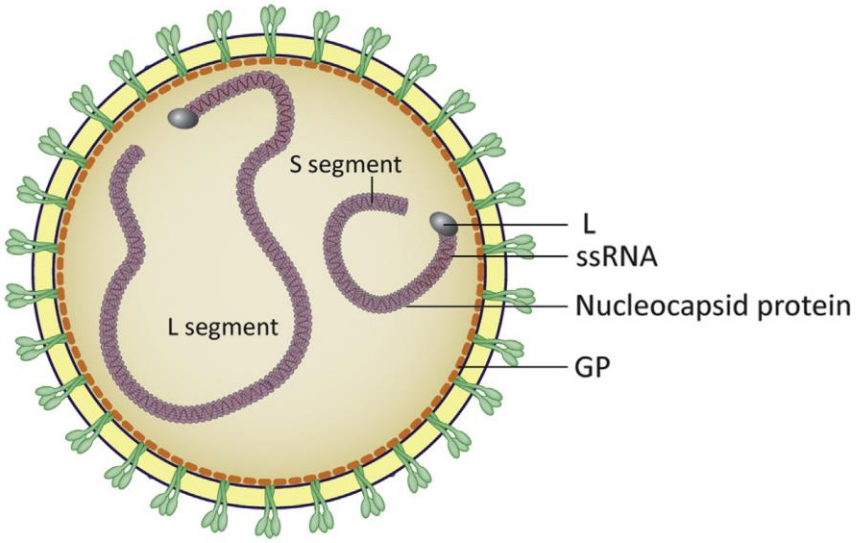


Figure 5: Schematic illustration of Arenavirus. (Payne, 2017)

The virus is stable in aerosol and 30% of relative humidity and at 32ºC (Stephenson et al., 1984), however infectivity is inactivated by lipid solvents, acids (pH< 5.5) and by ultraviolet and gamma radiation (Murphy, 1975), by organic solvents and common disinfectants (Olschewski et al., 2021).

The *Arenaviridae* family contains four genera, namely *Mammarenavirus* which contain viruses infecting mammalian hosts, *Antennavirus* with viruses infecting fish, *Hartmanivirus* and *Reptarenavirus* both restricted to viruses isolated in snakes. The archetype of the *Arenaviridae* family is the *Lymphocytic choriomeningitis virus* (King et al., 2012; Radoshitzky et al., 2019).

Based on geographical occurrence, genetic, and epidemiological features, the genus *Mammarenavirus* is further divided into New World group (NWG) and Old World Group (OWG) (Albariño et al., 1998; Bowen et al., 1997; Charrel et al., 2002) (**Figure 6**).

The NWG encompasses mammarenaviruses from South and North America. The NWG also called Tacaribe seromplex, contains the following mammarenaviruses: *Allpahuayo, Flexal, Paraguayan, Cali, Pirital, Serra do Navio, Chapare, Cupixi, Guanarito, Argentinian, Machupo, Brazilian, Tacaribe, Latino, Oliveros, Bear Canyon, Tamiami and Whitewater Arroyo mammarenaviruses* originated from American continent (Bowen et al., 1996; Charrel et al., 2003; Radoshitzky et al., 2015; Maes et al., 2018). The NWG mammarenaviruses associated with human diseases include Junín, Machupo, Guanarito, Sabiá, and Chapare (Charrel et al., 2002; Radoshitzky and de la Torre, 2019). Besides the above mammarenaviruses the NWG also contains the genus *Hartmanivirus* with one species (*Haartman*) and the genus *Reptarenavirus* with five species: *California, Giessen, Golden, Ordinary* and *Rotterdam* (Radoshitzky et al., 2015; Maes et al., 2018).

The OWG (Lassa-lymphocytic choriomeningitis serocomplex) contains viral species from the African continent including the *Lymphocytic choriomeningitis virus* (LCMV) with worldwide distribution (Hallam et al., 2018). To date more than 20 species of mammarenaviruses from the Lassa-LCMV serocomplex were recorded Africa mainland (**Table 4**) (Maes et al., 2018; Radoshitzky et al., 2015). The OWG also contains the *Wenzhou, Loei River* and *Ryukyu mammarenavirus* described in Asia (Pontremoli et al., 2017; Maes et al., 2018). Within the OWG mammarenaviruses, three members (*Lymphocytic choriomeningitis virus, Lassa virus* and *Lujo virus*) are associated with human diseases (Paweska et al., 2009; Radoshitzky and de la Torre, 2019).

2.2.2.2 Laboratory diagnostic and phylogenetic relationships within the family Arenaviridae

Cross-reactivity occurs between different species of mammarenaviruses as elucidated during the study of sympatric occurrence of three mammarenaviruses in Tanzania where serology only (indirect immunofluorescence antibody) assays did not distinguish *Morogoro virus* from the potential novel *Mammarenavirus* detected in *Lemniscomys rosalia* and *Kodoko virus* detected in *Mus minutoides* (Goüy de Bellocq et al., 2010). Genetic analysis based on the pan–OWG RT-PCR for identifying new Old World mammarenaviruses (Vieth et al., 2007) allowed the discrimination between the above three species of mammarenaviruses. The primers for the pan–OWG RT-PCR were designed to identify target sequences within the L segment are feasible for identification of both known and unknown OWG mammarenaviruses (Vieth et al., 2007).

Within the OWG viruses, phylogeny analysis (**Figure 6**) suggests that Lassa, Mopeia and Mobala viruses which are original from Western, Southern and Central Africa, respectively are closely related, whereas *Ippy virus* which occurs in Central Africa and *Lymphocytic choriomeningitis virus* are more distantly related (Bowen et al., 1997). The southern African *Lujo virus* is an OWG virus because of its geographical occurrence but the analysis of glycoprotein gene suggests that it has genetic features of the NWG virus (Briese et al., 2009; King et al., 2012).

The phylogenetic analysis of the NWG mammarenaviruses divides the group into three lineages A, B, and C (**Figure 6**). All human pathogenic viruses of the Tacaribe complex Machupo, Junín, Guanarito, Sabiá, and Chapare cluster together in lineage B. The monophyly of pathogenic viruses within the Tacaribe complex suggest that the highly pathogenic phenotype could be a result of evolutionary radiation from a common ancestor (Bowen et al., 1996; Burrell et al., 2017). However, monophyletic relationship could not be established among all the known pathogenic arenaviruses, suggesting that the pathogenic phenotype might be a result of long-term accumulation of mutation during virus evolution (Bowen et al., 1997). Notably, all the NWG pathogenic viruses are monophyletic and the natural host are rodents, so Brown et al. (1996) suggests that for the NWG the identification of new *Mammarenavirus* in rodents with genetic

features resembling lineage B viruses should be viewed as an indication of a potential zoonotic virus.

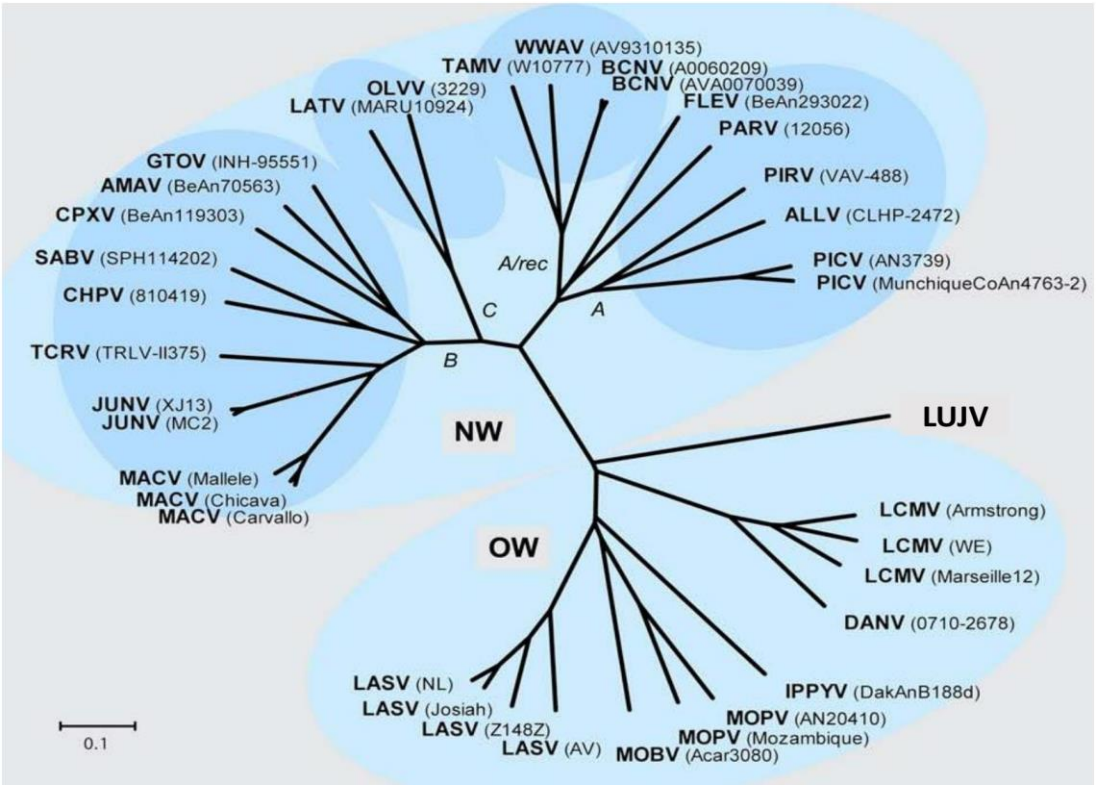


Figure 6: Phylogenetic analyses of old world (OW) and new world (NW) Mammarenavirus based on full L segment (Briese et al., 2009)

2.2.2.3 Mammarenaviruses and their natural rodent reservoirs

The important natural reservoir of the NWG are rodents from the family *Cricetidae* and subfamilies *Sigmodontinae* (genus *Calomys, Sigmodon, Neacomys* and *Oryzomys*) and *Neotominae* (genus *Neotoma*) (King et al., 2012) except *Tacaribe virus* described in *Phyllostomidae* bats (Cogswell-Hawkinson et al., 2012).

In Africa the OWG mammarenaviruses were recorded in approximately 20 rodent species (**Table 4**) Among these rodent species, the highest number of mammarenaviruses (at least six) were detected in *M. natalensis* (Goüy de Bellocq et al., 2010; Olayemi et al., 2018; Grobbelaar et al., 2021). This finding highlights the role of *M. natalensis* as the main reservoir of mammarenaviruses. The genus

Mus also hosts at least six mammarenaviruses including the pathogenic ones for humans (*Lassa virus and* LCMV) (**Table 4**) suggesting that the species of this genus should be considered as an important source of mammarenaviruses. Likewise, *Praomys sp*. should be accounted as a potential source of zoonotic mammarenaviruses because besides hosting *Lassa virus*, at least two more mammarenaviruses were identified from these rodents.

Table 4: The OWG mammarenaviruses from Africa, their distribution and rodent species where they were detected

Geographic distribution	***Mammarenavirus***	**Rodent species**	**Reference**
West Africa	*Lassa virus*	*Mastomys natalensis* *Mastomys erythroleucus* *Mastomys huberti* *Hylomyscus pamfi* *Praomys daltoni* *Praomys misonnei* *Praomys rostratus* *Rattus rattus* *Crocidura spp*** *Lophuromys sikapusi* *Malacomys edwardsi* *Mus baoulei* *Mus minutoides*	McCormick et al., 1987; Olayemi et al., 2016, 2018; Bangura et al., 2021
	Jirandogo virus * Natorduori virus *	*Mus baoulei* *Mus mattheyi*	Kronmann et al., 2013
	Kodoko virus	*Mus minutoides*	Lecompte et al., 2007
Mozambique	*Mopeia virus*	*M. natalensis*	Wulff et al., 1977
Zimbabwe	*Mopeia virus*	*M. natalensis*	Johnson et al., 1981
Tanzania	*Mopeia virus* *Morogoro virus* *Gairo virus* *Luna virus*	*M. natalensis*	Borremans et al., 2011 Günther et al., 2009 Gryseels et al., 2015 Cuypers et al., 2020
	Kodoko virus *	*Mus minutoide*	Goüy de Bellocq et al., 2010
Zambia	*Luna virus*	*M. natalensis* *Aethomys chrysophilus*	Ishii et al., 2011 Grobbelaar et al., 2021
	Lunk virus *Solwezi virus*	*Mus minutoides* *Grammomys sp.*	Ishii et al., 2012 Ishii et al., 2016
South Africa	*Mopeia virus* *Lujo virus* *Merino Walk virus*	*M. natalensis* Unknown *Myotomys unisulcatus*	Grobbelaar et al., 2021 Paweska et al., 2009 Palacios et al., 2010
Namibia	*Mariental virus* *Okahandja virus*	*Micaelamys namaquensis*	Witkowski et al., 2015
Central Africa Republic	*Mobala virus*	*Praomys jacksoni*	Emonet et al., 2006
Cameron	*Souris virus*	*Praomys sp.*	Grobbelaar et al., 2021
Worldwide	*Lymphocytic choriomeningitis virus*	*Mus musculus*	N'Dilimabaka et al., 2014

Angola	*Bitu virus* * *Kwanza virus* *	*Grammomys sp.* *Mus triton*	Těšíková et al., 2021
Côte d'Ivoire	*Gbagroube virus* * *Menekre virus* *	*Mus setulosus* *Hylomyscus sp*	Coulibaly N'Golo et al., 2011
Ethiopia	*Dhati welel virus* *	*M. natalensis*	Gouy de Bellocq et al., 2020

*Not classified into species by International Committee on Taxonomy of Viruses accepted taxonomy of the family Arenaviridae; ***Elephant shrew*

2.2.2.4 Diseases caused by Mammarenaviruses

Human pathogenic mammarenaviruses have been ordinarily identified after humans presented clinical diseases or as a result of laboratory workers' infections. In the absence of contact between rodents and humans, other pathogenic mammarenaviruses may not have been identified because of their asymptomatic infection in rodents (Bowen et al., 1997). Similarly, humans may undergo asymptomatic infections (Payne, 2017), highlighting the need for active surveillance in both rodents and humans. Rodent to human transmissions of mammarenaviruses occur through contact with infected rodents, contaminated rodent excreta such as urine, feces and saliva via inhalation of dust or aerosolized or ingestion of contaminated food (Charrel et al., 2003).

Human exposure to contaminated rodent excreta during crop harvesting or hunting activities and poor hygiene in households increases the risk of mammarenaviruses transmission from rodent to humans (Paweska, 2014). Mammarenaviruses transmissions between human beings can occur through direct contacts with contaminated human fluids such as blood, urine and pharyngeal secretions. (Paweska, 2014; Payne, 2017). Additionally, transmission among humans through solid-organ transplantation was recorded in Australia (Palacios et al., 2009).

As annotated above five members of NWG and three members of OWG are associated with haemorrhagic fever syndrome in humans (Radoshitzky and de la Torre, 2019), however two of them (*Lassa virus* and *Lujo virus*) are of major concern in Africa (Briese et al., 2009; Bangura et al., 2021).

Lassa fever

Lassa fever is a viral rodent-borne disease caused by *Lassa virus*. It is one of the aetiology of haemorrhagic fever disease in humans and the most important *Mammarenavirus* of public health

concern in Africa (Fichet-Calvet and Rogers, 2009; Mari Saez et al., 2018). The disease is endemic in some countries of West Africa (**Figure 7**) where up to 300.000 human cases with about 5000 deaths occur annually (Günther and Lenz, 2004; Olayemi et al., 2018; Mateer et al., 2018). Imported cases of *Lassa virus* were recorded in South Africa (https://www.nicd.ac.za/imported-case-of-lassa-fever-identified/), United States, Canada, United Kingdom, Japan, Germany, Netherlands, and Israel (Childs et al., 1995; Günther et al., 2000; Radoshitzky and de la Torre, 2019). The main natural reservoir of *Lassa virus* is *M. natalensis* and studies suggest that both vertical and horizontal transmission may play a role for viral maintenance (Demby et al., 2001; Fichet-Calvet et al., 2008). *Lassa virus* transmission from rodent to humans occurs through several ways including consumption of infected food or rodents, inhalation, direct contact with surface contaminated with rodent excreta such as urine, feces and saliva (Childs et al., 1995; Akhmetzhanov et al., 2019). Most *Lassa virus* infections are mild or even asymptomatic (Günther and Lenz, 2004; Radoshitzky and de la Torre, 2019). In endemic areas, transmission from human to human through exposure to contaminated blood or body fluids has been reported, however there are no indications of human to human transmission in cases of imported Lassa fever infections (Günther and Lenz, 2004; Lo Iacono et al., 2015; Radoshitzky and de la Torre, 2019).

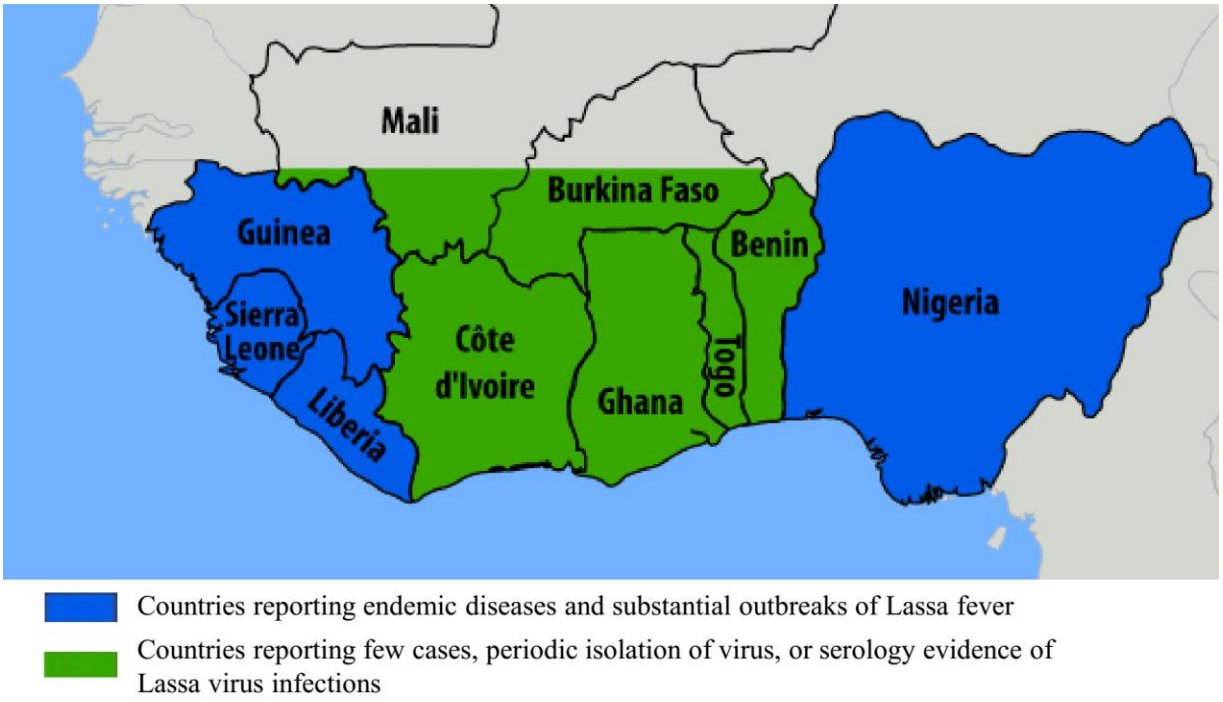


Figure 7: Map of Lassa fever outbreak distribution in west Africa. (https://www.cdc.gov/vhf/lassa/outbreaks/index.html)

Lujo virus

In 2008 a second viral rodent-borne disease caused by *Lujo virus* emerged in southern Africa. An infected patient by *Lujo virus* from Zambia and evacuated to South Africa transmitted the virus to additional four patients. Four out of the five patients died due to hemorrhagic fever (Briese et al., 2009). The *Lujo virus* nosocomial epidemic (Paweska et al., 2009) elucidated the potential of human-to-human mammarenaviruses transmission as described elsewhere with *Lassa virus* by (Monath et al., 1974; Kernéis et al., 2009) but not seen in the imported cases of Lassa fever.

As follow up of *Lujo virus* emergency, studies were carried out in Zambia in order to identify the potential reservoir but neither *Lujo virus* nor resembling viruses were detect in any of the Murinae rodents trapped and tested (Ishii et al., 2011; Ishii et al., 2012). The phylogenetic analysis showed a high degree of divergence between *Lujo virus* and other mammarenaviruses previously described in Zambia (*Luna virus and Lunk virus*), thus authors suspected that *Lujo virus* might have evolved from a different location in Zambia or in a different host. In order to get better inside further

surveys outside of Zambia and/or survey of host animals other than subfamily Murinae would eventually contribute to the identification of the ancestral strain of *Lujo virus* (Ishii et al., 2012).

2.2.3 Transmission ecology

2.2.3.1 Impact of host community structure on mammarenaviruses epidemiology

Disease transmission may be affected by interactions between host ecology, abiotic and biotic factors (Tong et al., 2008; Bordes et al., 2015). Predictions of the impact of global changes on emergence of zoonotic diseases indicated that both environmental and human population growth reacted positively as risk factors for the emergence of mammarenaviruses (Redding et al., 2016).

The relationship between host community structure and levels of pathogens circulation, as the link between host diversity and pathogens transmission, is intensely debated in the scientific community (Keesing et al., 2006; Huang et al., 2016; Levi et al., 2016). Some studies put forward the dilution effect to explain the link between host diversity and pathogens transmission where a higher host diversity is predicted to decrease disease transmission (Ostfeld and Keesing, 2000; Keesing et al., 2009; Young et al., 2013). Keesing et al. (2006) to support the dilution effect discussed several possible mechanisms and suggested that for diseases transmitted by direct contact the presence of non-susceptible hosts capable of cohabiting peacefully will reduce encounters between infected and susceptible individuals of susceptible hosts, thus lowering pathogen transmission. Also, in case the non-competent hosts compete for resources, it may reduce the abundance of individuals of susceptible species through deaths or limiting births thus reducing disease transmission (Keesing et al., 2006). To further support the negative correlation between biodiversity and disease risk it was postulated low species diversity may increase encounters among the susceptible hosts increasing pathogen transmission (Mills, 2006). In the same vein of the dilution effect, it is argued that the dilution effect only occurs under certain circumstances and its assessment need to consider several factors, including the identity effect of particular species, the scale of analyses, and the risk indicator used (Huang et al., 2016). Several rodent-borne diseases such as hantaviruses, Lyme disease and anaplasmosis appear to exhibit a pattern

consistent with a dilution effect (Bordes et al., 2015) and it may also be the case for mammarenaviruses in rodents.

On the other hand, there are studies reporting that the risk of emergence of zoonotic diseases is elevated in tropical forest regions due to a higher mammalian species diversity (Jones et al., 2008; Allen et al., 2017). This was then supported by observation indicating that the diversity of microbes increases proportionally with the biodiversity of their host (Intergovernmental science-policy platform on biodiversity and ecosystem services - IPBES, 2020). *Yersinia pestis* is an example of pathogen transmitted by rodents with reports of a positive relationship between rodent diversity and its occurrence in humans (Bonvicino et al., 2015; Sun et al., 2019). The possible explanation of positive correlation between biodiversity and disease risk could be that less disturbed habitats support a higher diversity of animals capable of harboring a higher variety of animal diseases that could be transmitted to humans (Dunn et al., 2010).

At global scale the habitats with a high biodiversity supports high diversity of potential emerging pathogens. However, within biodiversity hotspots, biodiversity loss induced by human activities increases potential contacts with wildlife and the risk of diseases emergence.

For diseases transmitted by direct contact, among other factors the host density is an important driver of pathogen transmission in a process designated density-dependent transmission. The density dependent hypothesis suggests that a higher host density increases pathogen transmission due to increased direct contact rates between uninfected hosts with infected individuals or materials (Altizer et al., 2006; Kamath et al., 2014; Bordes et al., 2015). Mammarenaviruses transmission occurs via direct contact (Childs et al., 1995), however, a study in Guinea did not find correlation between *M. natalensis* abundance and *Lassa virus* prevalence and they suspected that it was because *M. natalensis* was always more abundant inside houses and did not fluctuate enough to have a significant impact on the *Lassa virus* prevalence (Fichet-Calvet et al., 2007). Similarly, the study carried out in Tanzania where the multimammate *M. natalensis* was more abundant in agricultural fields did not find effect of host density on the occurrence of *Mopeia virus* (Borremans et al., 2011). A recent study that incorporated both personality and population density parameters in the analysis suggested that *Morogoro virus* infections were density dependent

(Vanden Broecke et al., 2019). Mammarenaviruses density-dependent transmission was used to explain the correlation between seasonality of *Morogoro virus* circulation and fluctuation of *M. natalensis* population (Mariën et al., 2020).

2.2.3.2 Impact of host characteristics on mammarenaviruses epidemiology

Juveniles are immunologically naive after having lost their protective maternal antibodies, and more susceptible to a variety of pathogens (Altizer et al., 2006; Peel et al., 2014). High prevalence of *Mopeia virus* in *M. natalensis* juveniles was reported in Tanzania and the authors suggested that it was related to both vertical and horizontal transmissions (Borremans et al., 2011). The hypothesis of very early infection through vertical transmission was supported by the higher proportion of very young individuals (10 to 20 days) positive for *Lassa virus* RNA detection in Guinea (Demby et al., 2001; Fichet-Calvet et al., 2008). These two studies also noticed an increase of prevalence with age within the juveniles' group and the authors indicated that horizontal transmission also played a role on *Lassa virus* transmission in this group.

Females and males display immunological differences and this may contribute to variations in disease susceptibility (Weinstein et al., 1984; Klein and Flanagan, 2016). *Mastomys* males use promiscuous mating strategy (Kennis et al., 2008; Borremans et al., 2014) and this may promote competition between males and possibly increase the risk of disease transmission between them compared to females (Fichet-Calvet et al., 2008). It has been suggested that reproduction costs may lower host immunity in rodents, birds, and humans due to activation of glucocorticoid hormones, particularly testosterone for males and for case of females due to pregnancy, offspring feeding, and care (Festa-Bianchet, 1989; Mougeot et al., 2005).

2.2.3.3 Transmission cycle of mammarenaviruses between rodents and humans

All contributing factors to mammarenaviruses circulation in rodents and presence of virus in other contamination sources such as aerosol, contaminated surfaces or food (Bonwitt et al., 2017; Garry, 2022), as well as those contributing to human exposure, play an important role in rodents and humans transmission cycle. Studies on *Lassa virus* transmission in West Africa indicate that the

ecology of *M. natalensis* is the epicenter of the transmission cycle to humans (Fichet-Calvet et al., 2008). The prevalence of the *Lassa virus* in both rodents and humans fluctuate over the year and more *M. natalensis* are infected during the rainy season (Fichet-Calvet et al., 2007) while cases of Lassa fever in human are higher in the dry season (Shaffer et al., 2014). During the dry season when food resources are scarce in the rodents migrate to inside houses increasing their densities, while during the rainy season the rodents return to the lowering the densities inside houses (Fichet-Calvet et al., 2007; Bangura et al., 2021). The higher rodent densities inside houses during the dry season increases direct contact between humans and infected rodents and aerosols. Furthermore, this high rodent density increases the level of surfaces and food contamination through rodent urine, saliva and feces, thus increasing the risk of human infections (Bonwitt et al., 2017; Garry, 2022).

2.3 AIMS OF THE STUDY

General objectives

Climatic conditions, host community structure, density, sex, age have been demonstrated as determinants for pathogen transmission. Moreover, biodiversity loss induced by human activities increases potential contacts with wildlife and the risk of emergence. This study was implemented to assess the circulation and the drivers of mammarenaviruses at the rodent/human interface to improve our knowledge about the risk of emergence of mammarenaviruses and other rodent-borne infectious diseases in Mozambique.

Specific objectives

- To describe the small mammal's community structure and study the ecology of *M. natalensis* in different habitats.
- To detect and characterize the mammarenaviruses circulating in the study area.
- To assess the ecological factors associated with mammarenaviruses circulation in rodent populations.
- To identify potential routes of transmission of mammarenaviruses from rodents to humans.

To achieve the above objectives this thesis starts with a theoretical chapter reviewing the literature to deepen the background of the current situation of our topic. Then follow a data collection chapter describing the study area and the sampling protocols. Four scientific chapters were set up to develop the research. In the first chapter we described the small mammal community structure and the ecology of *M. natalensis.* The detection and characterization of mammarenaviruses was performed in chapter two. The third chapter was dedicated for assessment of ecological factors associated with mammarenaviruses circulation in rodent populations. In chapter four we performed a sociological study to identify potential routes of mammarenaviruses transmission from rodents to humans. The thesis ends with an overall discussion of the main results obtained over the four chapters.

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3 DATA COLLECTION

3.1 INTRODUCTION

In this chapter we describe the study area, then we present and discuss the trapping protocols. We also describe how the rodents were processed in the field as well as in the laboratory, then we present and discuss the approach used for species identification. The specific materials are described and discussed in each scientific chapter.

3.2 THE STUDY AREA

The study was carried out in the Limpopo National Park (LNP) and its buffer zone. This park along with two other Mozambican national parks (Banhine and Zinave National Parks) form the Mozambican counterpart of the *Great Limpopo Transfrontier Conservation Area* linked to the Kruger National Park in South Africa and the Gonarezhou National Park in Zimbabwe (**Figure 8**).

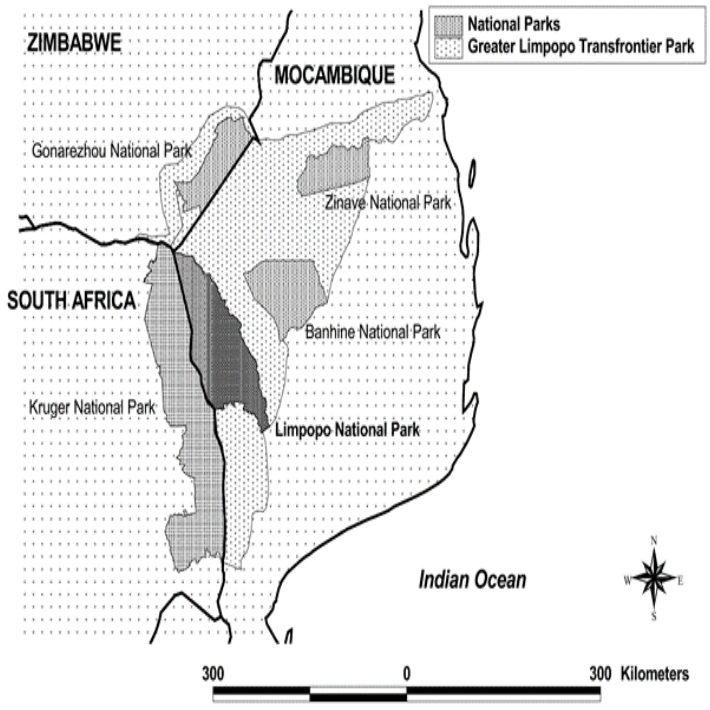

Figure 8: The LNP and the adjacent national parks. (Source: Stalmans et al., 2004)

The LNP is located between latitudes 22º25'S-24º10'S and longitude 31º18'E–32º39'E in Gaza province (**Figure 9 B**), south Mozambique and covers four districts, namely Massingir, Mabalane, Mapai and Chicualacuala (**Figure 9 C**) and our sampling was performed in the Massingir district

counterpart. Inside the LNP there are communities residing in four localities (Mavodze, Bingo, Chimangue, and Machamba). Macavene was a former inside the LNP, but the community that lived there was resettled outside of the LNP. A buffer zone was created to allow communities to access sustainable use of the park's resources to support existing traditional subsistence livelihoods, including agriculture and livestock production. The buffer zone covers 2,349 km^2 corresponding to 20.9% of the LNP (*Plano de Maneio e Desenvolvimento do LNP, 2003*).

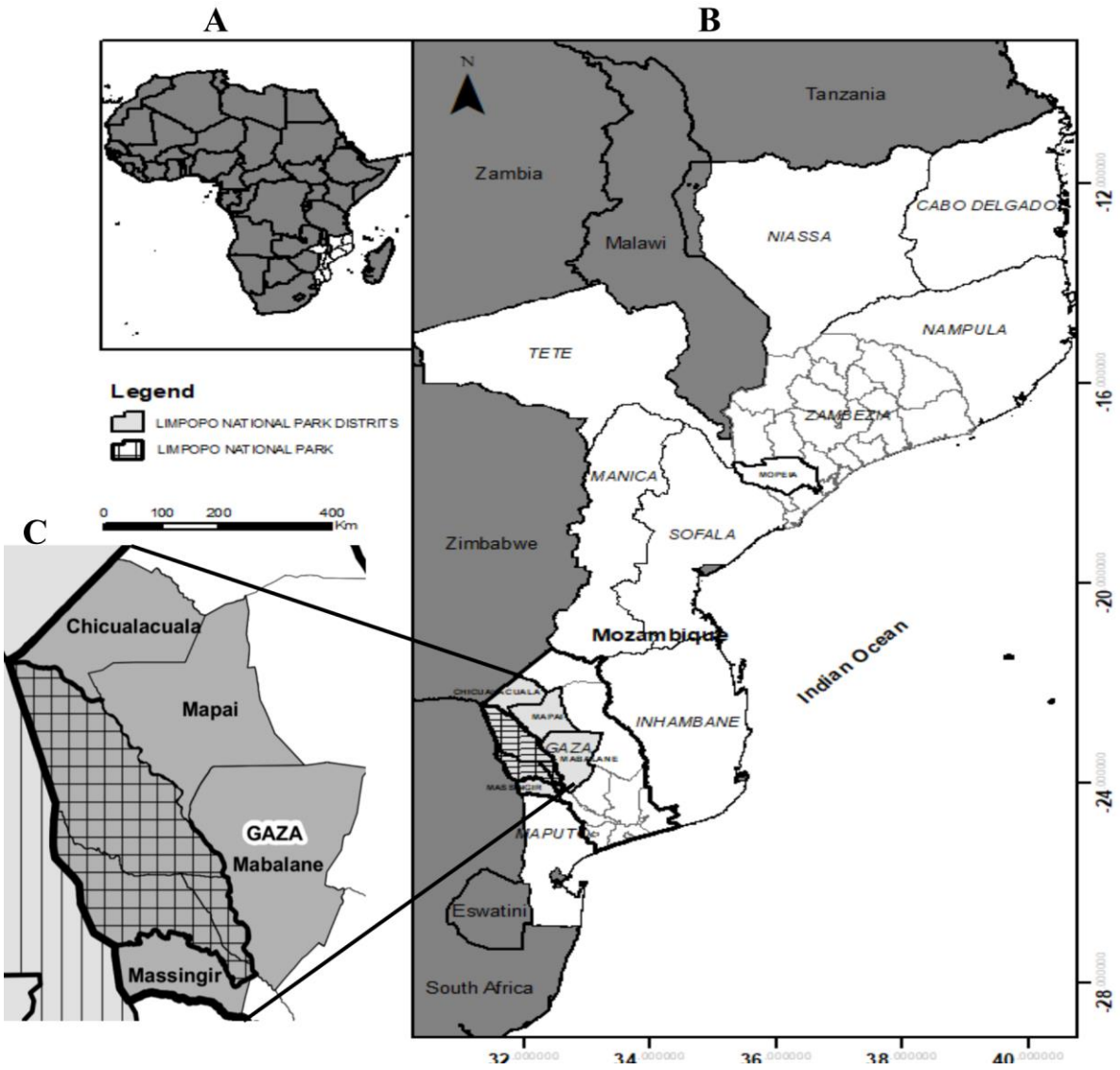

Figure 9: Map of Africa (A) locating Mozambique, national map (B) locating the LNP and zoom of the LNP area (C).

Rodents were trapped in four types of habitats in order to test the influence of the habitat on rodent community composition and mammarenaviruses circulation. The sampling sites in each type of habitat are described in (**Table 5**). Inside the LNP, rodents were trapped in the localities of Bingo, Mavodze, and Macavene while in the buffer zone we trapped in Chibotane, Machavule, and Madingane localities. We sampled inside the LNP and its buffer zone to cover different types of habitats from natural to human habitations. In Bingo, Mavodze, Chibotane, and Madingane we sampled village sites and croplands. In Machavule only the village was sampled while in Macavene we only sampled the mopane woodland (***Figure 10***; **Table 5**).

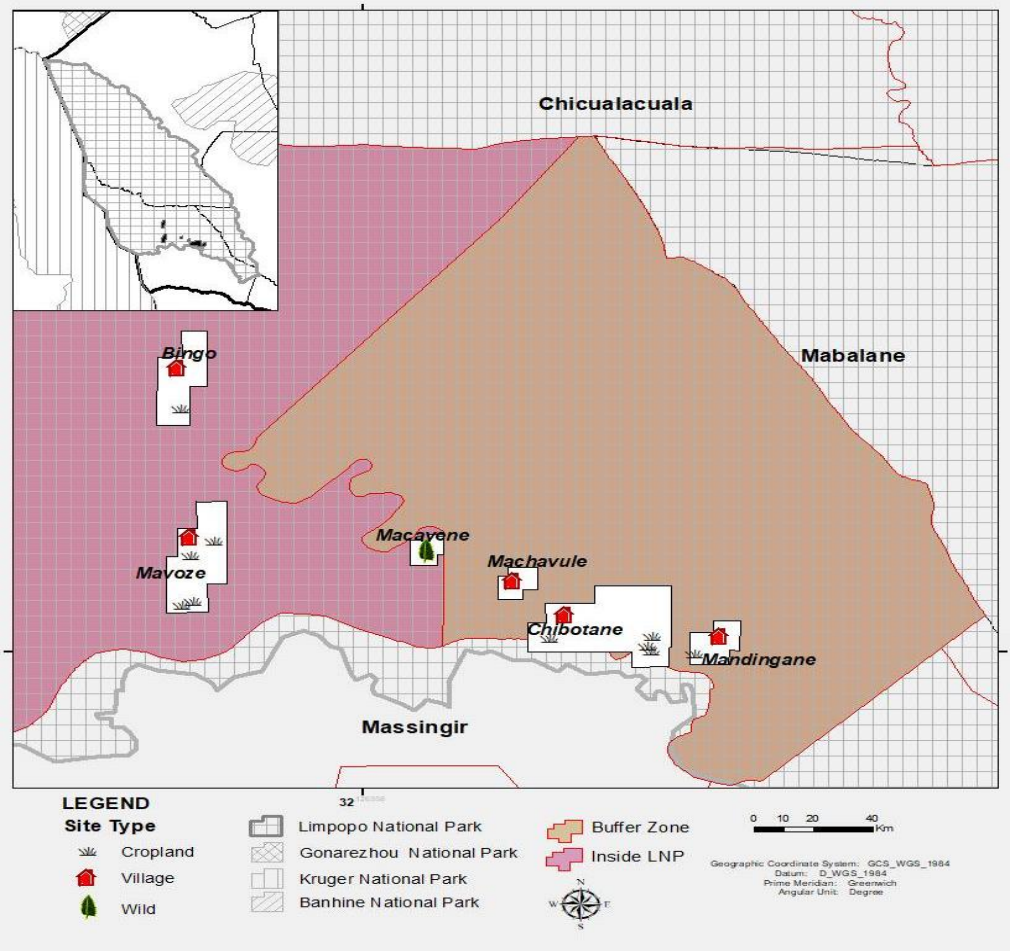

Figure 10: Map of study area showing the type of habitats sample in each locality

The sampled sites were coded by combining two letters from the locality's name, the first letter of habitat type, and a number. We expected to sample more than one site per habitat in each locality

so the number in the site code indicates whether it was the 1st, 2nd, 3rd, 4th or 5th site to be sampled in the locality (**Table 5**).

Table 5: Sampled localities per habitat inside the LNP and its buffer zone. The table also display the names and the codes of sampled sites

Location	Habitat	Locality	Protocol	Site name	Abbreviation
Inside the LNP	Rain fed cropland	Mavodze	CMR*	Mavodze cropland field 1	mzf1
			Removal	Mavodze cropland field 2	mzf2
	Irrigated cropland		Removal	Mavodze cropland field 4	mzf4
			Removal	Mavodze cropland field 5	mzf5
		Bingo	Removal	Bingo cropland field 1	bgf1
	Village	Mavodze	Removal	Mavodze village 1	mzv1
		Bingo	Removal	Bingo village 1	bgv1
	Mopane woodland	Macavene	Removal	Mopane woodland forest	mcw1
Buffer zone	Irrigated cropland	Chibotane	CMR*	Chibotane cropland field 1	cbf1
			Removal	Chibotane cropland field 2	cbf2
			Removal	Chibotane cropland field 3	cbf3
			Removal	Chibotane cropland field 4	cbf4
		Madingane	Removal	Madingane cropland field 1	mgf1
	Village	Chibotane	Removal	Chibotane village 1	cbv1
		Madingane	Removal	Madingane village 1	mgv1
		Machavule	Removal	Machavule village 1	mlv1

*CMR: Capture Mark Recapture

3.3 ABIOTIC CHARACTERIZATION OF THE STUDY AREA

The soils are predominantly sandy in high plains areas but rocky soils are found inside the LNP and wetland clays extracts occur on the slopes of rivers. The main rivers that cross the district are Elephant, Mazimulhpe (with the permanent flow), Chingedzi, Machapane, Benhuca, Zambalala, Chivambalane, Nhamvotso, Nhapombe, and Inhatcozoane (with the temporary flow). There are also 10 lagoons, namely Chileusse, Vele, Dzendzenfu, Inhaphessane, Malopane, Furene, Pumbe, Nhavalungo, Nhatindzau and Namagungo. The climate is subtropical hot dominated by semi-arid dry zones, with average annual temperatures of 30°C and rainfall of 600 mm (*Plano de Maneio e Desenvolvimento do LNP, 2003*; *Plano de Gestão Ambiental e Social do LNP*, 2017).

Mozambique has two seasons: the rainy (hot and humid) between October and April and the dry between May and September (https://www.inam.gov.mz). The precipitation data in the district of Massingir is collected through three rain gauges each located in Mavodze, Massingir headquarters, and Zulo (Source: *Serviços de Planeamento e Infraestruturas de Massingir*). The **Figure 11** presents

the monthly average precipitation from 2000 up to 2017 and separately the amount of precipitation for 2018 and 2019 to display the rainy season during the study. The 2018/2019 rainy season started in October 2018 (8.7 mm) and ceased in April 2019 (57.8 mm). Between May and September 2019, it was the dry season. Thus, the first primary trapping session (PTS) of our study, in March was the end of the rainy season. In this study, PTS correspond to one field trip mission with a maximum duration of 15 days. The PTS2 (in May) up to PTS4 (in August) were conducted during the dry season. The PTS5 implemented in November was already the rainy season.

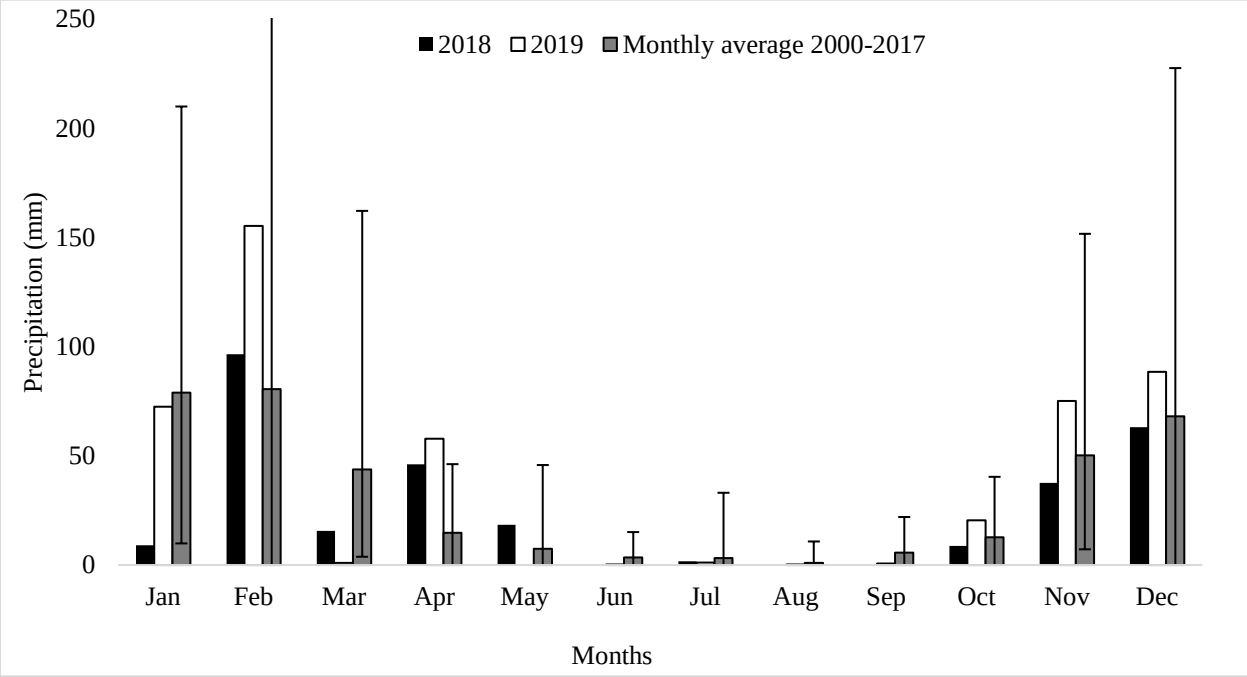

Figure 11: Monthly rainfall in Massingir in 2018, 2019 and the monthly average and its variation between 2000 and 2017.

3.4 DESCRIPTION OF HABITATS

The area supports subsistence farming dominated by rainfed cropping in highlands and small irrigation schemes close to rivers and the Massingir dam. Some farmers practice cropping close to the riverbanks and they were grouped as irrigated croplands in this study. In the irrigated croplands (**Figure 12 A**), they continuously cultivate maize, beans, sweet potatoes, pumpkin, and vegetables in association or intercropping. In the rainfed croplands (**Figure 12 B**) seasonally they grow maize, peanuts, and beans depending on the rain pattern. More specifically, the rainfed cropland site sampled in this study was left post harvesting (the main crop was peanut) from the first PTS up to

the last one. The sampled villages are typically rural areas where houses (**Figure 12 C**) and granaries (**Figure 12 D**) are constructed using local materials such as sticks and grass. In some cases, the houses are reinforced with stones between the sticks or are covered with wet red sand which after drying becomes very hard. The plant community in the mopane woodland is classified as *Colophospermum mopane - Panicum maximum* short woodland (Stalmans et al. 2004) and its main grass species is *Urochloa mossambicensis* (**Figure 12 E**).

Fig.12A: Irrigated croplands

Fig.12B: Rainfed croplands

Fig.12C: Houses

Fig.12D: Granary

Fig.12E: Mopane woodland

Figure 12: Types of habitats sampled during the study: irrigated croplands, rainfed croplands, villages and mopane woodland forest

3.5 TRAPPING PROTOCOLS

The following field protocol was approved by the Ethical Committee of VetAgro-Sup in Lyon, France (*Comité d'Ethique* de VetAgro Sup n°18, Avis 1905; Annex-I) and the credential for rodent capture in the LNP was obtained from the Mozambican National Administration for Conservation Areas (Credential Nr. 1/02/2021; Annex-II).

The fieldwork was implemented to cover the two main periods of the year, the rainy (from October to April) and the dry (from May to September). Five PTSs were implemented each in March (PTS1), May (PTS2), June (PTS3), August (PTS4), and November (PTS). Due to practical constraints, we mostly sampled the dry season. Two types of sites were included during this study: Capture Mark Recapture (CMR) and removal sites. The CMR sites were set to collect longitudinal data from the same population so we performed one capture session during each PTS. Rodents captured at the CMR sites were marked and released for potential recapture. Removal sites were

set for rodent diversity study and mammarenaviruses screening so they were sampled in one or two PTS and all rodents captured were sacrificed. The trapped sites were selected based on the information collected from the local population about the presence of rodents and we also checked signs of their presence (destroyed crops, holes, feces) before setting the traps (**Figure 13A**).

3.6 FIELD PROTOCOL FOR CMR SITES

Large and medium live-catch traps Sherman (H.B. Sherman, Inc., Tallahassee, FL, USA; **Figure 13C**)) baited with roasted coconut, oats, and peanut butter were deployed in an intercalated way, each medium trap followed by the small one. In both mzf1 and cbf1 sites, we set grids of 10m between the trap stations (**Figure 13B**) and after the initial setup, the trapping stations were marked with sticks for easy identification on the following PTSs. Grids were rectangular where rows and columns were respectively identified with letters and numbers, which allowed the identification of trapping stations with a combination of column numbers and row letters (**Figure 13C**).

The site mzf1 had 0.86 ha and rodents were trapped for three consecutive secondary trapping session (STSs) over the five PTSs with a constant number of traps (n=327), except when traps were stolen by the locals. Here, STSs it the number of nights the traps we left opened in one site. The site cbf1 had 1.21 ha and the number of traps (minimum=60, maximum=144) and the STSs (three to five days) were not constant. The number of traps and STS were adjusted during some PTSs due to the high number of captures which consequently increased the waiting time for processing and then rodent deaths. The traps were set up at the end of afternoon and checked once a day earlier in the morning. The positive traps (trap that captured at least one rodent) were replaced by others coded with the same trap identification (**Figure 13D**).

Fig. 13 A: Rodent demage (eaten peanuts)

Fig. 13 B: Setting trapping stations

Fig. 13 C: Armed trap identified with trapping station code

Fig. 13 D: Trap check and replacement

Figure 13: Inspecting signals of rodent presence, demarcation of trap station, trap setting, check and replacement

The rodents trapped in the CMR sites were processed in the field in the shade (**Figure 14 A**) the same day of capture and they were fed with a small piece of apple or tomato to avoid dehydration while waiting for processing. Only rodents morphologically identified as *Mastomys* were inserted with transponders. During the sampling, we grouped *Mastomys* individuals as adults (>20g) and juveniles (≤ 20g) (Leirs et al. 1989). Rodents weighing < 20g were not marked due to the difficulties to manipulate them and we were unsure if they will survive after inserting the

transponder (Kirkland, 1998; Smyth and Nebel, 2013). Rodents weighing ≥20 g were marked using a subcutaneous transponder (**Figure 14 B**) and the following samples were collected: a small piece of ear and tail, an oral swab, and dried blood spots (**Figure 14 C; D**).

A dried blood spot was obtained using a Whatman® protein saver card 903 x 100 after cutting a piece of tail (**Figure 14 D**). A piece of ear and oral swab were collected also from some juvenile individuals randomly selected. Each rodent processed was given an identification number (rodent ID) used to code the collected samples and the rodent form (Annex-III). Rodent data, i.e., transponder number, trapping station, weight, sex, and reproductive condition was documented in the rodent form. Because of a high number of captures during some PTSs, it was not possible to collect individual data of all rodents trapped, so for some rodents, we just checked if they were a recapture or not. However, each day we recorded the numbers of these non-processed rodents, but because individual data was not collected, they were not included in our main database. Finally, all the rodents were released back to the same trap station in the grid where they were caught (**Figure 14 E**). Rodents recaptured (**Figure 14 F**) on following STS of the same PTS were just recorded on the recapture form (Annex-IV). During the subsequent PTSs, the rodents were processed as explained above except that the recaptures were neither marked nor sampled for ear and tail but they were weighed and sampled for a dried blood spot from tail.

Fig. 14 A: Taking out the rodent from the trap

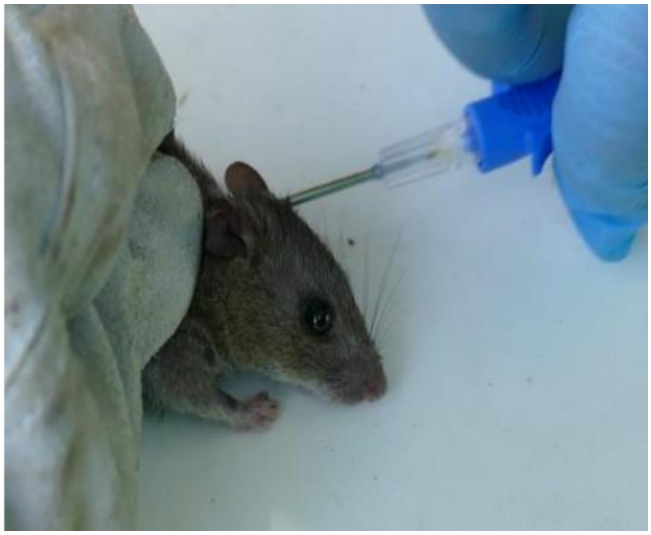

Fig. 14 B: Inserting the transponder under the skin of M. natanesis

Fig. 14 C: Sampling oral swab sample from M. natalensis

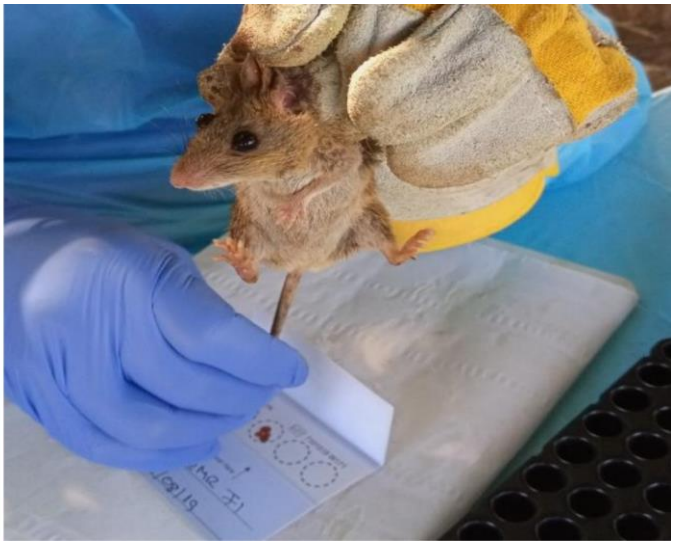

Fig. 14 D: Sampling dried blood spot sample from M. natalensis

Fig. 14 E: Releasing M. natalensis at the trap station

Fig. 14 F: Transponder checking from recaptured M. natalensis

Figure 14: Rodent processing in the CMR site

3.7 FIELD PROTOCOL FOR REMOVAL SITES

In villages, the rodents were trapped inside the houses and granaries using medium Sherman traps (baited as in the CMR) and Mesh traps (Manufacturer Directly Metal Trap Rat Cage Reusable Mouse Trap for Live Catch and Release) baited with chorizo (***Figure 15***). The ratio between Mesh and Sherman traps varied between 1:1 and 1:3 depending on the size of the house or granary. In the croplands (irrigated and rainfed) and the mopane woodland, the type of traps, baiting, grid

demarcation, traps inspection, and replacement process was as described in the CMR sites. The numbers of traps and STSs were adjusted according to the field, house, granary sizes, and capacity to deal with the number of rodents trapped (Kirkland, 1998). The bigger the size of the field, granary, or house, the more traps were deployed. Because of time constraints, most of the time we set up the traps on two sites at the same time, and depending on the number of captures versus our processing capacity in the laboratory, we decided to stop or go for more STS.

Due to logistics to ensure security personnel for protection against possible attack by wild animals (such as elephants and lions), a single mopane woodland forest was sampled four times inside the LNP. As in the CMR sites, traps in the removal sites were set up at the end of the afternoon and inspected the following morning. All rodents trapped in the removal sites were transported and processed within 48h in the local laboratory located at the headquarters of the LNP. They were fed the same way as in the CMR sites while waiting for processing. The rodents were euthanized with isoflurane, dissected, and sampled for lung, heart, liver, spleen, kidney, and brain puncture in addition to the samples collected in the CMR sites (**Figure 16 A; B**). The tail and ear were conserved in 70% ethanol and the other tissue samples and the oral swabs were preserved in home made RNAlater solution (http://www.protocol-online.org/prot/Protocols/RNAlater-3999.html).

A site form (Annex-V) was used to collect data such as geographical coordinates, number of traps deployed each date, number of positive traps, vegetation, etc.

Figure 15: Sherman (A) and mesh (B) traps

Fig. 16 A: Oral swab collection from euthanized M. natalensis

Fig. 16 B: M. natalensis dissection

Figure 16: M. natalenis processing in the laboratory

3.8 TRAPPING EFFORT AND RESULTS

The efficiency and precision of studies on population diversity may be influenced by low trapping effort due to constraints on sampling time, available personnel, and resources (Tull and Sears, 2007). Thus, in ecological studies, especially when performing temporal and special comparisons it is important to assess how the differences in trapping effort could potentially affect the interpretation of results.

Here we displayed the trapping effort over the different habitats and PTSs. A total of 7290 traps in 16 sites were deployed during the study. The sampling was performed using removal (n=14 sites) and CMR (n=2 sites) protocols. A total of 1997 traps were successful. The irrigated cropland with the highest number of trap nights (n=3331) had the highest number of successful traps (n=1679). The lowest number of traps (n=1100) were set in both mopane woodland and villages and the former habitat had the lowest number of successful traps (n=13). The highest number of captures was observed in the irrigated cropland (n=1762) and the lowest was in the mopane woodland (n=13) (Table 6). In the irrigated cropland we had multiple captures so the number of captures is superior than the number of successful traps.

Table 6: Trapping effort in different habitats in the LNP and its buffer zone

Habitat	Nr. of sites	STSs	Trap nights	Nr. of successful traps	Nr. of captures
Irrigated cropland*	8	34	3331	1679	1762
Rain fed cropland*	2	18	1759	110	110
Villages	5	18	1100	195	195
Mopane woodland	1	11	1100	13	13
Total	**16**	**81**	**7290**	**1997**	**2094**

*: The number of captures in the irrigated and rainfed croplands includes the recaptures from the CMR sites

The trapping effort was not homogeneous over the different sites and habitats (Annex-VI) and it was determined by practical imperatives. More irrigated croplands were sampled to get a significant number of *M. natalensis* tissue samples for mammarenaviruses screening. Due to funding limitations two weeks were allocated for each PTS and they were not sufficient to cover

many sites from all four habitats. Thus, the data obtained from the removal sites might not be suitable for analysis of some aspects of rodent ecology.

3.9 SMALL MAMMAL'S SPECIES IDENTIFICATION

3.9.1 Rodent morphological identification

In the CMR sites rodents were morphologically identified in the field while the small mammals trapped in the removal sites were initially identified in the laboratory during the necropsy. Based on the morphological characteristics (Herbreteau et al., 2011) we identified seven rodent species, among them *Gerbilliscus leucogaster*, *Mastomys natalensis*, *Rattus rattus*, *Rattus norvegicus*, *Saccostomus campestris, Mus musculus and Grammomys dolichurus* and *Macroscelide sp.*. Also, we forgot to record the species name for some rodents during the field work. Because species in some African rodent genera are difficult to recognize and the taxonomy of many groups is still not well resolved (Monadjem et al., 2015) we relayed on genotyping to confirm the species identification.

3.9.2 Rodent molecular identification

The molecular identification was done by partner laboratories at the Biology Center for Population Management (CBGP), France and at the Institute of Vertebrate Biology, Czech Republic. In both laboratories DNA barcoding approach was used to amplify and sequence (by the Sanger method) the complete mitochondrial cytochrome b gene (CYTB; 1140 bp) (Lecompte et al., 2005). The number of samples accepted by each partner laboratory depended on their interest in the rodent species and the availability of resources to carry out the tests. The CBGP was interested in helping with barcoding all rodents morphologically identified as *Rattus rattus* (n=134) and *Rattus norvegicus* (n=6). The Institute of Vertebrate Biology kindly allocated resources to assist with genotyping all rodents we missrecorded the species in the field (n=44), all *Mus musculus* (n=40), all *Grammomys dolichurus* (n=4) and limited number of *Gerbilliscus leucogaster* (n=5) and *Mastomys natalensis* (n=60).

We selected some *M. natalensis* and *Gerbilliscus leucogaster* over trapping sites and sessions and sent them for genotyping. *M. natalensis* were grouped into heavy (≥40g) and light (<40g) individuals and then selected 30 representants in each group. We selected 6% (49/880) in the irrigated cropland, 8% (4/48) in the rain fed cropland field, 50% (3/6) in the mopane woodland forest and 50% (4/8) in villages. Individuals were selected from all trapping sites and we picked between 1 and 8 representants per site according to the total number of individuals captured. *Gerbilliscus leucogaster* was caught in two sites, namely mcw1 (n=1) and mzf1 (n=10). For genotyping, we included the unique individual from the mcw1 site and four individuals from the mzf1 site (one individual in each PTS; *Gerbilliscus leucogaster* was not present in PTS1).

3.9.3 Morphological versus molecular identifications

The morphological identification was wrong with three species, namely *Mus musculus* (n=40), *Grammomys dolichurus* (n=4*)* and *Rattus norvegicus* (n=6) which the correct species names should be *M. natalensis*, *Thallomys paedulcus* and *Rattus rattus,* respectively. The second error was related to morphological identification of *Rattus rattus* where we failed to identify 14.9% (20/134) of individuals whose correct names should be *Rattus tanezumi* (n=17) and *M. natalensis* (n=4). The third error were the two *Aethomys ineptus* we identified as *M. natalensis* in the field giving an error of 3.3% (***Table 7***).

Table 7: Morphological and molecular identification of small mammals captured in the LNP and its buffer zone

Morphological identification	Nr. of captures	Nr. of individuals genotyped	Molecular identification			
			Incorrect	error (%)	Species	Nr. of individuals
Mus musculus	40	40	40	100	*M. natalensis*	40
Gerbilliscus leucogaster	11	5	0	0	*Gerbilliscus leucogaster*	5
Grammomys dolichurus	4	4	4	100	*Thallomys paedulcus*	4
M. natalensis	942	60	2	3.3	*M. natalensis*	58
					Aethomys ineptus	2
Rattus rattus	135	135	20	14.8	*Rattus rattus*	114
					Rattus tanezumi	17
					M. natalensis	4
Rattus norvegicus	6	6	6	100	*Rattus rattus*	6
Forgot to record identification in the field	44	44	Not applicable		*M. natalensis*	36
					Rattus rattus	7
					Saccostomus campestris	1
Saccostomus campestris	52	0				
Macroscelide sp.	1	0				
Total	**1235**	**294**				

Not all *Saccostomus campestris, Gerbilliscus leucogaster* and *M. natalensis* individuals were genotyped. We did not genotyped *Saccostomus campestris* due to limited resources and also because we were confident with the morphological identification particularly based on its short tail (Mikula et al., 2016). All *Gerbilliscus leucogaster* (n=5) genotyped were morphologically identified correctly, so we feel more confident that all individuals were correctly identified. All specimens of *Thallomys paedulcus* and of both species of genus *Rattus* were genotyped so there is no uncertainty about the identification of these three rodent species (***Table 8***).

Table 8: Number of individuals genotyped in each species

Species	Nr. of captures	Individuals genotyped	
		Number	%
Saccostomus campestris	53	1	1.9
Aethomys ineptus	2	2	100.0
Gerbilliscus leucogaster	11	5	45.5
Macroscelide sp.	1	0	0.0
M. natalensis	1020	138	13.5
Thallomys paedulcus	4	4	100.0
Rattus rattus	127	127	100.0
Rattus tanezumi	17	17	100.0
Total	**1235**	**294**	**23.8**

In total 13.5% (138/1020) of *M. natalensis* trapped during the whole study were genotyped (**Table 8**). All rodents trapped in villages were genotyped except four of them morphologically identified as *M. natalensis*, meaning that in villages we may have not correctly identified four individuals. The two *M. natalensis* genetically confirmed to be *Aethomys ineptus* were captured in the same rain fed cropland field (mzf1) during the same PTS. The site mzf1 rainfed cropland field, was left post harvesting all over the sampling period, located inside the LNP and surrounded by bush. Furthermore, the two *Aethomys ineptus* were caught by traps set up at the edges of the field. The identification of the 56 *M. natalensis* trapped in the irrigated cropland were all genetically confirmed to be correct. Given that *Aethomys ineptus* prefer natural habitats (Chimimba and Linzey, 2008), we think that the mopane woodland is the other site where we likely have misidentified *Aethomys ineptus*. The number of *M. natalensis* captured in the sites mzf1 (n=46) and mcw1 (n=6) corresponded to 5% out of 1020 *M. natalensis* trapped during the study (***Table 9***). Therefore, we think that other possible misidentifications, might be limited only to *M. natalensis* and we guess that the percentage should be lower than 5% and most likely limited to the mzf1 and mcw1 sites.

Table 9: Number of M. natalensis genotyped in each site across the four habitats

Habitat	Site ID	Nr. of individuals captured	Nr. of individuals not genotyped	Individuals genotyped	
				Number	%
irrigated croplands	bgf1	79	69	10	12.7
	cbf1	478	453	25	5.2
	cbf2	64	56	8	12.5
	cbf3	24	20	4	16.7
	cbf4	8	4	4	50.0
	mgf1	63	51	12	19.0
	mzf4	120	107	13	10.8
	mzf5	81	71	10	12.3
Rain fed croplands	mzf1	46	44	2	4.3
Villages	bgv1	12	0	12	100.0
	cbv1	20	4	16	80.0
	mgv1	9	0	9	100.0
	mlv1	4	0	4	100.0
	mzv1	6	0	6	100.0
Mopane woodland	mcw1	6	3	3	50.0
Total		**1020**	**882**	**138**	**13.5**

3.9.4 Conclusions

The most probable error in the identification of all species captured during our study may be the presence of *Aethomys ineptus* identified as *M. natalensis*. Therefore, it is important to barcode all individuals identified morphologically as *M. natalensis* in the rainfed cropland and in the mopane woodland as they are part of the preferred habitats for *Aethomys ineptus.* All rodents trapped in the villages were genotyped (except four) so these all remaining can be easly genotyped. However, the number of *M. natalensis* not genotyped in the irrigated cropland is large, thus, we suggest barcoding all individuals tested positive on *Mopeia virus* screening and in the future, all individuals' positive on other pathogens' screening should be genotyped.

3.10 REFERENCES

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4 CHAPTER-I: SMALL-MAMMAL COMMUNITY STRUCTURE AND *MASTOMYS NATALENSIS* POPULATION DYNAMIC IN DIFFERENT HABITATS IN THE LIMPOPO NATIONAL PARK AND ITS BUFFER ZONE

4.1 SMALL-MAMMALS' COMMUNITY STRUCTURE, DIVERSITY AND ABUNDANCE IN DIFFERENT HABITATS

4.1.1 Introduction

Host community structure may drive the transmission of EIDs among the reservoirs and transmission to humans (Tong et al., 2008; Bordes et al., 2015). Thus, better understanding of host community is needed to better understand the transmission ecology of pathogens. The hypothesis behind the mechanisms by which host community structure influences pathogen transmission include both negative and positive effects of host biodiversity on diseases risk transmission (see § 2.2.3). Moreover, to efficiently tackle multi-host pathogens EIDs that involve at least one free living wild animal it is important to integrate wildlife community structure studies to understand the dynamics of host-pathogen interactions (Portier et al., 2019). Mammarenaviruses are multi-host pathogens EIDs whose the wildlife hosts are rodents (Radoshitzky et al., 2019).

Mammarenaviruses transmission occurs via direct contact (Childs et al., 1995). For diseases transmitted by direct contact, density-dependent is an important driver of pathogen transmission (see § 2.2.3). Mammarenaviruses density-dependent transmission was used to explain the correlation between seasonality of *Morogoro virus* circulation and fluctuation of *M. natalensis* population (Mariën et al., 2020).

Rodent species composition and abundance may vary according to habitat type (Odhiambo et al., 2006; Chidodo et al., 2020). Natural habitats have a variety of resources and ecological niches to support high rodent diversity contrary to disturbed ecosystems where the lesser variety of resources will limit the diversity of residing species (Monadjem, 1999; Vera y Conde and Rocha, 2006; Agerie and Afework, 2015). Natural factors and human activities such as urbanization and agriculture are responsible for the disturbance of ecosystems (Massawe et al., 2005; Taylor et al., 2012; Ssuuna et al., 2020).

In this section we described the small-mammal's community structure, diversity and abundance in the cropland, villages and the mopane woodland in our study area. This information will be used

later to assess the relation between host diversity and abundance with mammarenaviruses circulation. We hypothesized that small-mammal diversity was high in natural habitats compared to disturbed ones and human settlements because natural habitats have a variety of resources and ecological niches to support a wide range of species contrary to disturbed ecosystems. We also expected a higher small-mammals' abundance in agricultural because of food availability in quantity to support a higher number of individuals.

4.1.2 Material and methods

For purposes of description and analysis, we define the small-mammal community as all the small-mammal species that can be trapped from different habitats in the study area (Odhiambo et al., 2006; Ssuuna et al., 2020). The species composition in different habitats was assessed using species richness which is the number of species trapped in a community or defined geographic unit (Magurran, 2003; Begon and Townsend, 2021:574). Thus, the species richness in each habitat corresponds to the number of species trapped in each habitat while for the whole study area we considered all species trapped during the study.

The use of species richness to describe species composition has the disadvantage that the number of species recorded depends on the sampling (duration, size and representativeness, area of habitat studied (Fleishman et al., 2006). Moreover, it does not consider the possibility of some species being more common while others are rare (Fleishman et al., 2006). On the other hand, the diversity indices incorporate richness, commonness, and rarity (Begon and Townsend, 2021:574).

We used Simpson's Diversity Index (SDI) to evaluate diversity between habitats. The Simpson's Diversity Index takes into account the number of species present and the abundance of each species. The formula $D = \Sigma\, n(n-1) / N(N-1)$ was used to calculate Simpson's index (D) and the formula $1-D$ was applied to obtain SDI (Simpson, 1949; Whittaker, 1972; Begon and Townsend, 2021:574). In the above formula, n is the total number of individuals trapped of a particular species and N corresponds to the total number of individuals trapped of all species in one habitat. The

bigger the value of *1-D,* the greater the diversity. A t-test (Makundi et al., 2010; Mayamba et al., 2019) was used to compare the differences of Simpsons Diversity Indices between habitats.

Different methods are used as estimator of rodent abundance. Initially, we set up CMR sites to obtain accurate estimation of population size by using CMR analysis programs. However, due to time constraints the student did not have sufficient time to learn CMR analysis programs. The number of captures relative to the trapping effort (**number of captures / trap nights x100**) was used as an estimator of population abundance (Granjon et al., 2005). Here we used relative abundance as estimator because the trapping effort was not homogeneous over the sites and PTSs. We thought that considering the trapping effort would allow a fair comparison between the habitats. For the purposes of comparing our estimator method, we also calculated the trapping success in the different habitats and the trends were similar (Annex-VIII). The χ^2 test for independence was performed to evaluate differences of relative abundance of small-mammal and of *M. natalensis* between habitats.

4.1.3 Results

With 7290 trap nights in irrigated cropland (n=3331), rainfed cropland (n=1759) villages (n=1100) and mopane woodland forest (n=1100), a total of 1235 small-mammals were trapped. The small-mammal species richness in the study area included seven rodent species, namely *Aethomys ineptus*, *Gerbilliscus leucogaster*, *M. natalensis*, *Rattus rattus*, *Rattus tanezumi*, *Saccostomus campestris*, *Thallomys paedulcus*, and an *Elephant shrew*. Overall, the irrigated cropland with two rodent species had low species richness (n=2) and the lowest Simpson Diversity Index (0.09). The other three habitats had the same species richness (n=4), however, the Simpson Diversity Index indicated that the mopane woodland with 0.68 had a relatively higher species diversity (**Table 10**). The Simpsons Diversity Index differed significantly between the four habitats (t=3.4; DF=3; *p*=0001), however, the differences were not significant among the three habitats with four species each (t=6.0; DF=2; *p*=0.0).

The highest abundance of rodents was recorded in the irrigated cropland 28.9% (n=961) and the lowest in the mopane woodland 1.2% (n=13) (**Table 10**). The differences of small-mammal's relative abundance observed between the habitats were significant (χ^2=755.9; DF=3; p <0.0001). *M. natalensis* was trapped from the four habitats and in overall was the most abundant rodent species 14.0% (n=1020) and over the sampled habitats (except in villages) (**Table 10**). The differences of *M. natalensis* relative abundance observed between the habitats were significant (χ^2=941.7; DF=3; p <0.0001). *Rattus rattus* was only trapped in villages where it was the most abundant rodent species 11.5% (n=127). *Rattus tanezumi* 1.5% (n=17) and *Thallomys paedulcus* 0.4% (n=4) were only present in villages. *Saccostomus campestris* were trapped from all habitats except in villages. *Aethomys ineptus* 0.1% (n=2) was caught only in the rainfed cropland field while *Gerbilliscus leucogaster* was found in both rainfed cropland 0.6% (n=10) and mopane woodland 1.0% (n=1). *Elephant shrew* 0.1% (n=1) was caught in the mopane woodland (**Table 10**).

Table 10: Small-mammal's community structure and relative abundance (RA) over four different habitats in our study area. n = the number of individuals trapped

Species	irrigated cropland n (RA)	Rainfed cropland n (RA)	Mopane woodland n (RA)	Villages n (RA)	Overall n (RA)
Aethomys ineptus	/	2 (0.1)	/	/	2 (0.0)
Gerbilliscus leucogaster	/	10 (0.6)	1 (1.0)	/	11 (0.2)
Mastomys natalensis	917 (27.5)	46 (2.6)	6 (0.5)	51 (4.6)	1020 (14.0)
Rattus rattus	/	/	/	127 (11.5)	127 (1.7)
Rattus tanezumi	/	/	/	17 (1.5)	17 (0.2)
Saccostomus campestris	44 (1.3)	4 (0.2)	5 (0.5)	/	53 (0.7)
Thallomys paedulcus	/	/	/	4 (0.4)	4 (0.1)
Elephant shrew	/	/	1 (0.1)	/	1 (0.0)
Total number captured	961 (28.9)	62 (3.5)	13 (1.2)	199 (18.1)	1235 (16.9)
Total trap nights	3331	1759	1100	1100	7290
Number of sites	8	2	1	5	
Species richness	2	4	4	4	
Simpson Diversity Index	0.09	0.43	0.67	0.45	

4.1.4 Discussion

The small-mammal community structure composed by eight species that was observed over the four habitats sampled during our study differed from the ones reported by previous studies in Mozambique. In our study we observed two out of eight rodent species recorded in a study which sampled only a dry savanna in Tete province, (Gliwicz, 1985). We trapped all the three rodent species recorded in villages, irrigated cropland and rainfed cropland field in Zambézia province (Belmain, 2009) and three out of ten rodent species recorded in villages and cropland in Tete, Zambézia and Maputo provinces (Leirs, 2006:22). In our study, more habitats were sampled than in these three other studies. Similar to our findings, a study conducted in Tanzania, Namibia and Swaziland recorded differences of species composition and the possible explanation given was habitat differences, heterogeneity and complexity between the geographical areas (Massawe et al., 2011).

The main difference of species diversity was between the irrigated cropland and the other three habitats. Similar to our findings a low species richness (n=1 spp), was recorded by a previous study in Mozambique from a paprika irrigated field (Belmain, 2009). Similarly to our findings, in Tanzania, species richness in the irrigated rice (n=3 spp) was lower than in the fallow land (n=5 spp) (Mulungu et al., 2012) and highest in agro-forestry habitats (n=6 spp) than in the extensively cultivated (n=1 spp) (Makundi et al., 2005). In Tanzania, like in our study, the Simpson Diversity Index was close between the rainfed cropland field (0.49) and the woodland (0.53), however the species richness was higher in the woodland (n=10 spp) than in the rainfed cropland (n=5 spp) (Makundi et al., 2010). Agriculture is one of the major disturbing factor of the ecosystem and the timing and intensity of cropping may affect the species richness (Massawe et al., 2005; Willig and Presley, 2018). The difference of species diversity we observed here between the irrigated cropland and the rainfed cropland may be explained by a continuo cropping system in the irrigated cropland, contrary to the rainfed cropland left post-harvest over our study. Non-disturbed habitats have a high diversity of plants and another source of food for small-mammals translated into increased food alternatives that attract animals of varied diets, leading to increased species richness (Leis et

al., 2008; Ssuuna et al., 2020). In our study this hypothesis could support the differences between the irrigated cropland with the mopane woodland forest and the rainfed cropland.

M. natalensis was present in all studied habitats. Like in our study, *M. natalensis* was reported to be an important member of the rodent community in a variety of habitats such as forest, agro-forestry, savanna grasslands, woodlands, cultivated and fallow land in Tanzania, Zimbabwe, Namibia, Eswatini, Ethiopia and Uganda (Linzey and Kesner, 1997; Odhiambo et al., 2006; Massawe et al., 2011, 2012; Agerie and Afework, 2015; Mayamba et al., 2019). *M. natalensis* is an omnivore feeding on grass stems, seeds, rhizomes, and insects and this foraging behaviour could facilitate its presence in a range of habitats (Coetzee, 1975.; Monadjem et al., 2015).

M. natalensis was the most abundant rodent species in our study area, especially in the irrigated cropland. This is not surprising because a higher abundance of this rodent species has been recorded in cultivated fields throughout Sub-Saharan Africa (Makundi et al., 2006; Monadjem et al., 2015; Lema and Magige, 2018; Ssuuna et al., 2020). The high abundance of *M. natalensis* had been attributed to its high breeding rate due to large litter size, short interval between litters, earlier sexual maturation, the length of the breeding season and availability of resources in this habitat (Coetzee, 1975; Leirs 1994: 76-78; Agerie and Afework, 2015).

The exclusive presence of *Rattus rattus* in village sites (inside houses and granaries) is not suprising because it is well known to reside inside human dwellings in sub-Saharan Africa (Bastos et al., 2011; Monadjem et al., 2015). A previous study in central Mozambique also observed a high abundance of this commensal rodent species inside houses (Belmain, 2009). However, other authors reported accidental records in rice 0.08% (n=1) and in fallow land 0.11% (n=2) in Tanzania (Mulungu et al., 2013), in rice in Madagascar (Rahelinirina et al., 2010) and peri-domestic areas in Mozambique (Belmain, 2009) and in Tanzania (Taylor et al., 2012). In our study the nearest site to the village was the rainfed cropland and was located far more than 3 km from the residences so it was not a surprise that we did not capture *Rattus rattus* outside villages.

Rattus tanezumi was trapped only in Bingo village in sympatric occurrence with *Rattus rattus* and *M. natalensis*. Across the villages, the trapping effort in Bingo did not differ much from

Madingane, while a relatively higher number of traps were set up in Mavodze and Machavule so we think that the probability of missing detection of *Rattus tanezumi* in other villages than Bingo was low. The absence of *Rattus tanezumi* in other villages sampled during our study suggests a restricted introduction of this species in the village of Bingo. To our knowledge, previous studies carried out in villages in Mozambique never reported occurrence of *Rattus tanezumi* (Leirs, 2006; Belmain, 2009). However, because of the morphological similarity between *Rattus rattus* and *Rattus tanezumi*, probably there was misidentification as genotyping was not performe. *Rattus tanezumi* is endemic to south-east Asia (Stuart et al., 2015) and in southern Africa, it was recorded in human settlements in Zambia, South Africa and Eswatini "former Swaziland" (Bastos et al., 2011; Nakayama et al., 2012; Taylor et al., 2012; Nakamura et al., 2013). One of the South African provinces where Bastos et al. (2011) recorded *Rattus tanezumi* is the Limpopo province bordering with Gaza province where lays our study area. Between Gaza and Limpopo provinces there is the border post of Giriyondo which registers migratory movements to both directions, but mainly of tourists from South Africa while Mozambicans, mainly from Gaza cross the border for work or commercial purposes. It is more likely that this movement was responsible for the introduction of *Rattus tanezumi* in Bingo from the nearest Limpopo province. To our knowledge, this is the first report of the occurrence of *Rattus tanezumi* in Mozambique.

The presence of *Thallomys paedulcus* we reported in villages contrasts with its preferred savanna habitat of where it builds nests under the frayed bark of acacia tree trunks and branches or in holes in the ground between the roots of the tree (Graaff, 1978; Linzey and Kesner, 1997). Indeed, *Acacia xanthophloea* occurs in the study area and the four *Thallomys paedulcus* were trapped in granaries so possibly they went there in search of food. As we mentioned above rodent movement from field to humans' settlements when food resources are scarce in fields is a common feature of *M. natalensis* in West Africa (Fichet-Calvet et al., 2007). *Thallomys paedulcus* feeds on *Acacia* seeds and leaflets but occasionally eats insects (Graaff, 1978; Taylor et al., 1995). The occurrence of *Thallomys paedulcus* in low densities as we observed in our study in villages was reported in the mopane woodland and miombo forests in Zimbabwe (Linzey and Kesner, 1997). Given what we know about this species these captures were unusual.

The two *Aethomys ineptus* were trapped from a rainfed cropland field left post-harvesting during the sampling period and this field was located in the middle of the mopane woodland. *Aethomys ineptus* despite feeding on insects is classified as unspecialized herbivores that occur in grassland to woodland savannas and it is found in low to moderate densities (Chimimba and Linzey, 2008; Monadjem et al., 2015). Our results resemble those reported in Tanzania where species of this genus (e.g., *Aethomys chrysophilus)* were trapped in a field that had been under cultivation and then left fallow (Makundi et al., 2010).

The presence of *Gerbilliscus leucogaster* we reported here in the woodland forest is consistent with the description of the preferred habitat (savanna and woodland) of this omnivorous rodent (McDonough et al., 2015). As mentioned above, the mzf1 rainfed cropland was left postharvest during the study and the presence of *Gerbilliscus leucogaster* in this field is comparable with reports from Tanzania where it was trapped from fallow land and maize field in Tanzania (Odhiambo et al., 2006).

Saccostomus campestris was very much trapped in the irrigated cropland and trapped in almost all other habitats in small numbers much as was *Mastomys natalensis,* except in human dwellings as reported in central Mozambique by Belmain (2009). This could be explained by its ability to utilize transformed habitats and its generalist foraging behavior (Jackson, 2019).

4.1.5 Conclusions

The small-mammal community structure in our study area included seven rodent species and one *Elephant shrew*. The irrigated cropland had high rodent abundance but low species diversity whereas the mopane woodland had low abundance of small-mammals but high species diversity. *M. natalensis* was present in all habitats and comprised the most abundant rodent species. *Rattus rattus* was the main rodent species in villages. Overall, our results are compatible with the hypothesis that the diversity of small mammals is lower in agrosystems than in more natural ecosystems. To our knowledge, we are reporting for the first time the occurrence of *Rattus*

tanezumi in Mozambique. *M. natalensis* was trapped in significant number in the irrigated croplands, thus the assessement of population dynamic should be performed only in this habitat.

4.2 *M. NATALENSIS* POPULATION FLUCTUATION IN THE IRRIGATED CROPLANDS

4.2.1 Introduction

In the previous section we have shown that *M. natalensis* was the most abundant rodent species in the irrigated cropland, rainfed cropland and mopane woodland, being second in villages. *M. natalensis* is our targeted species because is the main natural reservoir of mammarenaviruses (reasons detailed in chapter four), so we investigated its population fluctuation across the PTSs in our study area. Our analysis will focus on the irrigated cropland because of the low numbers of *M. natalensis* captured from other habitats.

Small-mammal populations fluctuate seasonaly and spacialy (David and Jarvis, 1985; Delany and Monro, 1986). Studies on *Mastomys* in Sub-Saharan African demonstrated both intra and inter annual fluctuations in population abundance. In Mali, maximum numbers of *Mastomys huberti* were recorded in October (Granjon et al., 2005) and December/January for *Mastomys erythroleucus* (Crespin et al., 2008). In Tanzania, peaks of population size of *M. natalensis* were recorded between July and October (Leirs et al., 1993; Makundi et al., 2010; Mulungu et al., 2013). In Eswatini, Monadjem and Perrin (2003), during a three-year study recorded peaks in *M. natalensis* numbers in winter and spring of the first year, in the summer of the second and third years and in the winter of the third year. In Zambia peaks of *M. natalensis* abundance were seen during the rainy season and another early in the dry season (Chidumayo, 1984).

The population dynamic is influenced by the recruitment through reproduction or immigration, local survival and emigration (Leirs 1994: 56). Data from previous studies have revealed that population abundance tends to increase as reproduction progresses (Massawe et al., 2011; Mulungu et al., 2013) so peaks of abundance are more likely to be correlated with peaks of breeding season.

In this section we assess the fluctuation of the *M. natalensis* population to predict its breeding season. Moreover, the population dynamics will be later used to assess the effect of host abundance fluctuation on *Mopeia virus* transmission. We expect the peak of *M. natalensis* population abundance in the middle of the dry season based on the data from neighboring Tanzania.

4.2.2 Material and methods

The assessment of fluctuation of *M. natalensis* population over the PTSs was performed using data obtained by both removal and CMR protocols (see § 3.5). The number of captures relative to the trapping effort (**number of captures / trap nights x100**) was used as an estimator of *M. natalensis* abundance (Granjon et al., 2005). Here we used relative abundance as estimator because the trapping effort was not homogeneous over the sites and PTSs. For the purpose of comparing our method of estimating rodent abundance, we also calculated the population density and the trends were similar (Annex-VII) and the trapping success (Annex-VIII) and the trends were similar. The χ^2 test for independence was performed to evaluate the differences of relative abundance of *M. natalensis* between the PTSs.

4.2.3 Results

M. natalensis abundance in removal irrigated croplands

The relative abundance of *M. natalensis* increased from May to June in both Chibotane and Mavodze localities then decreased toward August in Chibotane and toward November in Mavodze. Likewise, a declining trend of relative abundance was observed between June and August in Bingo. The highest relative abundances of *M. natalensis* were recorded in June in Mavodze (90.0%), Chibotane (75.0%) and Bingo (79.6%) (**Figure 17**). The differences of relative abundance observed between the PTSs were significant in Chibotane (χ^2=30.4; DF=2; p <0.0001), Mavodze (χ^2=17.2; DF=3; p=0.0007) and Bingo (χ^2 =56; DF=1; p <0.0001).

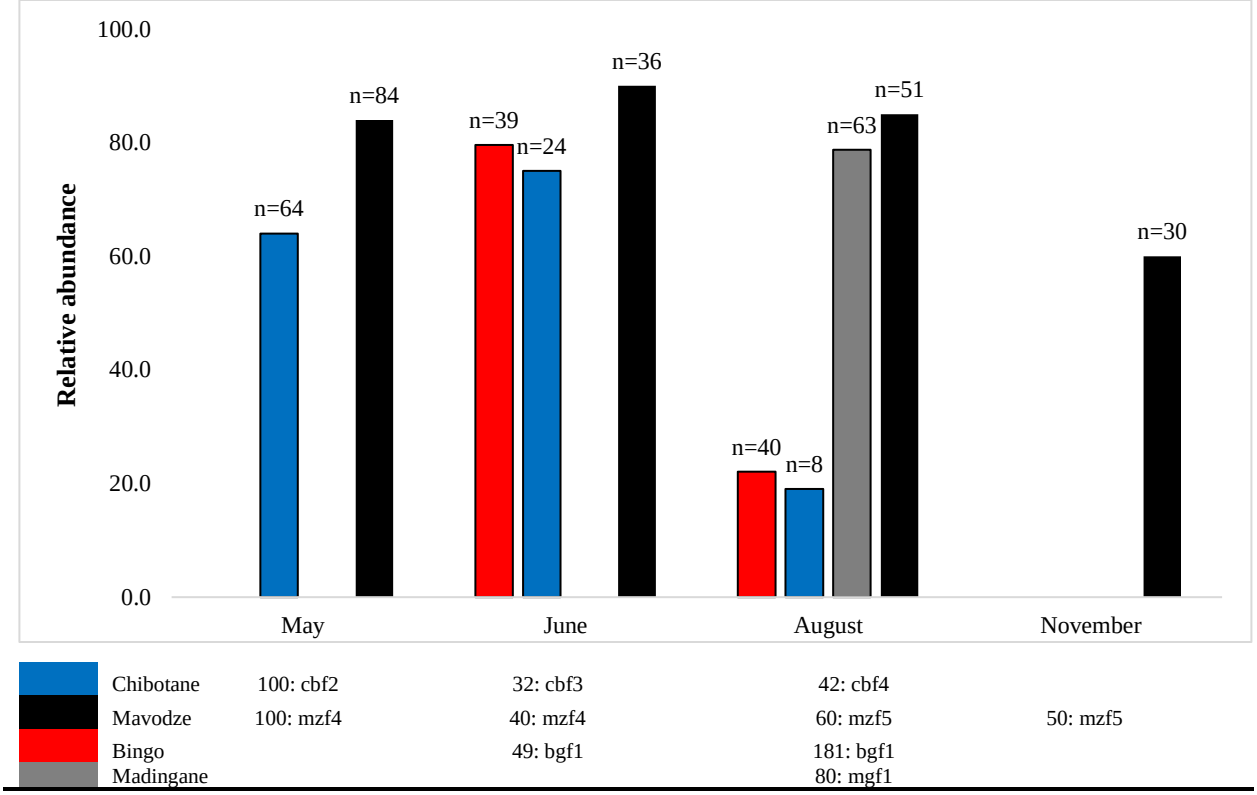

Figure 17: Relative abundance of M. natalensis in the removal irrigated cropland. The number of captures (n) are indicated outside end of the bars while the trap nights and the sampled sites are displayed below the PTSs

M. natalensis abundance in the cbf1 CMR irrigated cropland

In the cbf1 CMR irrigated cropland the relative abundance of *M. natalensis* increased from March (8.3%; n=53) to June (31.8%; n=161) in the middle of the dry season where we observed the highest peak. A deacrease trend was observed towards to November (17.6%; n=76) (***Figure 18***). The differences of *M. natalensis* relative abundance observed between the PTSs were significant (χ^2 =120.2; DF=4; p <0.00001).

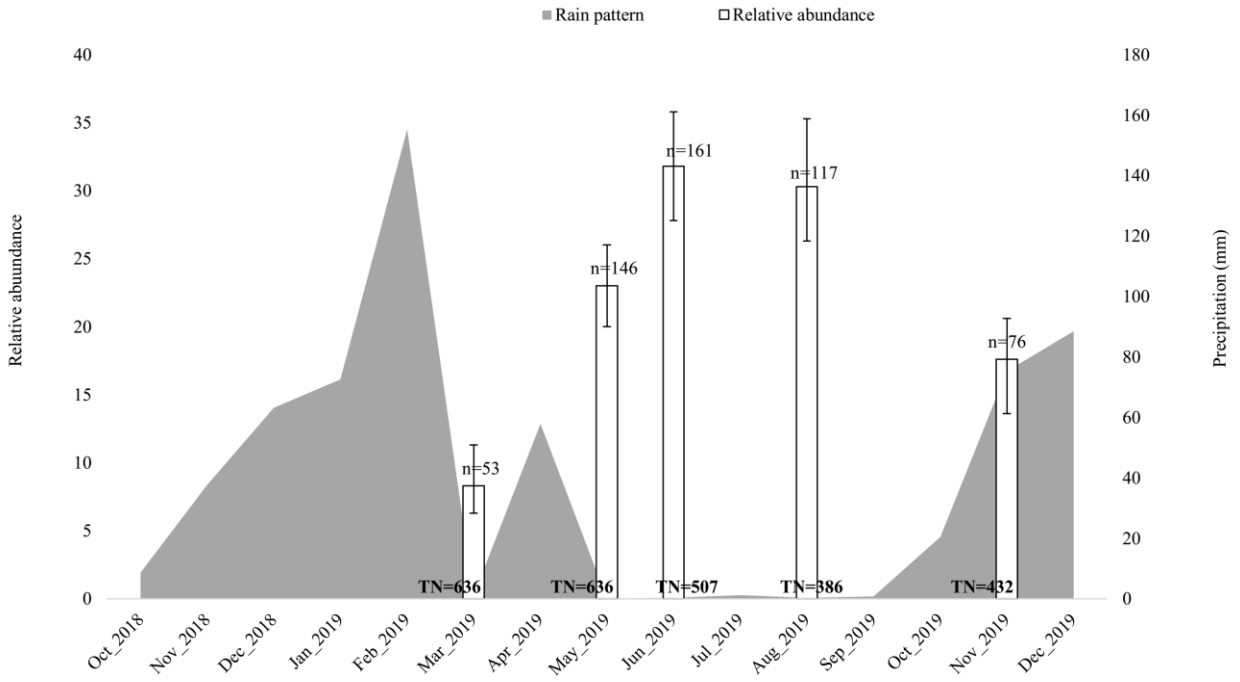

Figure 18: Relative abundance of M. natalensis (clustered column) in the CMR irrigated cropland (cbf1) and the 2018/2019 rain pattern (stacked area). The number of captures (n) are indicated outside end of the bars while the trap nights are displayed inside end of the bars. The rain data was obtained as explained in data collection (see §3.3)

4.2.4 Discussion

Both removal and CMR data indicated an increasing trend of *M. natalensis* population abundance towards the middle of the dry season followed by a declining trend towards beginning of rainy season. A higher abundance of the *M. natalensis* population that we observed around the middle of the dry season is comparable to that reported by previous studies in Mozambique (Gliwicz, 1985; Segeren et al., 1995). The study of Gliwicz (1985) was conducted in a dry savanna in central Mozambique and covered both rainy and dry seasons and noticed a higher abundance of *M. natalensis* population with its peak in the middle of dry season as well. Results comparable to our findings and that of Gliwicz (1985) were reported in fallow land in South Africa where *M. natalensis* population density raised just one month after the end of rainy season in April and the highest peaks were seen in middle of dry season in July and then decline toward the end of dry season and onset of wet season in South Africa (Leirs, 2006). However, unlike in our study where we caught *M. natalensis* at the end of the rainy season (in March), *M. natalensis* were completely

absent in April in the studies of Gliwicz (1985). Likewise, the results from South Africa presented by Leirs (2006) displayed complete absence of *M. natalensis* over the preceding rainy season. The differences between these two comparative studies and our finding could be the differences of habitat types. Our results were obtained from the irrigated cropland where there was a continuous crop production and this may have probably prevented *M. natalensis* population abundance fluctuation to zero captures. It has been indicated that vegetation cover and food resources are the main factors determining the maintenance of higher rodent densities (Mulungu et al., 2013).

A study conducted in fallow lands in Tanzania, Namibia and Swaziland reported highest population densities of *M. natalensis* over and after the rainy season (Massawe et al., 2011). This study highlighted that the local rainfall conditions have influenced *M. natalensis* breeding pattern. Moreover, the study of Massawe et al. (2011) showed that population densities increased in synchrony with the breeding progress. Further studies in Tanzania, recorded peaks of *M. natalensis* population abundance over different habitats during the dry season. But different from our study area the peaks of population abundance were seen towards the end of dry season between July and October (Odhiambo et al., 2006; Makundi et al., 2005, 2007; Massawe et al., 2011; Mulungu et al., 2013). These studies indicated that the higher population abundance they observed in the dry season was a result of high breeding activity during the rainy season.

The lack of data over the rainy season limited our analysis only to the dry season. It is therefore difficult to tell if the abundance of *M. natalensis* population in our study area started to increase just after the onset of rains as reported in Tanzania or just after the end of rains as reported in South Africa. We only have data of five months so it is difficult to tell if the pattern we described here is typical or not as *M. natalensis* population abundance fluctuates inter-annual (Monadjem and Perrin, 2003).

4.2.5 Conclusion

Despite the limitations of our sampling period, the highest abundance of *M. natalensis* we observed here in the dry season suggested that the main breeding season of this multimammate rodent most

likely occurred during the dry season. Like our results, previous studies reported peak of *M. natalensis* in the middle of the dry season as well and indicated that breeding season started during the rainy season. To ascertain this in our study area, *M. natalensis* reproduction parameters need to be assessed and this will be done in the following sections.

4.3 SEX RATIO

4.3.1 Introduction

The sex ratio is one of the most fundamental demographic parameters. Thus monitoring sex ratio specially in wildlife, can give an indication of sex balance and prediction of breeding potential of a population (Skalski et al., 2005). The whole population sex ratio can be defined as the ratio of males or females individuals out of whole population (Sapir et al., 2008), though some prefer to express it strictly as proportion of males in the whole population (Rosenfeld et al., 2003; Clutton-Brock and Iason, 2015). More broadly, sex ratio can be measured at different stages of the life cycle of a species, for example at conception (primary sex ratio), at birth (secondary sex ratio) or during adult life (adult sex ratio) (Székely et al., 2014). The primary and the secondary sex ratios rarely are translated to adult sex ratio and operational sex ratio which is the ratio of sexually active males to females, due to impact of ecological, life history and demographic process that occur in between (Székely et al., 2014). Identifying adult or sexually active individuals (male or female physiologically capable of producing offspring) in wildlife is challenging (Székely et al., 2014) and this could probably explain why there are more data on whole population sex ratio and less on adult sex ratio and operational sex ratio.

A single male *M. natalensis* can mate and impregnate several females; thus, the reproduction process on this species depends mainly on adult females (Coetzee, 1965; Leirs, 1994: 65). Thus, probably most of the studies on *M. natalensis* whole population sex ratio refer to the proportion of females out of the whole population (Mlyashimbi et al., 2020) and in our study, we also followed the same concept. Thus, from here sex ratio refer to the whole population sex ratio. More generally in mammals, if the costs and benefits of producing female or males are the same, the sex ratio is balanced (Rosenfeld and Roberts, 2004; Smanski and Zarkower, 2019). Spatial and temporal

variation of sex ratio was reported in rodents (Gomez et al., 2008; Monica et al., 2020). Sex ratio close to the parity across the year was reported for *M. natalensis* (Odhiambo et al., 2006; Mulungu et al., 2013). However, there are also records of preponderance of females during the breeding peak and sex ratio skewed toward males after the breeding season (Leirs 1994: 63; Mlyashimbi et al., 2020).

In this study we assessed the sex ratio of *M. natalensis* over the PTSs. The working hypothesis was that sex ratio is different among PTSs and females were in higher numbers during the reproductive season.

4.3.2 Material and methods

The assessment of sex ratio of *M. natalensis* population over the PTSs was performed using data obtained by both removal and CMR protocols (see § 3.5). As indicated before we recorded the gender of our rodent during the field and the laboratory work (see § 3.6) and here we usesed these records to assess the sex ration. Here sex ratio is the the proportion of females out of the whole population (Mlyashimbi et al., 2020). The χ^2 test for independence was used to compare the sex ratio between the PTSs in the cbf1 where the data displayed a clear trend.

4.3.3 Results

A total of 992 *M. natalensis* were trapped from the irrigated croplands, from which 52.9% (n=525) were females, 45.5% (n=451) were males and 1.6% (n=16) were not identified to sex and the overall sex ratio was 0.54 (525/976).

M. natalensis sex ratio in the removal irrigated croplands

In the removal irrigated croplands, the overall sex ratio was 0.45 (192/430). In May the proportion of females was higher than males in both sampled sites cbf2 (0.55; n=35) and mzf4 (0.65; n=54) (***Figure 19***). Between June and November, more males than females were trapped in all sites sampled, except in the site mzf5 (0.57; n=28) in August (***Figure 19***).

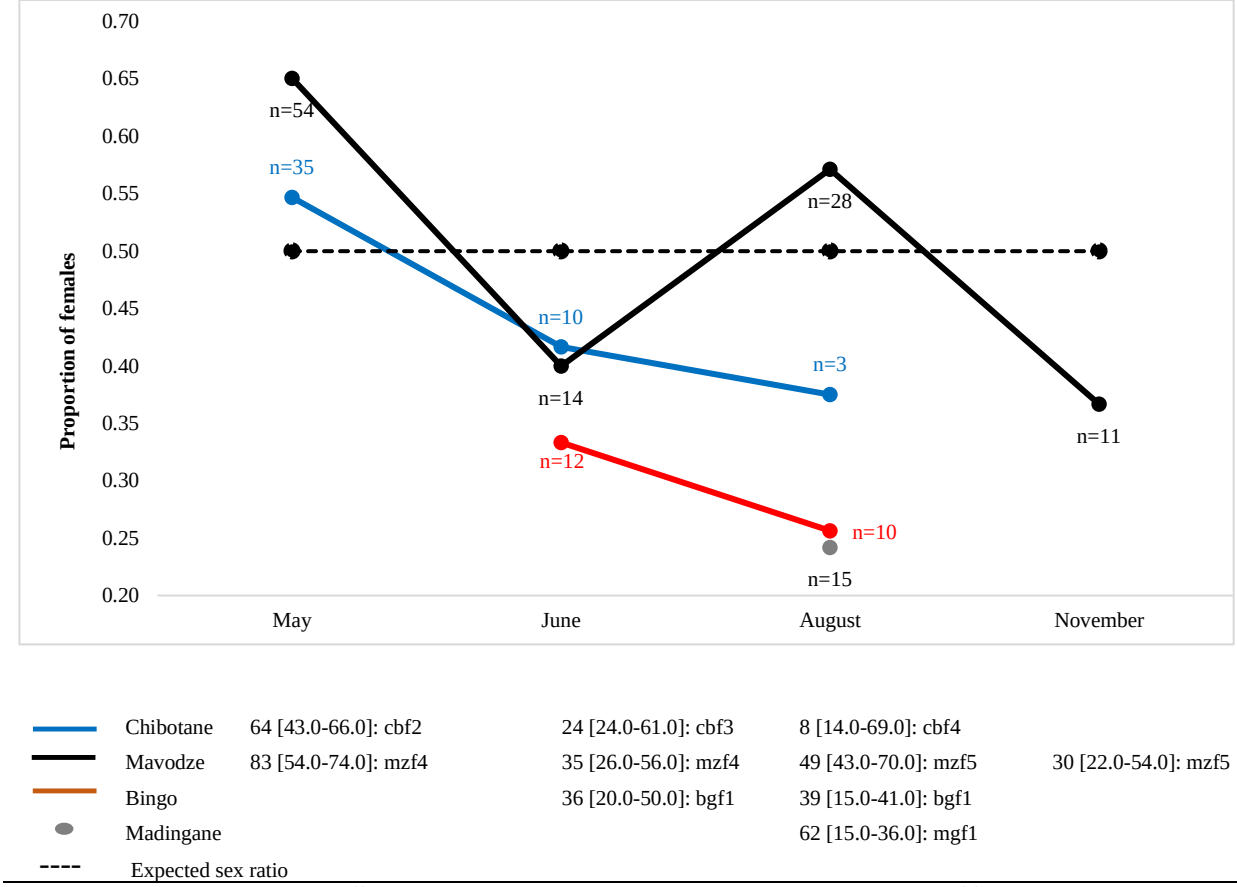

Figure 19: M. natalensis sex ratio in different removal irrigated cropland over the PTSs. The total number of females and males, the 95%IC of proportion of females and the sampled sites are displayed below the PTSs. On the graph n is the number of females captured

Sex ratio of *M. natalensis* trapped in the cbf1 CMR irrigated cropland

The overall sex ratio in the cbf1 CMR irrigated cropland was 0.61 (333/546). The proportions of females were higher than males between March and June (minimum = 0.64; maximum=0.68), but in August and November the proportion of females and males were close to the parity (**Figure 20**). The sex ratio differed significantly between the PTSs (χ^2=11.0; DF=4; p=0.03).

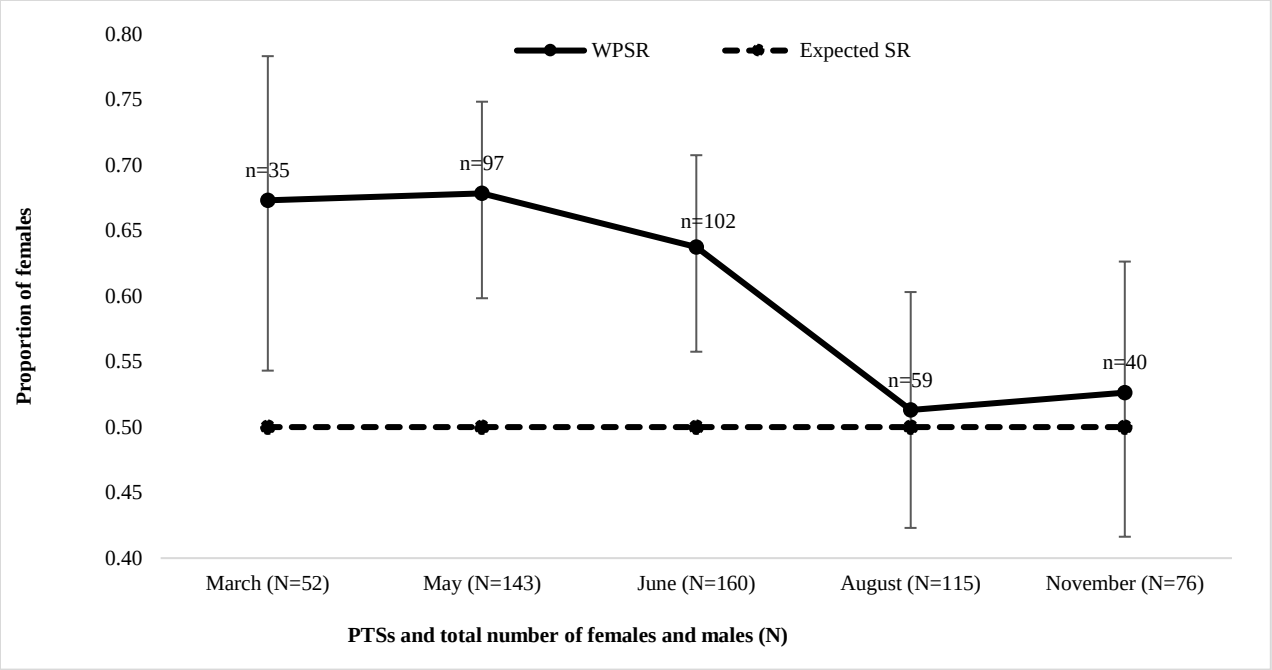

Figure 20: Evolution of sex ratio of M. natalensis trapped in the CMR irrigated cropland (cbf1) over the PTSs. The number of females (n) is display inside the graphic

4.3.4 Discussion

The non-agreement of the sex ratio trends between the removal and CMR sites from June towards November could be due to the possible disturbance of population structure we might have introduced in sites sampled in two PTSs for the removal sites (as mentioned before). Due to this our discussion will focus on the CMR data. The trend displayed by our CMR data is comparable to data from Tanzania where they also reported preponderance of females toward the middle of dry season while toward the onset of the rainy season the sex ratio was skewed towards males or close to parity (Leirs 1994: 63 & 84). According to Leirs (1994:65), the preponderance of females has been suggested for *Mastomys* during the breeding season and possibly indicates that the mating system is based on a harem structure or an organization in which the males are polygamous.

Another study from Tanzania recorded a higher proportion of females during the wet season and preponderance of males during the dry period (Mlyashimbi et al., 2020). The explanation that was put forward for the preponderance of females during the breeding season was that the catchability

of females was higher compared to males because sexually active females search for food and males for mating more widely (Mulungu et al., 2013; Mlyashimbi et al., 2020; Monica et al., 2020). Kokko and Rankin (2006), argued that although male mate searching prevails in nature, there is an interaction between the mating system and the population dynamics as in some animal species it has been suggested that in high population densities females searched more for mates than males.

According to Leirs (1994: 66), juvenile recruitment may have a diluting effect on the sex ratio if counted. In our study this is more likely to be the case if more juvenile females were recruited to the population as they will increase the total number of females used to compute the sex ratio. According to the Trivers-Willard hypothesis, mothers in poor conditions produce more female offspring (Trivers and Willard, 1973), however, in our study we did not assess the nutritional condition of trapped rodents.

The declining trend of the proportion of females toward August may be interpreted as possible evolution of the breeding season where more females were getting pregnant and subsequently delivered offspring. It has been suggested that pregnant females and those lactating and taking care of offspring reduce their movements and this may decrease their probability of capture (Mlyashimbi et al., 2020; Monica et al., 2020). On the same vein, the increment of proportion of females onward November may suggest a shift to out of breeding season and the probability of capture was not influenced by reproduction.

4.3.5 Conclusion

He reported variation of sex ratio over the PTS with preponderance of females toward the middle of dry season and this pattern was observed during the breeding season of *M. natalensis* in previous studies. In order to ascertain a possible ongoing breeding season in our study area, it is important to analyze the other reproductive parameters and this analysis will be performed over the the next sections.

4.4 SEXUAL ACTIVITY

4.4.1 Introduction

Reproduction depends mainly on individuals attaining sexual maturity. The reproductive cycle of *Mastomys* females consists of oestrous (perforated vagina), gestation and lactation (Leirs 1994:65; Johnston and Oliff, 2010). Signals of reproductive cycle (together or separately) have been used by several authors to describe the dynamics of *M. natalensis* populations (Odhiambo et al., 2006; Mulungu et al., 2012; Massawe et al., 2012). The catchability of reproductive females and males may vary over the breeding season. Pregnant females and those lactating spend most of their time taking care of offspring decreasing their probability of capture while sexually active females (but neither pregnant nor lactating) search more for food and males for mate, which increases their probability of capture (Leirs 1994; Mlyashimbi et al., 2020). The reproductive condition of *M. natalensis* females is more important for the timing of reproduction because sexually active males may be present over sampling period (Leirs 1994: 65). Here we used sexual activity to access the breeding season of *M. natalensis* in our study area. We expected a higher proportion of sexually active individuals during the breeding season.

4.4.2 Material and methods

The evolution of *M. natalensis* reproduction condition was assessed over the PTSs using data obtained by both removal and CMR protocols (see § 3.5). Here, sexual activity is defined as a physiological condition and not a typical behavior (Leirs, 1994:61). Females were considered sexually active when vagina was opened and or nipples were prominent while males were considered sexually active when testes were scrotal and or seminal vesicle was developed (Leirs, 1994:61; Herbreteau et al., 2011). The percent of sexually active individuals was calculated for each gender in the removal and CMR sites by PTS.

To better estimate the body weight threshold to distinguish between juveniles and adults in our studied M. natalensis population, we plotted sexual activity and body weight data in the same graphic (Granjon and Duplantier, 2009). We used this approach because of the youngs born

recently and not sexually active should have low weight (Granjon et al., 2005). The χ^2 test was performed to evaluate differences in proportions of sexually active females and males between different PTSs in the cbf1 CMR irrigated cropland.

4.4.3 Results

M. natalensis sexual activity in the removal irrigated croplands

The reproductivity condition of *M. natalensis* in the removal irrigated croplands was variable over the trapping sites and sessions. In May, the proportion of sexually active females and males were almost the same (≈ 60%) in the cbf2 site while in the mzf4 site the proportion of sexually active female was higher (40.7%; n=22) than the one of males. In June the proportion of females was higher in the sites bgf1 (41.7%; n=12) and cbf3 (20.0%; n=10) while in the mzf4 site we observed more males (57.1%; n=12) than females. The proportion of males was higher than the one of females in the four sites sampled in August. The mzf5 site showed a higher proportion of females (72.7%; n=8) in November (***Figure 21***).

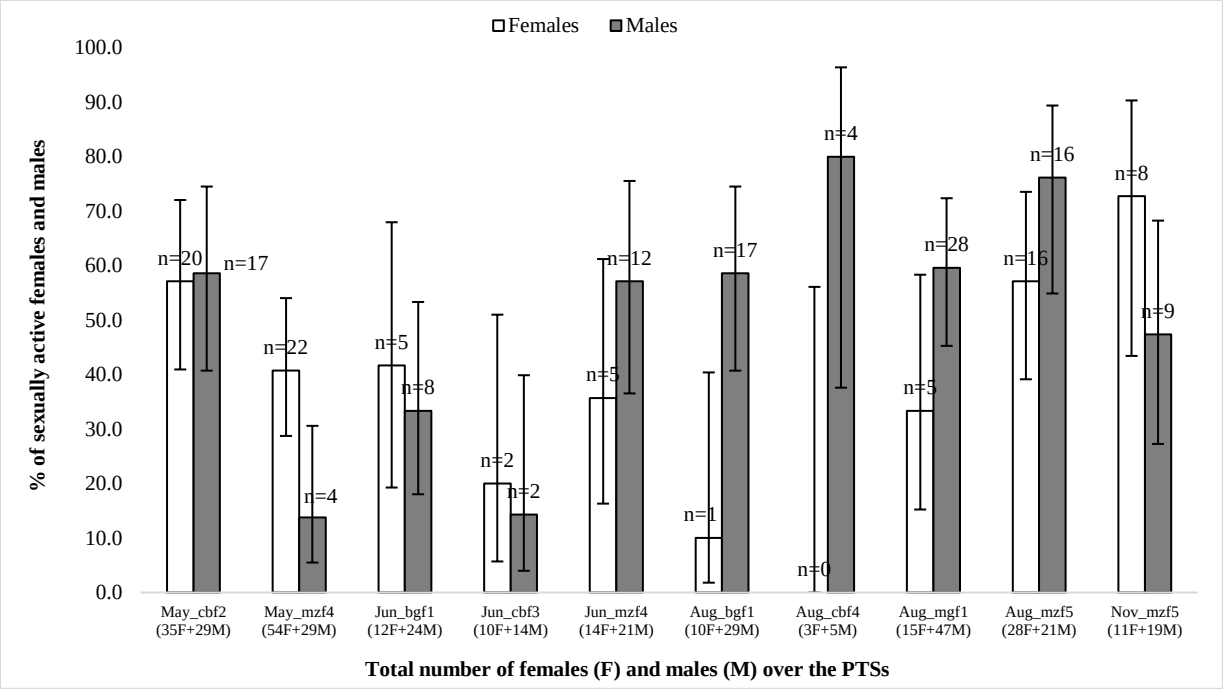

Figure 21: Reproduction conditions of M. natalensis females and males in the removal irrigated cropland. The numbers (n) of sexually active indiduals are displayed outside end of the bars while the total number of females and males are indicated bellow the PTSs

M. natalensis sexual activity in the cbf1 CMR site

In the cbf1 CMR site, the proportions of sexually active *M. natalensis* males were higher than those of females over the PTSs, except in March. The proportion of sexually active females decreased between March and May, then it remained static toward August (≈30.0%). The proportion of sexually active males did not show variation between March and May (≈76.0%). In June, the proportion of sexually active males registered a reduction by 29.5%, followed by a sharp rise towards November (***Figure 22***). The differences of *M. natalensis* sexual activity observed between the PTSs were significant for both females (χ^2= 72.2; DF=4; p<0.0001) and males (χ^2= 30.2; DF=4; p<0.0001).

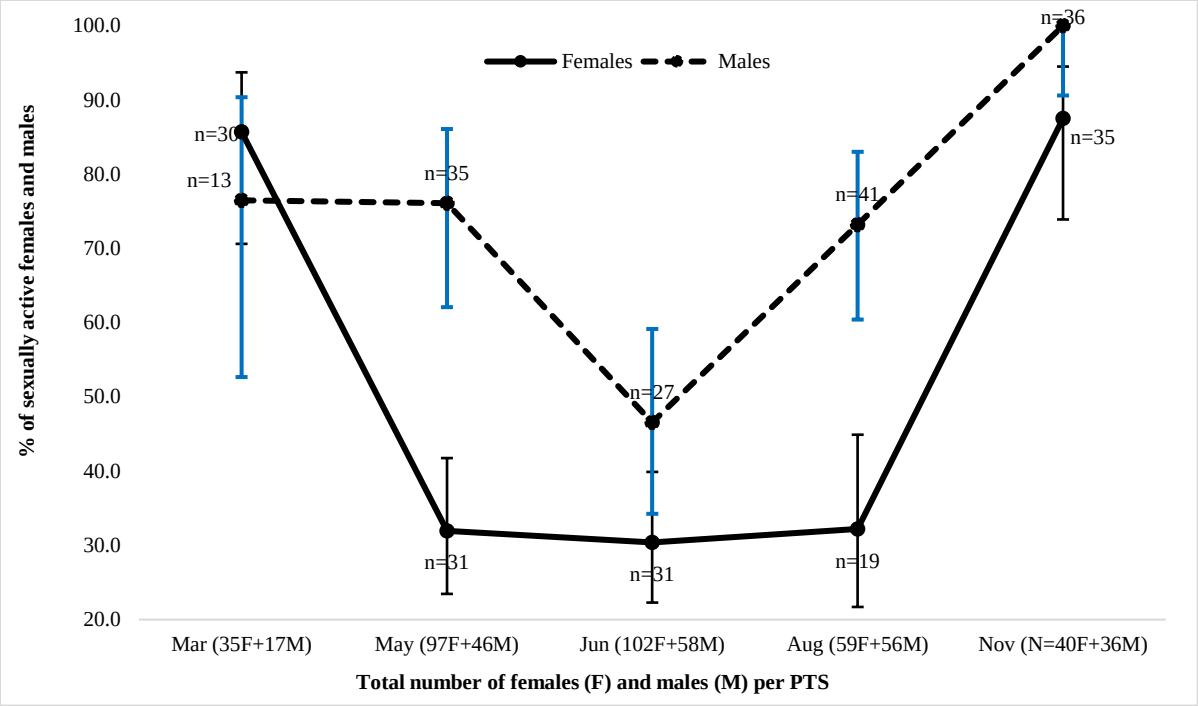

Figure 22: Reproduction conditions of M. natalensis females and males in the cbf1 CMR irrigated cropland

4.4.4 Discussion

Our data from removal sites did not display a clear trend of evolution of reproduction condition over the PTSs probably due to the possible disturbance of population structure we might have introduced in sites sampled in two PTSs for the removal sites (as mentioned before). Our data from the cbf1CMR irrigated cropland indicated the presence of sexually active *M. natalensis* individuals over the PTSs with significant temporal fluctuations. This observation as well as the trend of higher proportions of sexually active *M. natalensis* males than females observed in our study conforms with reports of previous studies in Tanzania (Leirs 1994:65) and Guinea (Fichet-Calvet et al., 2008). Similarly to our findings, studies in Tanzania recorded peaks of proportions of sexually active *M. natalensis* females between March and April with a declining trends during the dry season (Massawe et al., 2012; Mulungu et al., 2013). The hypothesis they put forward to explain the observed differences was that breeding is influenced by diet and the transition from wet to dry season brings changes in the quantity and quality of food resources.

Another possible explanation for the decline in the proportion of sexually active individuals observed in our study may be the recruitment of juveniles that increased the subpopulation of sexually inactive individuals. This hypothesis is justified because studies reported that in Southern Africa the peaks of *M. natalensis* breeding season and the higher recruitment of juveniles occur in the middle of dry season (Coetzee, 1975; Leirs, 2006). We suspect that the reproductive pattern in our study area was not different from that reported in the neighbouring countries. We guess that it would be more informative if we restrict the analysis of reproduction conditions to the adult population but we will not have studies for comparison as the studies available for *M. natalensis* often performed the analysis for the whole population.

The increase in the proportion of sexually active individuals toward November suggests that offspring born at the beginning of the reproducing season reached sexual maturity and reproduced in the same breeding season they were born. A study on seasonal variation in the growth of *M. natalensis* in Tanzania found that if the heavy rains start early during the wet season the reproduction season start at the beginning of the year and the resulting offspring reach their maximal size without growth stop and start reproducing immediately (Leirs et al., 1990). It has been suggested that *M. natalensis* females do not reach sexual maturity during the breeding season in which they were born, but aseasonality of rainfall may produce a generation of progenies off-seasonal which may be potential breeders in the main breeding season of the same year (Leirs et al., 1993).

When population densities are higher females group themselves to delay sexual maturation in young rodents and this keep them in anestrus or the groups of females or their urine delays puberty in young females (Drickamer 2007). Density dependent reproduction regulation was suggested for *M. natalensis* by Leirs (1994) but he noticed that low density alone was not correlated with onset breeding. A study on the effect of host density in maturation rates found positive correlation between low abundance estimates and low maturation rate of females *M. natalensis* (Sluydts et al., 2007).

4.4.5 Conclusions

Sexually active females and males were present during all the PTSs and in overall the proportion of sexually active males were higher than females. At the end of the rainy season, which according to the literature is the start of the breeding season for *M. natalensis*, the proportion of sexually active individuals was high relatively to the inactive ones. However, in the middle of the dry season the proportion of inactive individuals was higher than that of active ones, suggesting a higher recruitment of juveniles. Previous studies indicate that the middle of the dry season corresponds to the peak breeding season of *M. natalensis*. Towards the end of the dry season the number of sexually active individuals increased, suggesting that the recruited juveniles were reaching sexual maturity. We estimated 22g is the weight limit to differentiate juveniles and adults in our data set because with this cut off the number of mature individuals regarded as juveniles will be minimized.

4.5 AGE STRUCTURE

4.5.1 Introduction

The classic and reliable method to determine the age of living beings is to count the time since the date of birth. Unfortunately, it is not always possible to register or to know the date of birth, especially in wildlife such as rodents. However, knowledge of the age structure of a population is a very important indicator in population demography, hence the need to find alternatives to estimate age. The presence of juveniles in *Mastomys* population has been used as an indicator of a recent or ongoing reproduction process (Granjon et al., 2005; Mulungu et al., 2013). The methods that had been used to estimate the age of *Mastomys natalensis* included metric characters such as body length, body weight and eye lens weight (Coetzee, 1965; Leirs 1994: 111-119). The advantages and disadvantages of these three methods were discussed by Leirs (1994: 111-119). According to him, the eye lens weight technique is more accurate because the eye lens grows continuously regardless of the nutritional, social and reproductive status of the individual, but it cannot be used for live animals. The body length and body weight techniques can be applied to both dead and alive animals. However, these two measures are associated with growth that does

not only depend on age but also on other factors such as nutrition, social status, hydration and pregnancy. In our study, although we sacrificed the animals trapped in the removal sites, we did not collect and dry the eye lenses because of time and cost constraints. Furthermore, in the CMR sites we monitored live animals and it was difficult to measure body length. Non-metric methods such as tooth wear and coat have been used to group animals into age-classes (Coetzee, 1965; Chidumayo, 1984; Gliwicz, 1985). Opening the mouth of live rodents to describe tooth wear is very complicated, and on the other hand we felt that describing the skin color of animals would be subjective since the processing of rodents involved different persons.

The only measurement that was possible to collect from rodents captured on both the removal and CMR sites was the body weight. In addition to the shortcomings mentioned above, it is worth mentioning that the weight stops increasing when the animal reaches its maximum growth and at this stage the use of this parameter to estimate the age becomes inconvenient (Leirs et al., 1990). In this sub-section we used the body weight to evaluate the age composition of the population in the different sampling sites and months. The assessment of juveniles' temporal variation performed here, in chapter five will be used to assess the effect of age on *Mopeia virus* circulation in our study area. *M. natalensis* breeding is seasonal, starting a few months after the rainfall and it continues during the dry season (Leirs et al., 1994). This seasonality has been synchronized with fluctuations of age structure. We expected higher presence of *M. natalensis* juveniles in the middle of the dry season.

4.5.2 Material and methods

The outcome of a successful pregnancy is the delivery of offspring. Thus, besides pregnancy detection, the presence of young animals may give indication of the reproduction process in a population. The reliability of using this indicator depends on the survival of offspring which can be affected by several factors that can include parenteral care, environmental conditions and predators. The presence of light animals as evidence of reproduction in *M. natalensis* should be interpreted with care because young animals stop growing mainly at the end of dry season (Leirs et al., 1990). Still, we think the presence of juveniles will give an indication of the breeding season

of *M. natalensis* in our study area. Our data strongly shows that from 40g onwards all females were sexually active, while for males this limit rises to 50g (Annex-IX). If we use these weights as minimum thresholds to differentiate juveniles from adults, we will have a higher number of sexually active individuals classified as juveniles. We think that 22g may be an approximate weight to separate adults from juveniles because bellow this weight we will have a reduced number of adult individuals classified as juveniles for both males (n=22) and females (n=19).

The percentage of juveniles out of the whole population was calculated across the PTSs. Individuals with missing value for body weight measurement were excluded. The χ^2 test of independence was performed to evaluate the differences of percentage of juveniles between the PTSs in cbf1 irrigated cropland. We expected a higher proportion of juveniles in the middle of the dry season because previous studies in the neighboring countries indicated that the peak of *M. natalensis* breeding season occur in the middle of dry season (Mulungu et al., 2013).

4.5.3 Results

M. natalensis age structure in removal irrigated cropland

In the irrigated cropland, juveniles were present across all the sites sampled and over the PTSs. Across the PTSs, different trends were observed between May and June when the percentage of juveniles inceased from 46.9% to 58.3% in Chibotane while in Mavodze it declined from 48.8% to 33.3%. A declining trend of percentage of juveniles was observed between June and August in Chibotane to 50.0% and in Mavodze to 27.5% while in the same period it increased from 53.8% to 57.5% in Bingo. Finally, in November the percentage of juveniles increased to 43.3% in Mavodze (***Figure 23***).

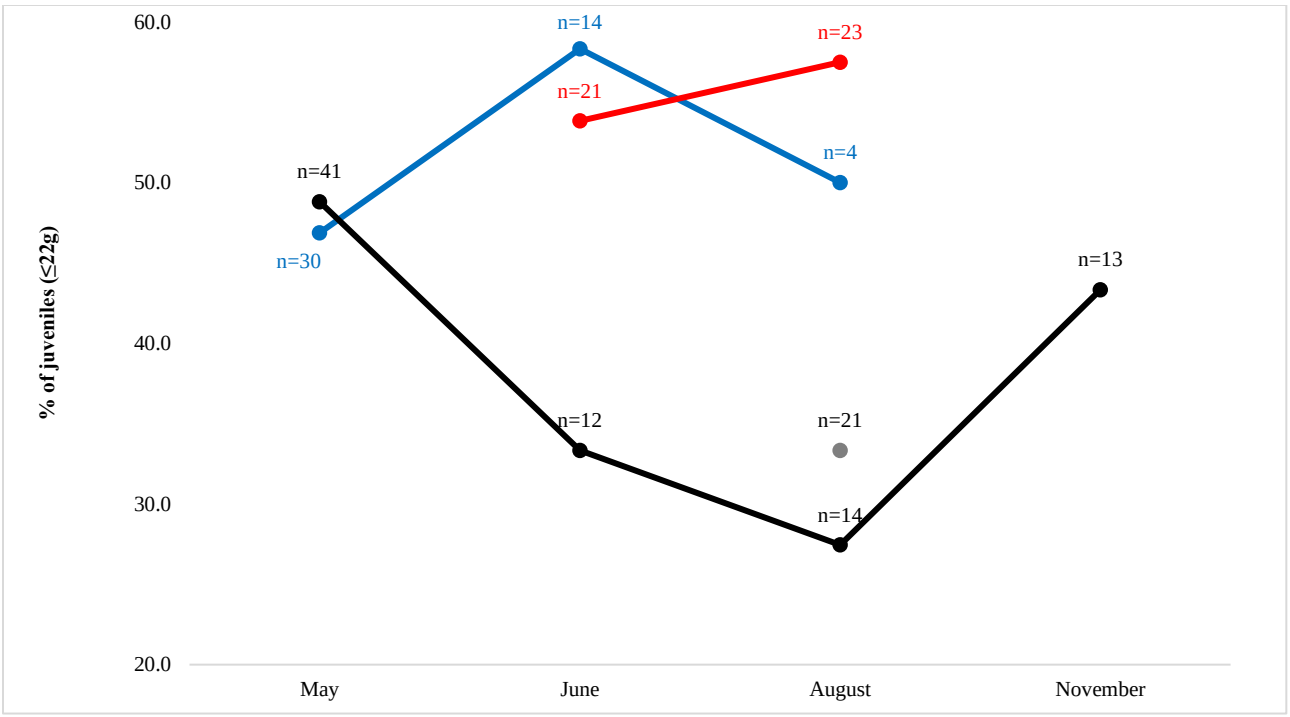

Figure 23: Distribution of M. natalensis juveniles in different removal irrigated cropland sampled in different localities over different PTSs. The total number of juveniles and adults, the 95%IC and the sampled sites are display below the PTSs

M. natalensis age structure in the cbf1 CMR irrigated cropland

In the cbf1 CMR site we observed an increament of percentage of juveniles between March and May. Around the middle of the dry season in May and June approximately 50.0% of the population was juvenile. It is worth mentioning that, based on our field observation in the site cbf1, we released more light individuals (so not documented) in June than May so we believe that there were more juveniles in June than May. From June towards, the juvenile population decreased as we approached the end of the dry season (***Figure 24***). The differences of percentage of juveniles observed between the PTSs were significant (χ^2 =59.2; DF=4; p <0.0001).

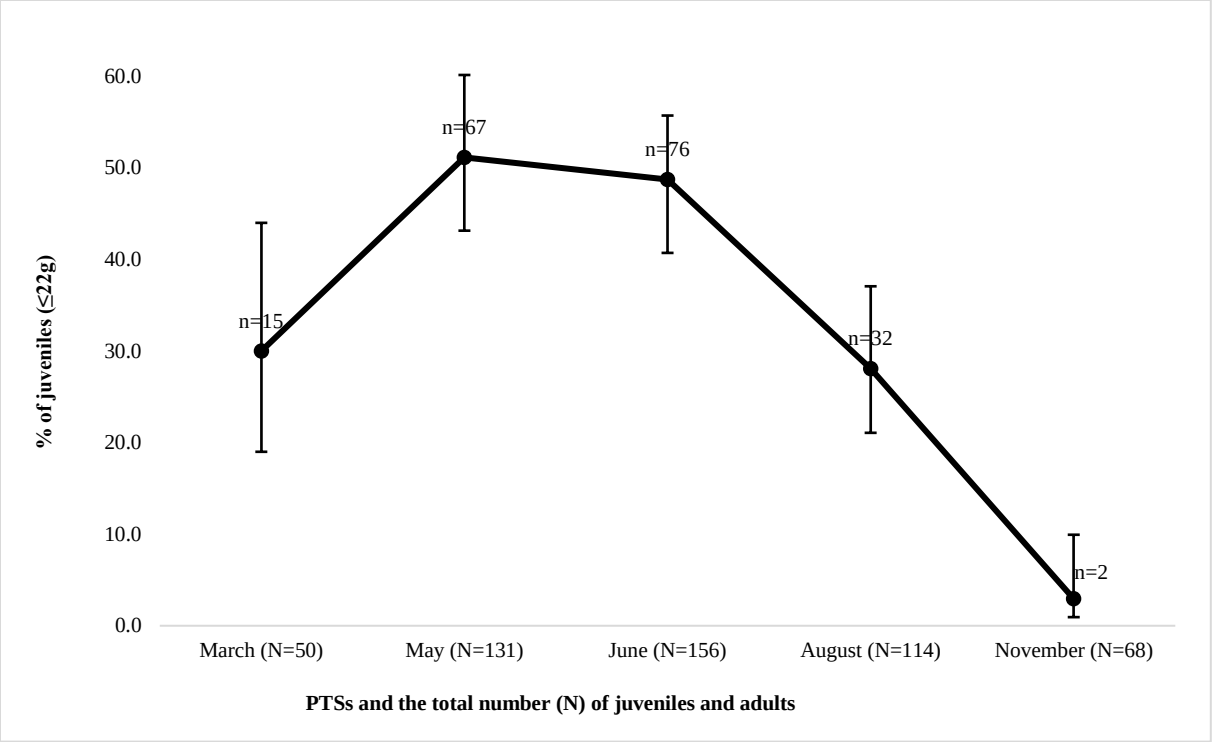

Figure 24: Distribution of M. natalensis juveniles trapped in the CMR irrigated cropland (cbf1) over the PTSs

4.5.4 Discussion

As previously mentioned, the removal sites sampled in two PTSs may have altered the population structure. Because of this possible disturbance in the population, our discussion will focus more on the data from the cbf1 CMR site. The presence of light individuals observed from the end of rainfall in March toward the end of the dry season in August in both removal and CMR sites could be interpreted as juveniles' recruitment process occurring in our studied population. Our findings are consistent with the results from a previous study in Mozambique that reported higher proportions of younger individuals from the end of the rainy season up to the middle of the dry season in July with a declining trend toward the end of the dry season (Gliwicz, 1985).

Across the Sub-saharan Africa, findings comparable to our results were reported in South Africa where juveniles were present towards the end of the rainy season, with a peak in the middle of the dry season followed by a declining trend toward the end of the dry season (Coetzee, 1975). In

Zambia and Tanzania, juveniles recruitment were observed from the rainy season toward the dry season with higher peaks in the dry season, however, in Tanzania the declining trends depended on the duration and intensity of the two rainy seasons (Leirs et al., 1993; Mulungu et al., 2013).

In our study we used body weight to distinguish between juveniles and adults and this method has been regarded as a poor estimator of age for *M. natalensis* because weight growth stop is common in this rodent species (Leirs et al., 1989). Because of the latter, the presence of juveniles will be interpreted altogether with other host parameters. Not all juveniles were processed in the cbf1 CMR site mainly in May and June PTSs due to higher number of captures so is it possible that the proportion of juveniles were underestimated in these months.

4.5.5 Conclusions

The recruitment of juveniles observed during the study suggested that a breeding event occurred in the population of *M. natalensis* in our study area. We presume that the breeding started during the previous months in the rainy season because we recorded the presence of young individuals at the end of rainfall in March. Our data also shows that the middle of the dry season is the peak of the breeding season which decreases as we approach the end of the dry season. However, the presence of light weight individuals itself may not be enough to conclude precisely about *M. natalensis* reproduction. Thus, below we jointly analyzed all reproductive parameters.

4.6 GENERAL CONCLUSIONS OF *M. NATALENSIS* POPULATION DYNAMIC

Reproduction is the major phenomena responsible for juveniles' recruitment and increment of population density (Leirs, 1994: 59). Moreover, the sex ratio and the proportion of sexually active individuals may fluctuate as the reproduction process, for example, more females than males were recorded during the breeding peak (Mulungu et al., 2013). Our data at CMR sites which is more appropriate for temporal monitoring because the same population was longitudinally sampled, showed preponderance of females, increasing trends of juvenile recruitment and population abundance from the end of rainy season toward the middle of dry season. These trends are compatible with an ongoing breeding season. So, our data suggested that the reproduction of *M.*

*natalensi*s started during the rainy season and its highest peak was in the middle of the dry season (June/July). The shift in the trend of the reproductive parameters mentioned above as we approach the end of the dry season suggests a decline in the reproductive season. Although we have limited data for the rainy season, the breeding pattern suggested by our data seemingly is comparable to those described in South Africa and Tanzania where the main breeding season for *M. natalensis* started during the rainfall and extended up to the dry season with spatial and annual variations depending on the differences in the annual rainy pattern Coetzee, 1975; (Leirs et al., 1993; Massawe et al., 2011; Mulungu et al., 2013). The link between *M. natalensis* reproduction and rainfall have been attributed to the influence of rains on availability of resources in quality and quantity for rodents.

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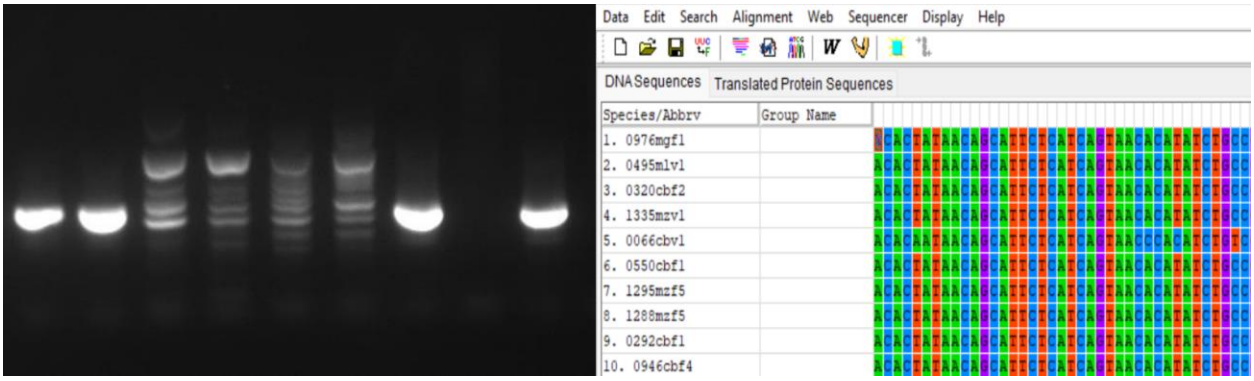
5 CHAPTER II: DETECTION AND GENETIC CHARACTERIZATION OF MAMMARENAVIRUSES IN *M. NATALENSIS* FROM DIFFERENT HABITATS IN THE LNP AND ITS BUFFER ZONE



5.1 INTRODUCTION

In the general introduction we indicated the *Lassa virus* (hosted mainly by *M. natalensis*) and *Lujo virus* (of unknown host) are the two mammarenaviruses yet implicated with haemorrhagic fever in humans in Africa. We also highlighted that *M. natalensis* also hosts several mammarenaviruses yet regarded as non-pathogenic for humans. In chapter three we reported a wide distribution of *M. natalensis* rodents in all four habitats sampled in our study area.

In this chapter we assessed the circulation of mammarenaviruses in *M. natalensis* trapped from different habitats, then we performed the phylogenetic analysis to identify the species of mammarenaviruses present in our study. This assessment will provide an indication of areas where humans are exposed to a potential risk of mammarenaviruses spill-over.

We found that the mammarenaviruses that circulated in our study area was *Mopeia virus* and it was detected in all sampled habitats. *Mopeia virus* was reported by previously studies in Mozambique (Wulff et al., 1977), Zimbabwe (Johnson et al., 1981), Tanzania (Borremans et al., 2011) and South Africa (Grobbelaar et al., 2021).

Mopeia virus is yet regarded as non-pathogenic for humans, despite the lack of surveillance studies in humans. The wide circulation of *Mopeia virus* in all habitats sampled during our study may suggest that humans are exposed to an eventual spill-over of mammarenaviruses in all areas. Therefore, awareness campaigns should be designed taking into account the risk factors in the different habitats. To this end, we recommend the assessment of the drivers of *Mopeia virus* circulation in the *M. natalensis* population and the risk factors for human transmission.

All contents of this chapter were published in the below manuscript and the results described here will be analyzed later together with the ecology of *M. natalensis* (chapter three) and human knowledge, attitudes and practices to rodents (chapter four) for better characterization of the risk of mammarenaviruses spill-over to humans.

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Research paper

Detection and genetic diversity of Mopeia virus in *Mastomys natalensis* from different habitats in the Limpopo National Park, Mozambique

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ABSTRACT

Mammarenaviruses have been a growing concern for public health in Africa since the 1970s when *Lassa virus* cases in humans were first described in west Africa. In southern Africa, a single outbreak of *Lujo virus* was reported to date in South Africa in 2008 with a case fatality rate of 80%. The natural reservoir of *Lassa virus* is *Mastomys natalensis* while for the *Lujo virus* the natural host has yet to be identified. *Mopeia virus* was described for the first time in *M. natalensis* in the central Mozambique in 1977 but few studies have been conducted in the region. In this study, rodents were trapped between March and November 2019in villages, croplands fields and mopane woodland forest. The aim was to assess the potential circulation and to evaluate the genetic diversity of mammarenaviruses in *M. natalensis* trapped in the Limpopo National Park and its buffer zone in Massingir district, Mozambique. A total of 534 *M. natalensis* were screened by RT-PCR and the overall proportion of positive individuals was 16.9%. No significant differences were detected between the sampled habitats ($\chi^2 = 0.018$; DF = 1; $p = 0.893$). The *Mopeia virus* (bootstrap value 91%) was the *Mammarenavirus* circulating in the study area sites, forming a specific sub-clade with eight different sub-clusters. We concluded that *Mopeia virus* circulates in all habitats investigated and it forms a different sub-clade to the one reported in central Mozambique in 1977.

1. Introduction

Mammarenaviruses are enveloped, ambisense or negative single-strand RNA microorganisms and their genome (≈10.5 kb) consists in two or three RNA segments named large (L), medium (M) and small (S). Currently, there are four genera in the *Arenaviridae* family (*Mammarenavirus, Antennavirus*, *Hartmanivirus* and *Reptarenavirus*). The genus *Mammarenavirus* infects mainly mammals including rodents (Radoshitzky et al., 2019). In Africa the viruses of the *Mammarenavirus* genus have been a growing concern for public health since the 1970s when the first cases of human disease associated with haemorrhagic fevers caused by the *Lassa virus* were described in west Africa (Buckley et al., 1970; Frame et al., 1970).Based on geographical occurrence, genetic and serological features, mammarenaviruses are divided into old world group (OWG) and new world group (NWG) (Bowen et al., 1997; Albariño et al., 1998). The OWG is restricted to the Eastern Hemisphere while the NWG occur in the Western Hemisphere. The *Lymphocytic choriomeningitis virus* an OWG *Mammarenavirus* has a worldwide

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[2] J. Cappelle and F. Liegeois were equally involved in the study.

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distribution (Gratz, 2006; Fornůsková et al., 2021). To date, 16 species of *Mammarenavirus* have been isolated from African mainland and all belong to the OWG (Burrell et al., 2017; Radoshitzky et al., 2019). Two of them, *Lassa virus* and *Lujo virus*, are associated with human lethal diseases. *Lassa virus* is responsible for 300,000 to 500,000 infection cases resulting in about 5000 deaths each year in west Africa (Ogbu et al., 2007) while a single outbreak of *Lujo virus* has been reported to date in South Africa in 2008 where four out of the five infected patients died (Paweska et al., 2009). The natural reservoir of *Lassa virus* is the rodent *M. natalensis* (Monath et al., 1974) while for the *Lujo virus* the natural host is yet to be identified (Simulundu et al., 2016). *M. natalensis* is largely distributed in sub-Saharan African (IUCN, 2016a), with a wide ecological range and can be found in savannas, grasslands, agricultural fields and houses (Coetzee, 1975; Monadjem et al., 2015). It hosts a number of non-pathogenic mammarenaviruses for humans such as *Mopeia virus, Luna virus*, *Lunk virus, Morogoro virus*, *Gairo virus* (Wulff et al., 1977; Taylor et al., 1981; Günther et al., 2009; Ishii et al., 2011; Gryseels et al., 2015; Cuypers et al., 2020).

The *Mopeia virus,* identified for the very first time in Mopeia district, Zambézia province (Fig. 1A), central Mozambique (Wulff et al., 1977), was the second African *Mammarenavirus* described in *M. natalensis* after the *Lassa virus.* No human cases of *Mopeia virus* diseases have been reported so far. However, this virus has been foreseen as an useful model to understand *Lassa virus* transmission ecology and immune responses (Borremans et al., 2011; Russier et al., 2012; Schaeffer et al., 2019). Grobbelaar et al. (2021), highlighted the importance of information on the occurrence and diversity of mammarenaviruses in Africa to understand possible risks to human health.A new *Mammarenavirus* non-pathogenic for humans (*Dhati Welel virus*) has been recently described in *M. natalensis* in eastern Africa (Gouy de Bellocq et al., 2020); which suggests that with further studies new mammarenaviruses could be identified in Africa and that a systematic mapping of *Mastomys* and *Mammarenavirus* deserves more attention. Indeed, apart from an early study from the 1970s (Wulff et al., 1977), there is a lack of data about *Mammarenavirus* circulation in Mozambique. Thus, this study was set out to investigate the diversity of mammarenaviruses circulating in *M. natalensis* trapped in villages, cropland fields and mopane woodland forest in the Limpopo National Park (LNP) and its buffer zone.

2. Materials and methods

2.1. Study sites

The study was carried out in the Massingir district counterpart of the LNP in Gaza province, south Mozambique (Fig. 1A). The rodents were trapped between March and November 2019 in three different habitats (villages, croplands fields and mopane woodland forest) from localities inside the LNP (Bingo, Macavene and Mavoze) and its buffer zone (Chibotane, Machavule and Madingane). (Fig. 1B). The Massingir district has 5893 km^2 of surface, 37,664 inhabitants, is dominated by dry semi-arid type zones with average annual temperatures of 30 °C and rainfall 600 mm. The study area falls within the *Great Limpopo Transfrontier Conservation Area* adjacent to the South African Kruger National Park (KNP) and the Zimbabwe's Gonarezhou National Park. The area supports subsistence farming dominated by rain-fed cropping in elevated land and with small irrigation schemes close to rivers and to Massingir dam. There are an extensive livestock production. Four localities are located inside the LNP with no physical barrier to separate wildlife and human. The wildlife mammal community includes large, medium and small sized terrestrial animals, such as elephants, buffaloes, lions, wild dogs, wild pigs and zebras.

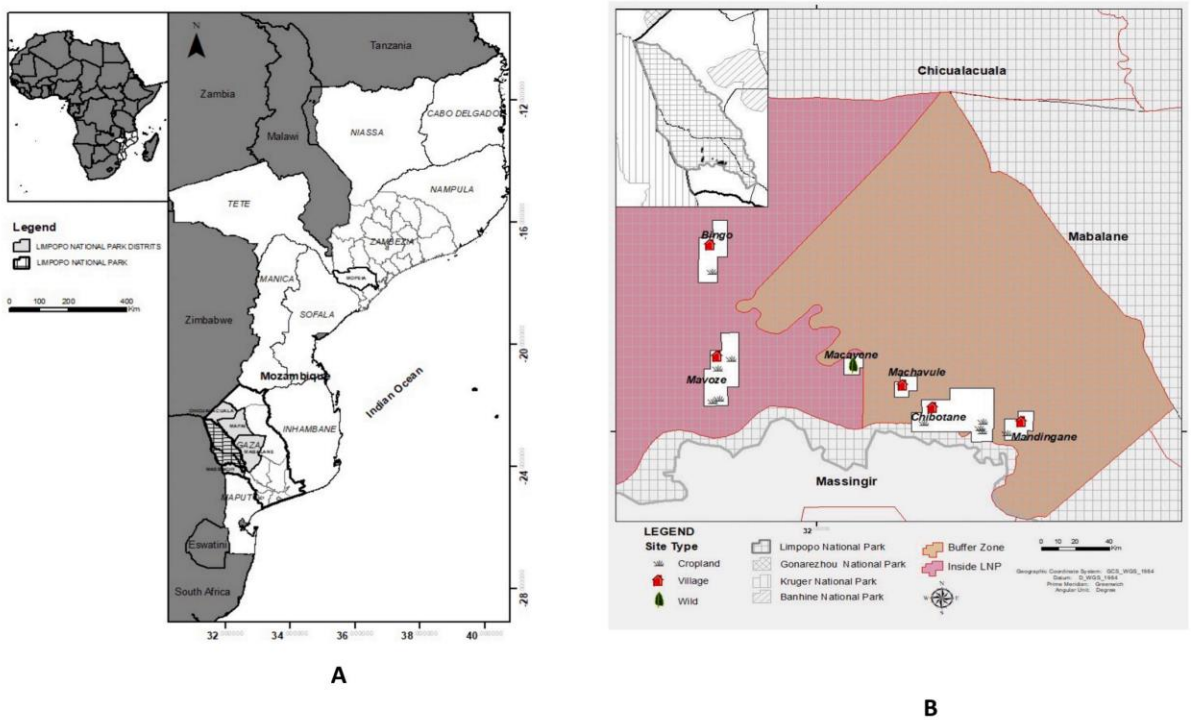

Fig. 1. Study area. A. Map of Africa in the upper left corner showing the location of Mozambique. On the right side the map of Mozambique indicating the location of the LNP and the districts covered by the park. The map of Mozambique also illustrates the location of Zambézia province where *Mopeia virus* was first described for the first time. B. The LNP map showing the sampled sites inside the park and in the buffer zone.

2.2. Rodent trapping and sampling

The following experimental protocol was approved by the Ethical Committee of VetAgro-Sup in Lyon, France (*Comité d'Ethique* de VetAgro Sup n°18, Avis 1905) and the credential for rodent capture in the LNP was obtained from the Mozambican National Administration for Conservation Areas (Credential Nr. 1/02/2021).

Two types of sites were included during this study: capture mark and recapture (CMR) sites and removal sites. In CMR sites trapped rodents were marked and released for potential recapture for rodent ecology studies (results not shown here). Only rodents found dead in the traps in the CMR sites were sampled for *Mammarenavirus* screening. By contrast in the removal sites, all *M. natalensis* rodents captured were sacrificed and the samples were screened for *Mammarenavirus* RNA detection. In villages, rodents were trapped inside houses and granaries. The captures in cropland fields included irrigated and rain-fed sites. The sites were selected based on the information collected from the local population about the presence of rodents and we also checked for signs of rodent's presence before setting the traps. During the sampling, crops (mainly bean and maize) were abundant in irrigated cropland fields while in the rain-fed cropland field, the sampling occurred after peanut harvesting.

In houses and granaries, the rodents were trapped using mesh traps baited with chorizo and Sherman (small and medium sizes) baited with toasted coconuts, peanut butter and oats. In cropland fields and mopane woodland only Sherman traps were set up. The number of traps deployed in each site was adjusted according to the house, granary and field size. In each house or granary, the number of traps varied between two and five while in cropland fields and mopane woodland the minimum number was 24 and the maximum 144 traps. Due to logistic and safety reasons (i.e., presence of dangerous wildlife) a single mopane woodland was sampled four times inside the LNP. The traps were armed at the end of the day and inspected the following morning. Traps were set for one to five consecutive nights, depending on the trapping success. Captured rodents were transported to the laboratory located in the LNP headquarters where the team was based during the trapping sessions. Rodents were euthanised with isoflurane, dissected and tissues (lung, heart, liver, spleen, kidney, cerebral, tail and ear samples) as well as blood (Dried Blood Spot, DBS) were collected. All tissue samples were conserved in home-made RNAlater solution (http://www.protocol-online.org/prot/Protocols/RNAlater-3999.html) and tail and ear for rodent species genetic identification were conserved in 70% ethanol (Herbreteau et al., 2011).

2.3. Rodent species genetic identification

Initially, morphological characteristics were used for rodent species in-field identification (Herbreteau et al., 2011). Because species in some African rodent genera are difficult to recognize and the taxonomy of many groups is still not well resolved (Monadjem et al., 2015), at least 10% of rodents morphologically identified as *M. natalensis* were selected for genetic identification at each capture site. We applied the DNA-barcoding approach to amplify and sequence (by the Sanger method) the complete mitochondrial cytochrome *b* gene (*CYTB*; 1140 bp) of selected individuals. We followed the protocols described by Bryja (2014) for DNA extraction, polymerase chain reaction (PCR) and sequencing. Species and intraspecific lineages were identified by a maximum likelihood (ML) analysis in RAxML 8.2.8 (Stamatakis, 2014), using GTRCAT substitution model recommended by the author. The robustness of the nodes was evaluated by the default bootstrap procedure with 1000 replications and new sequences were considered as part of previously defined taxa/lineages if the bootstrap support was >95%. For this study, we specifically compared the new sequences with those from the phylogenetic studies of the genus *Mastomys* (Colangelo et al., 2013; Martynov et al., 2020).

2.4. Mammarenavirus detection and phylogeny

Only *M. natalensis* individuals were considered for *Mammarenavirus* screening. RNA was extracted from lung tissues using Qiagen RNeasy Mini kit (Hilden, Germany). Briefly, 30 mg of lung tissue samples were disrupted for RNA release by adding 20 µL of proteinase K, one 2 mm inox bead and 500 µL of lysis buffer in a 2 mL tube and vortexed using a Qiagen TissueLyser LT system for 5 min at 50 Hz. The homogeneous lysate in the 2 mL tube from the above step was centrifuged at 11,000 $\times g$ for 3 min and then the supernatant was collected and used for viral RNA extraction following the manufacturer's instructions.

Two pairs of primers P1-LSF/R and P2-LSF/R (Li et al., 2015) targeted to conserved regions of the L segment of mammarenaviruses were used to amplify a 611 bp fragment. The total volume of the reaction mix for the first-strand cDNA synthesis was 20 µL containing 5 µL of template RNA, 20 pmol of *Mammarenavirus* genus-specific primer (P1-LSF), 0.5 mM of each dNTP, 4 µL of 5× RT Buffer, 20 units of RiboLock RNase Inhibitor and 100 units of Maxima H Minus Reverse Transcriptase. The thermo profile conditions were those described in the manufacture protocol (Maxima H minus Reverse Transcriptase, Thermo Scientific). The pair P1-LSF/R was used on the first amplification and the P2-LSF/R on the second PCR. The final volume of PCR mix for the two reactions was 50 µL and the volume or concentration of mix components were: 5 µL of cDNA, 1.25 units of Dream Taq Hot Start DNA Polymerase (Thermo Scientific, city and country), 1× Dream Taq Buffer (Thermo Scientific) with 2 mM $MgCl_2$, 0.2 mM of each dNTP (Thermo Scientific) and 3 pmol of each primer. The same thermal profile was used for each primer pair: initial denaturation at 95 °C for 3 min, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s and extension at 72 °C for 1 min. The final extension was at 72 °C during 5 min. The expected RT-PCR products were visualized on 1% agarose gel.

Positive RT-PCR amplicons including the positive control were purified using a Wizard SV Gel and PCR Clean-up system (Promega, Wisconsin, USA). Only purified amplicons with sufficient concentration were sent to LGC Genomics (Berlin, Germany) using the primer pair P2-LSF/R.

The final ML analysis of *Mammarenavirus* included 67 sequences of which 50 generated in this study, one *Mopeia virus* used in this study as positive control, 15 *Mammarenavirus* partial L gene fragment of representatives of the OWG and one representative of the NWG as outgroup from GenBank. *Mammarenavirus* nucleic acid sequences were aligned using MEGA 7 (Kumar et al., 2016), with minor manual adjustments. Sites that could not be unambiguously aligned were excluded and divergent regions were excluded from subsequent analysis. Thus, the final size of our alignment used for the phylogenetic analysis was of 468 bp. Phylogenies were inferred using ML method implemented in PhyML (Guindon et al., 2010). The reliability of branching orders was tested using the bootstrap approach (1000 replicates). The suited evolution model (GTR + I + G) was selected by Akaike's Information criterion (AIC) using Topali software (Milne et al., 2009). *Mopeia virus* nucleic acid sequences generated in this study were deposited in GenBank under accession numbers MZ512094 to MZ512143.

2.5. Statistical analysis

The proportion of *Mopeia virus* RNA positive individuals were calculated by habitat and locality with 95% confidence intervals (CI) using Wilson score test (Agresti and Coull, 1998). The χ^2 test of independence was performed to evaluate differences in proportions of positive individuals among the habitat types as well as among the localities.

3. Results

3.1. Rodent trapping and sampling

During a total of 7290 trap nights; 1235 rodents were captured and

82.5% ($n = 1019$) were morphologically identified as *M. natalensis*. In CMR sites we caught 541 *M. natalensis* individuals out of which 56 were found died in the traps and in removal sites we trapped 478 individuals. A total of 534 lung tissue samples were obtained from all *M. natalensis* trapped and sacrificed in removal sites and from all individuals found died in the traps deployed in CMR sites h. *M. natalensis* were captured in all the three types of habitats and across all the localities trapped. The highest number of *M. natalensis* was trapped from cropland fields and the lowest number of individuals was caught in the mopane woodland (Table 1 and Table S1).

3.2. Rodent species genetic identification

Mitochondrial CYTB genotyping of selected 101 out of 534 specimens (Table S2) confirmed all of them to be *M. natalensis* and all belonging to phylogroup B-VI (Colangelo et al., 2013).

3.3. Mammarenavirus molecular detection

The overall proportion of positive individuals for *Mammarenavirus* RNA detection was 16.9% [95% CI: 13.9–20.3]. Neither croplands nor villages were sampled in Macavene locality because the entire population that lived there was resettled outside the park. *Mammarenavirus* RNA was detected in 16.9% [95% IC: 13.8–20.5] and 16.3% [95% IC: 8.1–30.0] of specimens, respectively from cropland fields and villages. A single mopane woodland forest was sampled in the Macavene locality where 16.7% [95% CI: 3.0–56.4] of individuals tested positive. Nevertheless, the proportions of *Mopeia virus* positive rodents did not differ significantly between the habitats ($\chi^2 = 0.018$; DF = 1; $p = 0.893$). Across the localities, Machavule (only two individuals were caught) had the highest proportion 100% [$n = 2$; 95% CI: 34.2–100] of positive individuals followed by Bingo with 22.7% [95% CI: 15.2–32.5] and the lowest proportion 2.9% [95% CI: 0.8–9.8] was found in Madingane locality (Table 1).

Concerning the differences among habitats (or localities), five samples that had doubtful RT-PCR results were excluded from the chi-square test. Besides the mopane woodland forest habitat and two localities (Macavene and Machavule) were excluded from the analysis because of low expected values (McHugh, 2013). The proportion of positive individuals differed significantly among the localities ($\chi^2 = 12.512$; DF = 3; $p = 0.0058$).

3.4. Phylogenetic analysis

A total of 50 out of 90 purified amplicons had sufficient concentration for sequencing, thus the phylogenetic analysis was performed for all the positive samples from villages ($n = 7$) and mopane woodland forest ($n = 1$) and for 51% (42/82) of the positive samples from the cropland fields.

All our *Mammarenavirus* sequences clustered together with *Mopeia virus* (bootstrap value 91%) (Fig. 2). However, all the *Mopeia virus* identified in this study formed a specific *Mopeia virus* sub-clade with eight different sub-clusters. The sub-clusters I, III, IV, VI, VII and VIII were well sustained with bootstrap value >70%, whereas for sub-clusters II and IV the tree topology seemed unresolved with bootstrap values of 48% and 56%, respectively. The nodes of divergence between the sub-clusters I and II; and the sub-clusters IV, V, VI, VII were also not well supported with bootstrap value <70% (Fig. 2).

4. Discussion

In the present study, we screened *M. natalensis* by RT-PCR and we detected *Mopeia virus* in all habitats and localities across the study area at the interface between LNP and communal land in its buffer zone. We reported the presence of *Mopeia virus* in 16.9% (90/534) of tested *M. natalensis* with no significant differences between habitats. Phylogenetic analysis indicated that the viruses detected formed a specific *Mopeia virus* sub-clade with eight sub-clusters.

Several villages and cropland sites were surveyed inside the LNP and its buffer zone while a single mopane woodland was sampled four times inside the LNP. *M. natalensis* has a very similar external morphology with *Mastomys coucha* which is largely endemic in southern Africa (IUCN, 2016b). Sympatric occurrence of *M. natalensis* and *M. coucha* was reported in some regions of South Africa including the KNP (Kneidinger et al., 2014) which is adjacent to the LNP in our study area. The mitochondrial phylogenetic analysis split *M. natalensis* into six matrilineage phylogroups: A-I, A-II, A-III, B-IV, B—V, B-VI (Colangelo et al., 2013) and all were identified as natural reservoirs of mammarenaviruses (Gouy de Bellocq et al., 2020). The CYTB mitochondrial gene sequence phylogenetic analysis indicated that all captured *Mastomys* in our study area were *M. natalensis* belonging to the B-VI lineage. Previous studies reported that two lineages of *M. natalensis* (B—V and B-VI) occur in parapatry in Mozambique with the former being geographically restricted to the northernmost part of the country (Colangelo et al., 2013; Gouy de Bellocq et al., 2020).

Mammarenavirus detection in Mozambique dates back to 1977 when the *Mopeia virus* was first identified in *M. natalensis* population from Mopeia district in central Mozambique (Wulff et al., 1977). Studies have been carried out in southern Africa and indeed detected non-pathogenic *Mammarenavirus* such as *Mopeia virus, Morogoro virus, Gairo virus and Luna virus* in *M. natalensis* (Wulff et al., 1977; Günther et al., 2009; Ishii et al., 2011; Gryseels et al., 2015). Here, we detected the *Mopeia virus* in 16.9% (90/534) of tested *M. natalensis* samples by the viral RNA molecular detection system (RT-PCR). The proportion of positives is below the ones detected by previous serological studies: 21% in Mozambique (Wulff et al., 1977), 20% in Zimbabwe (Johnson et al., 1981) and the highest of 31.8% from retrospective samples collected South Africa and Zimbabwe (Grobbelaar et al., 2021). In Tanzania Borremans et al.

Table 1
Number of *M. natalensis* tested (*n*) and number of positives samples in different habitats and localities of the LNP and its buffer zone.

Locality	Habitat						Total	
	Cropland fields		Villages		Mopane woodland			
	Nr. of positives (*n*)	Proportion [CI, 95%]	Nr. of positives (*n*)	Proportion [CI, 95%]	Nr. of positives (*n*)	Proportion [CI, 95%]	Nr. of positives (*n*)	Proportion [CI, 95%]
Bingo	17 (78)	21.8 [14.1–32.2]	3 (10)	30.0 [10.8–60.3]	/	/	20 (88)	22.7 [15.2–32.5]
Chibotane	28 (144)	19.4 [13.8–26.7]	0 (19)	0.0 [0.0–16.8]	/	/	28 (163)	17.2 [12.2–23.7]
Macavene	/	/		/	1 (6)	16.7 [3.0–56.4]	1 (6)	16.7 [3.0–56.4]
Machavule	/	/	2 (2)	100.0 [34.2–100.0]		/	2 (2)	100.0 [34.2–100.0]
Madingane	1 (62)	1.6 [0.3–8.6]	1 (8)	12.5 [2.2–47.1]		/	2 (70)	2.9 [0.8–9.8]
Mavoze	36 (201)	17.9 [13.2–23.8]	1 (4)	25.0 [4.6–69.9]		/	37 (205)	18.0 [13.4–23.9]
Grand Total	82 (485)	16.9 [13.8–20.5]	7 (43)	16.3 [8.1–30.0]	1 (6)	16.7 [3.0–56.4]	90 (534)	16.9 [13.9–20.3]

Five individuals had dubious RT-PCR results in Bingo ($n = 1$), Chibotane ($n = 2$) and Mavoze ($n = 2$).

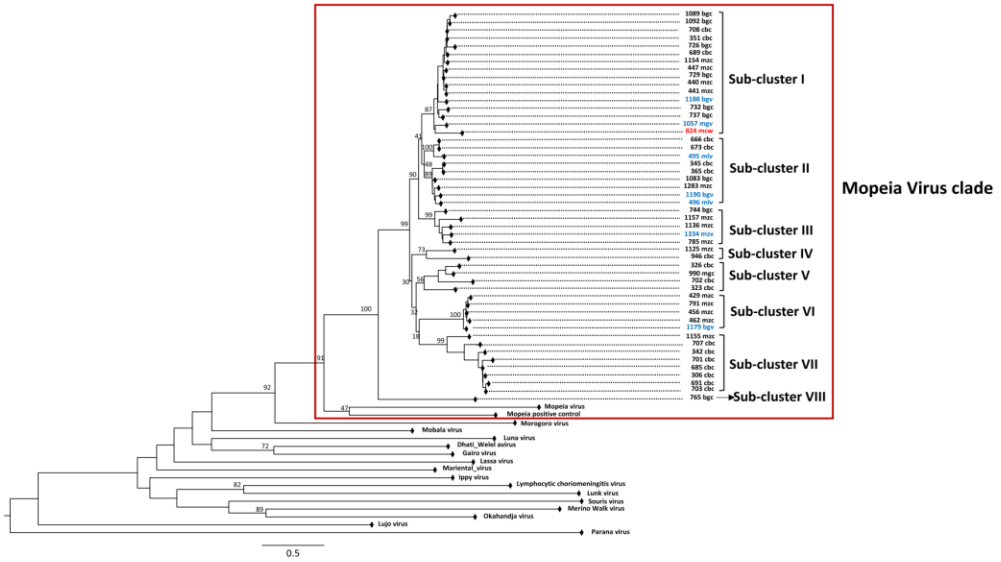

Fig. 2. Maximum Likelihood analysis, implemented in PhyML, of a partial nucleotide sequence of *Mammarenavirus* L gene fragment from the samples, our positive control genotyped, OWG *Mammarenavirus* reference sequences previously identified in Africa and available in the GenBank (Table S4). One *Mammare*navirus sequence of the NWG (Table S4) were used as outgroup. The asterisks at nodes represent the bootstrap values ≥70% calculated from 1000 bootstrap replicates. Scale bars indicate the number of base substitutions per site. The sample reference number is followed by three letters indicating the collection site as following: bgc = Bingo cropland; bgv = Bingo village; cbc = Chibotane cropland; mcw = Macavene mopan woodland; mzc = Mavoze cropland; mzv = Mavoze village; mlv = Machavule village; mgc = Madingane cropland and mgv = Madingane village. Village. The colors differentiate the types of habitats: black = cropland fields, blue = villages and red = mopane woodland. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(2011) found 12.1% [95% CI: 9.5–15.3] by serological testing and 8.4% [95% CI: 6.2–11.2] by the viral RNA molecular detection system. Due to the difference in the diagnostic tests used in these four studies, the comparison of prevalence is difficult. Comparing serological and molecular studies would require more studies in which both techniques are implemented on the same individuals (i.e., the presence of viral antibodies is supposed to be much long-lasting than viral RNA in individuals). At the molecular level (RT-PCR testing), the estimate of prevalence in our study was about twice the one reported in Tanzania, indicating a higher proportion of positive cases at the time of sampling in our study area. However, as these studies were carried out in different contexts, factors such as age of tested animals, habitat types and season may contribute to the difference in the proportion of positive individuals observed among them. Indeed, variation of *Mammarenavirus* prevalence estimators have been reported between geographic regions in Tanzania (Cuypers et al., 2020).

We sampled three different habitats (only two individuals were caught in the mopane woodland forest) and no significant differences were observed on the proportion of individuals positive for *Mopeia virus* RNA across the habitats. This observation suggests that the level of *Mopeia virus* circulation is the same among *M. natalensis* populations living in these three habitats. The possible explanation for this homogeneity could be that the studied habitats offer similar environmental conditions for viral viability and transmission. Borremans et al. (2011), also reported a great homogeneity in *Mopeia virus* prevalence across different habitat types in Tanzania and suggested that the abiotic environment may not be an important determinant of virus prevalence.

The proportion of positive individuals differed significantly among the localities; however, the 100% [95% CI: 34.2–100.0] found in Machavule is less informative because of the very small number of rodents (N = 2) captured in this locality. Thus, the 22.7% [95% CI: 15.2–32.5] detected in Bingo is referenced as the highest proportion of positive rodents across the localities in our study area. On the other hand, our statistical inference within the localities was influenced by the lowest proportion of *Mopeia virus* positive rodents detected in Madingane 2.9% [95% CI: 0.8–9.8]. The differences observed within localities suggests that in some localities humans are relatively more exposed to potentially zoonotic Mammarenaviruses. Nonetheless, the localities were sampled in different periods and this could possibly explain the differences of proportion of positive samples detected in Madingane locality. Rodent-host population dynamics is an important factor of the epidemiology of mammarenaviruses, whereby seasonal peaks of virus circulation may be associated with increased rodent populations and could explain prevalence differences between different months (Jay et al., 2005; Akhmetzhanov et al., 2019). The level of Mammarenavirus circulation in rodent population depend on the interaction between the virus, rodent-hosts and ecological variables (Childs and Peters, 1993). Therefore, a study incorporating an analysis of epidemiological and ecological parameters in our study area is needed to understand the homogeneity of virus circulation in habitats, the heterogenicity of prevalence in the localities and possibly during the different sampling months.

The mammarenaviruses detected in three different habitats (villages, croplands and mopane woodland forest) clustered with *Mopeia virus,* the unique *Mammarenavirus* species described so far in Mozambique (Wulff et al., 1977). The mammarenaviruses from the study area formed a specific sub-clade that did not include the reference *Mopeia virus* isolate from Mozambique (GenBank accessory Nr. AY772169.1) as well as the *Mopeia virus* positive control isolate ("Mopeia virus, strain UVE/MOPV/UNK/MZ/Mozambique 20410 | EVAg"). The mammarenaviruses detected in the study area formed eight sub-clusters and the sub-cluster I is the one most widespread with viruses from the three habitats and from five out of six localities included in the study. However, the sub-clusters II and V were not well sustained. Likewise, the bootstrap values at the roots of sub-clusters I and II as well as for sub-clusters IV to VII were low suggesting an unresolved tree topology. Consequently, it is probable that

the viruses could change the place between the sub-clusters I and II as well as among the sub-clusters IV to VII. This is likely due to the small size (468 bp) of the fragment analyzed. Therefore, we think that the analysis of the whole L segment would give better insight of the genetic diversity of the mammareviruses in our study area. Besides, the basal position of sample 765 from Bingo cropland field may suggest that in fact there is some degree of genetic variability of *Mopeia virus* in the study area. The detection of these probable different sub-clusters in the different habitats may indicate wide circulation of viruses of different sub-clusters within the study area. Evidence of *Mammarenavirus* circulation in other species than *M. natalensis* had been reported. *Mopeia virus* antibodies were detected in *Aethomys chrysophilus* in Zimbabwe (Johnson et al., 1981), *Mus minutoides* carries *Lunk virus* in Zambia and *Kodoko virus* in West Africa (Lecompte et al., 2007; Ishii et al., 2012). Recently, two novel mammarenaviruses were detected in Angola: the first one, *Bitu virus*, related to *Okahandja virus,* was found in *Micaelamys namaquensis*; the second one, *Kwanza virus,* related to *Mobala virus, was* detected in *Mus triton* (Těšíková et al., 2021). Results obtained in samples from South Africa and Zimbabwe indicated presence of mammarenavirues in 14 rodent species that included *Aethomys. chrysophilus*, *Mastomys. coucha*, *Rattus norvegicus*, *Rattus rattus*, *Saccostomus campestris* and *Gerbilliscus leucogaster* (Grobbelaar et al., 2021). The assessment of the potential circulation of mammarenaviruses in other rodent species from our study area is therefore important.

Mammarenaviruses transmission from rodent to humans occur through contaminated rodent excreta and secretions and crop harvesting or hunting activities, poor hygiene in households and rodent consumption increase the risk of transmission (Paweska, 2014; Akhmetzhanov et al., 2019) The human population in our study area practices agriculture, hunting (although prohibited by law), some consume rodents and like in other parts of the country the environment sanitation is deficient. Here we reported that *Mopeia virus* circulates in *M. natalensis* rodents, thus we hypothesize that humans in the study area may be exposed to mammareviruses. Unpublished data cited by Grobbelaar et al. (2021), indicated that antibodies to *Mopeia virus* were detected in humans' sera but with no association with clinical disease resembling mammarenaviruses. Serological and molecular investigation of *Mopeia virus* in humans will inform about viral exposure and the viral genetic profile in the study area.

5. Conclusions

Mopeia virus was detected in *M. natalensis* trapped in villages, cropland fields and mopane woodland from all the six surveyed localities. The *Mopeia virus* we detected formed a specific *Mopeia virus* sub-clade than the previously described in Mozambique. Habitat type does not have influence on the proportion of positive individuals in the study area. The genus *Mammarenavirus* contain zoonotic species and possibly others potentially zoonotic could emerge, thus, our results suggest that humans could be exposed to mammarenaviruses in all habitats, so awareness campaigns could be designed to sensitize local citizens about the potential risks of zoonotic disease spill over from rodents as well as further risk-based studies to explore which group of stakeholders are more exposed than others. We have expanded and updated the data on *Mopeia virus* in Mozambique and made more sequences available for this virus.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2022.105204.

Data availability

The authors declare that all the data analyzed here will be available without any restrictions.

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Competing interests

The authors have no competing interests to declare.

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6 CHAPTER III: ECO-EPIDEMIOLOGY OF *MOPEIA VIRUS* DETECTED IN *M. NATALENSIS* FROM THE IRRIGATED CROPLAND FIELDS IN THE LNP AND ITS BUFFER ZONE

6.1 INTRODUCTION

Hemorrhagic fevers caused by the emerging *Lassa virus*, an mammarenaviruses whose main host is *M. natalensis*, constitute a major public health problem in West Africa (Siddle et al., 2018; Karan et al., 2019). Studies from that region of Africa indicate that knowledge of the ecology of *M. natalensis* is a very important component for understanding the risk of transmission of the *Lassa virus* to humans, and therefore for designing better control strategies (Happi et al., 2022). However, despite the circulation of several mammarenaviruses in rodents in southern Africa (Günther et al., 2009; Cuypers et al., 2020; Grobbelaar et al., 2021), including the fatal *Lujo virus* from an unknown host (Paweska et al., 2009), few studies on the transmission ecology of mammarenaviruses have been conducted in the region.

Disturbed habitats change species community composition and induce a loss of biodiversity impacting disease transmission (Bordes et al., 2015). In China, it was found that the abundance of *Apodemus agrarius* in agricultural lands was higher and this may have increased the risk of human exposure to hantavirus (Yan et al., 2007). Similarly, a study in Tanzania suggested that the need for agricultural intensification to increase crop production may create conditions for colonization by *M. natalensis* (Makundi et al., 2007). Both increased risk of pathogen transmission with decreasing biodiversity (Schmidt and Ostfeld, 2001; Keesing et al., 2010) and increased risk of transmission when biodiversity is higher (Bonvicino et al., 2015; Dunn et al., 2010; Sun et al., 2019) have been intensively debated among scientific community. The details of the mechanisms to explain these hypotheses were explained in the literature review section (see § 2.2.3).

In southern Africa, *M. natalensis* display reproduction seasonality and it has been linked to the rainy season (Leirs et al., 1989; Monadjem et al., 2015). This reproduction phenology is more likely to be a key factor on disease transmission dynamic because of changes in population demography such as abundance, age structure, sex ratio and reproductive age individuals (Bordes et al., 2015).

Higher host abundance has been linked to higher rates of pathogen circulation according to the density-dependent transmission hypothesis. This hypothesis suggests that higher population

abundance increases the direct contact with infected individuals or materials (Altizer et al., 2006; Kamath et al., 2014; Bordes et al., 2015). In Guinea, the relative abundance of *M. natalensis* correlated positively with the high seroprevalence of the *Lassa virus* in humans (Lecompte et al., 2006). Fichet-Calvet at al. (2007) found a higher densities of *M. natalensis* in houses during the dry season, and suggested that this may explain higher encounter risks between humans and rodents, hence a higher prevalence of Lassa in humans. In Sierra Leone, both higher abundance of *M. natalensis* and a higher prevalence of *Lassa virus* were recorded inside houses (Bangura et al., 2021).

Juveniles are immunologically naive after having lost their protective maternal antibodies, and more susceptible to a variety of pathogens (Altizer et al., 2006; Peel et al., 2014). Among the mammarenaviruses, the hypothesis of very early infection through vertical transmission was reported for *Lassa virus* in Guinea (Demby et al., 2001; Fichet-Calvet et al., 2008) and *Mopeia virus* in Tanzania (Borremans et al., 2011). The above studies also noticed an increment of prevalence with age within the juveniles group highlighting the occurrence of horizontal transmission over time.

Females and males display immunological differences and this may contribute to variations in disease susceptibility (Weinstein et al., 1984; Klein and Flanagan, 2016). *Mastomys* male use promiscuous mating strategy (Kennis et al., 2008; Borremans et al., 2014) and this may promote competition between males and possibly increase the risk of disease transmission between them compared to females (Fichet-Calvet et al., 2008). Besides, this enlargement of the home range by males also increases the possibility of coming into contact with contaminated material with excreta from infected individuals. Reproduction costs may lower host immunity in rodents due to activation of glucocorticoid hormones, (Festa-Bianchet, 1989; Mougeot et al., 2005). A study in Tanzania suggested that reproduction conditions might increase the probability of *M. natalensis* becoming infected by mammarenaviruses (Mariën et al., 2017).

In this chapter we investigated how the factors in our dataset presented in chapter one could influence the circulation of the *Mopeia virus* in *M. natalensis* detected in chapter two over different habitats in the LNP and its buffer zone. Our aim was to identify factors and periods associated

with higher circulation of the *Mopeia virus* in *M. natalensis* in order to help implementing programs targeting the prevention and surveillance of hemorrhagic fevers in Mozambique.

6.2 MATERIAL AND METHODS

The study sites, rodent trapping and sampling were described in the data collection chapter (see §3.5). Rodent screening for mammarenaviruses detection was performed by RT-PCR after RNA extraction from lung tissues (see § 5.2).

6.2.1 Tested factors

6.2.1.1 Species diversity

The species diversity was determined by habitat using both species richness and Simpson Diversity Index (see § 4.1.2). The effect of host diversity on the proportion of individuals positive for *Mopeia virus* RNA detection was evaluated between habitats. Our data indicated that the irrigated croplands had the lowest species diversity compared to other habitats (see § 4.1.3). Thus, based on the dilution effect hypothesis, we expected a higher proportion of *Mopeia virus* RNA-positive individuals in the irrigated croplands compared to other habitats. The dilution effect hypothesis suggests that for pathogens transmitted by direct contact such as mammarenaviruses, the presence of non-susceptible hosts will reduce encounters between infected and susceptible individuals of susceptible hosts, thus lowering pathogen transmission. On the other hand, when biodiversity is low such as observed in our irrigated croplands, there is a high possibility of contact between individuals of the susceptible species which increases the risk of pathogen transmission (Keesing et al., 2009; Blasdell et al., 2011).

6.2.1.2 Rodent abundance

The relative abundance was used to estimate the rodent population abundance (see § 4.1.2). In our study area rodent abundance differed between habitats (see § 4.1.3) and in the irrigated croplands across the PTSs (see § 4.1.3). Thus, the effect of rodent abundance on the proportion of individuals positive for *Mopeia virus* RNA detection was assessed among habitats and between PTSs. We

hypothesized that the proportion of *Mopeia virus* RNA-positive samples was higher in habitats (irrigated croplands) and PTS (June) with higher rodent population abundance in light of density-dependent transmission hypothesis. This hypothesis suggests that for pathogens transmitted by direct contact such as mammarenaviruses a higher host density increase pathogen transmission due to increased direct contact rates between uninfected hosts with infected individuals or materials (Altizer et al., 2006; Lecompte et al., 2006; Kamath et al., 2014; Bordes et al., 2015).

6.2.1.3 Age

In this study the body weight was used to categorize *M. natalensis* individuals in adults (> 22g) and juveniles (≤22g). Our data indicated variation of age structure over the PTSs and the peak of proportion of juveniles was observed between May and August (see § 4.5.3). We hypothesized that the proportion of individuals positive for *Mopeia virus* RNA detection was higher when more juveniles were present in the population between May and August, because young individuals are considered to be immunologically naive (Altizer et al., 2006; Borremans et al., 2011; Peel et al., 2014). To assess this, we compared the evolution of proportions of adults and juveniles positive for *Mopeia virus* RNA detection over the PTSs.

6.2.1.4 Sex and reproduction condition

The individuals were categorized into mature and immature based on the condition of the genital organs. Our results displayed variations of sex ratio (see § 4.3.3) and proportion of mature and immature individuals (see § 4.4.3) over the PTSs, thus the effects of sex and reproduction condition were assessed by trapping. We hypothesized that during the breeding season more sexually active males were positive than females because it has been suggested that males move more searching for mate increasing the risk of viral horizontal transmission through direct contact with infected individuals or materials (Fichet-Calvet et al., 2008; Kennis et al., 2008; Mlyashimbi et al., 2020). In addition, young individuals are immature, so we expect a trend of positive samples in immature individuals similar to that of juveniles.

6.3 DATA ANALYSIS

A descriptive epidemiological analysis was performed based on the RT-PCR results for Mopeia virus detection. The proportion of *Mopeia virus* RNA positive samples was calculated with 95%CI using the Wilson score test (Agresti and Coull, 1998). The χ^2 test of independence was performed to evaluate the differences in proportions of *Mopeia virus* RNA positive samples between PTSs and host demographic parameters in the irrigated croplands. Whenever the expected value was very low (< 5) the data was excluded from χ^2 test analysis.

6.4 RESULTS

6.4.1 *Mopeia virus* circulation in *M. natalensis* across PTSs in different habitats

In our study area, the overall proportion of *Mopeia virus* positive samples was 17.0% (n=90) out of 529 *M. natalensis* lung tissues screened for viral RNA detection. The overall proportion of positive individuals across the habitats were presented and discussed in chapter two. Here, we showed that the differences of *M. natalensis* abundance over the habitats and differences of species diversity between the irrigated croplands and the other habitats did not have influence on the proportion of positive individual across the habitats (**Table 11**).

The variation of proportions of positive individuals across the trapping for the overall data followed the same trend of variation of proportion of positive individuals in the irrigated croplands because 90.0% (474/529) of tested individuals were trapped in the irrigated croplands. Due to this low number of individuals in villages, rainfed cropland field and mopane woodland forest, the effect of trapping period and host related characteristics was analyzed exclusively in the irrigated croplands. Across the PTSs in the irrigated croplands, the proportion of positive samples increased from March up to June when the highest proportion of positive individuals (26.4%; n=33) was recorded. Afterwards, the proportion of positive samples decreased toward November (8.3%; n=3) (**Table 11**). The differences of positive samples observed between the PTSs in the irrigated croplands were significant (χ^2=13.5; DF=3; p= 0.004).

Table 11: Proportion of M. natalensis positive samples in the four habitats and the evolution of positive samples over the five PTSs. N is the total number of M. natalensis tested and (n) is the number of positive individuals

PTSs		Habitat				Overall
		irrigated croplands *	rainfed cropland	mopane woodland forest	Villages*	
March	N (n)	1 (0)	/	/	19 (0)	20 (0)
	% [CI, 95%]	0.0 [0.0-79.4]	/	/	0.0 [0.0-16.8]	0.0 [0.0-16.1]
May	N (n)	152 (27)	/	/	2 (2)	154 (29)
	% [CI, 95%]	17.8 [12.5-24.6]	/	/	100.0 [34.2-100.0]	18.8 [13.4-25.7]
June	N (n)	125 (33)	6 (1)	6 (1)	/	137 (35)
	% [CI, 95%]	26.4 [19.5-34.8]	16.7 [3.0-56.4]	16.7 [3.0-56.4]	/	25.6 [19.0-33.5]
August	N (n)	160 (18)	/	/	18 (4)	178 (22)
	% [CI, 95%]	11.3 [7.2-17.1]	/	/	22.2 [9.0-45.2]	12.4 [8.3-18.0]
November	N (n)	36 (3)	/	/	4 (1)	40 (4)
	% [CI, 95%]	8.3 [2.9-21.8]	/	/	25.0 [4.6-69.9]	10.0 [4.0-23.1]
Overall	N (n)	474 (81)	6 (1)	6 (1)	43 (7)	529 (90)
	% [CI, 95%]	17.1 [14.0-20.7]	16.7 [3.0-56.4]	16.7 [3.0-56.4]	16.3 [8.1-30.0]	17.0[14.1-20.5]
Species richness		2	4	4	4	
Simpson Diversity Index		0.09	0.43	0.67	0.45	
Total number captured		1409	90	13	199	

*****The data from irrigated croplands was obtained from a pool of 8 sites and for villages was a pool of five sites

6.4.2 *Mopeia virus* circulation in *M. natalensis* in different localities sampled during different PTSs in the irrigated croplands

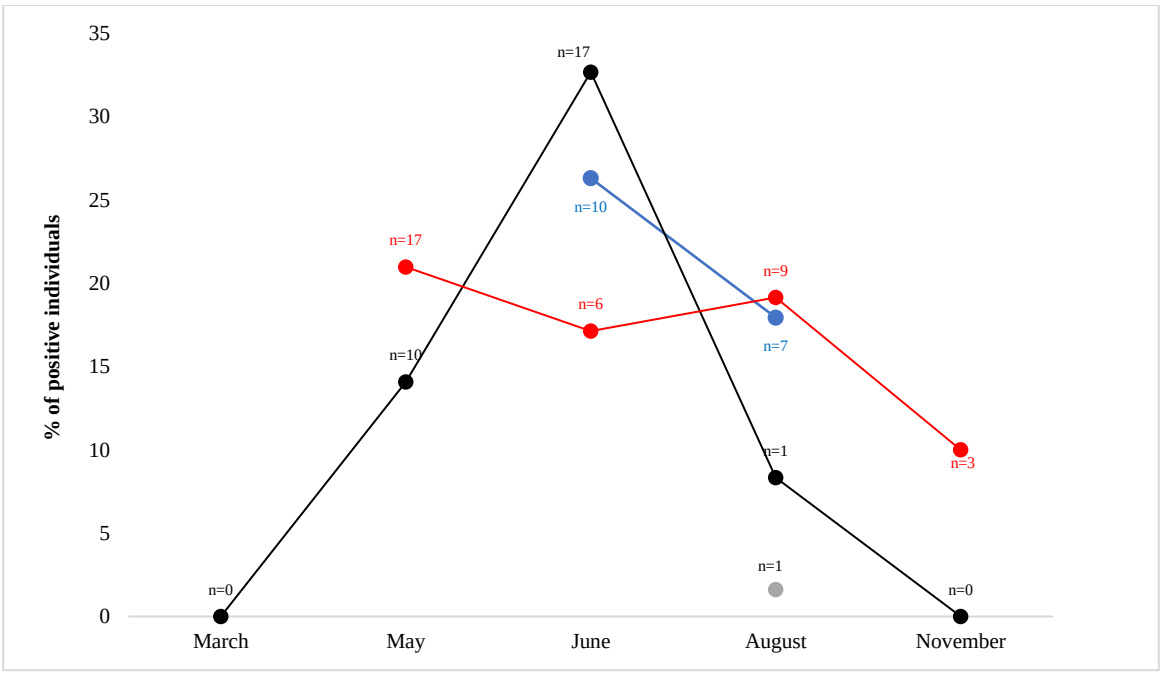
In our previous study (see § 5.2) we presented and discussed the overall proportion of positive individuals across the sample localities and we reported significant differences among localities. Here we displayed the results by trapping sites and PTSs in the different localities to get better inside of the variations observed in the overall results between localities.

The different sites sampled over different localities indicated differences of proportion of samples positive on *Mopeia virus* detection between localities over the PTSs (**Figure 25**). However, these diffrences were not significant in May between Chibotane and Macodze (χ^2=1.2; DF=1; p=0.2), in June between Chibotane, Bingo and Mavodze (χ^2=2.6; DF=2; p=0.3) and in August between Mavodze, Bingo and Madingane (χ^2=4.6; DF=2; p=0.1).

In Chibotane where different sites were sampled over the PTSs we observed an increase of *Mopeia virus* circulation between March and June (with the highest peak of 32.7%), followed by a decreasing trend toward November. However, the significance of the differences between the PTSs was not assessed due to the small number of samples in March, August and November. Nonetheless, the differences of positive samples were significant between May and June (χ^2=6.0; DF=1; *p*=0.01).

The data from Mavodze (mzf4 and mzf5) and Bingo (bgf1) sampled in two PTSs indicated a decrease in proportion of *Mopeia virus* positive samples between June (26.3%; n=10) and August (18.0%; n=7) for the site bgf1, from May (21.0%; n=17) to June (17.2%; n=6) in the site mzf4 and between August (19.2%; n=9) and November (10.0%; n=3) in the site mzf5 (**Figure 25**). This may suggest that the removal of individuals during the first PTS reduced the number of infected individuals that could serve as a potential source of virus transmission among the population, thus a potential bias that my interfere with density-dependence hypothesis testing.

As mentioned above, the differences of proportion of positive were no significant within the PTSs over the localities. In addition, the data by site over the PTSs displayed a similar trend (except in Mavodze between May and August) so we pooled the data by PTS to increase the power to investigate the effect of trapping period and host characteristics on *Mopeia virus* circulation in our study area. This comparison will be carried out in next sections.



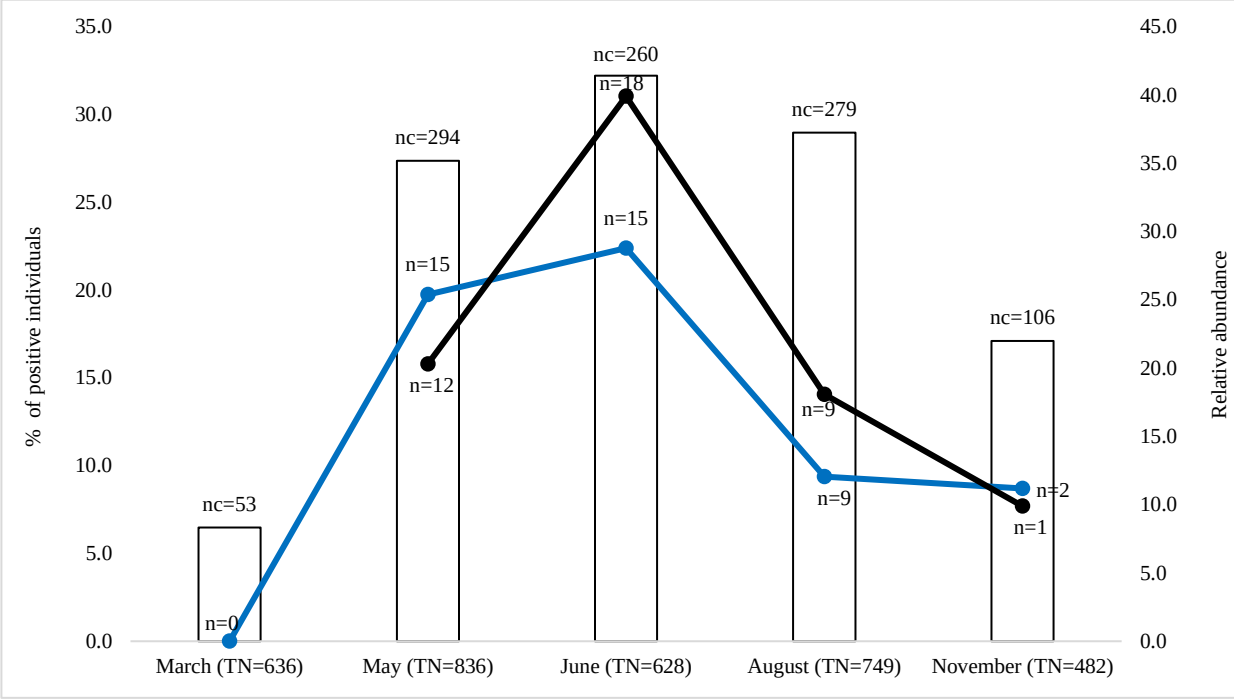
Chibotane	1 [0.0-79.4]; cbf1	71 [7.8-24.0]; cbf1, cbf2	52 [21.5-46.2]; cbf1, cbf3	12 [1.5-35.4]; cbf1, cbf4	6 [0.0-39.0]; cbf1
Mavodze		81 [13.5-31.1]; mzf4	35 [8.1-32.7]; mzf4	47 [10.4-32.5]; mzf5	30 [3.5-25.6]; mzf5
Bingo			38 [15.0-42.0]; bgf1	39 [9.0-32.7]; bgf1	
Madingane				62 [0.3-8.6]; mgf1	

Figure 25: Evolution of proportions of positive samples on RT-PCR for detection of Mopeia virus RNA in different sites and PTSs across the localities in irrigated cropland. The total number of samples tested (N), the 95%CI and the sampled sites in the localities are displayed below each PTS while the numbers of positive samples (n) are shown inside the graphic

6.4.3 *Mopeia virus* circulation in *M. natalensis* between age groups and rodent abundance over the PTSs in irrigated croplands

The overall proportion of *M. natalensis* positive samples on RT-PCR for *M. virus* RNA detection was 15.6% (n=41) for adults and 19.0% (n=40) for juveniles. The number of rodents captured, as well as the proportion of both adults and juveniles' individuals positive on RT-PCR for *Mopeia virus* RNA detection, displayed a similar trend across the PTSs. In May and November, more adult individuals were infected compared to juveniles but in June and August the trend shifted in favor of juveniles (***Figure 26***). The highest proportions of *Mopeia virus* positive samples were observed in June for adults (22.4%; n=15) and for juveniles (31.0%; n=18) (***Figure 26***). The differences of *Mopeia virus* positive samples were not significant among adults and juveniles (χ^2=0.7; DF=1; *p*=0.4),

within adult individuals over the PTSs (χ^2=5.8; DF=2; *p*=0.05) but significant across the PTSs for juveniles (χ^2=6.7; DF=2; p=0.03).



>22 g	1 [0.0-79.4]	76 [12.3-30.0]	67 [14.1-33.7]	96 [5.0-16.9]	23 [2.4-26.8]
≤22 g		76 [9.3-25.6]	58 [20.6-43.8]	64 [7.6-24.6]	13 [1.4-33.3]

Figure 26: Evolution of M. natalensis relative abudance (bars) and the proportions of positive samples (lines) on RT-PCR for detection of Mopeia virus RNA among adults (>22g) and juveniles (≤22g) over the PTSs in the irrigated croplands. The figure also shows the number of captures (nc), the numbers of positive samples (n), the total number of samples tested and the 95%CI.

Displaying *Mopeia virus* positive *M. natalensis* by different weight classes we observed viral circulation in both youngest and oldest individuals. The first three weight classes were relatively more infected compared to the last three weight classes; however, it is worth mentioning that higher number of individuals from the first weight classes were tested compared to the four last ones (***Figure 27***). Because RT-PCR was used to detect *Mopeia virus* RNA, the positive results in the youngest individuals in our data suggested occurrence of earlier infections in the studied *M. natalensis* population.

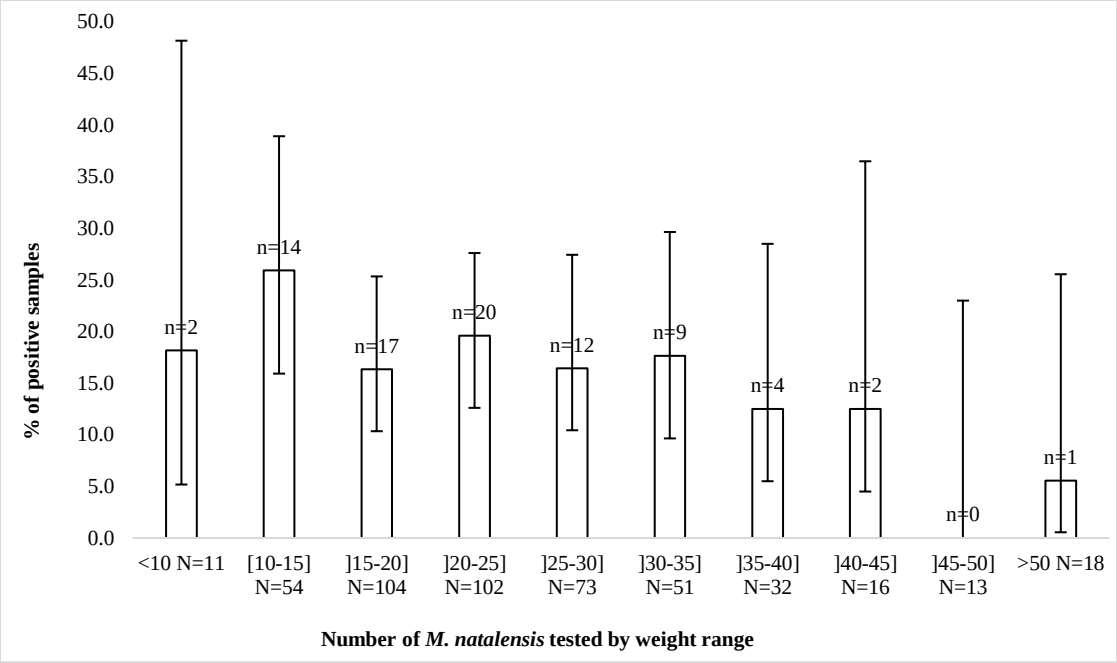
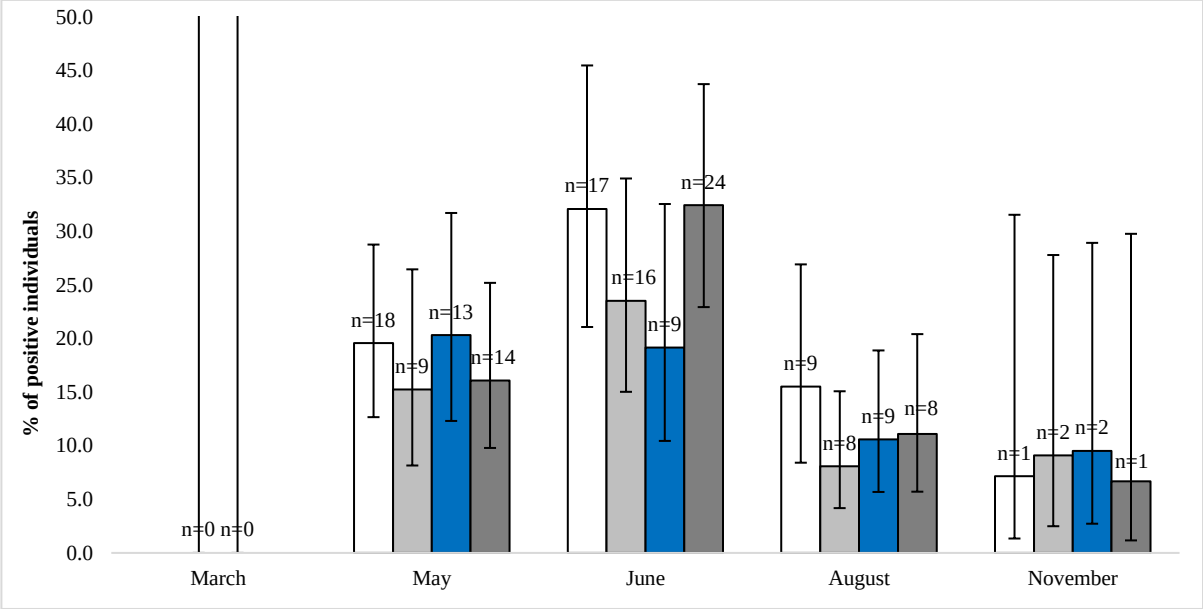


Figure 27: Distribution of Mopeia virus positive M. natalensis by weight groups in the irrigated croplands

6.4.4 *Mopeia virus* circulation in *M. natalensis* females, males, mature and immature individuals trapped over different PTSs in irrigated croplands

The overall proportion of *M. natalensis* positive samples on RT-PCR for *Mopeia virus* RNA detection was 20.7% (n=45) for females and 14.1% (n=35) for males. Between May and August, the proportions of positive females were higher compared to males while in November apparently, more males 9.1% (n=20) were positive than females 7.1% (n=10) (***Figure 28***). For both females and males, the proportions of *Mopeia virus* positive samples increased from May to June when the highest peaks of positive samples of 32.1% (n=17) for females and 23.5% (n=16) for males was observed, then decreased toward the last PTS in November (***Figure 28***). The differences between the number samples positive on RT-PCR for *Mopeia virus* RNA detection were not significant between females and males for the overall data (χ^2=3.2; DF=1; p=0.07 Between the trapping sessions, the differences of positive samples were not significant for females (χ^2=4.9; DF=2; p=0.09) but significant for males (χ^2=7.74; DF=2; p=0.02).

The overall proportion of mature *M. natalensis* samples positive on RT-PCR for *Mopeia virus* RNA detection was 15.1% (n=33) and for immature was 19.0% (n=48). In May and November, a higher percentage of mature and. immature individuals were infected, while in June we recorded a higher percentage of positive samples in the immature group. The level of infection was almost the same for both mature and immature individuals in August (***Figure 28***). The differences of *Mopeia virus* positive samples observed between mature and immature individuals were not significant for overall data (χ^2=1.2; DF=1; p=0.3), among mature individuals over the PTSs (χ^2=0.32; DF=2; p=0.85) but significant for the immature individuals across the PTSs (χ^2=29.4; DF=2; p<0.00001).

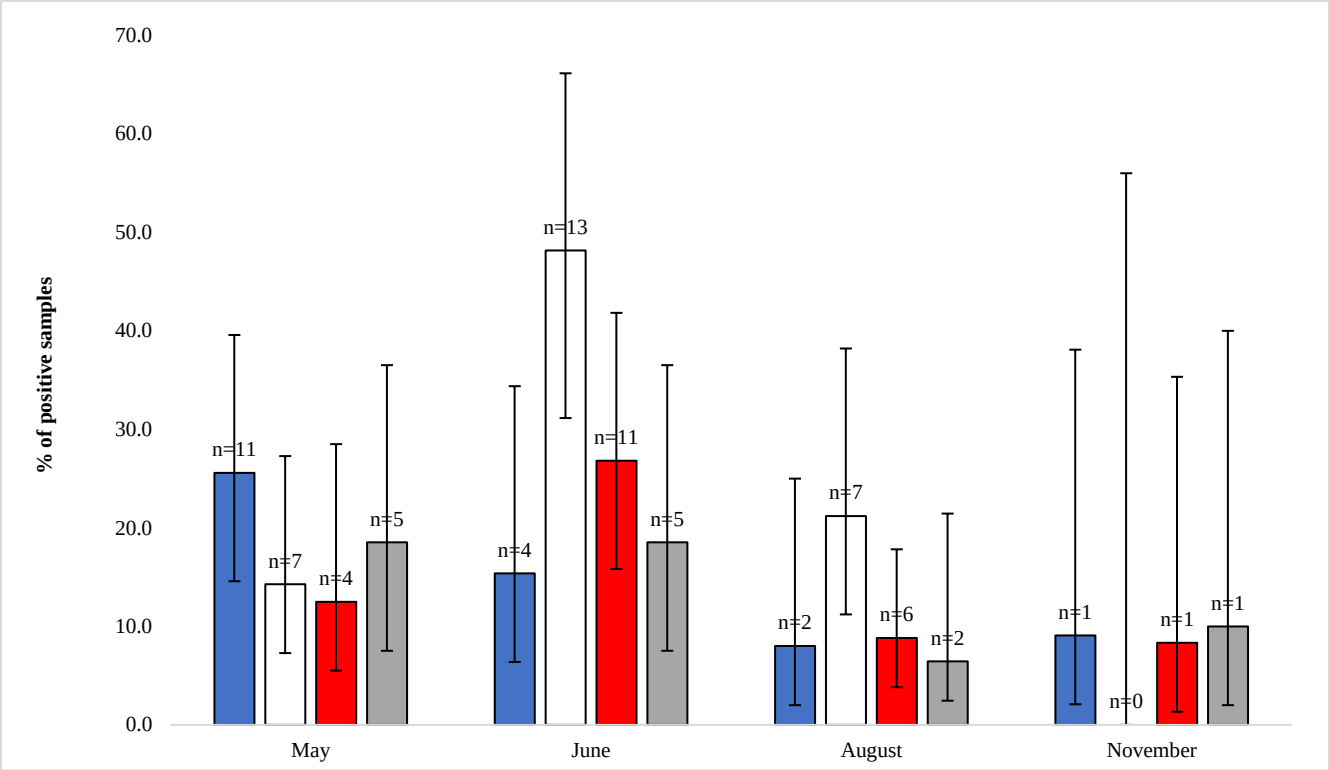


Female	0	92	53	58	14
Male	1	59	68	99	22
Mature		87	74	72	15
Immature	1	64	47	85	21

Figure 28: Evolution of proportions of positive samples on RT-PCR for detection of Mopeia virus RNA among females, males, immature and mature individuals over the PTSs in irrigated croplands. The total number of samples tested (N), are displayed below each PTSs while the numbers of positive samples (n) are shown along the graphics

Displaying *Mopeia virus* positive samples by sex and age, we observed that the number of positive adult females declined from May (n=11) toward November (n=1) and the majority of positive females in May were adults (61.1%; 11/18) while in June the majority of positive females were juveniles (76.5%; 13/17). Within the males, we noticed a rise of *Mopeia virus* positive samples from May (n=4) to June where the majority of positive males were adults (64.7%; 11/17) followed

by a slowdown pattern onward to November (n=1); finally, the number of positive juveniles' males (n=5) was the same in May and June (***Figure 29***). The results of X^2 significance analysis did not give enough support due to very low expected values (<5) for adult females and juveniles males.



Adult female	43	26	25	11
Juvenile female	49	27	33	3
Adult male	32	41	68	12
Juvenile male	27	27	31	10

Figure 29: Distribution of Mopeia virus positive M. natalensis females and males by age. The number of samples tested is displayed below the PTSs while the numbers of positive individuals (n) are indicated above the bars

6.5 DISCUSSION

The overall results did not show significant differences of proportion of *Mopeia virus* positive samples among age, sex and reproductive condition. Furthermore, the differences in rodent abundance and host diversity observed between the habitats were not reflected in differences in the proportion of individuals positive for *Mopeia virus* in the four habitats studied. Like in our study, abundance, age, sex and reproduction conditions did not correlate significantly with

occurrence of *Mopeia virus* in Tanzania (Borremans et al., 2011) and *Lassa virus* in West Africa (Fichet-Calvet et al., 2008, 2007; Olayemi et al., 2018).

However, in this study we observed that the main differences in *Mopeia virus* circulation was by PTSs suggesting a temporal variability of *Mopeia virus* transmission in our study area. Temporal variation in *Mammarenavirus* circulation was reported in a study in Guinea where the highest prevalence of *Lassa virus* in *M. natalensis* was recorded during the rainy season (Fichet-Calvet et al., 2008), however, contrary to our findings indicating peak of *Mopeia virus* positive samples in the middle of dry season. The likely possible explanation for the observed differences could be the differences in the ecology of *M. natalensis* between our study area and in Guinea, like in most of West Africa where *M. natalensis* is mostly commensal.

While attempting to link *M. natalensis* ecology and mammarenaviruses transmission, we observed a common feature about *M. natalensis* reproduction and mammarenaviruses positive samples in our study area and in Guinea. Our data on *M. natalensis* reproduction in our study area indicated that middle of dry season was the peak of breeding season characterized by peaks of rodent abundance and proportion of juveniles. The peak of *Mopeia virus* circulation in our study area was observed in the middle of dry season and coincided with the peak of infection in juveniles. In West Africa *M. natalensis* breed all over the year but their higher reproduction occurs in the rainy season (Balogun et al., 2021). The coincidence of *M. natalensis* breeding season with higher positive samples of *Mopeia virus* in our study highlights that *M. natalensis* demography deserves attention for better understanding of mammarenaviruses transmission.

Juveniles are regarded as immunologically naive so are more susceptible compared to adults (Altizer et al., 2006; Peel et al., 2014). As in our study, *Lassa virus* prevalence in Guinea differed between the adults and juveniles whereby RNA positive samples decreased as the age increased (Fichet-Calvet et al., 2008). The presence of *Mopeia virus* RNA in young individuals (body weight ≤11g); n=4 we recorded in our studied *M. natalensis* population was regarded as an indication of mammarenaviruses vertical transmission from chronically infected females *Lassa virus* RNA was detected in three young *M. natalensis* (body weight ≤11g; which correspond to 10 to 20 day old) in Guinea (Fichet-Calvet et al., 2008). Another study in Tanzania reported a higher presence of

Mopeia virus RNA in earlier ages and the possible explanations given included the lack of immunity in young individuals (Borremans et al., 2011).

There was a direct relationship between the variation in rodent abundance and the variation in the proportion of *Mopeia virus* positive individuals so density-dependent transmission is a hypothesis to be considered in the dynamics of *Mopeia virus* transmission in our study area. It has been suggested that the timing of the epidemic peak in a wildlife population could be associated with both the number of susceptible hosts and their rate of recruitment over breeding season (Begon et al., 2009). Mammarenaviruses density-dependent transmission was suggested by previous studies on *Morogoro virus* transmission (Vanden Broecke et al., 2019) and its seasonality circulation (Mariën et al., 2020).

During the study we came across some limitations that included low numbers of individuals captured and tested in habitats other than the irrigated croplands. This limited our assessment of the effect of habitat type and diversity on *Mopeia virus* transmission in our study area. Besides we sampled during the dry season, including the beginning and the end of the rainy season in a single year so our data was not enough neither to assess what is really happening over the rainy season, nor to tell whether the pattern of *Mopeia virus* transmission we observed is typical or not. A study in Guinea indicated *Lassa virus* transmission fluctuated in agricultural but not in villages (Fichet-Calvet et al., 2007). The implementation of removal protocol in the same site for several PTSs is likely to introduce a biased interpretation of the evolution of *Mopeia virus* transmission because the removal process may decrease population abundance (and the number of positive individuals) and then to reduce viral transmission. To alleviate this difficulty, we set removal sites with only one PTS. Moreover, after pooling the data by PTS we compared with the data displayed site by site and resulting trends were almost the same. In our study we used body weight to distinguish between juveniles and adults and this method has been regarded as a poor estimator of age for *M. natalensis* because weight growth stop is common in this rodent species (Leirs et al., 1989). However, the trends displayed by juveniles and by immature results were similar, thus we think the shortcomings of age were minimized.

6.6 CONCLUSIONS

The dry season could be the main season for *Mopeia virus* circulation in *M. natalensis* in the irrigated croplands in our study area. Our finding differs from the reports from West Africa in mostly commensal contexts where *Lassa virus* circulation in *M. natalensis* occur mainly in the rainy season. These differences highlight the need to assess the special and temporal dynamic of mammarenaviruses in *M. natalensis* over the different regions and biotypes. The dynamic of juveniles together with higher abundance seemingly drives the dynamic of *Mopeia virus* circulation over the sampling periods in our study area. Importantly, the reproduction of *M. natalensis* is the most important factor in the dynamics of *Mopeia virus* transmission in our area of study. The fluctuation of *Mopeia virus* positive samples over the sampling periods we described here, suggests existence of a specific period of higher risk for human's exposure to eventual infection or emerging zoonotic mammarenaviruses. To mitigate this, it is important to assess human practices and behavior to assess the exposure factors for better planning prevention and control strategies.

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7 CHAPTER-IV: STRUCTURAL DRIVERS OF VULNERABILITY AT THE HUMAN-RODENT INTERFACE IN THE LIMPOPO NATIONAL PARK, MOZAMBIQUE

All the contents of this chapter were used to prepare manuscript 2, thus the structure of this chapter followed the journal layout.

Title of manuscript: Vulnerability to zoonotic emerging diseases as an alternative framework to address human-rodent interface in light of the environmental history, in the Limpopo National Park, Mozambique.

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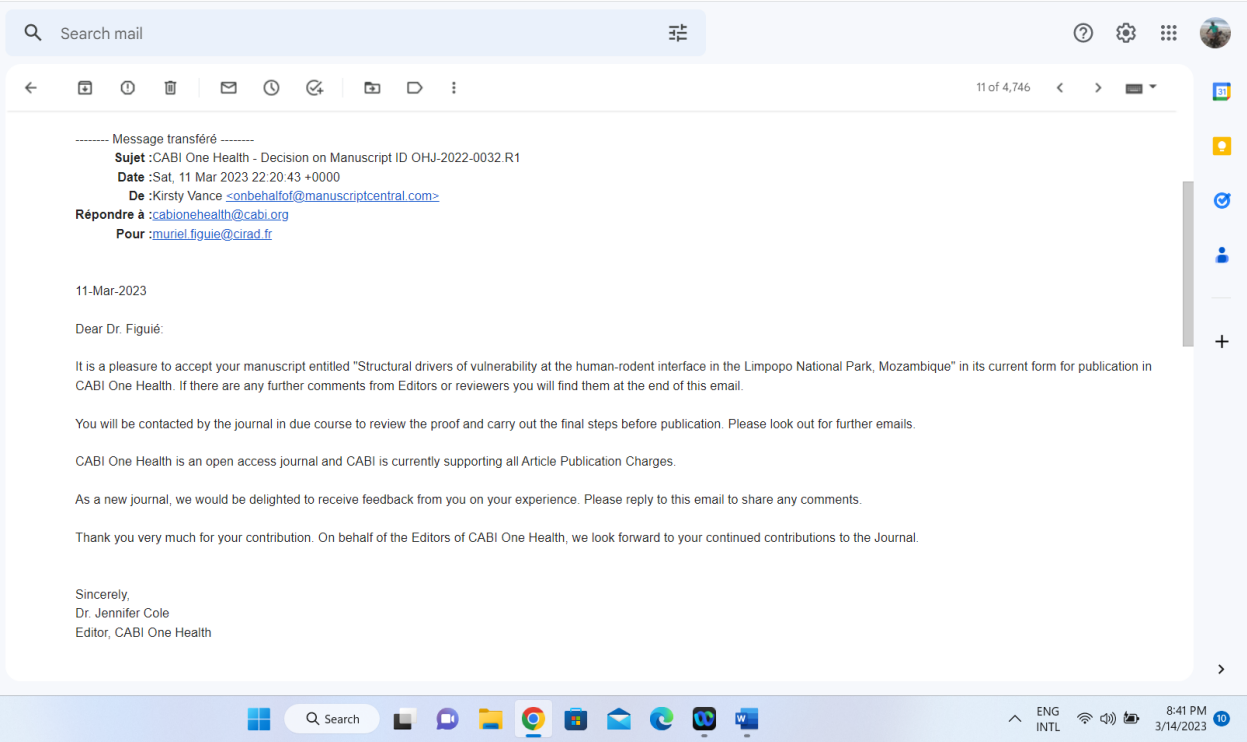

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7.1 ABSTRACT

This study investigates the human-rodent interface in a rural community of Mozambique. Initially designed in the framework of a research on Mammarenavirus, this socio-anthropological study explored the temporal, spatial, cultural and social dimensions of the rodent-human interface, complexifying and enriching the understanding of the socio-ecological dynamic, in which new viral transmission can occur and disease spread. A vulnerability-based methodology was used to assess the human exposure, sensibility and capacity of adaptation related to rodent-human conflicts. The main results of this study are: i) The added-value of using a vulnerability-based methodology, over the more classical KAP (Knowledge, Attitudes, Practices) methodology, to capture the lived and dynamic experience of this interface; ii) The transformations, observed locally during the last decades, with an increasing number of invasive rodents to the detriment of native species; iii) The social-ecological drivers attributed to these transformations: climate change, new infrastructures (e.g. construction of a dam), activities (e.g. development of irrigation) and policies (human resettlement); iii) the changing human-rodent interactions as a consequence of the shift in the rodent population, and the higher exposure of women to contacts with rodents;

iv) the multidimensional burden experienced locally of the human-rodent interface (i.e. crop losses, damaged goods) and the limited concerns for rodents related diseases; v) finally, despite the perceived high burden associated to new invasive rodents, the limited capacity to undertake individual and collective actions to mitigate the related damages. These results demonstrate how socio-ecological changes can create new routes for emerging viruses, and the vulnerability of the exposed population to address the potential related health risks.

Keywords: Animal, One Health, KAP study, Mozambique, socio-anthropology, zoonosis

One health impact statement

The present socio-anthropological study of the human-rodent interface comes in complement to a previous eco-epidemiological survey focusing on the rodent-virus interface. It explores the temporal, spatial, cultural and social dimensions of the rodent-human interface, complexifying and enriching the context in which viral transmission occurs and disease spread. It exemplifies, based on local knowledge, how socio-ecological changes can create new routes for emerging virus, and highlights the vulnerability of the exposed human population to address the potential related health risks. This multidisciplinary and dynamic approach of how human and rodents interact, and how these interactions are shaped by their changing environment is a contribution to a systemic approach of health, in line with the One health paradigm.

7.2 INTRODUCTION

Emerging zoonosis have been recognized as complex and multifactorial risks, and biomedical sciences working on the topic are putting an ever-greater attention to the social dimensions of these emergencies. The social dimension of health is often addressed through the lens of individual behaviours as a source of risks, and with an emphasis on laymen knowledge, perceptions and attitudes as determinants of these behaviours (Barennes et al., n.d.; Machila et al., 2003; LeBreton et al., 2006; Friant et al., 2015; Kamins et al., 2015; Gbogbo and Kyei, 2017; Moffo et al., 2020; Muriithi et al., 2021). The popularity of the tool “KAP” (Knowledge-Attitudes-Practices) illustrates this focus, including in the field of zoonosis, and in particular in low- and middle-income countries.

This behavioural-risk approach contrasts with the few social science studies on zoonosis adopting a broader perspective and based on the seminal works (Farmer, 1996; Nichter, 2008). Beyond the cognitive and psychological drivers of human behaviours, those studies (Dzingirai et al., 2017; Ebata et al., 2020) take into account the structural factors shaping human-animal or more broadly human-nature relationships (Envirosociety, 2015; Cabalion et al., 2018; Douno et al., 2021; Keck et al., 2021), and sometimes with a marked historical and environmental perspective (Nash, 2006).

Our paper is a contribution to this second set of works. Here we address the vulnerability of a rural community in Mozambique, to the risks related to rodents. The work was conducted by an interdisciplinary team (sociologist, veterinary, ecologist) and aimed at understanding the drivers of human exposure to rodent-borne infectious diseases (ideally, it could have been completed by collecting serological and virological data on humans, but this was not in the scope and resources of the project, which focused on rodents). The objective of the present study is also methodologic: proposing an approach based on the concept of vulnerability, as an alternative to behavioural approach based on the KAP tool.

First, we expose the limits of the KAP tool in addressing the social dimensions of zoonosis. Then, we present the context of our study (the periphery of the LNP, in Mozambique and the mammarenaviruses at the human/ rodent interface) and the framework developed for our analysis (vulnerability and its three components: exposure, sensibility and adaptive capacity). In the result section, we present successively the role of the local environmental history as a structural driver of human-rodent interface and the different components of the vulnerability. The discussion section highlights the potential of our analytical framework to overcome the limits of the approaches of human behaviours based on perceptions bias, cognitive deficit; and its potential, based on local knowledge, to document the socio-ecological changes that can create new routes for emerging viruses.

7.3 THE PROBLEMATIC: ADDRESSING THE SOCIAL DIMENSIONS OF ZOONOSIS

Preventing the risk of spillover of mammarenaviruses from rodents to humans

Rodents are a major source of current zoonosis and a threat for potential new emerging infectious diseases (Meerburg et al., 2009; Han et al., 2015; Nieto-Rabiela et al., 2019) Different rodents have been identified in our study area, such as *Mastomys natalensis, Rattus rattus, Saccostomus campestris, Gerbilliscus leucogaster, Thallomys paedulcus, Aethomys ineptus and Rattus tanezumi* (see § 4.1.3). A specific attention is given by researchers to *Mastomys natalensis* widely distributed in southern Africa (Monadjem et al., 2015) since the specie can host *Mammarenavirus*, an *Arenaviridae* virus family generally associated with rodents and including agents causing severe haemorrhagic fevers with high mortality in humans (Radoshitzky et al., 2019). One *Mammarenavirus*, the *Lassa virus*, is responsible for at least thousands of clinical cases each year in West Africa (Fichet-Calvet and Rogers, 2009). The *Lujo virus* is the second fatal *Mammarenavirus* in Africa, recorded in a single outbreak in South Africa (Paweska, 2014) but whose natural reservoir is yet to be discovered. *Mastomys natalensis* hosts other several mammarenaviruses regarded as non-pathogenic to humans, such as the *Mopeia virus* described for the first-time worldwide in Mozambique in 1977 (Wulff et al., 1977).

Our study site is located in the district of Massingir, Gaza province, neighbouring South Africa, in two villages, Bingo and Chibotane. The selection of these villages was based on the previous eco-epidemiological study on *Mammarenavirus*, accessibility and willingness of local authorities in participating. The study site lies in the LNP and its buffer zone inhabited mostly by small-scale farmers. Therefore, it provides the opportunity to explore a range of diverse habitats (villages, croplands and woodlands) harbouring different forms of human-rodent interfaces. *Mastomys natalensis* can be found in the different habitats, with higher number of captured individuals in the irrigated croplands and the lower number of captured individuals in the woodland (Mapaco et al., 2022). A sub-clad of the previously identified Mozambican *Mopeia virus* was detected by Mapaco et al. (2022), in around 17.0% of the local population of *M. natalensis*.

The history of the area is marked by the construction of the Massingir dam on the Elephant River in the 1970's (for irrigation and hydro-electric power), the war for independence (1964-1975; Massingir was at the frontline of the resistance against Portuguese colonizers), followed by the Mozambican civil war (1975-1992) and the creation of the LNP in 2001, previously a hunting area,

and now part of the Great Limpopo Transfrontier Park and Conservation Area. Currently, more than 10 000 people live inside the LNP and its buffer zone in small localities, depending mainly on small-scale agriculture and livestock farming, and remittances from South Africa. Contrary to Bingo, the village of Chibotane is located in the buffer zone and protected from most large wildlife (e.g., elephants, buffalos but not carnivores) by the park fences. However, being located between two rivers, Chibotane is exposed to flooding which impacts the dynamic of the population of rodents (as exposed later on).

Renewing the Knowledge-Attitudes-Practices approach

The KAP tool is a survey tool that emerged in the field of family planning in the 1950s (Cleland, 1973; Launiala, 2009). From then, its use rapidly extended to address a growing number of public health issues mostly in developing countries. These KAP studies aim to understand the cognitive (Knowledge) and psychological or emotional factors (Attitude) that determine health seeking practices and medical compliance (Practices). Since its inception, the scope of the KAP surveys has gradually widened its area of application to include various issues (climate change, pesticide exposure, organic farming) including farmers' practices in relation with animal health (Lambrou et al., 2020; Moffo et al., 2020; Moutos et al., 2022; Oloso et al., 2022).

The tool is often used in the study of risks in order to address how individuals perceive a given risk (Knowledge), their acceptability or aversion to that risk (Attitudes) and how they manage it (Practices). Nowadays, the KAP tool is a major instrument of expertise, used by many international organisations and NGOs such as WHO, UNICEF, FAO, USAID, Médecins sans Frontières, AICF. It is largely used in multidisciplinary research, as an easy way to include social issues, including in one health studies (Ahmed et al., 2018; Delgado-Hernández et al., 2021; Douno et al., 2021; Gbogbo and Kyei, 2017; Jumbam et al., 2020; Lambrou et al., 2020).

Despite the broadening scope and the large mobilisation of the KAP tool, it has not been adapted to the recent advances in anthropological sociology on survey techniques and to criticisms (Cleland, 1973; Launiala, 2009; Moatti et al., 1993) explains the attractiveness of this tool by its "*characteristics such as an easy design, quantifiable data, ease of interpretation and concise*

presentation of results (…)". As such, it can address social issues, and can be easily used by un-experimented researchers or experts, in social sciences. This stability also reveals the slowness by which some progress in a discipline communicates to another discipline, and into the practice of expertise.

The KAP tool emphasizes the cognitive deficits (Knowledge), the errors of judgment (Attitudes), and the lack of compliance with the recommended behaviours (Practices), to the detriment of experiences, values, and preferences. By focusing on the cognitive deficits as the main drivers of attitude and practices, the tool neglects the structural factors limiting individual choice, hardly values local knowledge and points of view; and tends to put the blame on the individuals, a position denounced in the field of political ecology of diseases (Farmer, 1996). As a consequence, the recommendations issued from KAP study narrowly focus on activities of communication, and give little space to other levers of intervention. In the specific area of emerging diseases, KAP study cannot highlight the social and structural dynamics contributing to new routes for "viral traffic" [using the term coined by (Morse, 1995), the social inequalities in risk exposure, and the potential local and collective capacity to innovate in order to face new health issues (Kleinman et al., 2008; Leach et al., 2010; Williams, 2016).

7.4 METHODOLOGY

Vulnerability-based approach

We propose here an alternative approach based on the concept of vulnerability. The concept is mobilized in many areas of interventions such as climate change, disaster events, food insecurity (Morrow, 1999; Dilley and Boudreau, 2001; Füssel and Klein, 2006). Tools used to assess vulnerability cover a diverse set of methods (including the construction of quantified vulnerability indexes) whose objective is to "*systematically integrate and examine interactions between humans and their physical and social surroundings*" (Hahn et al., 2009). Based on the definition adopted

by the IPCC (2012)[1], we considered three components for assessing, qualitatively, the vulnerability: exposure, sensibility and adaptative capacity.

Exposure relates to life-style and practices associated with a given risk, here to infectious diseases potentially transmitted by rodents. The diversity in exposure is addressed in the physical and social spaces, as well as its dynamics (inter and intra annual). The purpose is to identify based on local knowledge and points of view (i.e., based on an emic perspective) but also considering the differences in living conditions and activities: who is exposed (men, women, children, specific professional), when (seasonality, trend), where (home, field), why (transmission ways).

Sensibility to rodents' related risks is addressed in the broader context of human-rodent relationships, (note that, in a socio-anthropological perspective, we prefer to use "sensibility", rather than "sensitivity" used by IPCC, since "sensibility" refers to ability to perceive, "sensitivity" to a quality of being sensitive). It includes 1) Local knowledge on rodents and infectious diseases: ethnozoology, ethnomedicine, representation of rodents in the human-nature divide; 2) Perceived impacts of rodents on human livelihoods: benefits (e.g., meat, traditional drugs) and damages on goods (e.g., on crops, clothes) and human health; 3) Regimes of value that laymen mobilize in their relations with rodents: cultural metaphors used for rodents; feelings inspired by rodents (e.g., fear, disgust, compassion).

Adaptive capacity is about the capacity to adopt practices and to innovate in order to reduce perceived risks. This dimension recognizes laymen as potential risk managers, and aims at identifying concrete laymen experiences for the control of rodents, rodent conflicts and amongst them rodent-borne diseases. This includes control (individual and collective) of rodent population, control of contacts with rodents, mitigation of the negative impacts associated with the interactions with rodents.

[1] For IPCC (IPCC 2012, Ebi et al., 2006: ***Exposure*** is the magnitude and duration of the climate-related impact such as a drought or change in precipitation, ***Sensitivity*** is the degree to which the system is affected by the exposure, whether beneficial or detrimental and ***Adaptive capacity*** is the system's ability to withstand or recover from the exposure and to seize the opportunity.

Data collection

Background information was first collected on the socioeconomic and agroecological context of Massingir district (unfortunately, no sanitary information was available). A first set of exploratory interviews (11 interviews) was conducted with elders and traditional healers. One of the objectives was to list the rodents identified by the local population with their local names.

This task revealed a difficult match between the scientific and local taxonomies. Scientists recognize that species in some African rodent genera are difficult to recognize and the scientific taxonomy of many groups is still not well resolved (Monadjem et al., 2015). In our study site, the laymen taxonomy of rodents was complex and mixed criteria of appearance (size and colour), age (juvenile *vs.* adult), habitat (house, woodlands), and utility (e.g., edible, pest). To overcome these difficulties, we decided: i) to adopt the local taxonomy, including in our study non-rodent species, such as *Elephantulus sp*., a macroscelid, and refer to all of them as "rodents" ii) and to use pictures to clarify the animals referred to by the participants during the following discussions.

We collected information through focus groups (FG): 4 FG were organized in each of the two villages (8 FG in total) gathering 6 to 8 people by groups (49 participants in total). Based on the preliminary interviews, we hypothesized that exposure to rodents differs for men, women and children due to their distinct activities (with a supposed higher exposure for women). We decided then to conduct distinct FG in order to facilitate the expression of each category of respondents and to give more attention to the situation of women: 2 FG of women, 1 FG of men and 1 FG of children were then organized in each village. Guidelines (FG scripts) were adapted to the different activities addressed by the different groups: i.e., housekeeping, management of the granaries, agricultural field work, hunting, herding, wood and wild fruits gathering.

Participants were recruited with the support of the local authorities, the leaders of the women and farmers associations and the school teachers (for the 2 FG with children). Discussions were conducted in the local language, Xichangana, (with simultaneous translation in Portuguese for the non-Xichangana speakers of the team). The discussion was first introduced by a representant of the local authorities, who informed on the purpose of our research so that the participants could

confirm their willingness to participate by signing the consent form. Interviews and FG discussions were recorded. Discourse analysis was manual, and organized along the different topics exposed in the following sections. We used a triangulation methodology to assess the quality of the collected information (meaning that one information has to be collected from different focus groups, or informants to be considered as relevant). The quotations taken from interviews and FG are translated here in English.

7.5 RESULTS

Rodents in the local taxonomy

"Condjo" is a generic term used locally to name rodents found in homes and granaries. Respondents make the distinction between big Condjos (also named "ratazana") and the small ones. According to the pictures shown to the respondents, this group includes *Rattus sp., Mastomys natalensis, Mus musculus*. They are the most common rodents in the area according to our respondents (**Table 12**). "Mbeva" is a generic term used to name rodents found in the croplands. According to the pictures shown to the respondents, this group includes *Mastomys natalensis, Thallomys paedulcus* and *Aethomys ineptus.*

As a consequence, species considered as different for the researchers (*Mastomys natalensis, Thallomys paedulcus and Aethomys ineptus)* receive the same name in the local taxonomy: Mbeva. And one species for the scientists, *Mastomys natalensis*, receives different names in the local taxonomy depending on its habitat: small Condjo when found at home, and Mbeva when found in croplands.

The situation is simpler when it comes to rodents and related species found in woodland areas. Respondents make the difference between Vondo (cane rat/ *Thryonomys swinderianus*), Fucuzane (mole rat/ *Cryptomys hotentottus*), Nungo (porcupine/ *Hystrix africaeustralis*), Jengwa (South African springhare/ *Pedetes capensis*), Matxigane (squirrel, *Paraxerus cepapi*), Nadvitane (dormouse/ *Graphiurus murinae*) and Matoxo (bushveld gerbil/*Gerbilicus leucogaster*).

Other names were quoted that we found difficult to identify: Maduro (probably the *Elephant shrew*, *Elephantulus sp*.) and Sengane (probably the fat mouse, *Steatomys pratensis*) both said to be very common in homes, and woodlands. The difficulty of identifying these two species may have contributed to the erroneous indication of homes as their usual habitat.

We were not able to identify any mention of *Saccostomus campestris* (pouched mouse) during the FG discussions despite it having been found by the previous ecological study on quite an abundant number. By the same way, the FG members did not quote the presence of *Mastomys natalensis* (Natal multimammate mouse) in woodlands, although *Mastomys natalensis* have been found by the ecological study in villages, croplands and woodlands, but with a lowest number of individuals in the woodland.

Table 12: Rodents quoted in the area of the study. Laymen and expert taxonomy

Places	Local names	Scientific and common names	Respondents' comments
VILLAGES	Big Condjo	*Rattus rattus/* Black rat *Rattus tanezumi/ Asian rat*	not eatable invasive ("exotic" specie) common
	Small Condjo	*Mastomys natalensis/* Natal multimammate mouse *Mus musculus/* House mouse	not eatable invasive, from to villages (*Mastomys sp.)*
	Mbeva	*Mastomys natalensis/* natal multimammate mouse *Thallomys paedulcus/* Tree rat or Acacia rat *Aethomys ineptus/* Tete veld rat	bushmeat
WOODLANDS	Vondo	*Thryonomys swinderianus/* Great cane rat	bushmeat forbidden to hunt
	Nungo	*Hystrix africaeustralis/* Porcupine	bushmeat, medicine forbidden to hunt
	Jengwa	*Pedetes capensis/* South African springhare	bushmeat forbidden to hunt rare
	Fucuzane	*Cryptomys hotentottus/* Mole rat	medicines very scarce
	Matxingane	*Paraxerus cepapi* Squirrel	bushmeat
	Ndavitane	*Graphiurus murinae/* African dormouse	medicine

	Matxoxo	*Gerbilicus leucogaster/* Bushveld gerbil	-
MIX	Maduro	*Elephant shrew*	common
	Sengane	*Steatomys pratensis/* Fat mouse	bushmeat (depending where it is found) common

Data in columns 1, 2 and 4 are based on FGs and interviews; in column 3 on the expertise of the scientific team)

Exposure: invasion and inequalities

Condjos are depicted as invasive rodents whose population has been increasing in homes and granaries from the 2000', in particular for the big Condjo (*Rattus* sp./ Black rat or Asian rat). On the opposite, woodlands rodents (Vondo/ *Thryonomys swinderianus/* cane rat*;* Fucuzane/ *Cryptomys hotentottus/* mole rat*;* Nungo/ *Hystrix africaeustralis/* porcupine*;* Jengwa/ *Pedetes capensis/* South African Springhare) are considered as native and their population is said to be decreasing (despite the presence of the national park). Women and girls are considered as more exposed to contacts, mainly indirect contacts, with condjo, considering their role in the domestic tasks.

The local collective history of rodents' exposure: the story of an invasion

The different elements exposed by our respondents allow us to retrace the dynamic of the population of rodents in the area, during the last decades. The present population is the result of an historical process which provides a perspective on the structural and historical drivers of exposure to rodents, beyond individual behaviours.

In the past (years 1950'-60s'), rodents were said to be numerous in the area. But contrary to the present time, they were living in woodlands. Their presence was valued for bushmeat (such as Vondo/ *Thryonomys swinderianus*, highly valued for its tasty meat). But a decrease in the population of these types of rodents was observed and attributed to climate changes, marked by increasing temperature and decreasing rainfalls. These woodland rodents were replaced by more invasive rodents. Indeed, in 1972, with the construction of the dam of Massingir, big trucks arrived in the area bringing with them big black rodents (Condjo, most probably *Rattus rattus)* who

multiplied rapidly. At the same period, the human habitat changed, from scattered households to villages. These changes were driven by people displacement required by the creation of the dam and by the decision of the Frelimo Party that was ruling the area (the Frelimo Party led the movement leading to the independence of Mozambique in 1975). At that time, the Frelimo decided to gather the households for their protection (and control), a process named as "villagization".

From that time, progressively in the two villages, the population of these big invasive rodents (i.e., Conjo, *Rattus sp*.) increased and rodents from the croplands, i.e., Mbeva, also began to be more numerous (*Mastomys natalensis, Thallomys paedulcus, Aethomys ineptus?)* and to colonize households (*Mastomys natalensis)* suggesting that *Mastomys natalensis* did not live within houses before. Moreover, according to one of the respondents, the government promoted the production of sunflowers that would have benefited the population of these new invasive rodent (*Mastomys natalensis)*. But it was really in the 2000's that the population of rodents increased dramatically, in particular in Bingo. Following an important flood in 2000, trucks arrived with food aid but also with more "big black rodents" *(*probably *Rattus tanezumi* only found in Bingo*).* Moreover, the invasive rodents already present in the area moved from lower areas to higher ones, such as Bingo (Chibotane was said to be less exposed), invading and most houses. These rodents set up definitively in this most favourable environment, in houses and granaries. New floods in 2013 intensified the process. These rodents, invading the houses from 2000, are described as big, frightening and strange:

> *"They're big, scary rodents, we don't have any poison to kill them".*
> *"We have rodents in the field, in the house. But the rodents we have in the village are scary rodents".*

Floods would have benefited to the migration into the villages of these "new" commensal rodents. And periods of drought (2015 and 2019) would have badly affected the "local rodents", such as Vondo (great can rat*)* and Jengwa (South African springhare) more depending on the natural vegetation. These two trends explain the shift in the population of rodents, to the benefit mainly of *Rattus sp.*

Socio-cultural factors have also been underlined to explain this shift: Two women in a focus group explained the increasing population of rodents in the and houses as a consequence of a loss of tradition ("*The rodents started punishing us in the 2000s"*): with the development of "the religion" (meaning of Christianism, versus Animism), people are now neglecting their tradition, they prefer to go to the church and do not practice the *Upaxa*, a traditional ceremony aiming at preventing the crops from destruction by animals (rodents but also monkeys and elephants).

Inequal exposure to rodents: a gender focus

FG discussions also allowed us to identify people, places and activities contributing to contacts with rodents, based on how humans and rodents share common spaces (Annexs VI and VII). Participants of the FGs reported regular contacts (direct or indirect) with rodents: the same day or latest the week before. When we directly asked participants, who are the person's most exposed to contacts (direct or indirect) with rodents, women and girls were the ones the most often mentioned. Indeed, activities differ among women, men and children, and potential contacts with rodents too. All year long, women and girls spend a lot of time at home, performing domestic tasks. In particular, girls are the ones sent daily in the granaries where rodents are said to be numerous.

> *"I meet Sengane every day. Today we killed many. Some we can't even catch. If we go looking now, we will find them".*
>
> *"The people who are most exposed to rodents are the women because they look after the granary, then the girls because they help the women to look after the house".*
>
> *"It's the women because at the moment the men are in the village, since the rain stopped, they don't come to the croplands".*
>
> *"The rats come down in the kitchens. It even seems that they work together with the women".*
>
> *"The girls are more exposed than the boys because they work in the granary and in the kitchen while the boys go to pasture".*

We can also hypothesise (even if this was not emphasized directly by the respondents) that hunters, cattle herders (and in particular young boys) and traditional healers ("curandeiros") are probably

more exposed to woodlands rodents since they chase them for bushmeat (Vondo/cane rat, Fucuzane/mole rat, Nungo/porcupine, Jengwa/ South African springhare, Matxigane/squirrel) in the case of hunters, or in order to prepare traditional medicine (Fucuzane/mole rat, Nungo/porcupine, Ndavitane/African dormouse) in the case of the traditional healers.

Similarly, contacts may also differ between types of houses, as houses made of mud and straw are said to be less rodent-proof compared to houses made of concrete blocks. However, according to the respondents, this does not seem to make a great difference in terms of intensity of contacts with rodents.

Different types of contacts, mainly indirect

Contacts with rodents can take different forms, more or less direct. The main reported contact is through rodents spoiling food (in the kitchen or in the granaries) and clothes with their saliva and paws.

Rodents are said to seize any moment of inattention to rob food in the kitchen (in that case, the upper part of the meal is generally eliminated and given to dogs or cats). Numerous traces of urine and faeces can be found in the food stored in the granaries but also in the beds, blankets and clothes. Women and girls are the most exposed since they are the ones in charge of cleaning (and generally directly collecting faeces, what they do directly with their hands). More direct contacts occurred when women tried to trap the rodents in the houses. The trapped rodents are beaten with a wood and women catch the dead animal by the tail. Children can also have direct contacts when catching rodents, found dead, sometimes just to play with.

The presence of fleas is attributed to rodents (and sometimes chicken), and exposure to fleas is said to be a main issue, in particular in Bingo where respondents reported periods (whose seasonality could not be assessed) where they have to sleep outside of their home to escape from the fleas. Bites are rare and would affect babies, children playing with dying rodents, or people going to bed without washing hands (the smell of the food would attract rodents). Rodents are also said to bite dead bodies (exposed before the funeral). Some species found in woodlands (mainly Vondo/cane rat, Nungo/porcupine, Matxigane/squirrel) serve as bushmeat but this consumption is

said to decrease due to the decline of the population of edible species and the ban on hunting controlled by the park authorities (for this reason it was a sensitive issue difficult to address in the discussion). Contacts also occur for patients and traditional practitioners and healers through the preparation and use of traditional medicines based on rodent flesh, skin or faeces.

Sensibility: rodents and food

Not all respondents were able to name the different rodents they usually meet. But they all expressed a major concern for rodents living in the village (homes and granaries) and croplands due to the numerous damages caused. The FG of women emphasized the damages in homes and granaries, the FG of men those in the cultivated areas. On the contrary, rodents found in the woodlands are perceived positively as they can be useful for humans (as bush meat or traditional medicines). As mentioned by one respondent: "*the place of rodents is in the woodland*".

A threat to food security

Rodents living in human settings are perceived very negatively. The big rodents present inside the houses are described as frightening and scaring by many participants, in particular by the more exposed group, the women. Rodents obviously bring numerous many nuisances but the reasons for the inspired fear are not very clear. Rodents are perceived above all as a threat to food security: they first consume the seeds stored in the granaries and newly sowed in the croplands. They consume the crops during the pre-harvest period, the food stored in the granaries and the cooked meal in the kitchen. They also kill the chicks in the backyard. Respondents (woman FG in Chibotane) estimated the total food losses due to rodents up to 50%. Moreover, rodents destroy and spoil clothes, blankets, beds, and sometimes banknotes. They dig holes inside the house where other animals like snakes can live or nest.

> *While we are eating, the rodents are also eating. If there were no rodents, our crops could feed us a year. But when there are rodents, our crops cannot take up to a year".*
>
> *"Rodents do us a lot of damage, both in the house and in the croplands".*
>
> *"The crops that we have sown, the next day, they can all be uprooted".*
>
> *"The chickens in our houses… the rodents kill our chickens".*

"Our coexistence with rodents has been very difficult. Our complaint with rodents is that they are eating the food that we cook in our houses, as well as the food cultivated in our".

"Fleas come with the rodents. As long as rodents exist, fleas will exist. Disappearing rodents can help us with the disappearance of fleas".

"Rodents bring irritating insects through the skin; we can't sleep; we need to sleep outside because there are insects inside".

"The children end up crying, without us knowing what's happening, it's because of rodents chewing their fingers".

Potentially to health

When asked about rodents and diseases, what comes spontaneously to the mind of the respondents is the use of Fucuzane (the mole rat) in the traditional pharmacopeia. Participants were not able to mention any disease transmitted by rodents to humans, and most generally showed a limited and confused knowledge of zoonosis: one respondent quoted a disease transmitted by dogs (without naming rabies explicitly), another the risk of drinking the milk of cow with red urine (probably the Redwater disease, a tick-borne disease also named Babesiosis) and another a disease sexually transmissible by monkey (probably HIV). Nevertheless, respondents consider that rodents spoiling food can contaminate it with "poison" or unknown diseases. But no one was able to report any personal experience of health problems related to rodents.

"We have to dump the food for fear of diseases. We don't know what the diseases are".

"Sometimes when we have food and the rodents come and eat it; then we have to take it out from above because it contains poison".

Benefits associated with "woodlands rats" as a source of bush meat

Benefits associated with rodents are perceived as very limited. These are limited to food under the form of bushmeat and the supply of traditional medicines. Fucuzane is the word used to name a rodent species (the mole rat), a type of wound "with blood and water" on the leg or foot that doesn't heal easily, and also the traditional drug prepared with the flesh of the rodent to cure this kind of wound. Nungo (porcupine) is used for treating "fire of the night" (probably herpes zoster), to cure wounds due to bewitching, or vomiting. Ndavitane (African dormouse) can also be used to increase the skills of hunters and football players.

"I use to eat rodents...but the ones that exist today are no longer eatable.

"We use Fucuzane to cure a disease that comes out in a person's foot...we kill the Fucuzane, then we skin it, chop it on a stick and then bake it to dry. When someone appears who is sick, we burn the Fucuzane and take its ashes to put in the place where there are wounds.

Adaptative capacity: a feeling of powerless

Respondents agree on the need to reduce the population of rodents at home and in the cultivated. However, there is a mismatch between the level of complaints against rodents found in these areas and the paucity of measures taken to control them. Adaptive capacity to face the growing nuisances brought by rodents is limited.

Individual measures in the houses and granaries

Different measures to control rodents are reported by the respondents, who also underline their limited efficacy, their high cost and potential side effects. Traps or poison are only available in Massingir city (35 km from Bingo and 18 km from Chibotane, on a sandy road track, with no cheap transport facilities). They are sometimes used in the households and the granaries, but are said to lack efficiency: rodents are two big for these traps; and the rodenticides just "*fatten the rodents*". Moreover, people are reluctant to use them since they fear that other animals (e.g., chicken or cats) or even children could consume the rodenticides. Some of the households have cats, enclosed in the granaries, but cats are scarce, difficult to get, and considered as useless since rodents are bigger than they are, and even able to eat small cats.

Inside the homes, throwing boiling water in the tunnels excavated by rodents is seen as a more efficient option. Maintaining a clean house is also considered as a way to control the rodent population. Surprisingly, the architecture of the local granaries does not include any protection against rodents, as it may exist in other countries. Roofs made of zinc, rather than straw, in the houses and granaries would help to better control the rodent population according to respondents. Straw roofs are said to host rodent nets, fresh all year round. However, roofs made of zinc are considered as too expensive.

Attempt to collective actions in the cultivated areas

Measures are taken at individual level, but participants underline the need for a collective action in the. However, farmers hardly undertake collective action to control rodents. As mentioned above, in the past, people used to attend a traditional ceremony aiming at protecting the crops from rodents and other predators (monkeys and elephants) but this tradition is disappearing. Traps with bait are sometimes used in the fields, but as expressed by one respondent, as only few farmers use traps, he fears that this bait only attracts rodents from other in his own fields. Some burn the fields to clean them from rodents and other pests.

Support (financial and technical) is expected from the agricultural extension services rather than from the health services as rodents are perceived mainly as a threat for agriculture and food security, rather than for human health. In 2019, a group of farmers bought Tamaron, an insecticide-acaricide (pyrethroid) and used it (*surprisingly*) to eliminate rodents. This turned out to be very efficient according to the respondents but too expensive to repeat the operation.

No support is expected from the LNP. However, farmers living in a conservation area or in its proximity are exposed to potential crop damages from wildlife and compensation are generally expected from the park authorities. In the context of our study, rodents responsible for damages in the villages were not considered by our respondents as wildlife and their multiplication was associated to a process of anthropization (as exposed above) rather than to the proximity to a conservation area.

> *"The cats we have don't have the courage to catch the rodents present at home since they are big rodents".*
>
> *"We have no way to get rid of them".*
>
> *We don't have anything to reduce the rodents. If we had rodenticides, maybe ... Sometimes we put rodenticides, but the rodents don't eat, and if they do, they don't die, they just get fat".*
>
> *"We don't use it* (rodenticides*) in our houses because the cats will end up dying, and when rodents eat the rodenticide, they can then eat the chima* (traditional meal made of maize)*. Then it can also cause problems for us".*
>
> *"In the fields, we have nothing to reduce rats. Agriculture (i.e., agricultural services) has already offered us rat poison that looks like candy. The rats will eat and not die".*

7.6 DISCUSSION

Methodological inputs

Our study highlights the added-value of using a vulnerability-based methodology, to overcome the limits over more classical approaches (such as the KAP- tool) in addressing the social conditions framing the interface between human and animals, and the risk for zoonosis. The added value relies on the ability to collect local knowledge, previously unknown by researchers, as a source of information (whose reliability needs of course to be critically addressed, as for any collected data), rather than to oppose it to scientists' knowledge in the search for cognitive gaps as it is in the KAP studies.

Two main limits can be addressed on the reliability of the collected data. First, the context of the national park and the ban on hunting have probably limited the collection of information on risk exposure related to rodents hunting and consumption. But as the district of Massingir is a well-known place for criminal poaching, in link with international traffic of endangered species (in particular rhinoceros' horn), rodents hunting may also be seen locally as quite harmless and acceptable. Second, at the time of our survey (April 2021), agricultural works were limited and men exposure to rodents in irrigated areas may have been underestimated. These limits are contextual, rather than methodological.

Our study highlights the complexity of the rodent-human interface. This interface is not a static reality but a dynamic process influenced by the ecological and socio-political history of the place. Regarding the historical dimension, climatic (flood, drought) and anthropogenic (new infrastructure, new crops, process of "villagization", loss of tradition…) factors are said to influence the dynamic of the local rodent community. But, surprisingly, potentially impacting events such as the creation of the park (in 2001) and the development of irrigation schemes (in Bingo) were hardly mentioned. Regarding the ecological dimension, our initial hypothesis was one of a gradient in rodents' density, from the conservation area to the villages. But local knowledge brings new hypothesis that could be cross checked with further ecological research on the community structure of the rodents: rodents would be more numerous (and higher is the exposure) in highlands (e.g., Bingo) due to permanent presence of rodents compared to the

lowlands or flood-risk areas (e.g., Chibotane) where very often the rodents' nets are destroyed by flood and rodents tend to migrate to highlands. As a consequence, spatial variability in rodents' density may be highly variable depending on drought and flood years. Nevertheless when it comes to *Mastomys natalensis* the epidemiological study could trap more individuals in Chibotane than in Bingo (Mapaco et al., 2022).

Vulnerability to rodent borne infectious diseases

Mastomys natalensis (natal multimammate mouse) is identified by the scientists as a source of mammarenaviruses and a potential health threat. In terms of sensibility, the local perception of the species is contrasted, depending on the place where it is found. Rodents in general are not perceived as a source of infectious diseases. *Mastomys natalensis*, known as Condjo when found in a house, is seen as an issue in terms of hygiene (since it circulates from dirty areas to granaries, kitchens and beds), food loss, good damages, pleas and noise. When found in the fields, it changes name (from Condjo to Mbeva) and is seen in a more positive way as a potential source of bushmeat. This contrasted perception between home and bush rodents has been underlined by (Douno et al., 2021) studying young hunters' exposure to *Lassa fever*.

If people from Bingo and Chibotane do not know and do not feel exposed to rodent born disease (and such exposition to pathogenic rodents' virus, such as mammarenaviruses or Leptospirosis, is not confirmed by the available data), our study shows that people living in Bingo, and in particular women and girls, would be more exposed to rodents' contacts and potential rodent-borne existing or emerging zoonosis, than people from Chibotane and men and boys. This result could be useful to design a sampling strategy for further research of mammarenaviruses in humans (since the eco-epidemiological study of the present research focused on the search of this virus in rodents).

If rodents are perceived as a burden when found in houses, granaries or cultivated areas, the resources locally accessible to control them are limited. These resources are perceived as unsuccessful (cats, traps, rodenticide), and/or too expensive (roof in zinc, rodenticide) and/or with potential side effects on children and others species (people fear that children, poultry or cats may eat rodenticide). Measures are taken at individual level, but participants in our study recognized

the need for a collective action in the cultivated areas (that could be led by farmers association and/or local authorities). Women are the more concerned by the presence of rodents in the domestic area, but they may not be the decision makers when it comes to buy traps, rat poison, or to build rodent-proof granaries, etc. These elements indicate the points to address in order to address the risk of rodent born emergence.

7.7 CONCLUSIONS

Our study is a contribution to the understanding of the circulation of infectious diseases at the human/ animal interface. The present socio-anthropological study of the human-rodent interface comes in complement to a previous eco-epidemiological survey focusing on the rodent-virus one. It illustrates the benefit of a multidisciplinary collaboration in highlighting the socio-ecological dynamic, in which new viral transmission can occur and disease spread. From a social science perspective, this paper shows the limits of approaches focusing on individual perceptions and behaviours, and the added-value in addressing the cultural and social dimensions of the human-animal interface in time and space. Moreover, instead of focusing on knowledge gaps and perception bias, it shows how extensive local knowledge can be a rich source of information to document the local environmental history. As a result, it highlights that even in a quite remote place, such as the frontier of a conservation area, anthropogenic factors (new infrastructure, new crops, process of “villagization”) jointly with climatic ones (flood, drought) are importantly influencing the dynamic of the population of rodents and the human vulnerability to rodent-borne diseases. Capturing these dynamics, based on the lived experiences of potential exposed people, is essential when studying emerging diseases.

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Conflict of interest declaration:

The authors have no conflict of interest to declare

Ethics statement confirming that all relevant guidelines were followed

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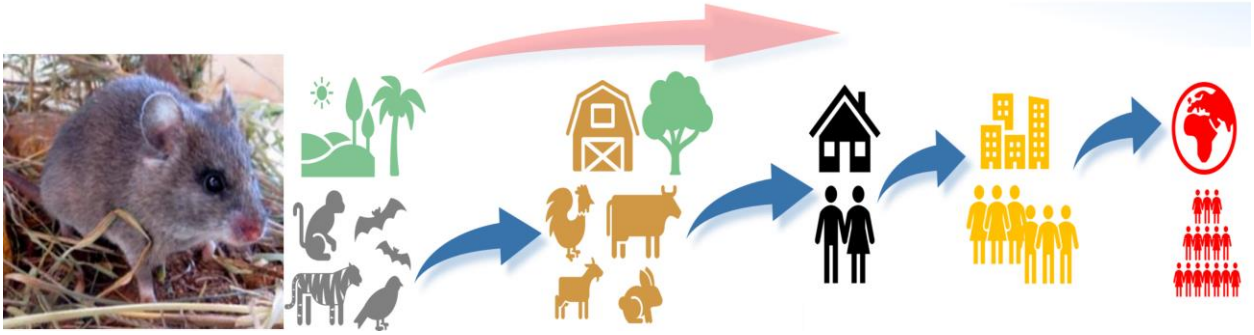
8 GENERAL DISCUSSION



8.1 INTRODUCTION

This exploratory study on the circulation of mammarenaviruses and their drivers at the human/rodent interface in Mozambique was carried out to improve our knowledge about the risk of the emergence of mammarenaviruses and other rodent-borne infectious diseases in Mozambique. To this end we studied the following four aspects: first, we described the small mammals' community structure in different habitats and studied the *M. natalensis* population dynamics; then we detected and characterized the mammarenaviruses in this species, followed by an eco-epidemiological study of mammarenaviruses; and finally, we assessed human vulnerability towards rodents and their pathogens. Below, we present and discuss the main findings of the study. When we studied the eco-epidemiology of *Mopeia virus* in *M. natalensis* (chapter three) we integrated and discussed the results of rodent ecology (chapter one) with the results of *Mopeia virus* detection and phylogenetic analysis obtained in chapter two. Nevertheless, we foresee a room for a further discussion of our results in the context of the description of the risk of emergency of mammarenaviruses or other rodent-borne diseases in humans.

8.2 PERSISTENT CIRCULATION OF *MOPEIA VIRUS* IN MOZAMBIQUE

The phylogenetic analysis performed in chapter two indicated that the *Mopeia virus* is the *Mammarenavirus* circulating in *M. natalensis* trapped in our study area located in southern Mozambique. A previous investigation on mammarenaviruses also described the occurrence of the *Mopeia virus* in central Mozambique (Wulff et al., 1977). Thus, the data so far available indicates that the *Mopeia virus* is the unique mammarenaviruses circulating in the country. This finding contrasts with results from Tanzania where *M. natalensis* besides *Mopeia virus* also host other mammarenaviruses such as *Morogoro virus*, *Gairo virus* and *Luna virus* (Günther et al., 2009; Borremans et al., 2011, 2015; Cuypers et al., 2020). This difference could be explained by a possible specificity of each *Mammarenavirus* to a distinct range of *M. natalensis* mitochondrial lineage (Cuypers et al., 2020).

The mitochondrial phylogenetic analysis sub-divided *M. natalensis* into six matrilineage phylogroups: A-I, A-II, A-III, B-IV, B–V, B-VI (Colangelo et al., 2013). Two of them (B–V and B-VI) occur in parapatry in Mozambique where the south and the central regions are dominated

by individuals with B-VI mitochondria while B-V is geographically restricted to the northernmost part of the country (Colangelo et al., 2013; Cuypers et al., 2020; Gouy de Bellocq et al., 2020). Indeed, our results indicated that all *M. natalensis* genotyped belong to the B-VI lineage. The B-VI mitochondria is known to be a specific host of the *Mopeia virus* (Cuypers et al., 2020), thus our findings highlighted the geographical overlap occurrence of *M. natalensis* phylogroup B-VI and *Mopeia virus* in the country.

M. natalensis phylogroup B-VI is also known to host the *Luna virus* in Zambia and Tanzania and the *Mopeia virus* in Zimbabwe (Cuypers et al., 2020). Thus, in our study, it would be expected the presence of the *Luna virus*. Neither the *Luna virus* was yet recorded in Zimbabwe nor the *Mopeia virus* was recorded in Zambia. The non-overlap occurrence of *Luna virus* and *Mopeia virus* both specific to phylogroup B-VI may suggest a possible geographic split in the distribution of these two mammarenaviruses or a possible further subdivision of phylogroup B-VI (Cuypers et al., 2020). In addition to these hypotheses, we think that the lack of systematic studies on mammarenaviruses in the region may contribute to the lack of clarity when attempting to make a comparison of host specificity over the countries.

Particularly in Mozambique, the north region dominated by the B-V mitochondrial lineage is under surveilled so it is difficult to conclude that the *Mopeia virus* in the country is restricted to the B-VI lineage. Moreover, the few surveillance studies on zoonotic rodent-borne pathogens carried out in both humans and rodents in the country have focused on bacteria agents (causing Leptospirosis and plague) and parasites agents (causing Toxoplasmosis) (Nala, 2006; Comia et al., 2018). The agents causing these diseases have gained interest over viral diseases because they have already been reported in humans in Mozambique (Barreto et al., 1995; Sitoe et al., 2010; Ribeiro, 2017). The lack of data on mammarenaviruses in Mozambique contributed to the 38th rank for Lassa fever on the one health zoonotic diseases prioritization list for multisectoral engagement in Mozambique (*Priorização de doenças zoonóticas da One Health para o envolvimento multisectoral em Moçambique, 2018).* This undervaluation indicates that if data is not produced, the risk of mammarenaviruses emergence will continue to be neglected in the country. For this reason, we believe that the data produced in our study constitute a call to attention about the existence of potential routes of transmission of mammarenaviruses at the rodent/human interface.

8.3 *MOPEIA VIRUS* TRANSMISSION DYNAMIC IN *M. NATALENSIS*

In chapter four we detected *Mopeia virus* RNA by RT-PCR and this indicates that the infection was active. When infection is active the virus could be shed and further viral transmission may occur and this allows monitoring the dynamics of *Mopeia virus* transmission across the five primary trapping sessions carried out between March and November 2019 and comparing it to the dynamics of *M. natalensis* demographic parameters.

In chapter one we saw that as more juveniles were recruited, population abundance increased and these variations were directly proportional to the variation in the proportion of individuals positive for *Mopeia virus* RNA detection over the trapping sessions (chapter three). Importantly, the overall proportion of positive individuals did not differ significantly between adults and juveniles but we observed a switch of a higher wave in adults (in May) to a higher wave in juveniles (between June and August). This wave of positive juveniles could be explained by their naive immunity or loss of their maternal antibodies (Altizer et al., 2006; Peel et al., 2014). As discussed in chapter three, the vertical transmission could also have played a role in the higher proportion of positive juveniles. These results suggest that the pulse of juveniles may have contributed to the amplification of *Mopeia virus* circulation in the population. Thus, the timing of *M. natalensis* breeding seems to be the key ecological factor for the *Mopeia virus* transmission dynamic in *M. natalensis*.

The peaks of population abundance and proportion of juveniles observed during the higher breeding period in our study may suggest that density-dependent transmission also played a role in *Mopeia virus* transmission because it increased contact with the naive juveniles and infected individuals or contaminated material. Comparable to our line of thought it has been suggested that the timing of the epidemic peak in a wildlife population could be associated with both the number of susceptible hosts and their rate of recruitment over the breeding season (Begon et al., 2009). Moreover, to support *Mopeia virus* horizontal transmission in juveniles a study in Tanzania indicated that the dispersal activity of juveniles is higher during the period of high population density and this may increase the contact rates, nonetheless lack of significant correlation between density and *Mopeia virus* prevalence (Borremans et al., 2011).

In conclusion, our data suggested that *Mopeia virus* transmission increased as *M. natalensis* breeding season progressed to its highest peak while viral transmission decreased as breeding season tended to cease. Thus, the planning and implementation of programs that aim for the prevention of mammarenaviruses transmissions between rodent and humans in our studied area should take into account the peak of the breeding season.

8.4 RISK TO HUMANS' INFECTIONS WITH *MOPEIA VIRUS*

8.4.1 *Mopeia virus* infections in humans

In chapter four we reported that the *Mammarenavirus* found in our study area was the *Mopeia virus* which is regarded as non-pathogenic for humans (Wulff et al., 1977) and non-human primates (Walker et al., 1982). These two studies claim that the *Mopeia virus* does not cause diseases in humans, but not necessarily that humans exposed to the virus will not get infected. However, there is a lack of studies on mammarenaviruses surveillance in humans out of West Africa. In this Lassa fever endemic region both asymptomatic and symptomatic *Lassa virus* infections in humans have been detected (Richmond and Baglole, 2003; Balogun et al., 2021). The aforementioned papers indicate that about 80% of *Lassa virus* human infections are asymptomatic, which means that these cases would not have been identified in absence of active surveillance in humans. This may suggest that there are possible asymptomatic human infections with the *Mopeia virus* in the Southern Africa that are not reported due to a lack of surveillance in humans.

A study recently published in South Africa (Grobbelaar et al., 2021) cited unpublished data indicating that antibodies to the *Mopeia virus* were detected in 16.1% (32/199) of humans' sera collected during the first investigation of *Mopeia virus* in the Mopeia district in Mozambique. According to Grobbelaar et al. (2021), antibodies to the *Mopeia virus* were detected in human sera from some regions in South Africa. However, none of the serology positive cases neither from Mozambique nor from South Africa was associated with a clinical disease resembling mammarenaviruses infection. All these findings may suggest that human asymptomatic infections by the *Mopeia virus* cannot be ruled out. Moreover, in the region including Mozambique, there are evidences of occurrence of other zoonotic pathogens transmission at the human / rodent interface

with transmission modes similar to those of mammarenaviruses, such as Leptospirosis (Nala, 2006; de Vries et al., 2014; Ribeiro, 2017).

The study we conducted in chapter four suggested that the communities in our study area are not aware of rodent-borne diseases. Although they considered that rodents spoil food and can contaminate it with poison or unknown diseases, little is done to prevent disease transmission. Low level of knowledge of the disease in the communities is partly responsible for the recurrent transmission of the *Lassa virus* in West Africa (Tobin et al., 2014; Gobir et al., 2020; Igetei et al., 2020; Balogun et al., 2021). A recent study in our study area indicated that poor levels of knowledge may contribute to a lack of adoption practices and attitudes to prevent human rabies (Mapatse et al., 2022). This study emphasises the vulnerability of communities to exposure to zoonotic diseases in our study area.

8.4.2 Risk groups and associated factors for humans' infections with mammarenaviruses and other rodent borne diseases in our study area

In chapter two we detected the *Mopeia virus* circulation in the croplands, villages, and mopane woodland forest in our study area. This result suggests that humans can potentially get infected in all these habitats. Our results about vulnerability to rodents (chapter four) indicated that social-ecological factors jointly are important in influencing the ever-changing human-rodent interactions in our study area and this could be influencing the dynamics of *Mopeia virus* transmission in our study area. Regarding this hypothesis, studies in West Africa indicated that the vulnerability for *Lassa virus* transmission from rodents to humans is exacerbated by poor environmental sanitation, poor housing quality, unhygienic conditions, poor food storage, land-use practices, deforestation, and rodent consumption (Bonner et al., 2007; Clark et al., 2021; Ilesanmi et al., 2021; Redding et al., 2021; Balogun et al., 2021; Izah et al., 2022). Our study area is typically a rural area where the aforementioned risk factors are common, highlighting a possible risk of occurrence of mammarenaviruses infections. However, the risk of human infections in the different habitats will depend on the degree of interaction between humans and rodents.

As we indicated in chapter four, women and girls constitute the higher risk group mainly in houses and agricultural fields but also woodland. The risk of exposure for this group in the home environment is linked to their main activities which include constant access to the granaries, cleaning the house, housewares, and washing clothes, and blankets. The demand for firewood for cooking is a factor of exposure for women, especially girls, in the woodlands. In Nigeria, a study also indicated that women and children were more exposed to *Lassa virus* infections due to high involvement in domestic activities (Izah et al., 2022). Additionally, in Guinea, it has been considered that contact with rodents in households is the main factor that sustains *Lassa virus* transmission between rodents and humans (Clark et al., 2021).

The above activities that pose a risk of exposure to rodents for women and girls occur regularly throughout the year. Our findings in chapter four (Annex-XI) indicated that rodent abundance did not vary throughout the year. This suggests that considering rodent abundance and timing of activities as exposure factors for this group to rodents, the risk of transmission of rodent-borne disease in households may not vary throughout the year. However, the limited data in June and over the rainy season provides limited support for the above hypothesis. Furthermore, this hypothesis contrasts with reports from Western Africa, where the transmission of *Lassa virus* from rodents to humans in houses is higher in the dry season when the density of rodents increases inside the houses (Fichet-Calvet et al., 2007).

In the irrigated croplands from our study area, both higher *M. natalensis* population abundance (chapter one) and a higher proportion of *Mopeia virus* (chapter three) were observed in the middle of the dry season. Additionally, our respondents (chapter four; Annex-XI) reported peaks of rodent abundance during the seeding and harvesting period in the dry season. Moreover, results from studies conducted in the neighboring countries of South Africa (Leirs, 2006) and Tanzania (Mulungu et al., 2013) indicate only one pronounced peak of *M. natalensis* density per year and in both countries, this peak occurs in the middle of the dry season. Thus, although we do not have enough data during the rainy season, we can hypothesize that the middle of the dry season (June/July) is the critical period for mammarenaviruses transmission between rodents and humans. The prediction suggested by our results is comparable to the results from Nigeria where humans

were more exposed to *Lassa virus* infection from rodents shortly after the onset of the dry season and this was associated with the breeding season of rodents (Akhmetzhanov et al., 2019).

When rodent abundance starts increasing at the beginning of the dry season as the breeding season progresses (chapter one) women and girls are in the fields weeding or harvesting, except in June. Harvesting is resumed in July and August (chapter four; Annex-X). Because the abundance of *M. natalensis* is higher between May and August, there is an increased possibility of direct contact between infected rodents and humans due to the above field activities. If not wearing protective gear such as boots, accidental bites by rodents may occur as a defense instinct, for instance, if they are accidentally stepped on them. Although during the study, our respondents reported that rodent bites are rare and are restricted to children at night due to lack of hand hygiene after eating, the hypothesis of rodent bites in the fields during harvest as a defense mechanism to accidental contact cannot be ruled out. Furthermore, during this period there is a high excretion of viruses (through feces, urine, and saliva) and contamination vegetables and cereals. After handling cereals, if hands are not cleaned properly, they can be a source of human infection. Similarly, if harvested vegetables or cereals are not properly cleaned before consumption, they can be a source of infection for humans. Lassa fever peaks in humans associated with agricultural activities such as planting and harvest periods were reported in Sierra Leone (Leach et al., 2017).

Men and boys are the risk groups in woodland areas because of hunting and cattle grazing. As mentioned in chapter four we did not get enough data about hunting because in the context of the national park hunting is forbidden by law so respondents were reserved to respond. However, hunting and rodent consumption was assumed by the studied communities, but not for all of them. *M. natalensis* is among the rodents indicated by our respondents in chapter four as eatable species when it is found in the fields. Indeed, previous studies in Mozambique indicated that rodents are eaten and in some areas constitute a large proportion of the protein source (Belmain, 2009; Heiberg, n.d.). In our study area, hunting has secondary motivation which is traditional medicine. Although *M. natalensis* was not mentioned as a species of medicinal interest we think that this motivation itself should be accounted as an exposure factor to be taken into consideration when mitigating the risk of rodent-borne diseases in our study area. Rodent hunting not only constitute the risk of exposure to diseases for the hunters during the hunting procedures, slaughtering, and

skinning but also for the other family member through contact with infected material during skinning, cooking and consumption (Douno et al., 2021; Izah et al., 2022). In West Africa, hunting and consumption of rodents are perceived as risk of *Lassa virus* primary transmission to humans (Bonwitt et al., 2016; Douno et al., 2021).

According to the calendar of activities in our study area (chapter four; Annex-X), in the middle of the dry season, the children have school holidays in July. The main activity indicated for the children was cattle grazing and our respondents did not make it clear who is involved in hunting rodents for consumption. We think that a higher rodent abundance observed in the middle of the dry season may attract more hunting activities most probably for boys during cattle grazing as they spend more in the fields during the school holidays. A study in Guinea found that rodent hunting by children is a childhood phenomenon and non-occupied boys during the dry season engage more in rodent hunting (Douno et al., 2021).

In our study area exposure to fleas from rodents is said to be the main issue as there are periods (not specified) where they have to sleep outside of their home to escape from the fleas. Rodent-borne diseases such as Plague can also be spread indirectly to humans by fleas (Meerburg et al., 2009). This highlights both direct and indirect transmission of rodent-borne pathogens at our studied human/rodent interface. Yet Leptospirosis, Toxoplasmosis, and Plague were detected in both humans and rodents in Mozambique (Nala, 2006). In addition to the above list, studies in Mozambique reported more than ten rodent-borne diseases in humans, such as Lyme diseases, Crimean-Congo hemorrhagic fever, hemorrhagic fever due to Hantavirus, Rift valley fever and Cryptosporidiosis (Salkeld et al., 2008; Augusto et al., 2009; Chau et al., 2017; Muianga et al., 2017; Messa et al., 2021; Rogier et al., 2022). However, little is known about the role of rodents in the transmission cycle of the above list of rodent-borne pathogens in the country. This highlights the need for futher studies on pathogens transmission at the human/rodent interface in Mozambique.

8.5 GENERAL CONCLUSIONS

Our results indicated that the small-mammal community in our study area included at least seven rodent species and *Elephantulus sp*. *M. natalensis* was present in all four habitats sampled during

the study, however, the irrigated croplands constitute the main habitat for this rodent species. *M. natalensis* most probably started breeding during the rainy season and its highest peak was observed in the middle of the dry season in June/July.

Mopeia virus circulates in all habitats investigated, and the proportion of positive samples (17.0%) was the same across all habitats. However, the low number of *M. natalensis* captures in other habitats other than the irrigated croplands may have not allowed us to detect differences in the proportion of positive samples over the habitats. Humans potentially are exposed to the risk of mammarenaviruses infections in all investigated habitats.

The risk of mammarenaviruses transmission between rodents and humans may be present all over the year in houses women and girls are the higher-risk group due to their domestic tasks. In croplands, the risk is even higher during the peak of *M. natalensis* breeding season in the middle of the dry season and again women and girls are the risk group because are the ones performing risks activity in the fields (weeding and harvesting) when the abundance of *M. natalensis* is higher. Girls are also exposed to rodents all over the year in the woodland when they look for firewood.

Men and boys are the main risk groups in the woodland due to hunting and cattle grazing activities. However, a higher rodent abundance in the fields and school holidays may attract boys to hunting activities, thus increasing the risk of exposure to rodents in the middle of the dry season.

8.6 RECOMMENDATIONS AND PERSPECTIVES

The results of this study indicated the circulation of the *Mopeia virus* in the Limpopo National Park and its buffer zone and described the risk of possible infections of humans with mammarenaviruses. However, due to resource and time limitations, our data is not enough to support all conclusions of our study, thus there are still several aspects requiring further clarification.

We recommend a long-term study for at least three years to cover more replicates of sites and seasons. In addition, more analyzis (other than abundance) should be performed (e.g., survival, movements, etc) to get better inside of rodent ecology. A minimum of three years is recomended to allow the collection of enough data to assess intra-annual variations in rodent ecology and

mammarenaviruses transmission dynamics. The replicates of sites from rainfed croplands and woodland should be increased. The sampling should cover all seasons of the year and during both rainy and dry seasons, and sampling should be implemented at the beginning, middle, and end of each season.

The level of exposure to rodents described in this study suggests that humans are at risk of contracting rodent-borne diseases. Therefore, were recommend an active surveillance to detect the *Mopeia virus* in humans and to se if there is a concordance between virus prevalence in rodents and in humans. Taking into account the transmission dynamics of mammarenaviruses in *M. natalensis* above described, this active surveillance should be set up around the middle of the dry season (June/July). Both serological and virological surveillance should be carried out in humans to detect and characterize possible mammarenaviruses circulating in humans and link them to the ones in rodents. Women and girls should be the main targeted groups, and whenever possible men and boys should also be included.

Community awareness campaigns should be implemented to fill the gap in knowledge about rodents and their diseases. More specifically for mammarenaviruses prevention emphasis should be given to address the risk factors (such as hygiene, food storage, hunting, agricultural practices, and others) including periods of higher risk. The communities expressed that the methods they use for rodent control in both croplands and houses are not efficient. Moreover, during the study, we noticed that the studied communities have limited access to the water supply source. Thus, advocacy at the governmental level is essential to address the need to assist the communities to improve rodent control methods in irrigated croplands and houses, water supply sources, and others that need authority intervention.

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9 ANNEX

Annex-I: Ethics committee VetAgro Sup

Revue Ethique d'un Projet d'utilisation d'animaux à des fins scientifiques

Comité d'Ethique de VetAgro Sup n°18

Numéro Comité d'Ethique : **1905**

Titre du Projet : «Mozambican Arenaviruses at the Rodent Human interface »

Demandeur : Julien Cappelle
Responsable de la mise en œuvre en conformité avec l'autorisation :
Etablissement Utilisateur :
Date de l'avis : 31/01/19

☒ Avis Favorable
☐ Avis Favorable sous réserve de modification de la version proposée (version 2)
☐ Avis Favorable sous condition d'apporter des réponses aux questions posées
☐ Avis Non Favorable en l'état

Commentaires :

Le Comité d'Ethique vous remercie pour la qualité de votre présentation et l'effort de traduction.

Nous vous signalons qu'au sens de la Directive 2010/63/UE, la limite acceptable pour la mise à mort d'un animal par disocatino cervicale est de 150 g.

Dans la mesure du possible, nous vous invitons à privilégier le recours à l'anesthésie pour les prélèvements de sang au sinus retro-orbitaires, pour garantir la réussite de l'opération.

L'avis pour ce projet est favorable.

Date limite de réception des réponses :

Signature du Président : p.o.

Annex-II: Credential from the National Administration of Conservation Areas

Administração Nacional das Áreas de Conservação
Credencial de Investigação/Levantamento/Recolha

Apenas para uso oficial:	
Nº da Credencial	**1/02/2021**
Tipo de actividade	**Investigação e Recolha de dados com amostras**
Data	**10 de Fevereiro de 2021**

Eu, **Mateus José Mutemba**, na qualidade de Director Geral da Administração Nacional das Áreas de Conservação, confirmo por este meio que foi concedida ao Sr. **Lourenço Paulo Mapaco**, da nacionalidade Moçambicana, funcionário na Direcção de Ciências Animais. Laboratório Central de Veterinária, Moçambique, uma Credencial de investigação e recolha para o projecto seguinte: Arenavírus na interface Roedores/Humanos em Moçambique para implementação no Parque Nacional de Limpopo.

Esta Credencial inicia no dia 1 de Junho de 2021 e expira no dia 30 de Outubro de 2021.

Igualmente por este meio solicito que as autoridades do Parque Nacional de Limpopo facilitem o desenvolvimento de quaisquer actividades relacionadas com este projecto, obviamente no total respeito pelas normas e orientações científicas da Área de Conservação.

lpmapaco@gmail.com, +258 84 4631511 / +258 86 4010290

O Director Geral

Mateus Mutemba

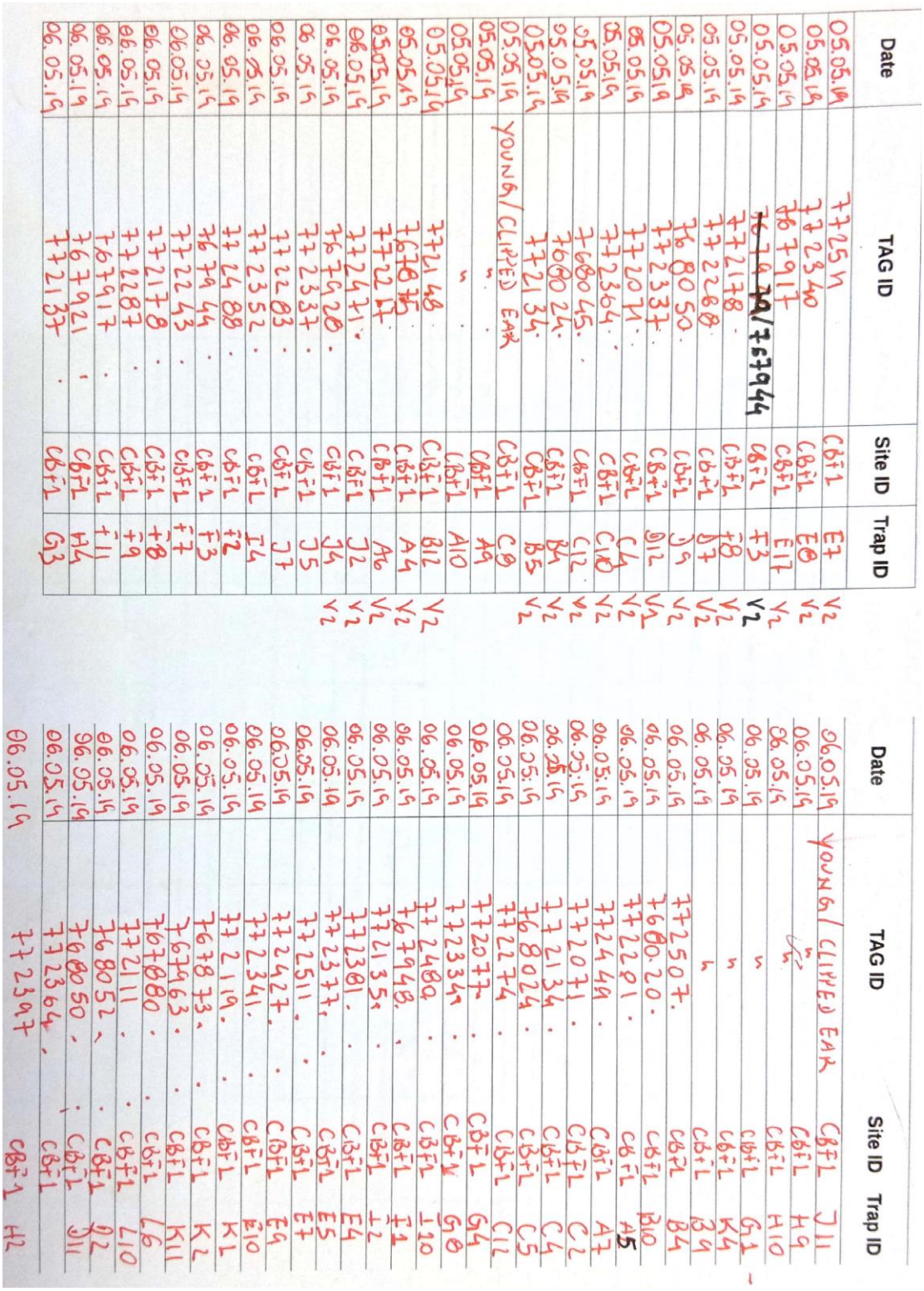
Annex-III: Example of filled rodent forms

Site ☒CMR ☐ One-shot	Trap location:			Date: 13.08.19	
SiteID: CBF1	Trap ID: J11	Rodent ID: 836	TAG ID: 772235		Recapt: Y N
Genus: MASTOMIS	Species: NATALENSIS		Certainty of species identification: ☒Yes ☐ No		
Trapped by: : ☒MozARH ☐ Locals ☐Other:		Trap arrangement: ☒ Line ☐ House ☐Other:			
Capture mean: ☐Mesh ☒Large Sh. ☐Small Sh. ☐Other:			☒ Alive ☐ Dead		Wounds # :
Weight: 41-8	Body:	Tail:	L.Foot:		L. Ear:
Sex:	☒Male ☐Female	**Vagina:** ☐Not open ☐Open			
Testes:	☐Abdominal ☒Scrotal	**Teats:** ☐Barely visible ☐Prominent			
Sem. vesicle	☐Developed ☐Undeveloped	**Embryos:** Nr.: Weight (g):			
Blood & Serum | **Ethanol** |		**RNA later**	**| Formol |**	**Dry Tubes**	
Filtr Paper Dry Tube Tail + Ear Spleen+Liver OralSwab Lungs Eyes LeptoTube ToxoTube Carcass					
Comments:					

Site ☒ CMR ☐ One-shot	Trap location:			Date: 13.08.19	
SiteID: CBF1	Trap ID: J10	Rodent ID: 837	TAG ID: 955000004767981		Recapt: Y (N)
Genus: MASTOMIS	Species: NATALENSIS		Certainty of species identification: ☒Yes ☐ No		
Trapped by: : ☐MozARH ☒ Locals ☐Other:		Trap arrangement: ☒ Line ☐ House ☐Other:			
Capture mean: ☐Mesh ☐Large Sh. ☒Small Sh. ☐Other:			☒ Alive ☐ Dead		Wounds # :
Weight: 34-11	Body:	Tail:	L.Foot:		L. Ear:
Sex:	☐Male ☒Female	**Vagina:** ☒Not open ☐Open			
Testes:	☐Abdominal ☐Scrotal	**Teats:** ☒Barely visible ☐Prominent			
Sem. vesicle	☐Developed ☐Undeveloped	**Embryos:** Nr.: Weight (g):			
Blood & Serum | **Ethanol** |		**RNA later**	**| Formol |**	**Dry Tubes**	
Filtr Paper Dry Tube Tail + Ear Spleen+Liver OralSwab Lungs Eyes LeptoTube ToxoTube Carcass					
Comments: ORELHA CORTADA (ANTIGO YOUNG) / NO EAR					

Site ☒ CMR ☐ One-shot	Trap location:			Date: 13.08.19	
SiteID: CBF1	Trap ID: J7	Rodent ID: 838	TAG ID: YOUNG		Recapt: Y N
Genus: MASTOMIS	Species: NATALENSIS		Certainty of species identification: ☒Yes ☐ No		
Trapped by: : ☒MozARH ☐ Locals ☐Other:		Trap arrangement: ☒ Line ☐ House ☐Other:			
Capture mean: ☐Mesh ☒Large Sh. ☐Small Sh. ☐Other:			☒ Alive ☐ Dead		Wounds # :
Weight: 27-12	Body:	Tail:	L.Foot:		L. Ear:
Sex:	☐Male ☒Female	**Vagina:** ☒Not open ☐Open			
Testes:	☐Abdominal ☐Scrotal	**Teats:** ☒Barely visible ☐Prominent			
Sem. vesicle	☐Developed ☐Undeveloped	**Embryos:** Nr.: Weight (g):			
Blood & Serum | **Ethanol** |		**RNA later**	**| Formol |**	**Dry Tubes**	
Filtr Paper Dry Tube Tail + Ear Spleen+Liver OralSwab Lungs Eyes LeptoTube ToxoTube Carcass					
Comments:					

Annex-IV: Example of filled recapture form



Date	TAG ID	Site ID	Trap ID	
05.05.19	772514	CBFL	E7	V2
05.05.19	772340	CBFL	E8	V2
05.05.19	767917	CBFL	E17	V2
05.05.19	~~767944~~ 767944	CBFL	F3	V2
05.05.19	772178	CBFL	F8	V2
05.05.19	772280	CBFL	F7	V2
05.05.19	768050	CBFL	J9	V2
05.05.19	772337	CBFL	J12	V2
05.05.19	772071	CBFL	C4	V2
05.05.19	772364	CBFL	C10	V2
05.05.19	768045	CBFL	C12	V2
05.05.19	768024	CBFL	B4	V2
05.05.19	772134	CBFL	B5	V2
05.05.19	YOUNG/ CLIPPED EAR	CBFL	C9	
05.05.19	" "	CBFL	A9	
05.05.19	772148	CBFL	A10	V2
05.05.19	767835	CBFL	B12	V2
05.05.19	772267	CBFL	A4	V2
05.05.19	772471	CBFL	A6	V2
06.05.19	767920	CBFL	J2	V2
06.05.19	772337	CBFL	J4	
06.05.19	772203	CBFL	J5	
06.05.19	772352	CBFL	J7	
06.05.19	772488	CBFL	I4	
06.05.19	767944	CBFL	I2	
06.05.19	772243	CBFL	F3	
06.05.19	772178	CBFL	F7	
06.05.19	772287	CBFL	F8	
06.05.19	767917	CBFL	F9	
06.05.19	767921	CBFL	F11	
06.05.19	772137	CBFL	H4	

Date	TAG ID	Site ID	Trap ID
06.05.19	YOUNG/ CLIPPED EAR	CBFL	J11
06.05.19	"	CBFL	H9
06.05.19	"	CBFL	H10
06.05.19	"	CBFL	G1
06.05.19	"	CBFL	K4
06.05.19	"	CBFL	B9
06.05.19	772507	CBFL	B4
06.05.19	760020	CBFL	B10
06.05.19	772281	CBFL	A5
06.05.19	772449	CBFL	A7
06.05.19	772071	CBFL	C2
06.05.19	772134	CBFL	C4
06.05.19	768024	CBFL	C5
06.05.19	772274	CBFL	C11
06.05.19	772077	CBFL	G4
06.05.19	772334	CBFL	G10
06.05.19	772480	CBFL	J10
06.05.19	767948	CBFL	F1
06.05.19	772135	CBFL	I2
06.05.19	772301	CBFL	E4
06.05.19	772377	CBFL	E5
06.05.19	772511	CBFL	E7
06.05.19	772427	CBFL	E9
06.05.19	772341	CBFL	E10
06.05.19	772119	CBFL	K1
06.05.19	767873	CBFL	K2
06.05.19	767963	CBFL	K11
06.05.19	767080	CBFL	L6
06.05.19	772111	CBFL	L10
06.05.19	768052	CBFL	J2
06.05.19	768050	CBFL	J11
06.05.19	772364	CBFL	
06.05.19	772397	CBFL	H2

Annex-V: Examples of filled site forms

Site Form: Cropland

Locality	Rivuep		CMR study site: Yes	No
Trapping Site Id#	Code: BGF1	Date: 22/08	Interviewer id:	
Study participation informed consent				
Geo position	Latitude: 23732	Longitude: 032.03893	Altitude: 11pm	
Traps (day1)	Number of traps: 50	Number of Captures: 16		
Traps (day2)	Number of traps: 60	Number of captures: 10		
Traps (day3)	Number of traps: 71	Number of captures: 14		
Crop type	**Main crop:** Maize	**Other crops:**		
Farm stage/description	Seeding	Vegetative	Reproductive	Harvesting	Post harvesting
Site size	140 m X m	60	**Trap map file:**	
Season	Dry	Wet		
Rodents Signs (by interviewer)	Yes	No		
Rodents presence (informant)	Yes	No		
Rodent Control	Yes	No		

Site Form: Village

Locality	KADINGANE		**CMR study site:** Yes	No
Trapping Site Id#	Code: MGV1	Date: 29/08	Interviewer id:	
Study participation informed consent				
Geo position	Latitude: 23.87105	Longitude: 32.29778	Altitude: 89m	
Village population	No data	**Households number:** 197		
Water access	River Well Water Pump	Other: using solar	**Irrigated Fields:** Yes	No
Traps (day1)	Number of traps: 51	Number of Captures: 23		
Traps (day2)	Number of traps: 44	Number of captures: 18		
Traps (day3)	Number of traps: 49	Number of captures: 09		
Traps (day4)	Number of traps:	Number of Captures:		
Traps (day5)	Number of traps:	Number of Captures:		

Site Form: Wild areas

Locality	Macavene	**CMR study site:** Yes	No
Trapping Site Id#	Code: MCW1 Date: 19/08	Interviewer id:	LPM
Geo position	Latitude: See last trapping session	Longitude:	Altitude:
Season	Dry Wet		
Traps (day1)	Number of traps: 100	Number of Captures:	0
Traps (day2)	Number of traps: 100	Number of Captures:	0
Traps (day3)	Number of traps: 100	Number of Captures:	100
Site size	100 m X 100 m	**Trap map file:**	
Habitat description	Grassland Shrubland	Wooded area	Other:
Rodents Signs	No Yes : Feces Burrows Ways	Leftovers	Other:
Human Signs	No Yes : Fireplace Charcoal	Other:	Grazing

Annex-VI: Trapping effort in different sites

Trapping effort in the removal (cbf2 to mgf1) and CMR (cbf1) irrigated cropland sites

Site ID	PTS	STSs	Trap nights	Nr. of successful traps	Nr. of individuals (Nr. of captures)
cbf2	May	1	100	71	75
cbf3	June	1	32	23	24
cbf4	August	1	42	8	8
mzf4	May	1	100	82	84
	June	1	40	35	36
mzf5	August	2	60	50	52
	November	1	50	27	30
bgf1	June	1	49	37	39
	August	3	181	39	41
mgf1	August	2	80	60	64
cbf1*	March	5	636	80	64 (81)
	May	5	636	337	169 (349)
	June	4	507	432	162 (466)
	August	3	386	232	117 (240)
	November	3	432	166	79 (173)

* For the cbf1 CMR site the number of individuals in each PTS include the recaptures from previous PTSs. The number of captures in brackets for each PTS included the recaptures from previous PTSs and those from the current PTS.

Trapping effort in the removal (mzf2) and CMR (mzf1) rainfed croplands

Site ID	PTSs	STSs	Trap nights	Nr. of successful traps	Nr. of individuals (Nr. of captures)
mzf2	March	3	140	1	1
mzf1	March	3	327	2	2 (2)
	May	3	321	23	19 (23)
	June	3	317	55	31 (56)
	August	3	327	16	7 (16)
	November	3	327	13	8 (15)

* For the mzf1 CMR site the number of individuals in each PTS include the recaptures from previous PTSs. The number of captures in brackets for each PTS included the recaptures from previous PTSs and those from the current PTS.

Trapping effort in the mopane woodland

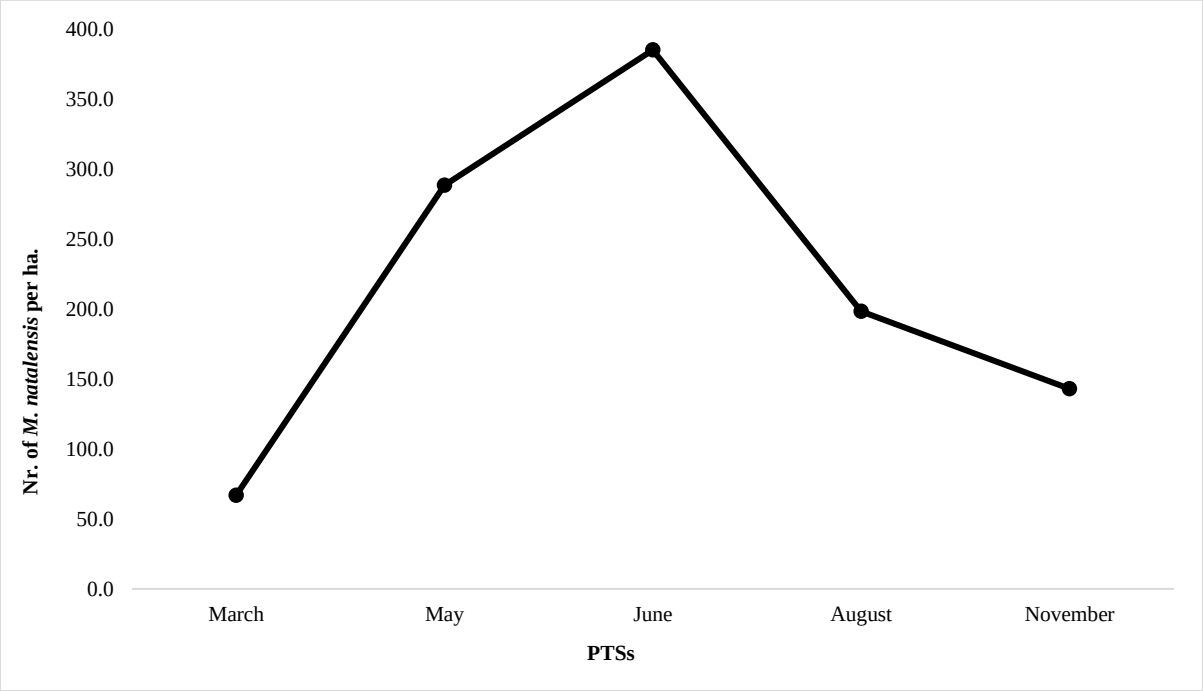
PTSs	STSs	Trap nights	Nr. of successful traps	Nr. of captures
March	3	300	4	4
May	2	200	1	1
June	3	300	8	8
August	3	300	0	0

Trapping effort in the village sites

Site ID	PTSs	STSs	Trap nights	Nr. of successful traps	Nr. of captures
mzv1	March	3	288	13	13
	November	2	103	19	19
bgv1	August	3	152	30	30
cbv1	March	3	204	39	43
mlv1	March	2	126	25	28
	May	2	84	21	22
mgv1	August	3	143	48	50

Annex-VII. *M. natalensis* density

Here population density (D) is the number of individuals (N) trapped in the total aera of sampled site (A): D=N/A (Parmenter et al., 2003).



M. natalensis density in the cbf1 CMR irrigated cropland. The area of this cropland field was 1.21 ha.

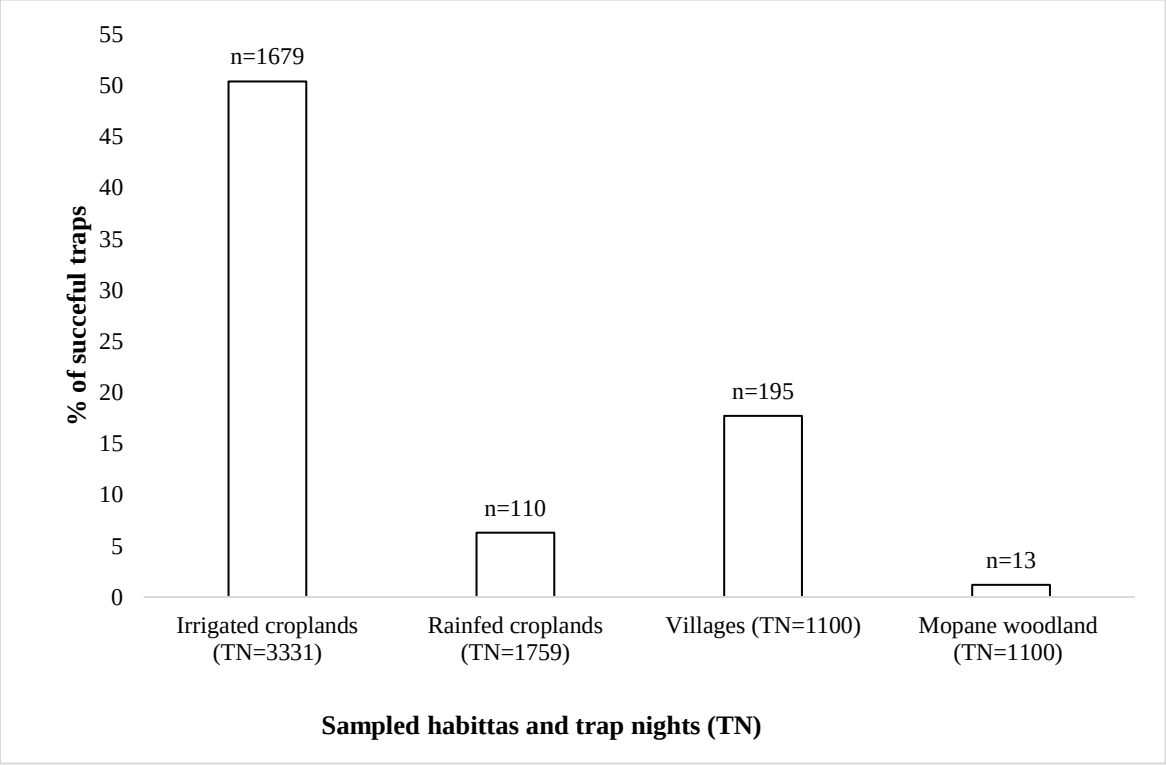
Reference

Parmenter, R.R., Yates, T.L., Anderson, D.R., Burnham, K.P., Dunnum, J.L., Franklin, A.B., Friggens, M.T., Lubow, B.C., Miller, M., Olson, G.S., Parmenter, C.A., Pollard, J., Rexstad, E., Shenk, T.M., Stanley, T.R., White, G.C., 2003. Small-Mammal Density Estimation: A Field Comparison of Grid-Based Vs. Web-Based Density Estimators. Ecological Monographs 73, 1–26. https://doi.org/10.1890/0012-9615(2003)073 [0001: SMDEAF]2.0.CO;2

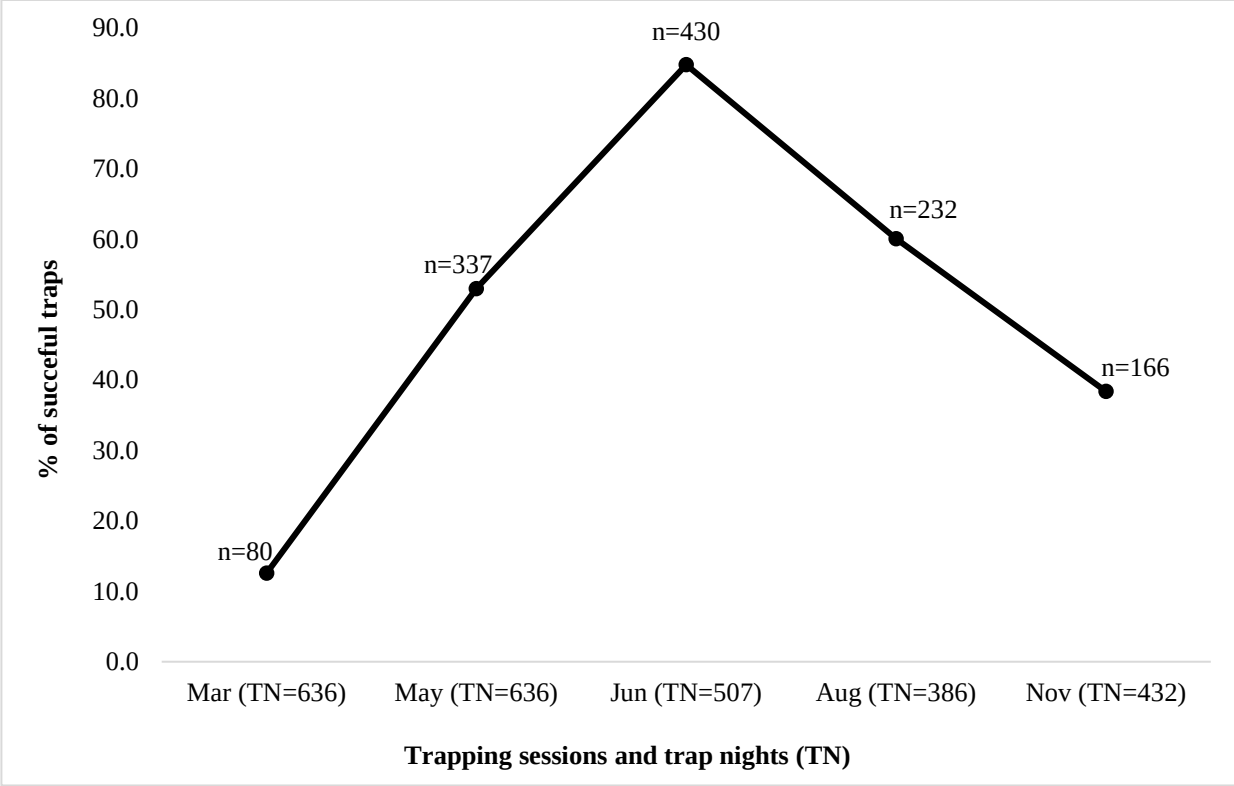
Annex-VIII: Trapping success

Trapping success is the percentage of traps that caught at least one small mammal (without considering their species) out of trap nights (Telford, 1989; Cavia et al., 2012).

$$\textbf{Trap success} = \frac{\textbf{Nr. of succeful traps}}{\textbf{Trap nights}} \textbf{ X 100}$$



Trapping success over different habitats. In the irrigated croplands and rainfed croplands the number of succeful traps (n) include all the recaptures



Evolution of trapping success in the cbf1 CMR irrigated cropland across the sampling sessions. The number of succeful traps (n) include all the recaptures

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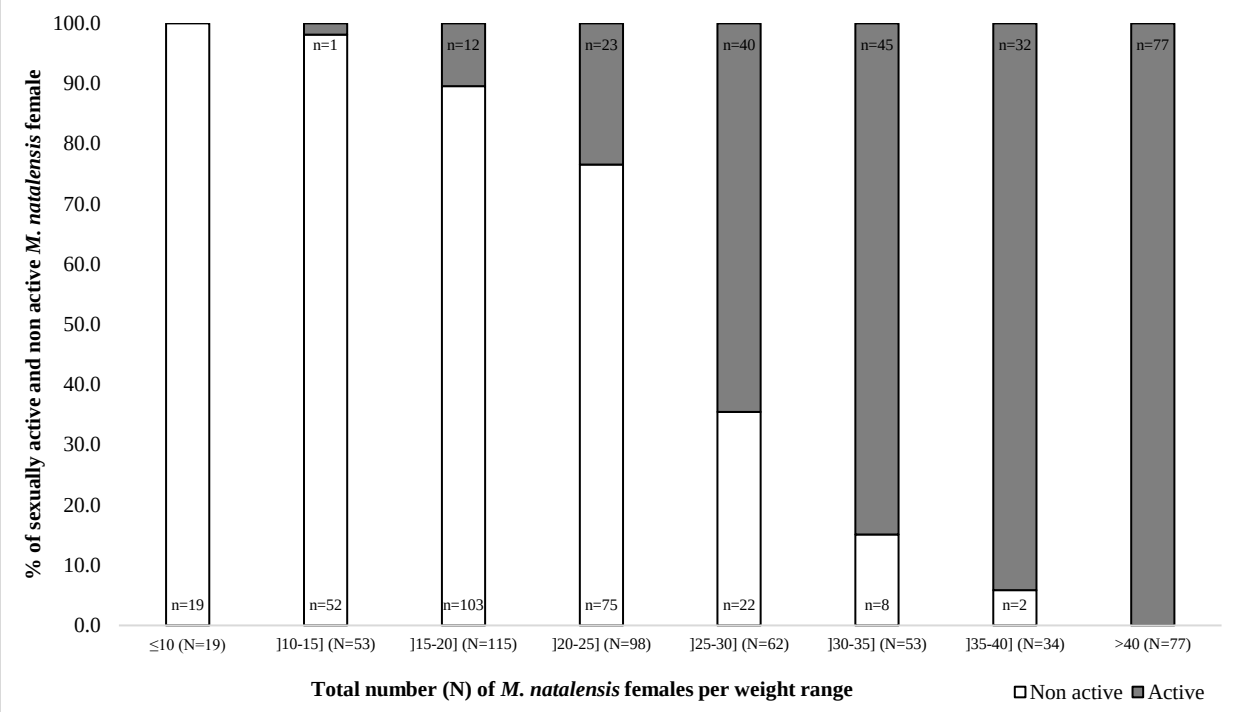
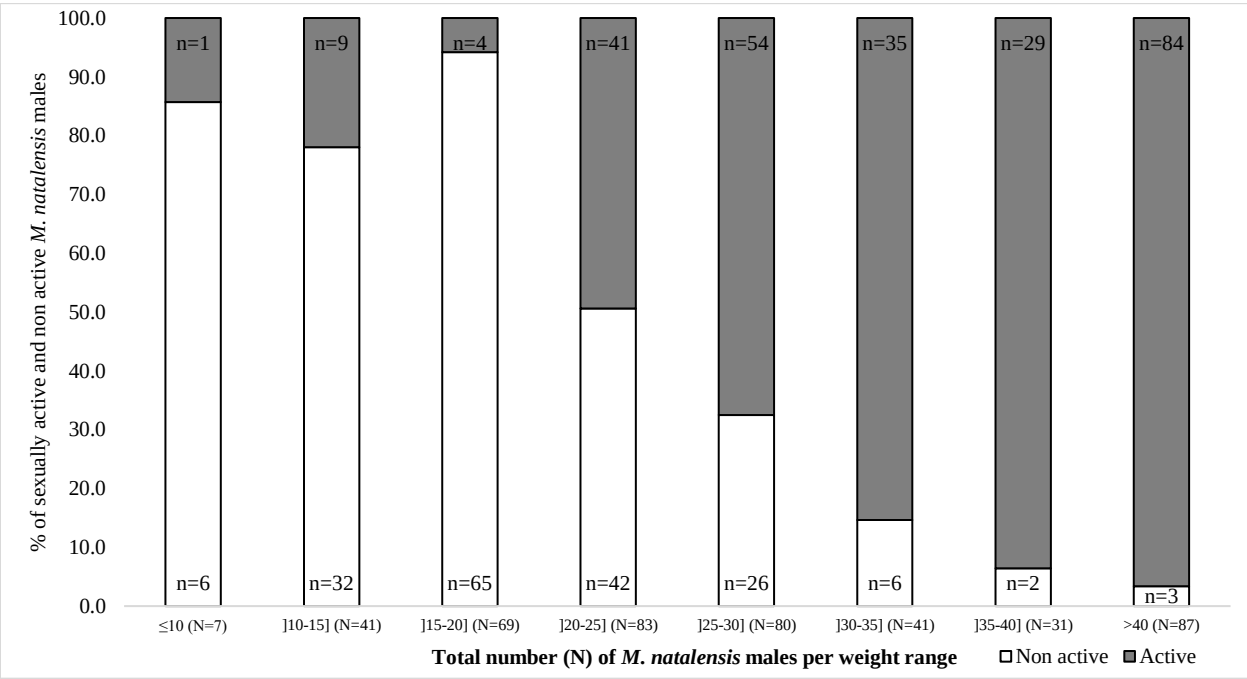
Cavia, R., Cueto, G.R., Suárez, O.V., 2012. Techniques to Estimate Abundance and Monitoring Rodent Pests in Urban Environments, Integrated Pest Management and Pest Control - Current and Future Tactics. IntechOpen. https://doi.org/10.5772/32541.

Telford, S.R. Jr. 1989. Population biology of multimammate rat, Praomys (Mastomys) natalensis at Morogoro, Tanzania 1981-1985. Bull Fl. State Mus. Biol. Sc., 34(6): 249-288.

Annex-IX Reproductive condition of *M. natalensis* in different weight classes



Weight classes (g) and distribution of sexually active and non-active *M. natalensis* females trapped in the irrigated croplands



Weight classes (g) and distribution of sexually active and non-active *M. natalensis* males trapped in the irrigated croplands

Annex-X: Calendar of activities in the Limpopo National Park and its buffer zone as reported by respondents.

	Months											
	Jan	Fev	Mar	Abril	May	Jun	Jul	Aug	Set	Out	Nov	Dec
Seasons												
Rainy season	x	x	x							x	x	x
Dry season												
Lean season												
Hazards												
Flooding												
Crops, pests, disease												
School holydays												
Crop production												
Land preparation	Men (in irrigated and wet areas)								men	men		
Sowing planting			Women in wet	Women/ men	(Women/ men) (beans)						x	x
Weeding	women			women	women							women
Harvesting			women	women	women		women	women				
Rodent perceived "density"												
Home (all year round)												
Granaries (decrease in the lean season)												
(peak during seeding and harvest times			+	+	+	?	+					
Forests (rainy season)												

Annex-XI: Main places, periods and perceived abundance as quoted by participants of the focus groups

Local name	Scientific name	Main place	Main period	Perceived abundance	Use
Big rat (black and brown rats "ratazana", "ratos da casa", "ratazao*"*	*Rattus sp. (and Cricetomys gambianus)*	Home, granaries and ("come milho")	All year round or "When there is maize"	Very common, met every day	Not eatable Allow to be hunted
Small Condjo / xicondjuane *(*Small rats with white spots) (Sometimes also named Mbeva when met in)	*Mastomys sp.* *Mus sp.*	Home and granaries	All year round	Last time met in January	Not eatable
Mbeva/ Mbewa (small, white or red patches)	*Mastomys sp.* *Thallomys paedulcus, Aethomys chrisophulus*	Field (Dry and irrigated areas*)*	Winter/ During land preparation During harvest time (maize)	Common (even today we killed one até hoje matamos um)	Bushmeat (but not for all) depending on the place where they are met
Vonduam, vondwane (small vondo)	Probably *Rattus norvegicus*	Home only	All year round	-	-
Vundo/ Mavondo (Greay cane rat)	-	Woodland (F7, F8) Eat maize and sugar cana	Winter (F8)	(Last week)	Bushmeat ("more delicious")
Vondo, (Big cane rat,)	*Thryonomys swinderianus*	Woodland Eat maize and sugar cana Swamps / farmland	Winter/ All year round April - June	Not very common	Bushmeat ("more delicious"), hunted (with dogs) but forbidden to hunt
Nungo (porcupine)	*Hystris africaeustralis*	Woodland / farmland	All year round Rainy season, when there is a lot of vegetation	Rare (but people find their spines and do not see it)	bushmeat but forbidden to hunt spines used to cure "fogo da noite" (Herpes zoster associated with HIV) sharp pain or nausea
***Maduro** (ratos)*	*Elephanturus* (is not a rodent)	Home, , woodlands "they stay in the bush, they don't eat much maize"	All year round Rainy season	Very common met daily when walking to	-
***Sengane/ Massengane** (red rats, whitish on chest)	*Steatomys pratensis*	Home/ Woodland / Savannah	All year round/ Winter	Very common, met daily ("today we kill a lot") are not many and do not cause us any problems.	bushmeat (but not for all)
Jenga/ Majengwa/magengo **Nphundla/**? Coelho-	*Pedetes capensis*	Woodland	All year round, Springtime	rare	bushmeat but forbidden to hunt

rabbit) (long back legs)			Rainy season		
Matxigane/ **Mathindane** (squirrel)	*Paraxerus cepapi*	Woodland	-	-	bushmeat
*** Matxoxo/ Matxoxa**	*Gerbilicus*	Woodland	All year round Springtime	Very rare	
Ndavitane (Looks like a cat, very smart)	*Graphiurus murinae*	Woodland / Savannah, arboreal	All year round springtime	Very rare	Used by traditional healers/ witch to increase the skills of hunters and football players
Fucuzane (mole rat, toupeira)	*Cryptomys botentottus*	Woodland / Savannah/ All places, subterraneous (eat sweet potatoes and peanuts)	All year round	Very rare. "You can go two years without seeing a Rare; people see the tracks"	Not consumed, used to prepare medicines
Ndlovene	-	Woodland / Savannah	All year round	-	-
Tenesse, "large-billed rat"	*Mus sp.*	-	-	-	-