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Morgane Labadie

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**THÈSE POUR OBTENIR LE GRADE DE DOCTEUR
DE L'UNIVERSITÉ DE MONTPELLIER**

En écologie de la santé

École doctorale GAIA

Unité de recherche Animal, Santé, Territoires, Risques et Écosystèmes

**Écologie des chauves-souris insectivores cavernicoles
dans le contexte d'émergence des zoonoses
en République du Congo**

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Le 14 mai 2024

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**UNIVERSITÉ
DE MONTPELLIER**

Abstract

With the Anthropocene, our human societies have created drastic and disproportionate pressures and disturbances on our environment. The consequences of these upheavals threaten animal, plant and human health and are causing a resurgence in the emergence of zoonoses. As a result, surveillance systems are recognizing the need to gain knowledge of the ecological mechanisms associated with emergence, particularly at the interface between wildlife and humans. However, there are still gaps in our knowledge of the ecology of these interfaces, particularly in countries such as the Republic of Congo. The main aim of this thesis is to improve our knowledge of the ecology and dynamics of cave bat communities, while contributing to a better understanding of interface ecology. This thesis begins with a characterisation study of cave bat communities in two caves in the Republic of Congo. This study shows the presence of six putative species using morphometric and acoustic data collected during trapping sessions spread over almost two years. The second study presents a semi-automatic method using a sonotype classifier coupled with a posteriori classification. Our study tested this method on the 2,375,956 acoustic files using ultrasonic recorders placed at the entrances to the two caves. The results allowed us to identify five acoustic groups, taking into account the acoustic superposition of certain species. The third study used the above methodology to describe the nocturnal activity patterns and annual phenology of the acoustic bat communities in the two caves. This study identifies a tri-modal activity with high activity from October to March. These variations in behaviour are linked to the periods when the young are reared (birth, lactation) and leave the caves. The fourth and fifth studies focus on the description of tropical chains and interfaces, including with humans, using a photographic trapping protocol. After describing the taxa that use the caves inside and outside, this study identifies predator-prey interactions, human activities and overlapping activities. Finally, the sixth study focuses on the determinants explaining the richness of viral families in African bats, based on existing data. Our study shows that this viral richness is mainly explained by sample size, which is characterized by a strong bias related to the life history characteristics of the bats.

This work allowed us to: (1) develop two non-invasive methods for collecting data on the temporal dynamics of faunal communities present in a habitat at the bat-wildlife-human interface, (2) contribute to the understanding of interactions in these particular interfaces, and (3) acquire knowledge on the dynamics and distribution of the communities studied. This knowledge is essential for understanding the ecology of zoonotic diseases, while also contributing to the conservation ecology of these species. Finally, these protocols can be replicated in other countries, contributing to the acquisition of knowledge for adequate prevention against the risks of zoonotic disease transmission.

Résumé

Avec l'Anthropocène, nos sociétés humaines ont engendré une pression et des perturbations drastiques et démesurées sur notre environnement. Les conséquences de ces bouleversements menacent la santé animale, végétale et humaine et causent la recrudescence d'émergences de zoonoses. Ainsi, les systèmes de surveillance voient la nécessité d'acquérir des connaissances sur les mécanismes écologiques associés à l'émergence, plus particulièrement à l'interface de la faune sauvage – et des humains. Cependant, les connaissances sur l'écologie des interfaces surtout dans des pays comme la République du Congo sont encore lacunaires. L'objectif principal de cette thèse est d'améliorer les connaissances sur l'écologie et la dynamique des communautés de chauves-souris cavernicoles tout en contribuant à l'amélioration du savoir sur l'écologie des interfaces. Cette thèse débute par une étude sur la caractérisation des communautés de chauves-souris cavernicoles dans deux grottes en République du Congo. Cette étude montre la présence de six espèces putatives à l'aide de données morphométriques et acoustiques récoltées lors de sessions de captures s'échelonnant sur près de deux ans. La deuxième étude présente une méthode semi-automatique d'un classificateur de sonotypes couplé à une classification a posteriori. Notre étude a testé cette méthode sur les 2,375,956 fichiers acoustiques à l'aide d'enregistreurs d'ultrasons disposés à l'entrée des deux grottes. Les résultats permettent d'identifier cinq groupes acoustiques en prenant en compte la superposition acoustique de certaines espèces. La troisième étude s'appuie sur la méthodologie présentée précédemment pour décrire les modèles d'activité nocturnes et la phénologie annuelle des communautés acoustiques de chauves-souris dans les deux grottes. Cette étude identifie une activité trimodale avec une forte activité du mois d'octobre au mois de mars. Ces variations dans le comportement sont reliées aux périodes d'élevage des jeunes (mise bas, lactation) et à l'envol de ceux-ci en dehors des grottes. Les quatrième et cinquième études se concentrent sur la description des chaînes tropiques et des interfaces, y compris avec les humains, en s'appuyant sur un protocole de pièges photographiques. Après une description des taxons utilisant l'intérieur et l'extérieur des grottes, cette étude identifie les interactions prédateurs - proies, les activités humaines et les chevauchements d'activités. Enfin, la sixième étude se concentre sur les déterminants expliquant la richesse des familles virales chez les chauves-souris africaines à partir des données existantes. Notre étude montre que cette richesse virale s'explique majoritairement par la taille de l'échantillonnage marqué par un fort biais lié aux traits de vie des chauves-souris.

Cette thèse a permis : (1) la création de deux méthodes non invasives de collecte de données de dynamique temporelle des communautés fauniques présentes dans un habitat d'interface entre chauves-souris – faune sauvage – humains, (2) de contribuer à la compréhension des interactions dans ces interfaces particulières et (3) d'acquérir des connaissances sur la dynamique et la distribution des communautés étudiées. Ces connaissances sont essentielles pour la compréhension de l'écologie des maladies zoonotiques tout en contribuant à l'écologie de la conservation de ces espèces. Enfin, ces protocoles peuvent être répliqués dans d'autres pays contribuant à l'acquisition de connaissances pour une prévention adéquate contre les risques de transmission de zoonoses.

Liste des productions scientifiques

Articles acceptés et en cours de publication

Labadie, M., Morand, S., Bourgarel, M., Niama, F. R., Nguilili, F., N’Kaya, T., Caron, A., De Nys, H., 2024. Habitat sharing and interspecies interactions in caves used by bats in the Republic of Congo

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Articles soumis

Labadie, M., Morand, S., Caron, A., De Nys, H., Niama, F. R., Nguilili, F., N’Kaya, T., Bourgarel, M., Roemer, C., 2024. Passive acoustic monitoring of cave-dwelling bats with a sonotype classifier.

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Articles en cours de préparation

Labadie, M., Morand, S., Niama, F. R., Caron, A., Nguilili, F., N’Kaya, T., Bourgarel, M., De Nys, H., 2024. Characterization of insectivorous bat communities in Republic of Congo using morphometric and acoustic data.

Labadie, M., Morand, S., Caron, A., De Nys, H., Niama, F. R., Nguilili, F., N’Kaya, T., Bourgarel, M., Bas, Y., Roemer, C., 2024. Listen, who is it? A novel method for studying the nightly and annual phenologies of cave bat communities in data deficient areas of the world.



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« Seul, on ne peut pas faire grand-chose ; ensemble, on peut tellement. »

Helen Keller

« Je vous apprécie davantage en raison du chemin que j'ai parcouru. Mon histoire m'a conduit jusqu'à vous et je ne changerais pas un instant mon passé s'il me conduisait ailleurs qu'à votre porte. » -**Aaron Polson**

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Introduction

L'homme est l'unique espèce vivante ayant modifié le fonctionnement de la planète dans sa globalité de l'exosphère à la pédosphère. À une échelle mondiale, on relève une multitude de changements dont les principaux sont la destruction des habitats (déforestation, urbanisation), l'intensification des modes d'élevages et de culture, une surexploitation des ressources naturelles et une forte pollution (Chaudhry & Malik, 2017; Delgado et al., 2001; Manisalidis et al., 2020; Mirsal, 2008; Myers, 2023; Peres, 2006; Zabel et al., 2019). Les scientifiques ont annoncé l'avènement d'une nouvelle ère géologique, l'Anthropocène, qui reflète la pression et les changements qu'occasionnent les activités humaines sur notre environnement (Crutzen & Stoermer, 2000). Encore aujourd'hui, la communauté scientifique débat de l'évènement clef qui représenterait le début de l'Anthropocène. Est-ce la première utilisation de la bombe atomique ? La création de machines nous permettant d'utiliser les énergies fossiles ou les métaux lourds ? L'invention du plastique ? Dans tous les cas, ces événements sont la représentation d'une ère en « grande accélération », où les activités humaines et leurs perturbations n'ont jamais été aussi rapides, drastiques et démesurées (Bradley et al., 2020; Head et al., 2022; Shoshitaishvili, 2021; Steffen et al., 2015).

Toutes ces perturbations entraînent des changements climatiques (Carlson et al., 2022; Jagadesh et al., 2022; Naicker, 2011), une perte massive de la biodiversité animale et végétale (Dobson et al., 2021; IPCC, 2023; Keesing et al., 2010; Keesing & Ostfeld, 2021; Patil et al., 2017) qu'on nomme « la sixième extinction massive » (Ceballos et al., 2017) et une augmentation des interfaces entre faune et homme (Ahmed et al., 2019; Dobigny & Morand, 2022; Hilderink & de Winter, 2021; Keusch et al., 2022; Lindahl & Grace, 2015; Morand & Lajaunie, 2021; Plowright et al., 2017; Rulli et al., 2017; Tajudeen, Oladunjoye, et al., 2022). Les conséquences de ces bouleversements impactent la santé animale, végétale et humaine avec une augmentation de la détection des maladies infectieuses depuis environ deux décennies. L'IPBES allant jusqu'à déclarer l'Anthropocène comme étant aussi l'ère des pandémies (Daszak et al., 2020).

Près des deux tiers des maladies infectieuses émergentes (MIE) ont une origine zoonotique et parmi elles la majorité proviennent des animaux sauvages (Han et al., 2016; Jones et al., 2008; Kruse et al., 2004; McArthur, 2019; Morse, 1995; Morse et al., 2012; Olival et al., 2017; Smith et al., 2014; Woolhouse & Gowtage-Sequeria, 2005). Le terme de zoonose comprend toutes les maladies ou infections qui se transmettent naturellement entre un animal et l'homme et inversement qu'elles soient parasitaires, virales ou bactériennes (Rahman et al., 2020; WHO, 2020). Une zoonose implique généralement une ou plusieurs espèces réservoirs qui vont assurer la maintenance et la transmission de l'agent infectieux. Les zoonoses sont une préoccupation majeure pour la santé publique mondiale en raison de l'impact qu'elles ont sur nos sociétés (Bao et al., 2017; Fernandes, 2020; Liu et al., 2014; Rahman et al., 2020; Singh & Singh, 2020).

L'un des exemples les plus marquants est la dernière pandémie mondiale, causée par le virus SARS-CoV-2 déclenchant la COVID-19, probablement acquis par un contact rapproché de personnes avec des animaux sauvages en Chine (Malik et al. 2020 ; Li et al. 2020a). Elle a engendré l'une des pires crises sanitaires, économiques et sociales de notre époque contemporaine en confinant une grande partie de la population humaine, infectant presque 10 pour cent de la population mondiale et en causant la mort de plus de 18 millions de personnes (Adam, 2022; WHO, 2023b; Worldometers, 2023). Malheureusement, ce type de pandémie n'est pas nouveau, nos sociétés ayant déjà été marquées par d'autres événements épidémiques majeurs. En 1918, la grippe espagnole aurait causé la mort d'au moins 25 millions de personnes (Morens & Taubenberger, 2020). En 2009, l'apparition du H1N1pdm09, provenant d'une mégaferme Nord-Américaine de cochons a causé la première pandémie du 21^{ème} siècle, le nombre exacte de personnes décédées restant inconnu (CDC, 2019; Markin et al., 2023; Mena et al., 2016; Viboud & Simonsen, 2012). Entre 2014 et 2015, la dernière épidémie d'Ebola a entraîné le décès d'environ 11 000 personnes en Afrique de l'Ouest (WHO, 2016). Ces exemples témoignent du risque d'émergence des zoonoses même si celui-ci diffère en fonction des continents. Ainsi, entre 1996 et 2009, 53% des MIE globales reportées provenaient du continent africain (Muzemil et al., 2018) qui dispose d'un système de surveillance lacunaire comparé à d'autres continents (Chan et al., 2010). L'Afrique est aussi un des continents qui abritent une forte biodiversité. De plus, une grande partie de sa population

dépend fortement de la viande de brousse comme source de protéines et de revenus (Friant et al., 2020; Kurpiers et al., 2016; Trefon, 2023; Van Velden et al., 2018).

Historiquement, les systèmes de surveillance sont tournés vers une approche de diagnostics et de réponses rapides (vaccination, médication). Plus récemment, afin de se prémunir contre les zoonoses, nos sociétés se sont tournées principalement vers une approche associant biosurveillance et biosécurité (ex : prélèvement et identification des pathogènes sur la faune sauvage, surveillance des filières de viande de brousse, ..) (National Research Council, 2010). Cette approche engendre une dégradation de nos relations avec le monde sauvage ; pour exemple la perception fortement négative des chauves-souris dans nos sociétés (Bonilla-Aldana et al., 2020; Cros, 2020; MacFarlane & Rocha, 2020; Straka & Voigt, 2022; Tsoucalas et al., 2021). Cependant, les récentes émergences zoonotiques (COVID-19, Mpox anciennement nommé Monkey Pox) ont mis en lumière les lacunes des systèmes de surveillance et le rôle crucial de la prévention en amont et de l'implication des populations vivant dans des zones à fort risque d'émergence (Ammirato et al., 2021; Bernstein et al., 2022; Callaby & Gordon, 2023; Shanks et al., 2022; Tajudeen, Oladipo, et al., 2022; Vora et al., 2022). Pour réaliser une prévention adéquate, il est primordial de comprendre les mécanismes écologiques associés aux risques d'émergences afin d'éclairer scientifiquement les institutions internationales ou nationales pour développer des systèmes performants de contrôle des MIE et organiser une prévention adéquate (Coker et al., 2011). De plus, il est primordial d'évoluer vers un système de surveillance des zoonoses plus holistique. Pour répondre à cette volonté, la communauté scientifique applique de plus en plus l'approche « One Health ». Une « seule santé » est une démarche globale qui prend en compte de manière intégrée la santé humaine, la santé animale et la santé des écosystèmes (OHHLEP et al., 2022). L'écologie de la santé est aussi un domaine scientifique qui veut contribuer à cette approche multidisciplinaire en participant à la compréhension des problèmes de santé à l'interface animal (sauvage et domestique), homme et environnement.

Dans ce contexte global, l'Union européenne a financé le projet EBOSURSY « Renforcer les capacités face aux fièvres hémorragiques virales et améliorer la surveillance des virus ». Ce projet est dirigé par l'Organisation Mondiale de la Santé Animale (OMSA) en collaboration étroite avec 10 pays cibles et trois instituts de recherche. Ce consortium scientifique est

composé du Centre de coopération internationale en recherche agronomique pour le développement (CIRAD), de l'Institut de recherche pour le développement (IRD) et de l'Institut Pasteur (OMSA, 2023). Le CIRAD se concentre en particulier sur la compréhension des mécanismes de transmission, l'écologie, le comportement et l'habitat des « réservoirs zoonotiques » en s'appuyant sur des partenaires locaux comme le Laboratoire National de Santé Publique (LNSP) de Brazzaville et la Direction Générale de l'Élevage (DGE) en République du Congo.

Bénéficiant de l'immersion dans une équipe interdisciplinaire, ce travail s'inscrit dans une volonté d'étudier l'écologie des chauves-souris insectivores cavernicoles afin de contribuer à l'amélioration des connaissances sur l'utilisation de l'habitat et le recouvrement de niche écologiques associées à des maladies zoonotiques préoccupantes pour les populations humaines. Ce travail est abordé à l'échelle des communautés et des populations dans deux grottes en République du Congo. Les différents aspects de cette question sont présentés dans quatre volets distincts.

Le premier volet de cette thèse a pour objectifs de synthétiser les connaissances actuelles sur l'écologie des chauves-souris en Afrique et d'identifier les communautés insectivores présentes dans les deux grottes de cette étude. Le premier chapitre présente l'écologie de ces mammifères évasifs et encore bien mystérieux. Voisins silencieux et fantômes de nos nuits que nous, humains, craignons en raison des récentes émergences zoonotiques, occultant les bénéfices écologiques que ces animaux nous offrent. Le deuxième chapitre caractérise les communautés de chauves-souris cavernicoles dans deux grottes en République du Congo à l'aide de données de capture et d'analyse morphométrique.

Dans un second volet, l'étude de ces deux communautés de chauves-souris insectivores cavernicoles est complétée par une méthode passive de surveillance acoustique. Ce volet est composé d'un troisième chapitre exposant la méthodologie utilisée pour caractériser les communautés de chauves-souris à l'aide de l'acoustique. Le chapitre quatre complète ce volet en appliquant les données acoustique pour analyser les effets des variations saisonnières sur l'activité et la composition des communautés de chauves-souris insectivores cavernicoles.

Le troisième volet de cette thèse identifie les niches écologiques et les interfaces entre les chauves-souris et les autres animaux à l'aide d'un dispositif de pièges photographiques. Ce volet est composé de deux chapitres. Le premier (chapitre cinq) identifie le partage des niches

écologiques et les interfaces avec les autres animaux et l'homme. Le chapitre suivant (chapitre six) présente des comportements peu renseignés de prédation de genette sur les chauves-souris et en discute leurs potentielles implications pour la transmission de pathogènes.

Enfin, le quatrième volet composé du chapitre 7, présente les biais de collecte de données des chauves-souris africaines et la nécessité d'améliorer leur échantillonnage afin d'améliorer les connaissances sur leur écologie et leur rôle de réservoirs.

Introduction (English version)

Man is the only living species to have changed the way the planet functions as a whole, from the exosphere to the pedosphere. On a global scale, there have been a multitude of changes, the main ones being the destruction of habitats (deforestation, urbanization), the intensification of breeding and farming methods, the over-exploitation of natural resources and heavy pollution (Chaudhry & Malik, 2017; Delgado et al., 2001; Manisalidis et al., 2020; Mirsal, 2008; Myers, 2023; Peres, 2006; Zabel et al., 2019). Scientists have announced the advent of a new geological era, the Anthropocene, which reflects the pressure and changes caused by human activities on our environment (Crutzen & Stoermer, 2000). The scientific community is still debating the key event that would represent the start of the Anthropocene. Is it the first use of the atomic bomb? The creation of machines enabling us to use fossil fuels or heavy metals? The invention of plastics? In any case, these events represent an era of “great acceleration”, in which human activities and their disruptions have never been so rapid, drastic and disproportionate (Bradley et al., 2020; Head et al., 2022; Shoshitaishvili, 2021; Steffen et al., 2015).

All these disturbances are leading to climate change (Carlson et al., 2022; Jagadesh et al., 2022; Naicker, 2011), a massive loss of animal and plant biodiversity (Dobson et al., 2021; IPCC, 2023; Keesing et al., 2010; Keesing & Ostfeld, 2021; Patil et al., 2017) known as the “sixth mass extinction” (Ceballos et al., 2017) and an increase in wildlife-human interfaces (Ahmed et al., 2019; Dobigny & Morand, 2022; Hilderink & de Winter, 2021; Keusch et al., 2022; Lindahl & Grace, 2015; Morand & Lajaunie, 2021; Plowright et al., 2017; Rulli et al., 2017; Tajudeen, Oladunjoye, et al., 2022). The consequences of these upheavals are having an impact on animal, plant and human health, with an increase in the detection of infectious diseases over the last two decades or so. The IPBES has even declared the Anthropocene to be the era of pandemics (Daszak et al., 2020).

Nearly two-thirds of emerging infectious diseases (EIDs) have a zoonotic origin, and the majority of these come from wild animals (Han et al., 2016; Jones et al., 2008; Kruse et al., 2004; McArthur, 2019; Morse, 1995; Morse et al., 2012; Olival et al., 2017; Smith et al., 2014;

Woolhouse & Gowtage-Sequeria, 2005). The term zoonosis includes all diseases or infections that are naturally transmitted between animals and humans and vice versa, whether parasitic, viral or bacterial (Rahman et al., 2020; WHO, 2020). A zoonosis generally involves one or more reservoir species that maintain and transmit the infectious agent. Zoonoses are a major concern for global public health because of the impact they have on our societies (Bao et al., 2017; Fernandes, 2020; Liu et al., 2014; Rahman et al., 2020; Singh & Singh, 2020).

One of the most striking examples is the latest global pandemic, caused by the SARS-CoV-2 virus triggering COVID-19, probably acquired through close contact between people and wild animals in China (Malik et al. 2020; Li et al. 2020a). It has created one of the worst health, economic and social crises of our time, confining a large part of the human population, infecting almost 10 per cent of the world's population and causing the deaths of more than 18 million people (Adam, 2022; WHO, 2023b; Worldometers, 2023). Unfortunately, this type of pandemic is nothing new, as our societies have already been marked by other major epidemic events. In 1918, Spanish flu caused the death of at least 25 million people (Morens & Taubenberger, 2020). In 2009, the outbreak of H1N1pdm09, originating from a North American pig mega-farm, caused the first pandemic of the 21st century, the exact number of deaths remaining unknown (CDC, 2019; Markin et al., 2023; Mena et al., 2016; Viboud & Simonsen, 2012). Between 2014 and 2015, the last Ebola epidemic resulted in the deaths of around 11,000 people in West Africa (WHO, 2016). These examples illustrate the risk of zoonoses emerging, even if this differs from continent to continent. For example, between 1996 and 2009, 53% of reported global EIDs originated in Africa (Muzemil et al., 2018), where the surveillance system is deficient compared with other continents (Chan et al., 2010). Africa is also one of the continents with the greatest biodiversity. In addition, a large proportion of its population relies heavily on bushmeat as a source of protein and income (Friant et al., 2020; Kurpiers et al., 2016; Trefon, 2023; Van Velden et al., 2018).

Historically, surveillance systems have focused on diagnosis and rapid response (vaccination, medication). More recently, in order to guard against zoonoses, our societies have turned mainly to an approach combining biomonitoring and biosecurity (i.e sampling and identification of pathogens in wildlife, monitoring of bushmeat, ...) (National Research Council, 2010). This approach is leading to a deterioration in our relationship with the wild world, as

illustrated by the highly negative perception of bats in our societies (Bonilla-Aldana et al., 2020; Cros, 2020; MacFarlane & Rocha, 2020; Straka & Voigt, 2022; Tsoucalas et al., 2021). However, recent zoonotic outbreaks (COVID-19, Mpox formerly known as Monkey Pox) have highlighted the shortcomings of surveillance systems and the crucial role of upstream prevention and the involvement of people living in areas at high risk of emergence (Ammirato et al., 2021; Bernstein et al., 2022; Callaby & Gordon, 2023; Shanks et al., 2022; Tajudeen, Oladipo, et al., 2022; Vora et al., 2022). In order to achieve adequate prevention, it is vital to understand the ecological mechanisms associated with the risks of emergence in order to provide international and national institutions with the scientific information they need to develop effective EIM control systems and organise adequate prevention (Coker et al., 2011). In addition, it is vital to move towards a more holistic zoonoses surveillance system. To meet this need, the scientific community is increasingly applying the "One Health" approach. One Health" is a comprehensive approach that takes into account human health, animal health and the health of ecosystems in an integrated manner (OHHLEP et al., 2022). Health ecology is also a scientific field that aims to contribute to this multidisciplinary approach by helping to understand health problems at the interface between animals (wild and domestic), humans and the environment.

In this global context, the European Union has funded the EBOSURSY project "Strengthening capacities to deal with viral haemorrhagic fevers and improving virus surveillance". This project is led by the World Organisation for Animal Health (OIE) in close collaboration with 10 target countries and three research institutes. The scientific consortium is made up of the Centre de coopération internationale en recherche agronomique pour le développement (CIRAD), the Institut de recherche pour le développement (IRD) and the Institut Pasteur (WHO, 2023). CIRAD is focusing in particular on understanding the transmission mechanisms, ecology, behaviour and habitat of 'zoonotic reservoirs', with the support of local partners such as the National Public Health Laboratory (LNSP) in Brazzaville and the General Livestock Directorate (DGE) in the Republic of Congo.

Immersed in an interdisciplinary team, this work is part of a desire to study the ecology of cave-dwelling insectivorous bats in order to contribute to improving knowledge of habitat use and ecological niche coverage associated with zoonotic diseases of concern to human populations. This work is approached at the scale of communities and populations in two caves

in the Republic of Congo. The various aspects of this question are presented in four separate sections.

The first part of this thesis aims at summarizing current knowledge on the ecology of bats in Africa and at identifying the insectivorous communities present in the two caves included in this study. The first chapter presents the ecology of these elusive and still mysterious mammals. Silent neighbours and ghosts of our nights that we humans fear because of recent zoonotic outbreaks, overshadowing the ecological benefits that these animals offer us. The second chapter characterizes cave bat communities in two caves in the Republic of Congo using capture data and morphometric analysis.

In a second section, the study of these two cave-dwelling insectivorous bat communities is supplemented by a passive acoustic monitoring method. This section consists of a third chapter setting out the methodology used to characterize bat communities using acoustics. Chapter four completes this section by applying the acoustic data to analyze the effects of seasonal variations on the activity and composition of cave-dwelling insectivorous bat communities.

The third part of this thesis identifies the ecological niches and interfaces between bats and other animals using a photographic trap system. This section is divided into two chapters. The first (chapter five) identifies the sharing of ecological niches and the interfaces with other animals and man. Chapter six) presents little-known genet predation behaviour on bats and discusses its potential implications for the transmission of pathogens. Finally, part four of this thesis (Chapter 7), presents biases in data collection on African bats, the need to improve their sampling and, at the same time, our knowledge of their ecology and their role as reservoirs.

PART I

**SUMMARY OF THE CURRENT STATE OF KNOWLEDGE ON BATS
AND CHARACTERISATION OF TWO CAVE BAT COMMUNITIES**

A la quête de l'unité,
Un jour un animal possédant des dents, des poils et capable de voler, partit
vers les oiseaux et leur dit « Bonjour ! »
Les oiseaux l'ont reçu mais quand il a ri,
Ils remarquèrent qu'il avait des dents.
Ils lui dirent, tu n'es pas des nôtres, toi, tu as des dents et même des poils.
N'ayant pas trouvé sa place,
Il s'en alla vers les animaux des champs et leur dit « Bonjour ! »
Et les animaux des champs l'ont reçu.
Mais quand il a fallu se déplacer, il s'envola.
Les animaux des champs lui dirent,
Tu n'es pas des nôtres, car nous, nous marchons.
N'ayant pas trouvé sa place,
Il s'en alla habiter dans le noir et les autres l'appelèrent "*Nguembo*",
C'est-à-dire, animal volant à dents et à poils qui vit dans l'obscurité...

**Classification morphologique mitigée de la chauves-souris,
Hosanna Lenguiya**

CHAPTER 1

ECOLOGY OF AFRICAN BATS SPECIES AND LINK TO ZONNOSES

1.1 Introduction

Bats, the only flying mammals, are seen as the ghosts of our nights, demons or animals linked to evil witchcraft in many local cultures, and more recently fingered for their role in infectious disease ecologies. Yet, bats do not deserve their bad reputation. They are fascinating animals because of their biology, ecology and their importance for the ecosystem services they provide.

This chapter aims at summarizing their various facets, focusing particularly on African bats, with particular emphasis on the key themes of this thesis project. In addition, we present the various factors linking them to emerging infectious diseases. Bats are merely the "silent messenger" that can promote diseases, but one could point out that the changes we make to our environment are the primary causes of these zoonotic emergences.

Finally, this chapter highlights some of the many gaps that remain in research on bat ecology. Recent technological advances in the study of bat behavior offer new opportunities to try and elucidate many aspects of bat ecology. At a time when habitats are constantly changing due to increasing human disturbance, the acquisition of this missing data will promote the conservation of bats while helping to prevent potential risks of zoonotic disease transmission to human populations.

1.2 Taxonomy, biology and ecology

1.2.1 Phylogeny of African bats

Phylogeny of bats used to be separated in two big families: the "Megachiroptera" with all larger frugivorous bats and the "Microchiroptera" with all small bats, predominantly insectivorous bats. Recently, with the improvement of genetic tools, a phylogenetic relationship has been confirmed between Pteropodidae (Old World fruit eating bats) and other bat families coming from the Microbats. Teeling et al. (2005) defined this new suborder within

the Chiroptera as Yinpterochiroptera (also named as Pteropodiformes) and Yangochiroptera (also named as Vespertilioniformes) that accommodates all the other families of bats (Teeling et al. 2018). In Africa, scientists have reported the presence of 390 species and subspecies divided into thirteen families (**Fig. 1**). The main morphological characteristics of the different bat families are given in Appendix 1. In the Republic of Congo, only 17 studies have been carried out, and no identification key is available for bats. The latest study confirmed the presence of 43 different species from eight families (Bates et al., 2013). However, Simmons & Cirranello (2023) suggests the presence of 63 different species (**Fig. 2**), which seems reasonable compared to neighbouring countries such as the Democratic Republic of Congo, which is home to 128 species, and Gabon, with 47 species (Simmons & Cirranello, 2023).

Figure 1: Phylogenetic tree of African bat families

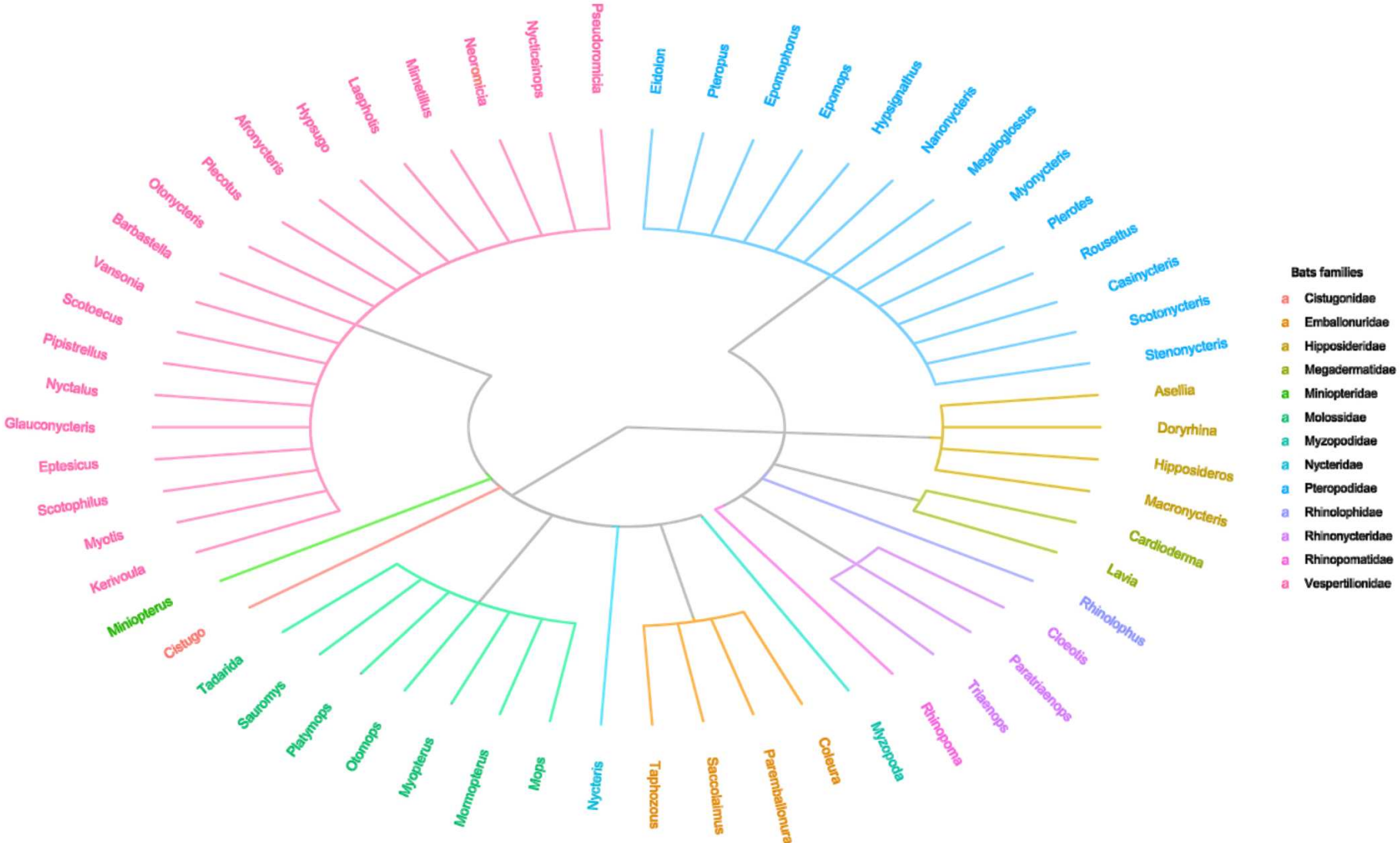
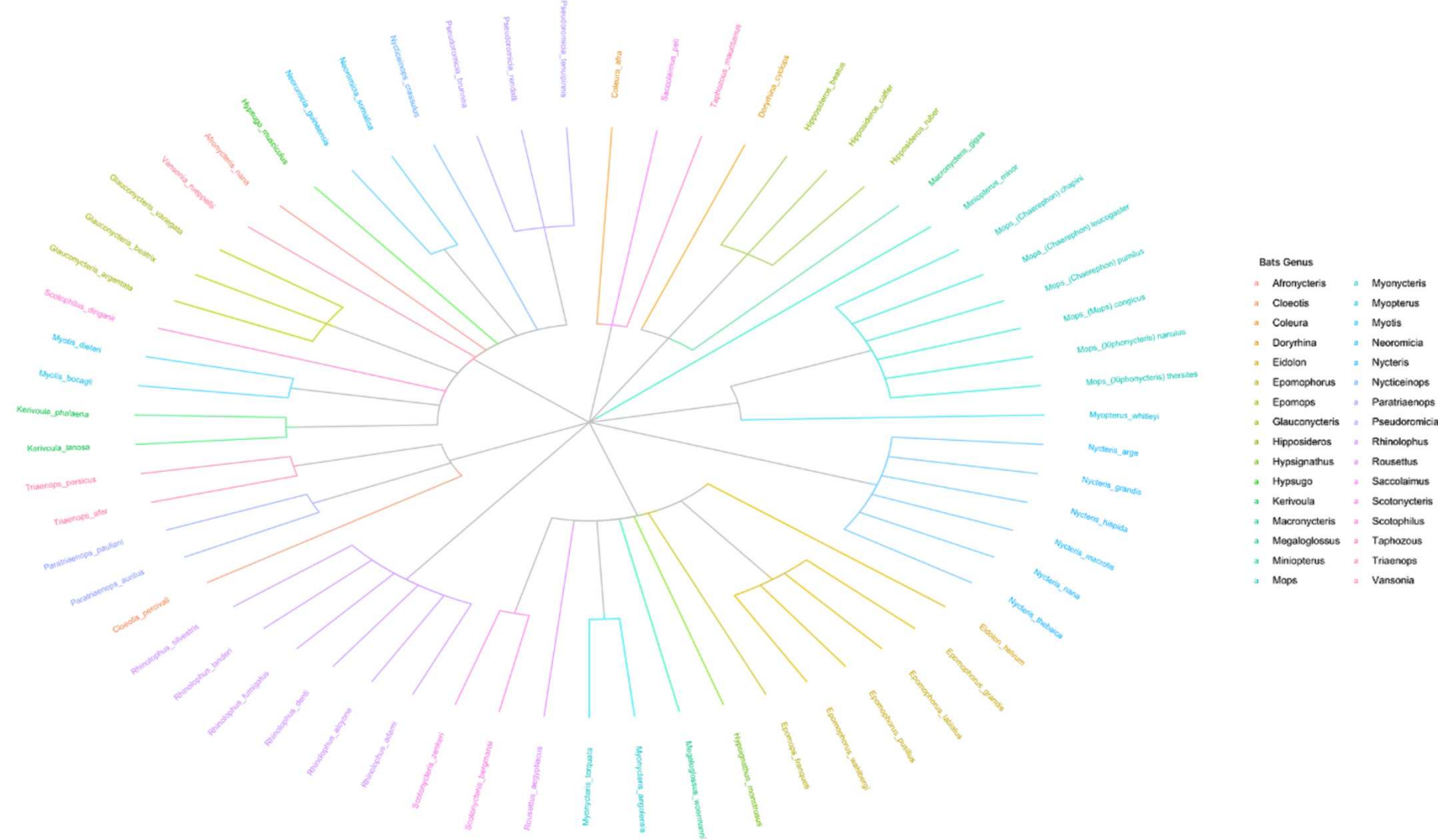


Figure 2 : Phylogenetic tree of bat genera reported (Simmons & Cirranello, 2023) in the Republic of Congo



1.2.2 Bat diversity in the world and in Africa

Bats are the only mammals who have conquered the skies (Gunnell & Simmons, 2005; Thewissen & Babcock, 1992) and the leading mammals in terms of total biomass (Greenspoon et al., 2023) with the exception of domestic mammals. Chiropterans' name derives from ancient Greek, χείρ - cheir, πτερόν - pteron , "hand-wing" (Bolling et al., 1926). This order has been present on all continents except for the poles for 52 million years and represent, with 1,470 species, the second mammalian order after rodents by the number of species (Kunz et al., 2011; Simmons & Cirranello, 2023). In Africa, there are 390 species of bats (with subspecies), with a high richness of species around the equator (**Fig. 3**). Ten percent of African bats have been classified as "most threatened" according to IUCN Red List (**Fig 4**). However, this number may be underestimated due to the limited information available on African bat populations and the new threats they are facing. For example, 48% of fruit bat species in Africa have been the focus of several research studies on bat-borne zoonoses and viral surveillance. Due to their wide-ranging diet, which includes insects, fruit, flowers, leaves, nectar, blood and vertebrates, bats are found in almost all terrestrial ecosystems and habitats (Fenton & Simmons, 2015; Kingdon, 2014; Monadjem et al., 2020). They are found from grasslands to deep forests of South America, Asia and Central Africa, or in harsh habitats such as mountains and deserts (Kingdon, 2014).

Figure 3: Gradient in the number of bat species on the African continent

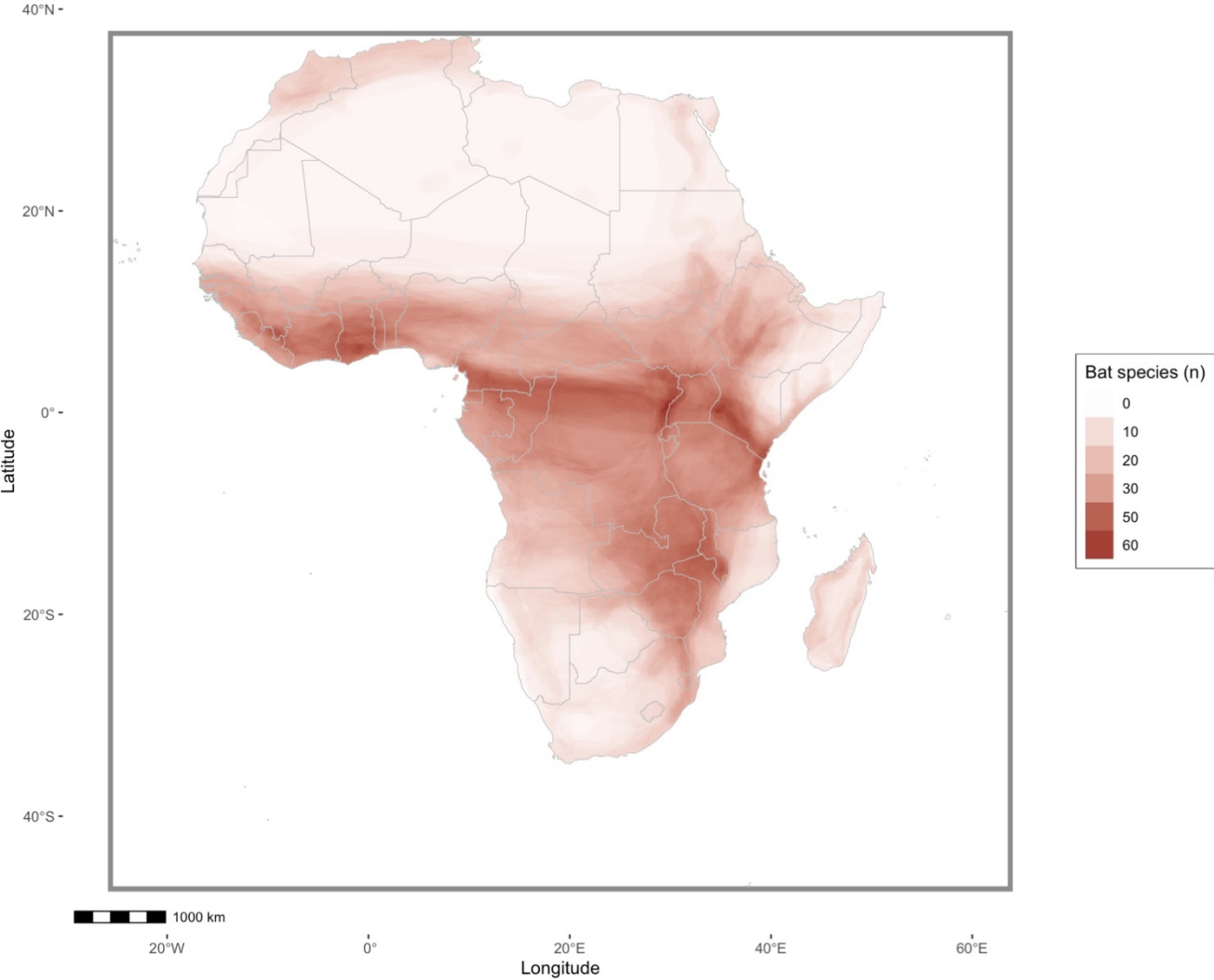
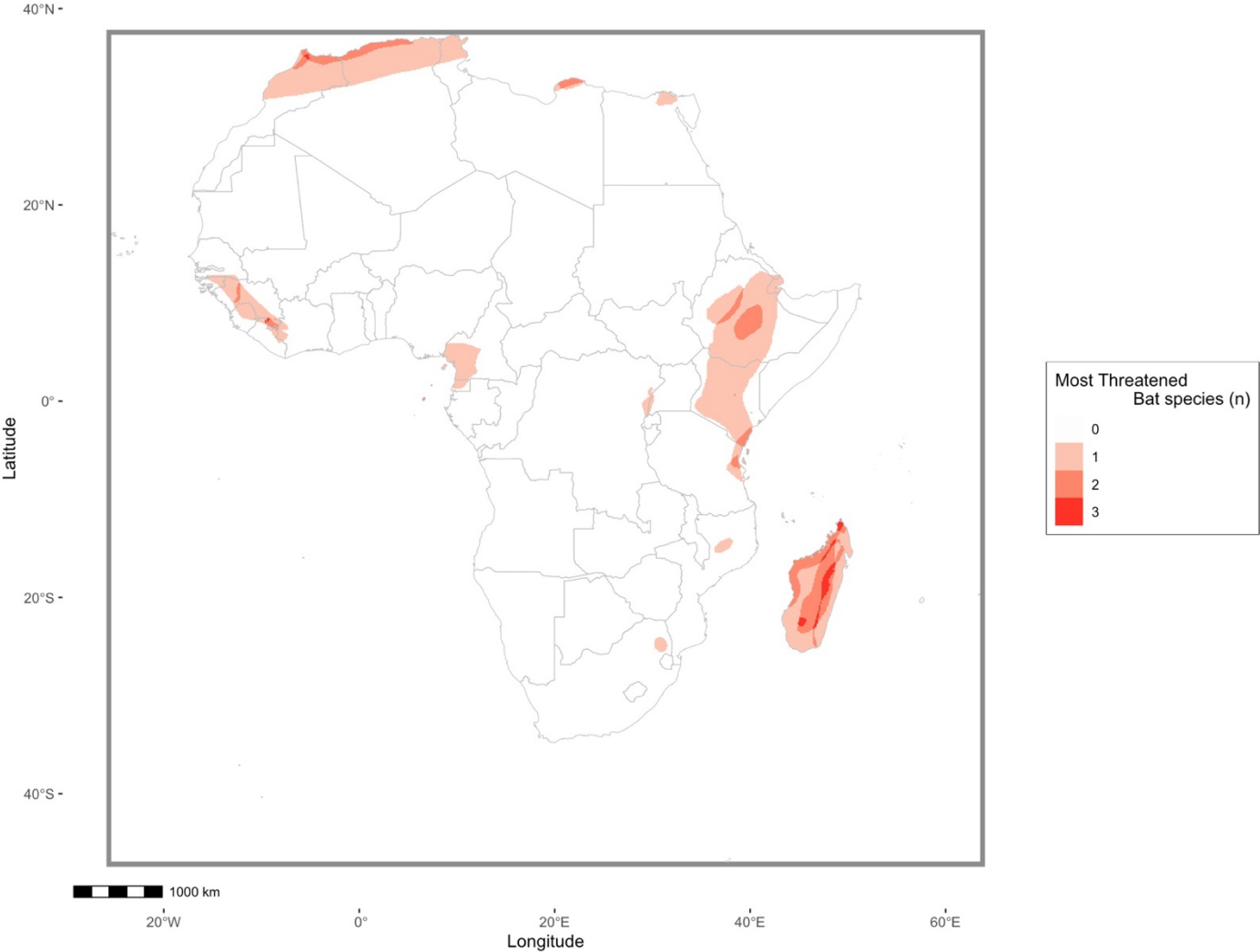


Figure 4: Gradient of the number of bat species with IUCN most threatened status (on the African continent).



1.2.3 Most essential activity for animals

Every living organism has three fundamental needs: foraging, rest and reproduction (Alcock, 2013). The behaviour and movements of chiropterans are no exception to this rule. By meeting these first two needs, each individual will maintain its overall fitness and vital functions (Schoener, 1971). In this way, the individual will improve its chances of reproductive success and pass on its genes to the next generation (Alcock, 2013; Crews & Moore, 1986). In addition, to meet these needs, the spatial movements of individuals are affected by seasonality and potentially generating variations in interactions between them and other species, including humans.

i. Diet

In Africa, 85.6% of species are insectivores, 10% are frugivores and the rest are nectarivores or have a varied diet (insectivores - carnivores) (GBIF, 2023; IUCN, 2023; Monadjem et al., 2020). Foraging is generally a daily activity, although this activity can vary (decrease or increase at specific times) due to physiological (reproductive state), biotic (predation, competition) and/or abiotic (climatic conditions, moonlight) factors (Appel et al., 2019; Jones & Rydell, 1994). Thus, at the end of gestation, female bats will reduce their hunting time due to the increase in body weight (Rydell, 1993). On the other hand, during the parturition and lactation periods, female bats may reduce their flight distance in search of food while increasing their activity due to the energy costs associated with milk production. (Cumming & Bernard, 1997; Henry et al., 2002; Kurta et al., 1989; Rydell, 1993). In general, the feeding habits of a species are defined by its morphology, its potential adaptations (echolocation system in insect-hunting species or development of a long tongue in nectar-eating bats) (Griffiths, 1978; Howell, 1974) and its physiological needs (Salinas-Ramos et al., 2021). In a habitat with same taxa, evolution has led many species to specialize in a specific diet in order to limit competition (Colwell, 1973). However, most bat species are generalists and adapt their diet according to the availability of food resources or potential environmental changes. For example, Gambian epauletted fruit bat (*Epomophorus gambianus*) eats a wide variety of fruit throughout the year, even if certain fruits seem to be recurrent in their diet, such as figs (Amponsah-Mensah et al., 2019). During the dry season, with fewer fruit available, this species eats leaves and flowers (Amponsah-

Mensah et al., 2019). Recent studies have also shown that some species of fruit-eater deliberately feed in anthropized habitats (crops, towns, etc.) because of the high abundance and diversity of available fruits, which results in increased contacts and interactions with other species, including humans (Egert-Berg et al., 2021; Schloesing et al., 2023). Finally, in the tropics, the rainy season is a period of abundant food resources (insects or fruits), which encourage large numbers of individuals to congregate in specific habitats (Fahr et al., 2015; Richter & Cumming, 2006a).

ii. Roosting behavior

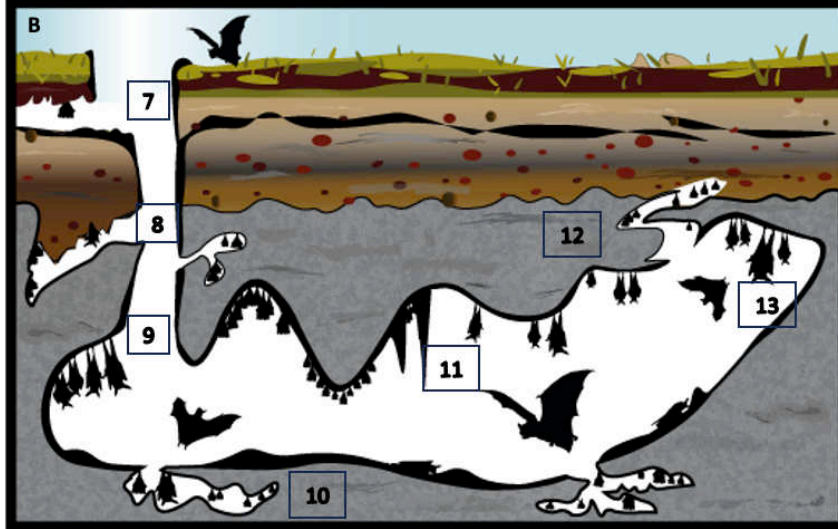
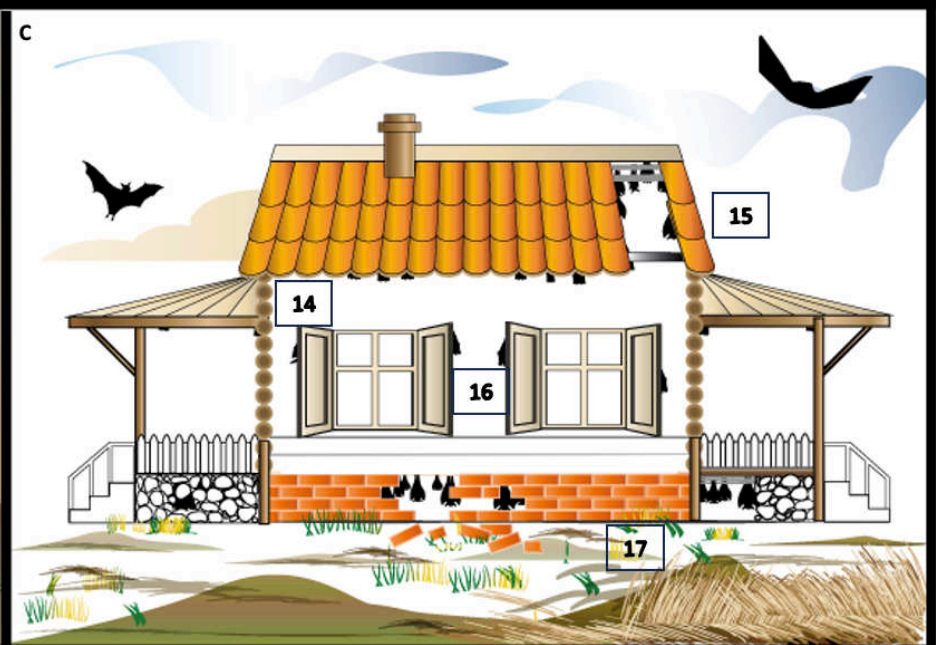
Bats are active at night and rest during the day in places called “roosts”. Some species are our silent neighbors. First, the type of roost used by bats is part of the natural habitat, such as tree branches, canopy cover, leaves, holes in tree trunks, dead trees, crevices in rocks or cavities in caves. (Keeley & Tuttle, 1999; Kunz, 1982; Kunz et al., 2003; Mering & Chambers, 2014; Russo & Ancillotto, 2015; The Protection of Bat Roost Guidelines Subcommittee et al., 1992) (**Fig. 5**). The second type of roost used by bats is man-made structures (such as bridges or roofs) to rest during the day, including the walls of our homes, cellars and attics. Around 504 bat species roost in caves, including 98 of the 316 African bats (IUCN, 2023). In tropical environments, most bat species use caves as permanent roosts, which makes this environment so special (Barros et al., 2020; Lewis, 1995; Ormsbee et al., 2007; Struebig et al., 2009).

Caves with bat colonies provide favorable conditions for the creation of ecological niches for many cave-dwelling animal, plant and micro-organism species (Gabriel & Northup, 2013; Kosznik-Kwaśnicka et al., 2022; Kováč, 2018; Pacheco et al., 2020; Tomczyk-Żak & Zielenkiewicz, 2016). In addition, the presence of a bat colony in a cave is an interface conducive to direct or indirect interactions between these colonies and other animals (wild or domestic), and human populations (Furey & Racey, 2016; Moyes, 2012; Straus, 1979). Throughout human history, people have used caves as refuges or sacred places before exploiting them for their resources (minerals, guano) or using them for entertainment (sporting activities, tourist visits, etc.) (Okonkwo et al., 2017; Simons, 1998). Events involving the transmission of pathogenic micro-organisms (fungi, viruses, bacteria or parasites) between cave bats and animals, including humans, have been recognized (Agustin et al., 2019; Federici et al., 2022; Jurado et al., 2010; Karunarathna et al., 2023). Transmission may be due to direct

contact with bats (i.e. bites, consumption). The other route of transmission of micro-organisms is indirect via (i) aerosols present in the air, such as histoplasmosis (Amona et al., 2021; Gughani & Denning, 2023; Jülg et al., 2008), (ii) body fluids present in the habitat and (iii) via a relay host which may then interact with other animals and/or humans. For example, many parasites can be transmitted between different bat species, but also to other animals likely to come into contact with humans, such as dogs and cats (Obame-Nkoghe et al., 2016, 2017; Stevens et al., 2014).

Caves play a key role in a bat's life, providing an ideal resting place and protected habitat for giving birth, rearing young and breeding (Barros et al., 2020; Ormsbee et al., 2007; Struebig et al., 2009). Depending on the configuration of the cave and its microclimate, several species can cohabit together by hundreds or thousands (Kunz, 1982; Monadjem et al., 2020), each using a specific niche that depends on three main factors: luminosity, hydrometry and temperature (Czenze et al., 2022; Willis & Brigham, 2007). In Africa, members of the *Rhinolophus* family cling to the ceiling or the walls of caves, that are characterized by a lack of light, cool temperatures and high humidity. Conversely, cave-dwelling Pteropodidae such as Egyptian fruit bat (*Roussettus aegyptiacus*) can live in fairly open caves with more light and higher temperatures (Monadjem et al., 2020). The diversity and high abundance of individuals in the same habitat encourage intra- and inter-species interactions. For example, during the rearing of young, when the mothers go hunting, it is possible to observe nurseries of several species grouped together improving social interaction and limiting predation and heat loss (Ancillotto et al., 2015; McDonald, 1990; Wilkinson et al., 2016).

Figure 5: Schematic comparison of the diversity of different daylight roosts selected by African bats with (A) in tree, (B) cave and (C, D) anthropogenic hollows-roosting. With (1) free-hanging from main boughs such as *Eidolon helvum*; (2) clinging under exfoliating bark (*Laephotis spp.*), in hollow boles high up in tree (e.g. *Mops condylurus*), hollow branches (e.g. *Chaerephon pumilus*) or free-hanging in large cavity of tree trunk (e.g. *Nycteris grandis*); (3) clinging within foliage (e.g. *Glauconycteris variegata*); (4) clinging on tree trunk (e.g. *Taphozous mauritanus*), exfoliating bark or in narrow holes (5) free-hanging under exposed roost (e.g. *Nycteris spp.*); (6) deep in dense foliage or free-hanging in shrubs (e.g. *Scotoecus spp.* or *Lavia frons*); (7) partially lit and well-ventilated boulder caves and overhangs ; (8) crevices in vertical or horizontal precipices; (9) free hanging in dimly illuminated caverns, often near entrance (e.g. *Rousettus aegyptiacus*); (10) crevices in boulders at ground level (e.g. *Chaerephon major*); (11) free hanging from ceiling in pitch darkness or space within roof timbers or crevices in roof materials; (12) cavities in roof (high or not) with walls or not and in contact or not (13) free hanging from low ceiling, hot and humid; (14) under eaves or crannies in roofs; (15) attic spaces and surfaces or dark abandoned rooms (e.g. *Rhinolophus blasii* or *Scotophilus dinganii*); (16) gaps inside wall or suspended floor; sheltered window ledges; (17) inside cellars, hollow cement bricks, crevices and cracks in masonry or low wall; (18) suspended under the deck carpet and (19) inside the deck crevices, cracks or gaps inside wall of the bridge. Created by Françoise Chirara, CIRAD.



iii. *Reproduction*

Among mammals, bats are considered the slowest reproducing mammals relative to their size (Barclay & Harder, 2004). Some females mature late (at two or three years old) and give on average one young per year (Barclay & Harder, 2004; Racey & Entwistle, 2000). When hibernating, some species can interrupt their reproduction through three different mechanisms: delayed embryo development, sperm storage in the uterus or oviducts, and delayed embryo implantation. The average gestation period for bats is two months, but can last several months longer depending on the fertilization strategy.

In addition, the majority of non-hibernating bats have a seasonal monoestry (single gestation and birthing season) reproduction (Bernard & Cumming, 1997; Happold & Happold, 1990).

However, some tropical species with a wide distribution (across the equatorial part) have a seasonal polyoestry (several gestations and birthing seasons) reproduction such as *Rousettus aegyptiacus* (Coe, 1975; Happold & Happold, 1989, 1990; Wolton et al., 1982). Some species of Vespertilionidae can commonly give birth to twins and triplets (i.e. *Neoromicia nana*, *Pipistrellus rusticus*) (Monadjem et al., 2020).

In general, bat species have synchronized the parturition and lactating periods for their young during a specific season (often a little before the period with the greatest availability of food resources). This behavior has a number of advantages, limiting the energy cost for the female and/or encouraging social behavior (nursery, presence of a "nanny" female) (Kunz & Hood, 2000; Rensel, 2021; Stirnemann, 2009). In gregarious species, pregnant females typically form maternity colonies (for example inside a cave) to raise their young together. Babies may be carried for the first two weeks, then left in nursery groups because of their size and weight. The energy cost of raising a young bat is very high for a female from gestation to weaning. In general, newborns may weigh 20-30% of maternal mass at birth, and youngsters are usually weaned when they have reached around 75% of maternal mass (Barclay & Harder, 2004).

Mating systems are highly variable. A common pattern is polygyny (or harem), in which a dominant male mates with a group of females, sometimes with a second "satellite male" on the periphery (McWilliam, 1987). However, it is estimated that only six percent of mating strategies have been studied in bats (Murray & Fleming, 2008). Male behavior and physiology to attract females vary, with mating territory selection (e.g. *Hypsignathus monstrosus with lek*) (Bradbury, 1977; Toth & Parsons, 2013), hormone secretion (Monadjem et al., 2020;

Murray & Fleming, 2008), display of specific colored fur, and/or courtship with singing and vocalize (audible or acoustic) (Middleton et al., 2022; Park et al., 1996).

1.2.4 Echolocation

The vast majority of nocturnal animals use their vision to move, perceive their environment and hunt. Insectivorous bats (70 percent of bats species worldwide) (Kunz, 2013), unlike other species have developed an alternative sensory mechanism: echolocation. The use of acoustics by the Pteropodidae family is absent, with the exception of three species of *Rousettus*, which use it mainly to fly inside dark cave (Rossborough et al., 2021; Waters & Vollrath, 2003). Other animals in the animal kingdom, such as marine mammals, certain rodents or insects, can also use acoustics, but this "echo-perception" is much more elaborated in bats (Denny & McFadzean, 2011). They are the only animals that have developed echolocation in the air, this specificity enables them to use and exploit a nocturnal environment where competition with other taxa is low (Arthur & Lemaire, 2015).

Acoustic calls can be divided into three distinct purposes: (1) to move in space by identifying – and avoiding – obstacles, (2) to locate specific objects for hunting, and (3) to communicate (social calls). Bat echolocation involves emitting a pulse of sound and analyzing the returning echoes using their auditory system (Fenton, 1990; Fenton et al., 2016; Kalko et al., 2008). Most bat calls are ultrasonic (above 15 kilohertz), although some calls may be within the audible range (Fenton et al., 2016). Depending on the bat family, these acoustic calls are emitted through the larynx, the mouth (Emballonuridae, Miniopteridae, Molossidae and Vespertilionidae) or nose (e.g. Hipposideridae, Nycteridae, Rhinolophidae and Rhinonycteridae) (Monadjem et al., 2020). In addition, acoustic calls have characteristics that are unique to a bat family and species.

i. The use of acoustics according to behaviour

To save energy, acoustic calls are usually synchronized with wing beats (about ten per second during flight). Each time the wings beat downwards, the muscles contract, exerting pressure on the thoracic cage and inducing a breath per exhalation. This breath crosses the larynx and passes through the vocal cords (Arthur & Lemaire, 2015). During this movement phase,

emission calls are very brief. However, during the hunting phase, bats need greater precision to visualize the position of their prey in space, and to anticipate their possible flight movements. Hence, during this particular phase, there is a desynchronization of the beat/acoustic call, as the number of acoustic calls increases (up to 40 to 50 calls per second during the capture phase) and their duration becomes shorter the closer they get to the prey (Arthur & Lemaire, 2015). The final phase before the prey is intercepted is called the "buzz", due to the burst of calls (up to 20 over a period of 0.2 milliseconds). Finally, during the capture phase, bats use tactile hairs to orient themselves on the best way to catch the prey they are hunting.

ii. Strategies and type of echolocation

Two echolocation strategies have evolved independently: high and low duty cycle echolocation (Eick et al., 2005) (**Fig. 6**). The first strategy is high duty-cycle echolocation that transmits long, narrow-band pulses separated by shorter inter-pulse intervals. The transmitted signals often overlap with the return echo but this overlap does not produce masking effects, due to the Doppler effect (**Fig. 7A**) (Appendix 2) (Schnitzler & Kalko, 2001). This mechanism keeps the target echo at few kilohertz above the peak frequency of the transmitted signal (Fenton, 1990; Neuweiler, 1990).

The second echolocation strategy is low-cycle echolocation. Bats emit narrow-band or broadband sound pulses separated by inter-pulse intervals that are much longer than the duration of the emitted pulses. Species using this strategy therefore separate the transmitted pulse from the return echo in time (Fenton, 1990).

Bats use three types of emission: (1) frequencies modulated (FM), which covers a wide range of frequencies (start from high frequencies and drop to low frequencies in a few milliseconds), (2) quasi constant frequency (QCF) concentrate energy in a relatively narrow band (3) constant frequencies (CF), which are close to a pure signal that varies very little. QCF helps detect reflections in echoes caused by sound reflecting off the wings of moving insects, but this reduces localization performance. FMs use echoes to provide precise information on the angle and distance to the target. CFs with Doppler shift compensation can still detect prey in clutter using echolocation (**Fig. 7B**) (Fenton, 1990, 2013; Fenton et al., 2016).

Most bat species alternate between complex frequencies, alternating or combining FM and FC, using harmonics of the initial sound, varying frequencies at the start or end of the call, flattening them out or dropping them sharply (Jones, 1999). These variations in acoustic calls may be due to the type of environment in which they are evolving and the cause of the acoustic call (feeding search, approach to prey, capture of prey, etc.) (see below).

In addition, a sound signal loses its power as it travels, so bat species use different strategies in the use of call finesse and intensity. The higher the frequency (kHz), the greater the resolution in the perception of small objects will be. On the other hand, high-pitched calls do not propagate over long distances (100 kHz does not carry beyond ten meters, whereas a 30 kHz call will travel to a distance of around 30 meters). This means that bat species have to make a compromise between the intensity which determines the reach of the call, and the finesse of resolution to discern precisely small prey and obstacles (Fenton et al., 2016).

iii. Evolutionary adaptation and specialization of echolocation

When flying, and especially when hunting, bats face a major environmental constraint: the number of obstacles they have to detect and avoid (also known as clutter) (**Fig. 7B**) (Fenton, 1990). In a natural habitat, this clutter is strongly influenced by the vegetation in the habitat. Taking this environmental clutter into account, each bat family must use its sensory abilities (echolocation, hearing, etc.) to detect, analyze and locate prey. The echolocation technique has a number of weaknesses: (1) echolocation does not work over long distances, (2) sound displacement also depends on atmospheric conditions (greatly attenuating echo intensity) and (3) echolocation depends on the size and shape of the target (Barclay & Brigham, 1991; Fenton, 2013; Lawrence & Simmons, 1982; Pye, 1980; Waters et al., 1995). The different families of insectivorous bats are often specialized on different groups of insects and on a particular stratum in the habitat.

The various intrinsic criteria influencing the specialization of the type of acoustics in bats are body size, wing shape, jaw strength, tooth type and echolocation type (Arita & Fenton, 1997; Jacobs, 2000; Schoeman & Jacobs, 2003; Schoeman & Jacobs, 2010). In general, the type of preys a bat species can capture depends on two main criteria: shape of their wings (Fig. 6) and call structure (Aldridge & Rautenbach, 1987; Jones, 1999; Neuweiler, 1989; Norberg & Rayner, 1987; Roemer et al., 2019; Schoeman & Jacobs, 2010). Among bat species, size is the primary

factor limiting exploitation of an insect group as preys. Large bats are able to consume a wide variety of insects while small bats feed only on small insects (Aldridge & Rautenbach, 1987; Jacobs & Barclay, 2009). Another factor is jaw type: long canines and a powerful jaw allow the consumption of insects with thick shells, while a weak jaw limits them to the consumption of insects with a "soft" body (Freeman, 1979, 1981).

Insectivorous bats have been categorized into three major foraging groups: open, edge clutter and clutter foragers (Crome & Richards, 1988; Fullard et al., 1991; Schnitzler & Kalko, 2001; Schoeman & Jacobs, 2008) (**Fig. 7**). Bat species foraging in open habitats often emit low frequency calls (cross long distance), generally of long duration (no problem with overlapping echoes as the prey is distant) and often with a QCF structure (which maximizes range and improves detection) (Schnitzler & Kalko, 1998).

Bats foraging in edge habitats emit calls that combine location accuracy and detection performance (FM/QCF with FM for accuracy and QCF for performance). Bats foraging in clutter can adopt two echolocation call structure strategies. First, low-cycle bats species emit FM calls for orientation, and often switch off echolocation to rely on other sensory signals such as hearing for prey detection and location (and movement) (Marimuthu, 1997). Secondly, high-use-cycle CF bats can detect flapping targets among clutter echoes using Doppler shift compensation (**Fig. 7A**).

Most species of Vespertilionidae, Emballonuridae and Molossidae families hunt in areas using their fast flight, with acoustic calls that are effective at long range (Aldridge & Rautenbach, 1987). Gleaning families, such as Hipposideridae and Rhinolophidae, are able to capture stationary prey on branches and leaves or on the ground using their wing morphology adapted to slow, maneuverable flight and echolocation signals capable of distinguishing prey from echoes in the burrow (Aldridge & Rautenbach, 1987).

However, inside this families, some species (such as *Hipposideros caffer* and some *Rhinolophus* species) are generalists, capable of aerial hunting as well as gleaning or stalking (positioning themselves on a branch to scan their surroundings within a 360 degree radius using Doppler effect, detecting prey and attacking it rapidly) (Jones, 1999; Monadjem et al., 2020). Species of the *Plecotus* genus use hovering flight to concentrate on their prey and limit echo problems due to their environment (Jones, 1999). All the acoustic call characteristics (frequency, intensity, shape, duration, number of pulses, etc.) presented above are family and species-specific indicators. Like a bird call, acoustic calls can be used to identify species, and in some

cases to identify the sex of a bat (G. Jones & Siemers, 2010). Finally, the use of social calls, which vary in complexity and intensity, can help identify bat species (Middleton et al., 2022).

iv. Social call

The acoustic calls of bats are not only used to create a mental map of their environment or to hunt, they are also a means of communication, whether deliberate or not, to express information to other nearby individuals (Knörnschild et al., 2012; Middleton et al., 2022). Numerous studies have shown that certain bat species (e.g. Greater mouse-eared bat) can listen to other species in order to locate potential prey or competitors (Barclay, 1982; Fenton, 2003; Yovel et al., 2011). The social vocalizations of bats are used in a very large number of circumstances, and are often louder and more complex than the 'normal' calls of a species. Chiropterans are social animals, they can exchange a wide variety of information depending on the situation. This communication is all the more essential when you live in a colony with several thousand individuals in the same habitat. Bats communicate with each other using audible or acoustic calls that can be categorized into four distinct categories: (1) distress calls, (2) territorial calls in a hunting or roosting habitat (Barlow et al., 2015; Bartonička et al., 2007; Pfalzer & Kusch, 2003), (3) social interactions between individuals in a colony (mother - pup, between individuals from the same social group to maintain group cohesion) (Altringham, 2011; Chaverri et al., 2010; De Fanis & Jones, 1995; Gustin & McCracken, 1987; Jones et al., 1991; Kunz & Hood, 2000; Liu et al., 2007; Loughry & McCracken, 1991) and (4) territorial calls related to reproduction. In the category of distress calls, bats in the same social group seem either to be warning their fellow bats against danger (to move away or to be cautious) or to be asking for potential "assistance" from other individuals (to distract a predator) (Russ, 2012; Russ & Montgomery, 2002). As for the territorial calls of males during the breeding season, they seem to have three different purposes: (i) protecting a roosting site, (ii) defending a male's harem and (iii) signaling promiscuity to a female (Bartonička et al., 2007; Furmankiewicz et al., 2013; Middleton et al., 2022; Swift, 1998). Understanding how bats communicate using social calls is still relatively unexplored. This field of study requires a great deal of additional research in order to discover the different categories and functions of social calls for each bat species. It opens up a wide range of future prospects, since it gives indications of the behavior of an individual of a species.

Figure 6: Diagram showing sonograms of southern African bats that use high duty-cycle and low duty-cycle echolocation calls. From Monadjem et al (2020).

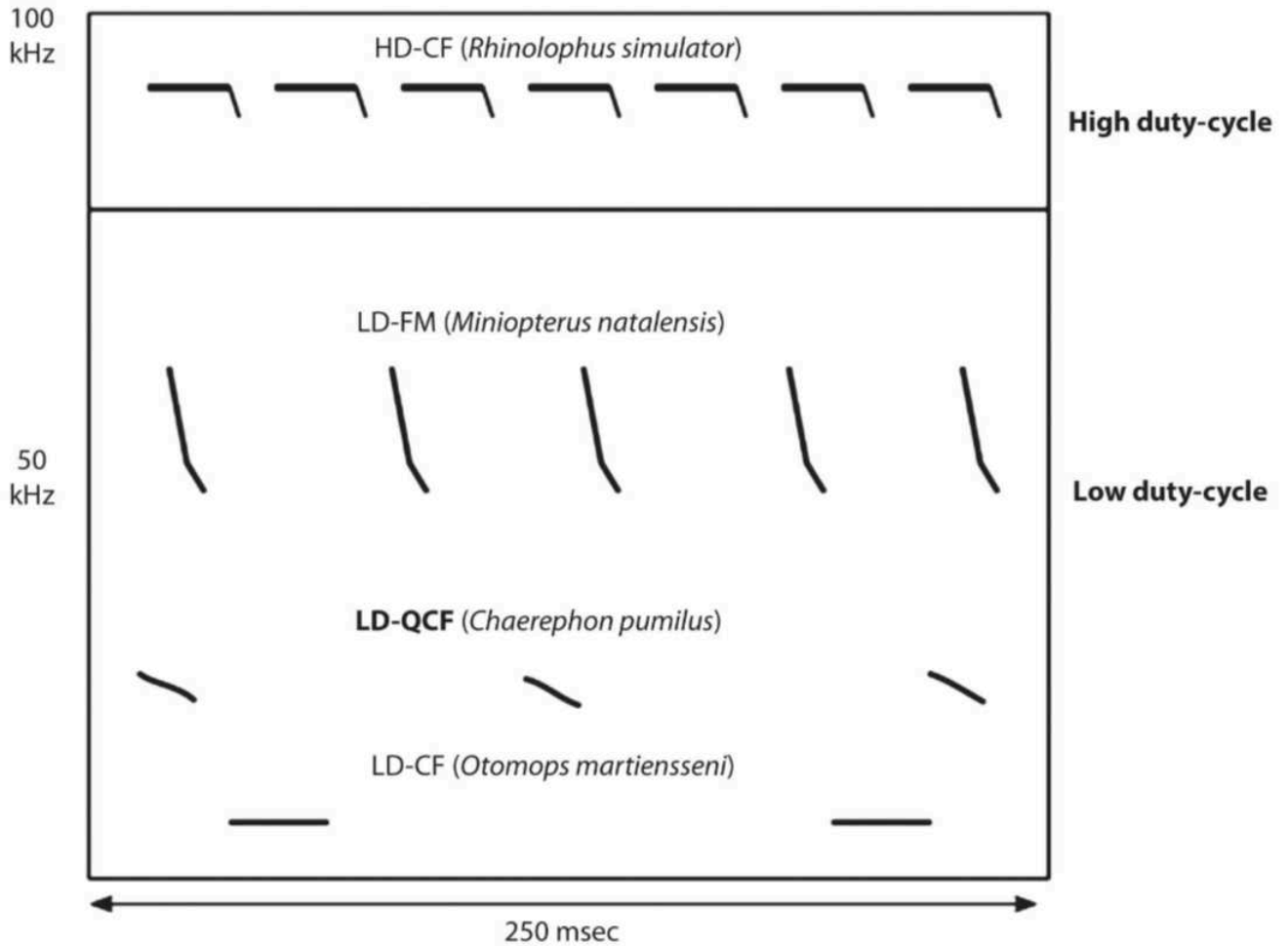
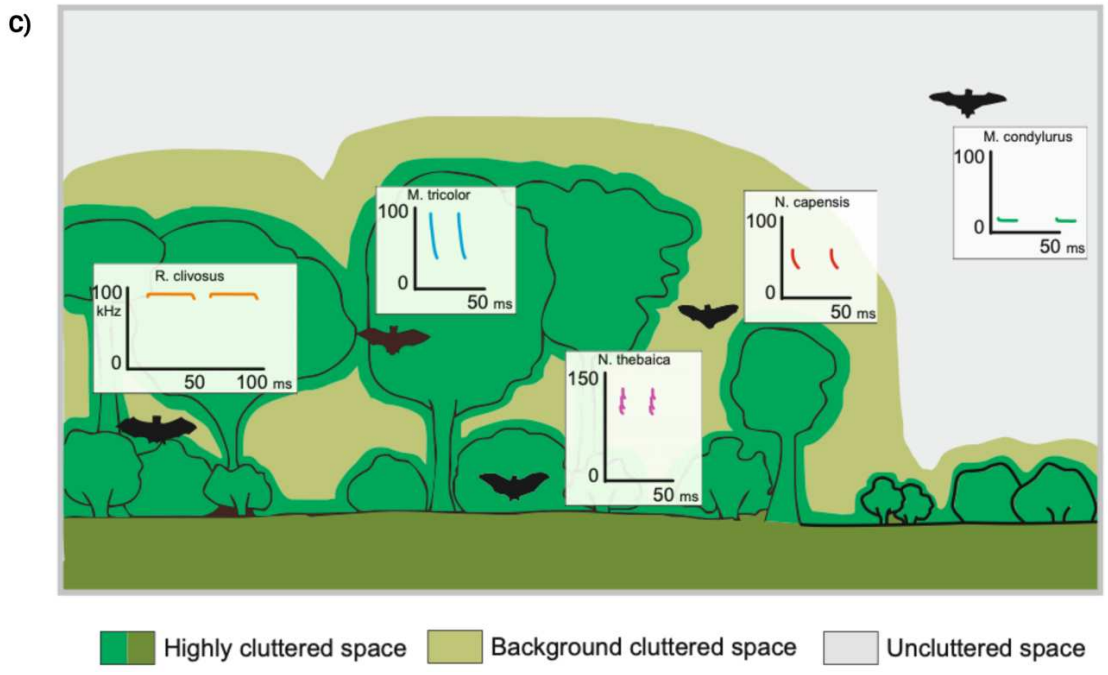
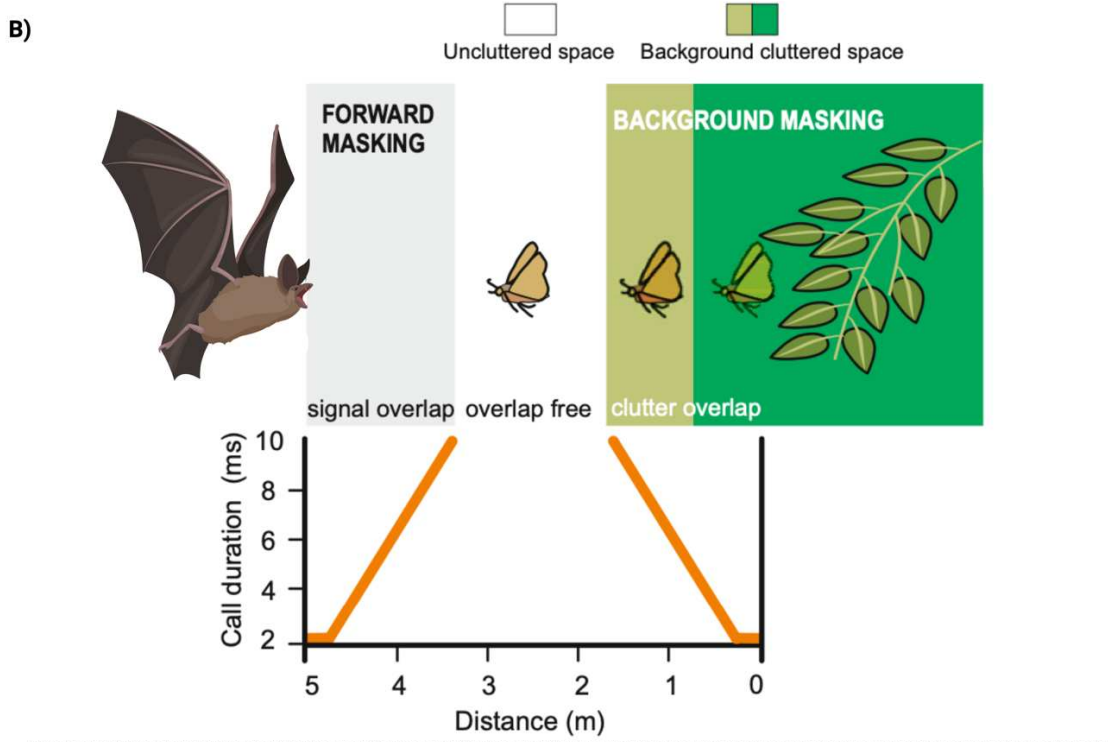
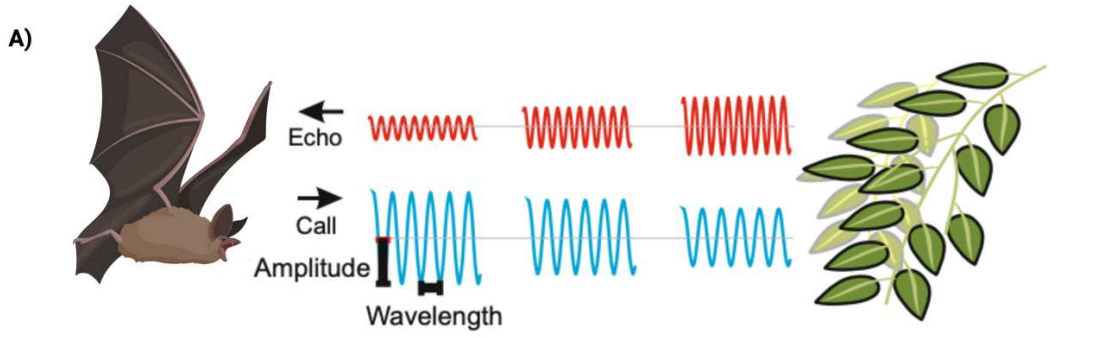


Figure 7: (A) Atmospheric attenuation and Doppler-shift compensation. Atmospheric attenuation: The emitted call (in blue) travels through the atmosphere in the form of sound pressure waves. As each pressure wave is formed, some of the energy in the sound is dissipated (indicated by the reduction in the amplitude of wave). When the sound is reflected off the object and travels back to the bat as an echo (in red), the echo is also attenuated. The echo that the bat hears is therefore much softer than the call that impinges on the object. Doppler-shift compensation: The bat emits its call (in blue) at a slightly lower frequency than the reference frequency (the frequency of its acoustic fovea). Because of the relative motion of the bat, the echo from the object is shifted up in frequency as a result of the Doppler effect indicated by the shorter wavelengths in the echo in red. The bat compensates for the Doppler effect so that the echo always returns at the reference frequency.

(B) Masking effects. Forward masking is the overlap of call and echo and occurs in the zone immediately in front of the bat (grey area). Backward masking is the overlap of target echo with echoes from the background (green areas). There is an overlap-free zone some distance from the bat and the vegetation. This zone increases in size (orange lines) as the bat decreases the duration of its calls.

(C) Bat foraging habitat and duty cycle. Depicted are three habitat categories based on the challenges to echolocation with the wing shape and echolocation calls associated with each category. With the exception of *R. clivus* which is a high-duty-cycle echolocator (indicated by the high ratio between call duration and call period) all the other bats are low-duty-cycle echolocators. Adapted from Jacobs 2016, Schnitzler and Kalko (2001). Created with BioRender.com.



1.2.5 Migration and movement

Bats, like birds, use their evolutionary advantage of the "flight" mechanism to travel large distances. We can define three types of movement behavior: sedentary, nomadic and migratory. Some African bat species have a sedentary behavior moving no more than 50 km around their roost (Fleming, 2019). Some species are nomadic, following an irregular pattern of movement throughout the year, in response to unpredictable regional availability in resources (Teitelbaum & Mueller, 2019). Migration is a seasonal behavior of moving from one habitat to another habitat, and often over a distance over 50 km (Bisson et al., 2009; Fleming, 2019). We consider that only 3% of bats species worldwide show a migratory behavior (Bisson et al., 2009). Bats may migrate for three main reasons: (1) to avoid unfavorable climatic conditions (too cold) and avoid hibernation (which is not very common for tropical bats), (2) to follow food resources very far away and (3) reach a suitable place for breeding or rearing young. In addition, bats may exhibit occasional nomadic behavior in order to escape environmental events such as human disturbance, fires (and smoke) and natural disasters (e.g. volcanic eruptions) (Alerstam et al., 2003; Dingle & Drake, 2007; Fleming et al., 2003; Jachowski & Singh, 2015). Several species of fruit-eating bats (Pteropodidae family) including *Eidolon helvum*, *Nanonycteris veldkampii* and *Myonycteris torquata* are known to migrate between countries of Africa to take advantage of seasonal variations in fruit availability, traveling up to 2,518 km (Richter & Cumming, 2008; Thomas, 1983; Webala et al., 2012). In East Africa, several species of Rhinolophidae and Vespertilionidae bats have been observed migrating short distances between inland and coastal habitats due to seasonal changes in available food resources (O'Shea & Vaughan, 1980). In South Africa during summer, females of Natal long-fingered bat (*Miniopterus natalensis*) migrate (up to 260 km) to warmer maternity caves to give birth and move to colder caves during winter for mating and hibernation (Van der Merwe, 1973, 1975).

i. Fission – fusion on bats: roost switching

Bat population dynamics are complex due to the many factors that can give rise to fission-merger mechanisms. In chiropterans, this term is defined as the dynamics of individuals in a group present in a common roost during the day. Fission-merger dynamics depend mainly on

(i) the quality of the roost, (ii) their life trait (i.e. type of roost, species behaviour), (iii) phylogeny, (iv) reproductive strategy and (iv) food resources, which are interconnected with seasonality (Barclay & Kurta, 2007; Kunz et al., 2003; Patriquin & Ratcliffe, 2016; Patterson et al., 2007; Postawa & Gas, 2009). Thus, a change of roost (if available) can not only reduce predation activity but also minimise travel costs by moving closer to a feeding area (Bartonička et al., 2008; Feyerabend & Simon, 2000; Kunz et al., 2003; Lima & O'Keefe, 2013; Trevelin et al., 2013). The disadvantage of several groups of bats congregating at a single location is that they may be in close proximity to one another, thus increasing the risk of parasite and pathogen transmission (Davies et al., 2012; Kashima et al., 2013). On the other hand, the individuals will also benefit from certain advantages, such as cooperation between individuals in nursing their young and sharing their body heat by grouping together, which reduces the cost of thermoregulation (Boyles et al., 2008; Carter & Wilkinson, 2013a, 2013b; Willis & Brigham, 2007). In this way, the fission fusion mechanisms of bats are impacted by the size of the group, but also on an individual scale by the sex, age and reproductive status of the individuals (Krause & Ruxton, 2010; Olson & Barclay, 2013; Terrien et al., 2011). Finally, some research tend to show that some of the fission fusion movements of a bat colony could be linked to an anti-predation strategy (Barclay & Kurta, 2007; Lima & O'Keefe, 2013). Some predators specialize in hunting bats as they leave a roost (Ancillotto et al., 2013; Mikula et al., 2013).

1.2.6 Mortality and predation

Bats are incredibly long-lived mammals for their body size, with a longevity up to 3.5 times greater than a mammal of comparable size (Austad & Fischer, 1991; Herreid, 1964; Wilkinson & Adams, 2019; Wilkinson & South, 2002). Lifespans of 41 years have been recorded (Podlutsky et al., 2005), although the average longevity is 14.6 years (Tuttle & Stevenson, 1982). Several hypotheses have been put forward to explain this longevity pattern, such as their hibernation behavior, which reduces their metabolism, the use of caves as roosts, their lower allocation to reproduction and their ability to fly, which enables them to reduce mortality (Austad & Fischer, 1991; Barclay et al., 2004).

In general, as with other wild mammals, the mortality rate is very high in the first year, decreasing considerably as the years go by (Cumming & Bernard, 1997; Monadjem, 2005;

Monadjem et al., 2015; Ransome, 1995). Monadjem et al. (2015) showed that Egyptian slit-faced bat (*Nycteris thebaica*) had only 34% of female and 43% male juveniles that survived the first year, the survival rate increasing up to 75% per year afterwards. The longevity record in an African wild bat is 13 years for *Miniopterus natalensis* in Southern Africa (Van der Merwe, 1989). However, knowledge of bat mortality is still patchy due to the difficulty of tracking individuals over the long term.

Many animals can prey on bats, some specializing in this type of prey such as snakes, certain birds including raptors, but also insects and carnivorous animals such as genet or domestic cats and dogs (For more details on predation on bats, see the article discussing genet predation in this thesis). In Central and Southern Africa, several bat species including the large slit-faced bat (*Nycteris grandis*) have been observed consuming other bat species (Fenton et al., 1993; Gual-Suárez & Medellín, 2021). While bats are an essential link in the food chain for many predators, they also bring many benefits to human populations.

1.3 Key roles of bats in ecosystems

Bats have been identified as providing at least three major ecosystem services (Appendix 2): pollination and dispersion of seeds, guano as fertilizer, biological pest control (Kunz et al., 2011; Ramírez-Fráncel et al., 2022). However, research on this subject is highly disparate across biogeographical regions, with Africa being one of the least studied continents.

Frugivorous and nectarivorous bats are key species in the dispersal and pollination of a minimum of 549 plant species (Castillo-Figueroa, 2020; Ramírez-Fráncel et al., 2022). Chiropterans play an important role in plant pollination, as their fur has the capacity to hold and shed a great quantity of pollen compared to other taxa (up to four times more pollen transported than a bird) (Ratto et al., 2018). In addition, their ability to fly long distances allows for optimal plant dispersal, while enabling exchanges between individuals from different populations, thus promoting genetic mixing within these plants (Ratto et al., 2018; Williams-Guillén et al., 2016). On a global scale, no comprehensive study has estimated the economic benefits of bats as pollinators. However, a few studies, especially in the Paleotropics region, have estimated a large economic benefit of bat pollination of certain crops of interest, such as durian, agave, bananas, jackfruit, bitter beans and dragon fruit (Acharya et al., 2015; Aziz et al., 2017; Bumrungsri et al., 2009; Lim et al., 2018; Sheherazade et al., 2019; Trejo-Salazar et

al., 2016; Tremlett et al., 2019; Zalipah et al., 2016). In the Ethiopian region, baobabs are pollinated by bats (Karimi et al., 2021; Start, 1972). A recent study in Mexico showed that pollinated flowers of a cactus (*Stenocereus queretaroensis*) by Mexican long-nosed bat (*Leptonycteris yerbabuenae*) were 35% more likely to become mature fruit than flowers pollinated by diurnal animals (Tremlett et al., 2019). This difference can be explained by two factors: their higher visitation rate than birds and their feeding mode, which offers a higher probability of contact with the reproductive parts of the cactus flower than that of birds (Tremlett et al., 2019). As a result, bats have a greater capacity to absorb and retain pollen on their fur than birds (Tremlett et al., 2019).

Bats are also key animals in the long-distance dispersal of seeds (Lobova et al., 2009). By moving the seeds away from the parent tree, they increase the likelihood of the plant species colonizing a new patch of forest and encouraging reforestation (Kasso & Balakrishnan, 2013; Lim et al., 2018; Macgregor & Scott-Brown, 2020; Saldaña-Vázquez et al., 2019; Tsoar et al., 2010). Studies also suggest that digesting the seeds of certain plant species may also promote germination (Sánchez & Giannini, 2018; Schupp et al., 2010; Traveset & Verdú, 2002).

Secondly, bat guano, extracted from caves, is an excellent crop fertilizer (Shetty et al., 2013; Sothearen et al., 2014). It is considered the best natural fertilizer, providing nutrients and organic material that improve plant growth compared to other fertilizers (Ramírez-Fráncel et al., 2022; Sludge et al., 2008). Guano contains high levels of nitrogen, phosphorus, potassium nitrate and numerous bacteria, improving soil fertility (Shetty & Sreepada, 2013; Tuttle & Moreno, 2005; Ware et al., 2020). Demand for guano has exploded in recent years due to its effectiveness and the expansion of organic farming practices (Saleque et al., 2004). In some continents, such as Africa, access to fertilizers can be complicated and very costly for local farmers (Dimande et al., 2023; Hatibu et al., 2021). Using guano can reduce costly, highly polluting chemical fertilizers and avoid pest resistance to insecticides (Marwa et al., 2021). Depending on the country, the price of guano can vary from 1 dollar to over 12 dollars per 500 g (Kunz et al., 2011; Tuttle & Moreno, 2005).

Thirdly, insectivorous bats are very good regulators of insects and other arthropods. Consumption of arthropod pests can also have a significant economic benefit (Bohmann et al., 2011; Boyles et al., 2011; Kunz et al., 2011; Long et al., 1998; Maas, 2013; Maas et al., 2015; McDonald, 1990; Noer et al., 2012). Some of these insects are considered pests of a wide variety of commercial crops, such as rice, maize, cotton, avocado, sugarcane, nuts and apple.

In Thailand, Wanger et al (2014) showed that a single species of insectivorous bat (*Mops plicatus*) prevented the annual loss of 2,900 tons of rice and avoided a loss of 1.2 million USD annually.

Every year, between Northern Mexico and Texas, billions of pest insects (corn earworm moths (*Helicoverpa zea*), fall armyworms (*Spodoptera frugiperda*) and other insects) migrate at high altitude (up to 3 km above the ground). These insects are responsible for the massive destruction of crops in Southern and Central United States, causing billions of dollars in losses every year (Boyles et al., 2011; McCracken, 1996). Texas is home to a population of around 100 million *Tadarida brasiliensis*, distributed throughout the Bracken cave and other roosts, and eating around 1,000 tons of insects every night. Recent studies have shown that the consumption of just 10% of these insects by this population of *Tadarida* would represent 340 million moths eaten every night, saving millions of dollars in lost crops (Kalko et al., 2008; McCracken, 1996; McCracken & Westbrook, 2002).

South Africa is the world's largest exporter of macadamia nuts. Weier et al (2019) reported that insectivorous bat species fed on four insect species harmful to these crops (*Thaumatotibia batrachopa*, *Cryptophlebia peltastica*, *Nezara viridula*, *Bathycoelia distincta*), limiting drastically the damage to crops. Weier et al (2018) also demonstrated that the maintenance of bocage and hedges in crops favors the consumption of insect pests by bats.

Finally, many bat species feed on mosquitoes, vectors of many diseases; for example, the genus "*Myotis*" is known to consume up to 600 mosquitoes per hour (Griffin et al., 1960). Although no scientific evidence has been found to demonstrate the impact of bat predation on the spread of malaria, it is clear that the absence of bats would have an impact on mosquito populations (Puig-Montserrat et al., 2020).

All the above examples demonstrate the benefits that bats can bring to us. However, literature and research on the subject remain rather poor, despite the immense ecological and economic impact that a decline in bat populations would have.

1.4 Conservation of bat population

With the rise of anthropogenic disturbances, bats are facing new factors that can increase their mortality. Among the threats facing bat populations, the first one is the destruction of their

habitat where they can breed, rest and/or feed (Almeida et al., 2021; Bennett & Hale, 2018; Schanzer et al., 2022; Stahlschmidt et al., 2017). More recently, bats have also been affected by new threats such as pesticides, wind turbines, light pollution and climate change (Frick et al., 2020; Patriquin et al., 2022). In some countries, they are also eaten by local populations for their meat or killed to protect fruit harvests or out of fear because of their link with certain zoonoses (Frick et al., 2020; López-Baucells et al., 2018; Mickleburgh et al., 2009; Mildenstein et al., 2015; Openshaw et al., 2017; Rocha et al., 2020; Williams-Guillén et al., 2016). Finally, human activity has spread a fungus to North America, causing an epidemic of white-nose syndrome that has led to the destruction of a very large number of bat colonies (Escobar et al., 2014; Hoyt et al., 2021; Langwig et al., 2017; Miller-Butterworth et al., 2014). On the other hand, some bat population species appear to benefit from the use of human infrastructures in urban areas and the high availability of food resources (Patriquin et al., 2022). However, this type of behavior may increase the interactions between bats and human populations.

1.5 Role of bats in the ecology of zoonotic viruses

1.5.1 Bat-borne pathogens

Among mammals, bats harbor the highest proportion of zoonotic viruses (Luis et al., 2013; Olival et al., 2017) (**Appendix 2**: glossary of scientific terms). Bats host a wide range of viral agents, from coronaviruses to rabies virus and filoviruses (Gupta et al., 2021). Recently, more than 200 viruses have been detected in bats due to the recent intensification of viral studies on this taxon and advances in pathogen detection techniques (Chen et al., 2014; Markotter et al., 2020; Moratelli & Calisher, 2015). However, robust evidence for bats as reservoir hosts is lacking in most African virus studies (Weber et al., 2023). Several bat species are known to be reservoirs for viruses of the genera *Lyssavirus* (rabies virus) and *Henipavirus* with Nipah virus (NiV) and Hendra virus (HeV). Bats are also reservoirs for viruses of the *Coronaviridae* family, including Severe Acute Respiratory Syndrome Virus (SARS-CoV), Middle East Respiratory Syndrome Virus (MERS-CoV), and probably the original host of SARS-CoV-2 (Latinne et al., 2020; Zhou et al., 2020). They are also the primary hosts of viruses from the *Filoviridae* family such as Marburg virus and suspected to host Ebola viruses as well (Rodhain, 2015; Wood et al., 2012). In Africa, over the last few decades, several bat-transmitted zoonotic viruses have

created outbreaks in humans, resulting in large (filoviruses) or sporadic spillovers (lyssaviruses and paramyxoviruses) (Banyard et al., 2014; Drexler et al., 2012; Schuh et al., 2017; Tian et al., 2022). One of the main causes of the transmission of zoonoses from bats to the human population is human activity and the human disturbance it causes (Daszak et al., 2001; Gibb et al., 2020; Keesing et al., 2010). The destruction of habitats and biodiversity, their exploitation and the increase in interfaces between bats, wildlife and humans increase the risk of spreading zoonotic diseases (Ellwanger et al., 2022; Ellwanger & Chies, 2021; McMahon et al., 2018). Despite current research efforts, many questions remain unanswered as to the causes and factors behind the emergence of zoonoses from bats or other reservoirs (Caron et al., 2018). We present below several examples of zoonoses.

i. Example of zoonoses: Coronaviruses

Following the recent global SARS-CoV-2 pandemic, our societies have recently become aware of the close link between coronaviruses and bats. However, pathogenic coronaviruses which belong to four genera (Alphacoronavirus, Betacoronavirus, Gammacoronavirus and Deltacoronavirus) (Wong et al., 2019) had already caused several zoonotic epidemics over the past twenty years (**Fig. 8**). Human coronaviruses (HCoVs) such as HCoV229E, severe acute respiratory syndrome (SARS), HCoV-NL63, Middle East respiratory syndrome (MERS), swine acute diarrhoea syndrome coronavirus (SADS-CoV) and potentially the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are respiratory pathogens transmitted primarily by aerosols (Hu et al., 2015; Wong et al., 2020). These coronaviruses all have a zoonotic origin, having been transmitted via a secondary host before reaching human populations. In each case, the coronavirus virus most closely related to these zoonoses was detected in bat species.

Global surveillance activities have led to the detection of a wide diversity of coronaviruses in bats (mainly from the Rhinolophidae and Hipposideridae families) (Wong et al., 2019).

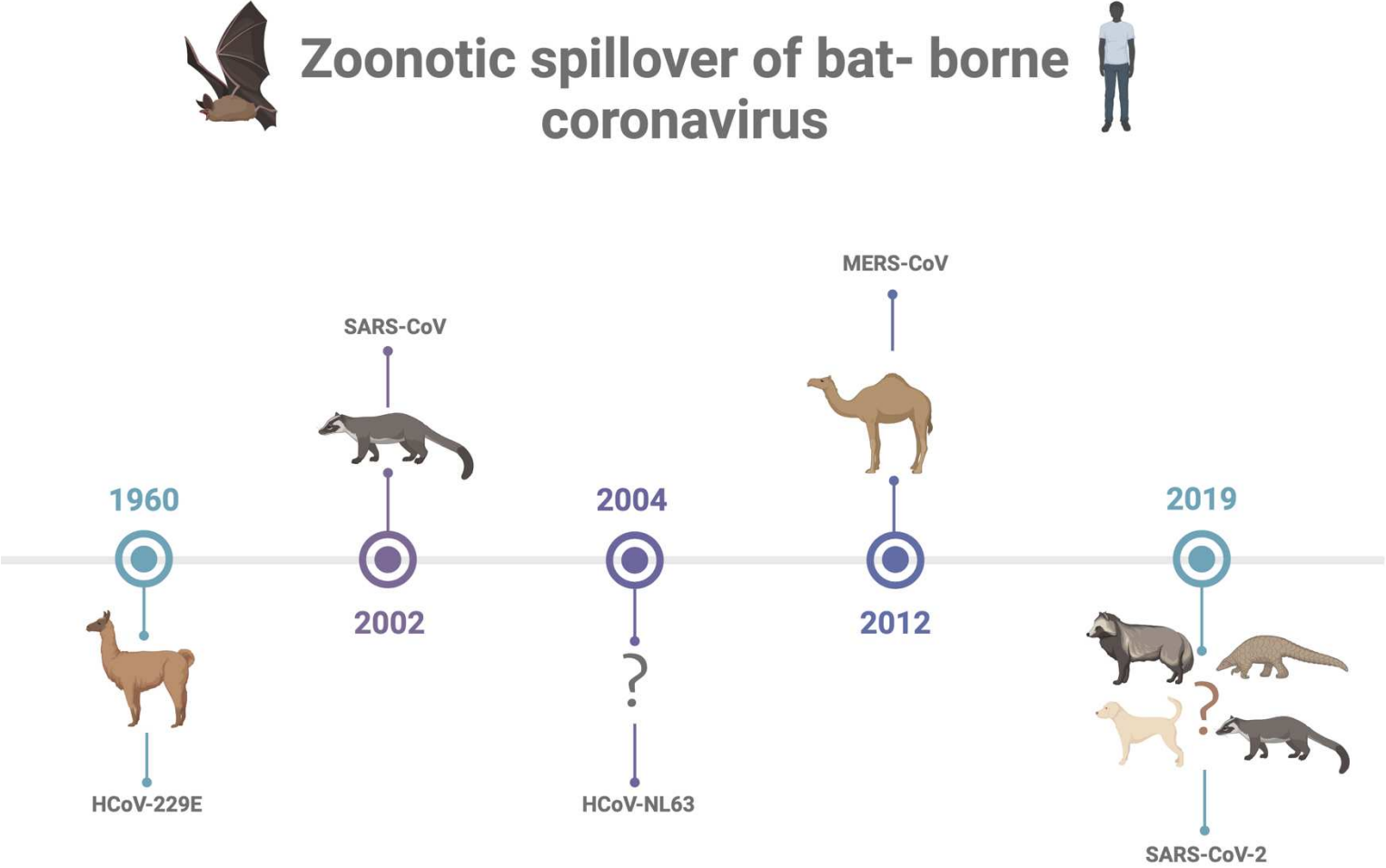
A large proportion of bat coronaviruses are excreted in faeces, creating numerous opportunities for inter- and intra-species exposure and transmission. Bat coronaviruses have also been reported at low frequencies in saliva, urine and different organs (Anindita et al., 2015; Geldenhuys et al., 2018; Mendenhall et al., 2016; Shehata et al., 2016). Coronaviruses can therefore also be transmitted to other species, especially since the great diversity of host

species would favor the capacity of these pathogens to adapt to new hosts (frequent mutation and recombination) (Anthony et al., 2017; Hu et al., 2017; Leopardi et al., 2018). However, bats are not the only animals that are coronavirus carriers such as different mammals (rodents, cat, dog, ...), birds or cattle (Bonilauri et al., 2021).

ii. Example of zoonoses: Lyssaviruses

Rabies is an ancient disease in human societies, which is still present in over 150 countries, causing the death of tens of thousands of people, mainly in Africa and Asia (WHO, 2023a). On the other hand, infection rates and mortality in bats are generally low in the species studied (Aguilar-Setien et al., 2005). This zoonosis is one of the most widespread viral zoonoses, affecting a wide range of bat species worldwide. However, the vast majority of rabies cases are caused by dogs (WHO, 2023a). In recent decades, even in several countries considered rabies-free (Australia, Spain, UK), rabid bats and human cases of bat-associated rabies have been identified (Banyard et al., 2011; Ceballos et al., 2013; Fooks et al., 2003; McElhinney et al., 2013; Picard-Meyer et al., 2013; Schatz et al., 2013)

Figure 8: Timeline of spillovers of coronaviruses originally hosted by bats which led to the emergence of epidemics in the human populations (Created with BioRender.com).



In the USA, rabies remains a major public health problem due to the number of cases associated with variants of the bat rabies virus (Patyk et al., 2012), although cases of canine rabies are more significant. Between 2001 and 2009, they detected rabies virus in 6.7% of bats diagnosed out of 205,439 bats tested. In Latin America, several studies have identified more cases of human rabies linked to exposure to bats than to dog bites (Condori-Condori et al., 2013; Schneider et al., 2009). For example, in Peru, between 2002 - 2007, 380 bat-transmitted human cases were recorded, with 77% of diagnosed rabies cases associated with vampire bats, while 23% were linked to canine rabies virus variants (Condori-Condori et al., 2013).

In addition, Streicker et al. (2012) demonstrated that the bite of rabid bats on cattle in several South American countries led to cattle paralysis and sometimes death, with economic consequences for farmers (Hoar et al., 1998). Due to the absence of "vampire" bats on the African continent, there is little bat-associated rabies, but the continent is heavily affected by canine rabies (WHO, 2023a). Despite the numerous studies on the subject due to the impact on human health, many aspects of lyssavirus ecology in bats are still under investigation, such as mechanisms of maintenance, excretion and potential survival rate to rabies infection.

iii. Example of zoonoses: Henipavirus

In the Henipavirus (HNV) genus, the two viruses, Hendra virus (HeV) and Nipah virus (Niv), are highly pathogenic for humans, causing respiratory diseases, acute encephalitis and the death of many patients (mortality rate of up to 90%) (Marsh & Wang, 2012; Pernet et al., 2014). The genus *Pteropus* (large frugivore) has been identified as a reservoir for this virus (Field et al., 2007; Halpin et al., 2011; Hasebe et al., 2012). Its range corresponds in part to the distribution of the species in Asia. Studies have identified the major factors that could explain the spread of HNV. The places where HNV emerges used to be natural habitats for bat species of the *Pteropus spp.* family, before they were severely disturbed by human activity. The disappearance of their habitat and the extension of agricultural production have encouraged direct interactions with bats. Those characteristics are common to other continents, including Africa. However, the emergence of HNV has only been reported in South Asia and Australia. Recent studies, though, have detected the presence of HNV antibodies in several species of fruit bats (*Eidolon dupreanum*, *Pteropus rufus* and *Eidolon helvum*) throughout the African continent.

A total of 19 new forms of HNV virus have been identified in Africa. In addition, positive seroprevalence for the Hendra virus was detected in human samples (Drexler et al., 2009; Hayman et al., 2008; lehlé et al., 2007; Peel et al., 2012; Richter & Cumming, 2006b, 2008; Weiss et al., 2012). Those humans lived in highly disturbed habitats (undergoing deforestation) and consumed bat bushmeat (Pernet et al., 2014). This example shows that a combination of several specific factors is responsible for the spread of HVN epidemics in the Indian Ocean. However, identical factors do not seem to be enough to trigger an epidemic in Africa.

As a result, many questions remain about the complexity of the mechanisms behind the emergence and re-emergence of zoonotic diseases. Another concrete example is the re-emergence of the EBOLA virus in the DRC caused by Ebola survivors, which calls into question all the hypotheses about the sole involvement of bats in the maintenance ecology of this virus (Jain et al., 2023; Lacroix et al., 2021; Leendertz, 2016).

1.5.2 Bats and their immune systems

In recent decades, chiropterans have become known for their role as a reservoir for a large number of viruses with only few that can be highly lethal in other species (Marburg virus (Towner et al., 2009), Hendra virus (Leendertz, 2016)), including humans, but rarely cause clinical consequences in bats (Middleton et al., 2007; Munster et al., 2016; Swanepoel et al., 1996; Watanabe et al., 2010). This particularity, between bats and other mammals, has sparked renewed interest in the scientific community to understand one of the most efficient immune systems in the animal kingdom, balancing enhanced host defense and immune tolerance (Irving et al., 2021; Schneider & Ayres, 2008; Schountz et al., 2017). Firstly, like other mammals, bats have an innate immune system that enables them to detect the molecular patterns associated with pathogens (viruses, bacteria, parasites) (Collins & Mossman, 2014; Janeway & Medzhitov, 2002; Koyama et al., 2008). Thus, during a viral infection, infected cells will trigger a series of reactions enabling the excretion of antiviral cytokines, such as interferons (IFNs), by activating the expression of IFN-stimulated genes (ISGs) which inhibit virus replication by various mechanisms (see Schoggins & Rice (2011) for more details) (Janeway & Medzhitov, 2002; Kawai & Akira, 2006; Lee & Kim, 2007). In addition, bats have an enhanced viral immune defense that facilitates a more effective response to infection (five

times more immunoglobulin segment diversity than humans, larger naive immunoglobulin repertoire which facilitates a more effective response to infection (Schountz et al., 2017).

Secondly, recent genome research on bats has identified that only 2.7 to 3.5% of genes are related to immunity (Lee et al., 2015; Papenfuss et al., 2012; Shaw et al., 2012) in contrast to the 7% in humans (Kelley et al., 2005). Yet, despite the loss of certain immune genes, other genes appear to be more present in the bat genome, such as those responsible for DNA damage checkpoints, repair pathways (Zhang et al., 2013) and encoding antiviral protein (Benfield et al., 2020; Fernandes et al., 2022; Jacquet et al., 2020, 2022; Jebb et al., 2020). Also, the evolution of flight in this mammal, and the energy cost involved, is thought to account for this difference in physiology (O'Shea et al., 2014; Zhang et al., 2013). Bats have adapted their immune systems to intense and prolonged physiological stress (oxidative stress), which leads to severe DNA damage and the release of DNA fragments into the cytoplasm (Barzilai et al., 2002), equivalent to a viral infection. Zang et al. (2013) argued that bat evolution has enabled the development of an efficient DNA repair mechanism to cope with induced cellular damage. Such mechanisms would have the added benefit of fighting viral infections, limiting aging, combating cancerous cells and participating in the mechanisms promoting bats' unique longevity (Brunet-Rossinni & Austad, 2004; Dammann, 2017; Munshi-South & Wilkinson, 2010; Pavlovich et al., 2018; Wilkinson & Adams, 2019).

Thirdly, the addition of certain immune-related genes would reduce the inflammatory response. For example, Ahn et al. (2016) showed that loss of the PYHIN gene (which serves as important immune sensors of intracellular self and foreign DNA and activators of the inflammasome and/or interferon pathways) resulted in a reduced inflammatory response. Conversely, in the bat species *P. alecto*, the important role of the NLR family pyrin domain-containing 3 (NLRP3) inflammasome sensor was associated with virus-induced inflammation (Ahn et al., 2019). By reducing or suppressing the inflammatory response, bats will promote a balanced immune system reaction and contribute to their immune tolerance. Indeed, immune system dysregulation in humans appears to be responsible for increased disease severity during the acute phase of viral illness (Hope & Bradley, 2021). Bats, on the other hand, cope better with deadly viruses and, despite a delayed reaction time, eventually overcome these viruses to achieve complete recovery and elimination of the pathogen.

However, given the diversity of bat species (size, diet, longevity, morphology, social interactions and ecological niches), studies carried out on one species are not representative of all bats, and some mechanisms may be unique to one family or species. Besides, certain bat traits or behaviours appear to play a more important role than immunity in the maintenance and transmission of pathogens from bats to other species.

1.5.3 Maintenance and transmission of pathogens in bats. Risks of emergence of new bat borne pathogens

Their longevity and strong social behavior (aggregation of thousands of individuals in a roost – increase contact rate) might favor multiple transmission events and co-infection (and also intra-interspecies transmission) over several generations (Hoarau et al., 2021; Luis et al., 2015; Markotter et al., 2020; Olival et al., 2017; Willoughby et al., 2017). A long period of aggregation of females in maternity colonies could be at the root of pathogen amplification. (Dietrich et al., 2015). During these specific periods, a massive influx of a new generation of juvenile bats can be observed in these colonies. Juveniles are vulnerable to pathogen transmission at the end of the lactation period (3 to 6 months after the birth of the young), when their immunity, which is not yet native (Amman et al., 2012; Plowright et al., 2008) is no longer protected by maternal immunity. In addition, during the suckling period, females may be less likely to groom themselves (Christe et al., 2007), and hairless young are more vulnerable to ectoparasites than hairy individuals (Lewis, 1995).

Current research has identified two transmission routes for emerging bat viruses in humans. The first route is direct, via contact with live animals (body fluids, feces, bites, airborne and droplet transmission) or the handling and consumption of dead (e.g., hunted) animals. The second route is indirect, with the pathogen being transmitted from the bat to a bridge host (Caron et al., 2015) such as wild animals, livestock, blood feeding or by contamination of environment (fruit, guano, ..) used by infected hosts (**Fig. 9**) (Han et al., 2015; Hayman et al., 2013; Joffrin et al., 2018; Wong et al., 2007).

The presence of an active virus in bats does not mean that it will automatically be transmitted to other animals, including humans. Normally, the transmission of a zoonosis from a reservoir animal to human populations requires the conjunction of a multitude of conditions (biological, ecological and anthropogenic). These factors rarely come together for the pathogen to

multiply and create an epidemic in human populations (Ellwanger & Chies, 2021; Markotter et al., 2020; Plowright et al., 2015, 2017) (**Fig. 10**). In recent decades, however, there has been a sharp increase in emerging diseases originating in wildlife (Fauci, 2005; Ferreira et al., 2021). Several environmental and anthropogenic factors have been identified as potential drivers for the emergence of zoonotic diseases in bats (Plowright et al., 2015). On a global scale, the increase in human populations has led to intensification of farming, heavy deforestation and increased hunting pressure on many bat species (Allen et al., 2017; Pernet et al., 2014).

These disturbances not only lead to habitat destruction and species extinction, but also to increased contact between human populations and wildlife, including bats. For example, the spread of the Hendra virus was caused by the destruction of their environment, which led to a change in bat behavior (Eby et al., 2023). These rapid changes in the ecology of fruit bats amplified contacts at the bat-human interface (Eby et al., 2023). In addition, contact between bats and humans is increasing with the growth in human populations, the exploitation of natural resources (mining and guano extraction) and human activities (cave tourism, caving, prayers) (Arita, 1996; Bonsall & Tolan-Smith, 1997; Fernández-Llamazares et al., 2018; Furey & Racey, 2016; Moyes, 2012; Simons, 1998).

Habitat disturbance can also lead to stress on biodiversity. The consequences of this stress on bats can lead to changes in viral circulation or excretion. Several studies have also shown that countries with high levels of biodiversity and anthropogenic disturbance, such as Asia and Africa, are at greater risk of zoonosis emergence (Allen et al., 2017; Friant et al., 2020; Jagadesh et al., 2022).

Figure 9: Possible transmission routes of bats-borne pathogens to humans (modified from Joffrin et al., 2018)

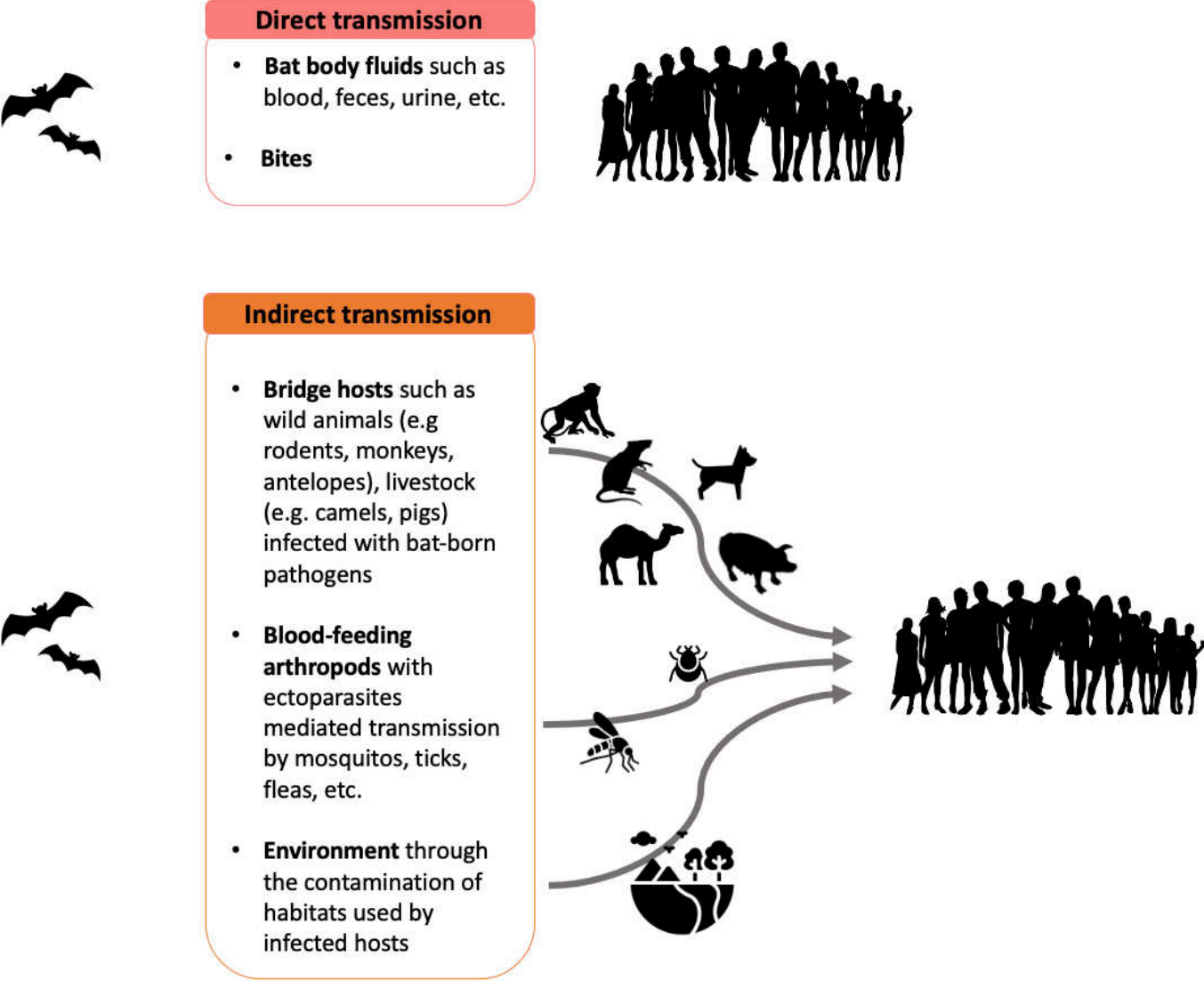
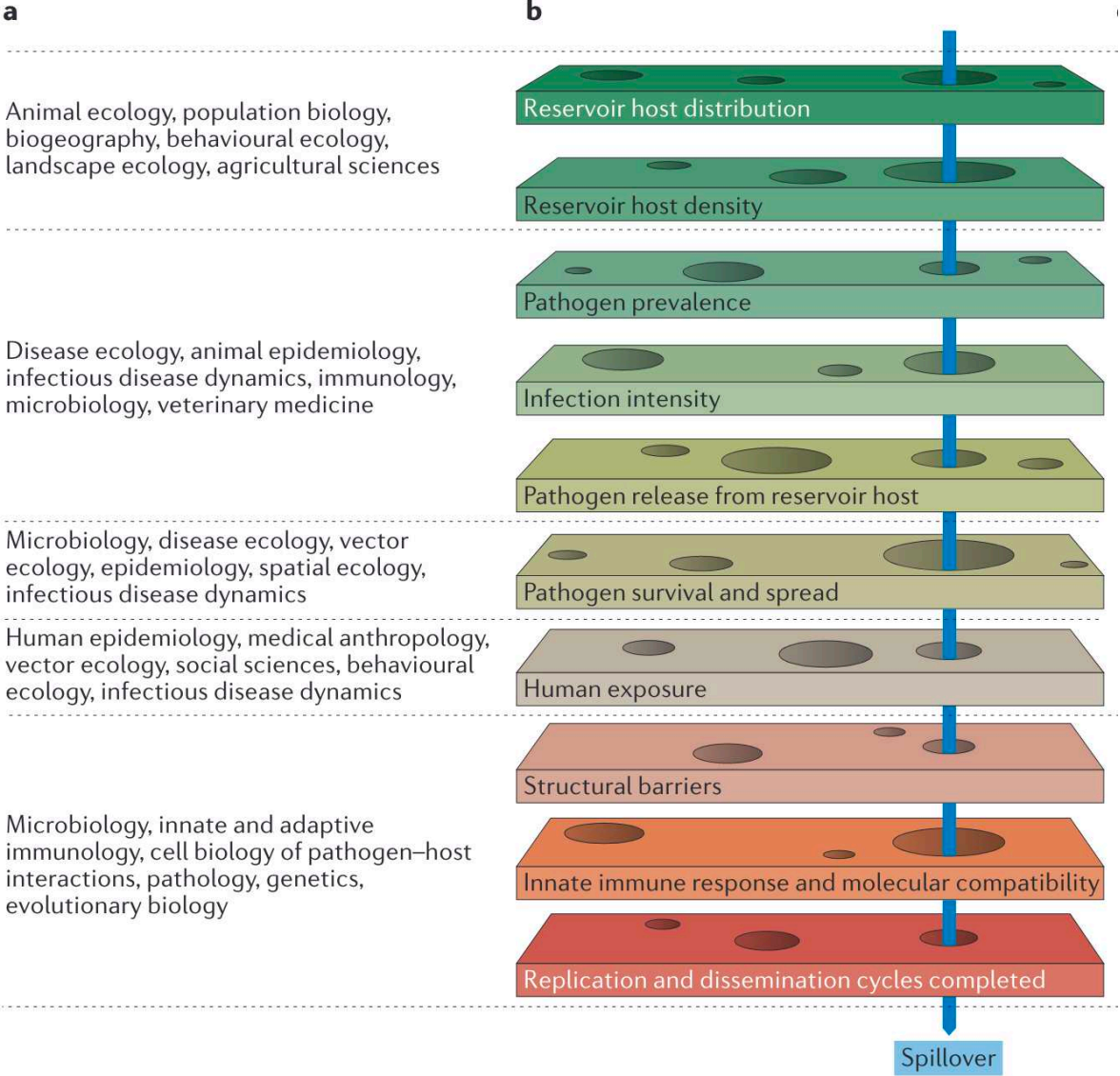


Figure 10: Barriers to spillover and dose–response relationships. a | Determinants of spillover are being studied by researchers in many disciplines. b | A pathogen must overcome a series of barriers to transmit from one species to another. If any of these barriers is impenetrable, spill over cannot occur. Spillover of some pathogens requires that gaps (depicted as holes) in all of the barriers align within a narrow window in space and time (indicated by the blue arrow). For other pathogens, protracted survival in the environment (for example, *Bacillus anthracis* spores¹⁰⁹), or wide dissemination (for example, the spread of aerosolized *Coxiella burnetii* by wind³⁵), may stagger the alignment of barriers to spillover. From (Plowright et al., 2017)



In Africa, bats often live in close contact with human populations (inside house, cave, ...), due to the capacity of some species to live in large numbers in highly anthropized habitats such as urban areas. This proximity between bats, potential secondary hosts (livestock, domestic animals) and human populations can create contact and facilitate the spread of disease. In addition, bats, particularly large ones (almost all fruit bats and large insectivorous bats: *Macronycteris gigas*), are consumed in many African countries (Kamins et al., 2011; Mickleburgh et al., 2009). These animals can be hunted using nets, blowpipes, rifles or shaking pins. Some populations go even further, hunting bats in caves by enclosing the cave with a plant wall before catching them by hand, or by intoxicating them by setting fire inside cave. This hunting activity can be carried out periodically by a few adults or children, or seasonally by an entire village in the case of large-scale bat hunts (pers. obs 2019 - 2023). In general, bats are killed and/or smoked for transport or sale. In some cases, when they are caught in nets, they are then transported in cages with several dozen individuals and sold on the market alive. Hunters and sellers generally take few or no protective measures when handling or killing bats. In addition to being consumed, bats are also used in many religious practices or as medicinal ingredients (Gbogbo & Daniels, 2019; Mildenstein et al., 2015; Rocha et al., 2021; Sieradzki & Mikkola, 2022).

The Anthropocene era is creating and accelerating global changes, such as habitat loss, forcing bats to find new places to live and feed. Finally, recent climate change and habitat destruction are forcing bat species to adapt, which could lead to changes in their distribution and encourage contact and the introduction of new pathogens (Hayes & Piaggio, 2018; Sherwin et al., 2013). However, these epidemics underline the importance of knowing bat species and their ecology. Unfortunately, we tend to blame bats for everything, especially when it comes to their role as pathogens. However, given the number of contacts between bats and humans worldwide, the spread of disease by bats remains anecdotal. We need to continue our research into this taxon in order to unveil the mysteries of this fascinating animal.

1.6 New technologies usher a new era in bat ecology

The study of bat ecology has always been an arduous science, due to several particularities of this taxon. They are nocturnal, secretive, fast and high flying, sometimes living in hard-to-reach places, with often small body sizes and high-altitude flights. Historically,

studies on bat communities have been carried out using invasive and non-invasive methods. Invasive method such as bat capture remains necessary to better assess species inventory, verify individual reproductive status, collect biometric data or samples (i.e., tissues, feces, ectoparasites and reference specimens) (Appel et al., 2021; Barnhart & Gillam, 2014; Flaquer et al., 2007). Handling bats is necessary to attach telemetry devices allowing monitoring behavior and movement (Castle et al., 2015; Epstein & Newman, 2011; O'Mara et al., 2014). Direct observation of individuals in their roost (e.g., cave, tree) or as they emerge to hunt has also provided information on their behaviour, abundance and movements (Thomas & West, 1989).

However, the emergence of numerous technologies and their miniaturization have opened up various opportunities to better understand the ecology and behavior of these species. It is now possible to track bat species using GPS devices fixed on the animal body to explore their movements and contacts with other species and to make long-term individual markings using microchips (Racey, 2011) Visual surveillance of individuals or colonies can also be carried out using cameras (thermal or otherwise), drones, aircraft, or even other animals (use of birds of prey trained to track bats with an on-board camera), offering multiple implications for population movements and behavioral studies (Buxton et al., 2018; Cilulko et al., 2013; Hristov et al., 2008; Krivek, 2023; McCarthy et al., 2021). In recent years, many researchers have begun to favor non-invasive methods such as acoustics and guano collection, with the double benefit of limiting field costs and the impact of human disturbance on bats (Browning et al., 2017; Zamora-Gutierrez et al., 2021).

Acoustics is one of the fastest-growing techniques used by researchers to study insectivorous bats (Browning et al., 2017; Gibb et al., 2019). Advantages are numerous : it is non-invasive, applicable in all environments (even the most remote), can collect a large amount of information and has a variety of applications (Hill et al., 2019) (**Fig. 11**). Lower costs for recording equipment and the democratization of deep learning. Automatic monitoring of populations and ecosystems over the long term has become essential, but it generates vast quantities of data that can no longer be processed efficiently by humans. Deep learning has therefore become a powerful key reference tool for the research community, including ecologists (Borowiec et al., 2022; Christin et al., 2019). Examples of acoustics applications include: inventorying species in a habitat using call identification, habitat use by species,

deciphering the social calls of bats, species phenology and colony counting (or individual density estimation).

1.7 Conclusion

This chapter describes most of the complex ecology of the chiropteran taxon and its important roles in natural processes that impact our life and health. At present, our societies focus mainly on the "harm" they bring to our societies. However, it is vital not to forget the many yet invaluable benefits they bring to our environment and indirectly to our economy. The conservation of bats is vital for the ecosystem services they provide, and all the more so in this period of the Anthropocene, which is putting the survival of all living things at great risk. Bats often suffer from a bad reputation due to a lack of information on these species and their link with the emergence of certain zoonoses. Today, it is much easier to obtain research funding for studies on bats in relation to zoonotic diseases than for studies on their ecology and/or ecosystem services. This chapter also highlights the many unanswered questions and the need for further research, particularly in countries where few studies have been carried out on bats. Protecting bat populations and understanding them will help protect them while benefiting the health of human populations.

1.8 Appendix Chapter 1

Appendix 1: Morphological and acoustical characteristics of African bat species (taken and modified from (Monadjem et al., 2020); * species present in Congo Brazzaville)

Bat family	Morphological characteristics of the bat family	Acoustic characteristics of the bat family	Species name of the bat family
Pteropodidae	<ul style="list-style-type: none"> • Face: dog or fox faces with elongated muzzles • Claws on wings: two claws on the wing • Eyes: Large eyes • Other criteria: often large size 	<p>This family is not known for using acoustics, with the exception of one species, <i>Rousettus aegyptiacus</i>.</p>	<p><i>Eidolon dupreanum</i> <i>Eidolon helvum</i>* <i>Eidolon helvum helvum</i> <i>Pteropus aldabrensis</i> <i>Pteropus livingstonii</i> <i>Pteropus niger</i> <i>Pteropus rodricensis</i> <i>Pteropus rufus</i>* <i>Pteropus seychellensis</i> <i>Pteropus subniger</i> <i>Pteropus voeltzkowi</i> <i>Epomophorus angolensis</i> <i>Epomophorus anelli</i> <i>Epomophorus crypturus</i> <i>Epomophorus dobsonii</i> <i>Epomophorus gambianus</i> <i>Epomophorus gambianus gambianus</i> <i>Epomophorus gambianus pousarguesi</i> <i>Epomophorus grandis</i>* <i>Epomophorus intermedius</i> <i>Epomophorus labiatus</i>* <i>Epomophorus minimus</i></p>

Bat family	Morphological characteristics of the bat family	Acoustic characteristics of the bat family	Species name of the bat family
			<i>Epomophorus minor</i> <i>Epomophorus pusillus*</i> <i>Epomophorus wahlbergi*</i> <i>Epomops buettikoferi</i> <i>Epomops franqueti*</i> <i>Hypsignathus monstrosus*</i> <i>Nanonycteris veldkampii</i> <i>Megaloglossus azagnyi</i> <i>Megaloglossus woermanni*</i> <i>Myonycteris angolensis*</i> <i>Myonycteris angolensis angolensis</i> <i>Myonycteris angolensis goliath</i> <i>Myonycteris angolensis petraea</i> <i>Myonycteris angolensis ruwenzorii</i> <i>Myonycteris angolensis smithii</i> <i>Myonycteris brachycephala</i> <i>Myonycteris leptodon</i> <i>Myonycteris relict</i> <i>Myonycteris torquata*</i> <i>Myonycteris torquata torquata</i> <i>Myonycteris torquata wroughtoni</i> <i>Plerotes anchietae</i> <i>Rousettus aegyptiacus*</i> <i>Rousettus madagascariensis</i> <i>Rousettus obliviosus</i> <i>Casinycteris argynnis</i> <i>Casinycteris campomaanensis*</i> <i>Casinycteris ophiodon*</i>

Bat family	Morphological characteristics of the bat family	Acoustic characteristics of the bat family	Species name of the bat family
			<i>Scotonycteris bergmansi*</i> <i>Scotonycteris bergmansi bergmansi</i> <i>Scotonycteris bergmansi congoensis</i> <i>Scotonycteris occidentalis</i> <i>Scotonycteris zenkeri*</i> <i>Stenonycteris lanosus</i>
Hipposideridae	<ul style="list-style-type: none"> • Face: not dog-like face • Eyes: small • Noseleaf: complex with half-moon shape; sella and connecting process absent • Ears: triangular and large • Tragus: none • Tail: medium length tail enclosed within membrane • Claws on wings: one claw on the wing • Other criteria: none 	High duty cycle (HD) – Constant frequency (CF) of long duration (10 – 100 ms)	<i>Asellia italosomalica</i> <i>Asellia patrizii</i> <i>Asellia tridens</i> <i>Doryrhina camerunensis</i> <i>Doryrhina cyclops*</i> <i>Hipposideros abae</i> <i>Hipposideros beatus*</i> <i>Hipposideros caffer*</i> <i>Hipposideros cf. centralis</i> <i>Hipposideros curtus</i> <i>Hipposideros fuliginosus</i> <i>Hipposideros jonesi</i> <i>Hipposideros lamottei</i> <i>Hipposideros marisae</i> <i>Hipposideros megalotis</i> <i>Hipposideros ruber*</i> <i>Hipposideros tephurus</i> <i>Macronycteris commersoni</i> <i>Macronycteris cryptovalorona</i> <i>Macronycteris gigas*</i> <i>Macronycteris vittata</i>
Megadermatidae	<ul style="list-style-type: none"> • Face: not dog-like face 	Low duty cycle (LD) – FM	<i>Cardioderma cor</i>

Bat family	Morphological characteristics of the bat family	Acoustic characteristics of the bat family	Species name of the bat family
	<ul style="list-style-type: none"> • Eyes: large • Noseleaf: enveloped by pointed spike • Ears: very long and with shape of rabbit ears • Tragus: divided and well developed • Tail: none • Claws on wings: one claw on the wing • Other criteria: second finger has one phalanx and the third finger has two phalanges 	Signal of short duration (1 – 3 ms) at low intensities	<i>Lavia frons</i>
Rhinolophidae	<ul style="list-style-type: none"> • Face: not dog like face • Eyes: tiny • Noseleaf: complex – pointed tip • Ears: triangular and large • Tragus: none but well antitragus • Tail: medium size for the tail that is enclosed within membrane • Claws on wings: one claw on the wing • Other criteria: none 	High duty cycle (HD) – Constant frequency (CF) of long duration (10 – 100 ms)	<i>Rhinolophus adami*</i> <i>Rhinolophus alcyone*</i> <i>Rhinolophus blasii</i> <i>Rhinolophus capensis</i> <i>Rhinolophus cf. hillorum</i> <i>Rhinolophus clivosus</i> <i>Rhinolophus cohenae</i> <i>Rhinolophus damarensis</i> <i>Rhinolophus darlingi</i> <i>Rhinolophus deckenii</i> <i>Rhinolophus denti*</i> <i>Rhinolophus eloquens</i> <i>Rhinolophus euryale</i> <i>Rhinolophus ferrumequinum</i> <i>Rhinolophus fumigatus*</i>

Bat family	Morphological characteristics of the bat family	Acoustic characteristics of the bat family	Species name of the bat family
			<i>Rhinolophus gorongosae</i> <i>Rhinolophus guineensis</i> <i>Rhinolophus hildebrandtii</i> <i>Rhinolophus hilli</i> <i>Rhinolophus hillorum</i> <i>Rhinolophus hipposideros</i> <i>Rhinolophus horaceki</i> <i>Rhinolophus kahuzi</i> <i>Rhinolophus landeri*</i> <i>Rhinolophus lobatus</i> <i>Rhinolophus mabuensis</i> <i>Rhinolophus maclaudi</i> <i>Rhinolophus maendeleo</i> <i>Rhinolophus mehelyi</i> <i>Rhinolophus mossambicus</i> <i>Rhinolophus rhodesiae</i> <i>Rhinolophus ruwenzorii</i> <i>Rhinolophus sakejiensis</i> <i>Rhinolophus silvestris*</i> <i>Rhinolophus simulator</i> <i>Rhinolophus smithersi</i> <i>Rhinolophus swinnyi</i> <i>Rhinolophus willardi</i> <i>Rhinolophus ziama</i> <i>Rhinolophus "incertae-sedis"</i>
Rhinonycteridae	<ul style="list-style-type: none"> • Face: not dog like face • Eyes: tiny • Noseleaf: complex – tridentate 	High duty cycle (HD) – Constant frequency (CF) of long duration (10 – 100 ms)	<i>Cloeotis percivali</i> <i>Paratriaenops auritus</i> <i>Paratriaenops pauliani</i>

Bat family	Morphological characteristics of the bat family	Acoustic characteristics of the bat family	Species name of the bat family
	<ul style="list-style-type: none"> • Ears: triangular and large • Tragus: none • Tail: medium size for the tail that is enclosed within membrane • Claws on wings: one claw on the wing • Other criteria: none 		<p><i>Triaenops afer</i> <i>Triaenops menamena</i> <i>Triaenops persicus</i></p>
Rhinopomatidae	<ul style="list-style-type: none"> • Face: not dog like face • Eyes: small • Noseleaf: simple nose with a simple noseleaf with vascular nostrils • Ears: large • Tragus: well developed • Tail: very long hairless tail, nearly same length that the body • Claws on wings: one claw on the wing • Other criteria: fat repository between the caudal penduncle and the hind legs 	Narrowband of rather long duration (8 – 25 ms) with a low frequency (< 30 kHz)	<p><i>Rhinopoma cystops</i> <i>Rhinopoma macinnesi</i> <i>Rhinopoma microphyllum</i></p>
Myzopodidae (endemic to Madagascar)	<ul style="list-style-type: none"> • Eyes: small • Noseleaf: long pointed snout • Ears: large • Tragus: well developed • Tail: near the midpoint, the tail protrudes from the upper half 	Complex frequency modulated (FM) echolocation calls, each being composed of two to four harmonics	<p><i>Myzopoda aurita</i> <i>Myzopoda schliemanni</i></p>

Bat family	Morphological characteristics of the bat family	Acoustic characteristics of the bat family	Species name of the bat family
	<p>of the membrane</p> <ul style="list-style-type: none"> • Claws on wings: vertigial claw • Other criteria: none 		
Emballonuridae	<ul style="list-style-type: none"> • Face: not dog like face • Eyes: large • Noseleaf: none • Ears: medium length • Tragus: well developed • Tail: near the midpoint, the tail protrudes from the upper half of the membrane • Claws on wings: one claw on the wing • Other criteria: elongated muzzles, between base of five finger and forearm pocket-like skin pouch (except for <i>Coleura afra</i> and <i>Saccolaimus peli</i>) 	<p>LD – QCF and LD – CF with narrowband echolocation calls at low frequencies (30 kHz) with long duration (8 ms)</p>	<p><i>Coleura afra</i>*</p> <p><i>Coleura gallarum</i></p> <p><i>Coleura kibomalandy</i></p> <p><i>Coleura seychellensis</i></p> <p><i>Paremballonura atrata</i></p> <p><i>Paremballonura tiavato</i></p> <p><i>Saccolaimus peli</i>*</p> <p><i>Taphozous hamiltoni</i></p> <p><i>Taphozous hildegardeae</i></p> <p><i>Taphozous mauritanus</i>*</p> <p><i>Taphozous nudiventris</i></p> <p><i>Taphozous perforatus</i></p> <p><i>Taphozous perforatus perforatus</i></p> <p><i>Taphozous perforatus sudani</i></p>
Nycteridae	<ul style="list-style-type: none"> • Face: not dog like face • Eyes: tiny • Noseleaf: vertical slit in face, covering noseleaf beneath • Ears: very long and with shape of rabbit ears • Tragus: well developed • Tail: medium size for the tail that is enclosed within membrane and ending in a T shape 	<p>LD – FM</p> <p>Signal of short duration (1 – 3 milliseconds) at low intensities</p>	<p><i>Nycteris arge</i>*</p> <p><i>Nycteris aurita</i></p> <p><i>Nycteris cf. parisii</i></p> <p><i>Nycteris gambiensis</i></p> <p><i>Nycteris grandis</i>*</p> <p><i>Nycteris hispida</i>*</p> <p><i>Nycteris intermedia</i></p> <p><i>Nycteris macrotis</i>*</p> <p><i>Nycteris major</i></p> <p><i>Nycteris nana</i>*</p> <p><i>Nycteris parisii</i></p>

Bat family	Morphological characteristics of the bat family	Acoustic characteristics of the bat family	Species name of the bat family
	<ul style="list-style-type: none"> • Claws on wings: one claw on the wing • Other criteria: none 		<i>Nycteris thebaica*</i> <i>Nycteris vinsoni</i> <i>Nycteris woodi</i>
Molossidae	<ul style="list-style-type: none"> • Face: not dog like face • Eyes: small • Noseleaf: none • Ears: co-joined with an intermediate to large length • Tragus: none or small, well developed anti-tragus • Tail: end of the tail that protrudes beyond hind margin of tail membrane • Claws on wings: one claw on the wing • Other criteria: none 	LD – QCF and LD – CF with narrowband echolocation calls at low frequencies (30 kHz) with long duration (8 ms)	<i>Mops (Chaerephon) aloysiisabaudiae</i> <i>Mops (Chaerephon) ansorgei</i> <i>Mops (Chaerephon) atsinanana</i> <i>Mops (Chaerephon) bemmeleni</i> <i>Mops (Chaerephon) bivittatus</i> <i>Mops (Chaerephon) chapini*</i> <i>Mops (Chaerephon) gallagheri</i> <i>Mops (Chaerephon) jobimena</i> <i>Mops (Chaerephon) leucogaster*</i> <i>Mops (Chaerephon) major</i> <i>Mops (Chaerephon) nigeriae</i> <i>Mops (Chaerephon) nigeriae nigeriae</i> <i>Mops (Chaerephon) nigeriae spillmanni</i> <i>Mops (Chaerephon) pumilus*</i> <i>Mops (Chaerephon) pusillus</i> <i>Mops (Chaerephon) russatus</i> <i>Mops (Chaerephon) tomensis</i> <i>Mops (Mops) condylurus</i> <i>Mops (Mops) congicus*</i> <i>Mops (Mops) demonstrator</i> <i>Mops (Mops) leucostigma</i> <i>Mops (Mops) midas</i> <i>Mops (Mops) midas miarensis</i> <i>Mops (Mops) midas midas</i> <i>Mops (Mops) niangarae</i>

Bat family	Morphological characteristics of the bat family	Acoustic characteristics of the bat family	Species name of the bat family
			<p> <i>Mops (Mops) niveiventer</i> <i>Mops (Mops) trevori</i> <i>Mops (Xiphonycteris) bakarii</i> <i>Mops (Xiphonycteris) brachypterus*</i> <i>Mops (Xiphonycteris) nanulus*</i> <i>Mops (Xiphonycteris) petersoni</i> <i>Mops (Xiphonycteris) spurrelli</i> <i>Mops (Xiphonycteris) thersites*</i> <i>Mormopterus acetabulosus</i> <i>Mormopterus acetabulosus acetabulosus</i> <i>Mormopterus acetabulosus natalensis</i> <i>Mormopterus francoismoutoui</i> <i>Mormopterus jugularis</i> <i>Myopterus daubentonii</i> <i>Myopterus whitleyi*</i> <i>Otomops harrisoni</i> <i>Otomops madagascariensis</i> <i>Otomops martiensseni</i> <i>Otomops martiensseni icarus</i> <i>Otomops martiensseni martiensseni</i> <i>Platymops setiger</i> <i>Platymops setiger macmillani</i> <i>Platymops setiger setiger</i> <i>Sauromys petrophilus</i> <i>Sauromys petrophilus erongensis</i> <i>Sauromys petrophilus haagneri</i> <i>Sauromys petrophilus petrophilus</i> <i>Sauromys petrophilus umbratus</i> </p>

Bat family	Morphological characteristics of the bat family	Acoustic characteristics of the bat family	Species name of the bat family
			<i>Tadarida aegyptiaca</i> <i>Tadarida aegyptiaca aegyptiaca</i> <i>Tadarida aegyptiaca bocagei</i> <i>Tadarida fulminans</i> <i>Tadarida lobata</i> <i>Tadarida teniotis</i> <i>Tadarida ventralis</i>
Cistugonidae	<ul style="list-style-type: none"> • Face: not dog like face • Eyes: small • Noseleaf: none • Ears: separate • Tragus: well developed • Tail: long tail completely within uroplagium • Claws on wings: one claw on the wing • Other criteria: fur long with yellow wash, gland in each wing membrane 	Intermediate frequencies (30 – 70 kHz) ranging from broadband to narrowband of intermediate duration (3 – 8 ms)	<i>Cistugo lesueuri</i> <i>Cistugo seabrae</i>
Miniopteridae	<ul style="list-style-type: none"> • Face: not dog like face • Eyes: small • Noseleaf: none • Ears: usually separated • Tragus: well developed • Tail: long tail completely within uroplagium • Claws on wings: one claw on the wing • Other criteria: second phalanx 	Intermediate frequencies (30 – 70 kHz) ranging from broadband to narrowband of intermediate duration (3 – 8 ms)	<i>Miniopterus aelleni</i> <i>Miniopterus africanus</i> <i>Miniopterus ambohitrensis</i> <i>Miniopterus arenarius</i> <i>Miniopterus brachytragos</i> <i>Miniopterus cf. fraterculus</i> <i>Miniopterus cf. inflatus</i> <i>Miniopterus egeri</i> <i>Miniopterus fraterculus</i>

Bat family	Morphological characteristics of the bat family	Acoustic characteristics of the bat family	Species name of the bat family
	<p>of third digit three times longer than first phalanx</p>		<p><i>Miniopterus gleni</i> <i>Miniopterus griffithsi</i> <i>Miniopterus griveaudi</i> <i>Miniopterus inflatus*</i> <i>Miniopterus inflatus inflatus</i> <i>Miniopterus inflatus rufus</i> <i>Miniopterus maghrebensis</i> <i>Miniopterus mahafaliensis</i> <i>Miniopterus majori</i> <i>Miniopterus manavi</i> <i>Miniopterus minor*</i> <i>Miniopterus mossambicus</i> <i>Miniopterus natalensis</i> <i>Miniopterus newtoni</i> <i>Miniopterus nimbae</i> <i>Miniopterus petersoni</i> <i>Miniopterus schreibersii</i> <i>Miniopterus sororculus</i> <i>Miniopterus villiersi</i> <i>Miniopterus wilsoni</i> <i>Miniopterus "incertae-sedis"</i></p>
<p>Vespertilionidae</p>	<ul style="list-style-type: none"> • Face: not dog like face • Eyes: small • Noseleaf: none • Ears: usually separated • Tragus: well developed • Tail: long tail completely within uroplagium 	<p>Intermediate frequencies (30 – 70 kHz) ranging from broadband to narrowband of intermediate duration (3 – 8 ms)</p>	<p><i>Kerivoula africana</i> <i>Kerivoula argentata</i> <i>Kerivoula cuprosa</i> <i>Kerivoula eriophora</i> <i>Kerivoula lanosa*</i> <i>Kerivoula phalaena*</i> <i>Kerivoula smithii</i></p>

Bat family	Morphological characteristics of the bat family	Acoustic characteristics of the bat family	Species name of the bat family
	<ul style="list-style-type: none"> • Claws on wings: one claw on the wing • Other criteria: second phalanx of third digit not particularly lengthened 		<i>Myotis anjouanensis</i> <i>Myotis bocagii*</i> <i>Myotis bocagii bocagii</i> <i>Myotis bocagii cupreolus</i> <i>Myotis capaccinii</i> <i>Myotis dieteri*</i> <i>Myotis emarginatus</i> <i>Myotis goudoti</i> <i>Myotis morrisi</i> <i>Myotis mystacinus</i> <i>Myotis nimbaensis</i> <i>Myotis punicus</i> <i>Myotis scotti</i> <i>Myotis tricolor</i> <i>Myotis welwitschii</i> <i>Myotis zenatius</i> <i>Scotophilus altilis</i> <i>Scotophilus andrewreborii</i> <i>Scotophilus borbonicus</i> <i>Scotophilus dinganii*</i> <i>Scotophilus ejetai</i> <i>Scotophilus leucogaster</i> <i>Scotophilus livingstonii</i> <i>Scotophilus marovaza</i> <i>Scotophilus nigrita</i> <i>Scotophilus nigritellus</i> <i>Scotophilus nucella</i> <i>Scotophilus nux</i>

Bat family	Morphological characteristics of the bat family	Acoustic characteristics of the bat family	Species name of the bat family
			<i>Scotophilus robustus</i> <i>Scotophilus tandrefana</i> <i>Scotophilus trujilloi</i> <i>Scotophilus viridis</i> <i>Scotophilus "incertae-sedis"</i> <i>Eptesicus (Cnephaeus) bottae</i> <i>Eptesicus (Cnephaeus) hottentotus</i> <i>Eptesicus (Cnephaeus) isabellinus</i> <i>Eptesicus (Cnephaeus) platyops</i> <i>Eptesicus (Rhinopterus) floweri</i> <i>Glauconycteris alboguttata</i> <i>Glauconycteris argentata*</i> <i>Glauconycteris atra</i> <i>Glauconycteris beatrix*</i> <i>Glauconycteris cf. beatrix</i> <i>Glauconycteris cf. humeralis</i> <i>Glauconycteris curryae</i> <i>Glauconycteris egeria</i> <i>Glauconycteris gleni</i> <i>Glauconycteris humeralis</i> <i>Glauconycteris kenyacola</i> <i>Glauconycteris machadoi</i> <i>Glauconycteris poensis</i> <i>Glauconycteris superba</i> <i>Glauconycteris variegata*</i> <i>Nyctalus azoreum</i> <i>Nyctalus lasiopterus</i> <i>Nyctalus leisleri</i>

Bat family	Morphological characteristics of the bat family	Acoustic characteristics of the bat family	Species name of the bat family
			<i>Pipistrellus (Pipistrellus) aero</i> <i>Pipistrellus (Pipistrellus) hanaki</i> <i>Pipistrellus (Pipistrellus) hesperidus</i> <i>Pipistrellus (Pipistrellus) hesperidus broomi</i> <i>Pipistrellus (Pipistrellus) hesperidus hesperidus</i> <i>Pipistrellus (Pipistrellus) hesperidus subtilis</i> <i>Pipistrellus (Pipistrellus) inexpectatus</i> <i>Pipistrellus (Pipistrellus) kuhlii</i> <i>Pipistrellus (Pipistrellus) maderensis</i> <i>Pipistrellus (Pipistrellus) nanulus</i> <i>Pipistrellus (Pipistrellus) permixtus</i> <i>Pipistrellus (Pipistrellus) pipistrellus</i> <i>Pipistrellus (Pipistrellus) pygmaeus</i> <i>Pipistrellus (Pipistrellus) raceyi</i> <i>Pipistrellus (Pipistrellus) rusticus</i> <i>Pipistrellus (Pipistrellus) simandouensis</i> <i>Pipistrellus *(Pipistrellus)* sp. aff. *kuhlii*</i> <i>Pipistrellus (Pipistrellus) "incertae-sedis"</i> <i>Scotoecus albigula</i> <i>Scotoecus albofuscus</i> <i>Scotoecus hindei</i> <i>Scotoecus hirundo</i> <i>Vansonia rueppellii</i> <i>Vansonia rueppellii fuscipes</i> <i>Vansonia rueppellii pulcher</i> <i>Vansonia rueppellii rueppellii</i> <i>Vansonia rueppellii senegalensis</i>

Bat family	Morphological characteristics of the bat family	Acoustic characteristics of the bat family	Species name of the bat family
			<i>Vansonia rueppellii vernayi</i> <i>Barbastella barbastellus</i> <i>Barbastella leucomelas</i> <i>Otonycteris hemprichii</i> <i>Plecotus balensis</i> <i>Plecotus christii</i> <i>Plecotus gaisleri</i> <i>Plecotus kolombatovici</i> <i>Plecotus teneriffae</i> <i>Afronycteris helios</i> <i>Afronycteris nana*</i> <i>Hypsugo ariel</i> <i>Hypsugo cf. eisentrauti</i> <i>Hypsugo musciculus</i> <i>Hypsugo savii</i> <i>Laephotis angolensis</i> <i>Laephotis botswanae</i> <i>Laephotis capensis</i> <i>Laephotis humbloti</i> <i>Laephotis kirinyaga</i> <i>Laephotis malagasyensis</i> <i>Laephotis matroka</i> <i>Laephotis namibensis</i> <i>Laephotis robertsi</i> <i>Laephotis stanleyi</i> <i>Laephotis wintoni</i> <i>Laephotis "incertae-sedis"</i> <i>Mimetillus moloneyi*</i>

Bat family	Morphological characteristics of the bat family	Acoustic characteristics of the bat family	Species name of the bat family
			<i>Mimetillus thomasi</i> <i>Neoromicia aff. guineensis</i> <i>Neoromicia anchietae</i> <i>Neoromicia bemaity</i> <i>Neoromicia guineensis*</i> <i>Neoromicia somalica*</i> <i>Neoromicia zuluensis</i> <i>Nycticeinops bellieri</i> <i>Nycticeinops crassulus*</i> <i>Nycticeinops eisentrauti</i> <i>Nycticeinops grandidieri</i> <i>Nycticeinops happoldorum</i> <i>Nycticeinops macrocephalus</i> <i>Nycticeinops schlieffenii</i> <i>Nycticeinops "incertae-sedis"</i> <i>Pseudoromicia brunnea*</i> <i>Pseudoromicia isabella</i> <i>Pseudoromicia kityoi</i> <i>Pseudoromicia nyanza</i> <i>Pseudoromicia rendalli*</i> <i>Pseudoromicia roseveari</i> <i>Pseudoromicia tenuipinnis*</i>

Appendix 2: Glossary of key terminology

Terminology	Definition	References
Doppler effect	Change in frequency of a sound wave perceived by a listener moving relative to the source of the sound. The most commonly used example to explain this mechanism is the frequency of the siren on a moving emergency vehicle. Initially, it starts out high, slides down as it passes by the person perceiving the sound, and decreases as the emergency vehicle moves away.	(M. B. Fenton et al., 2016)
Ecosystem service	“The benefits people obtain from ecosystems”	(IPBES core glossary, 2024)
Zoonoses/zoonosis	An infection or disease from a pathogenic agent (virus, bacteria, parasite, fungus, ..) that is naturally transmitted between animals and human.	(WHO, 2020)
Emerging infectious diseases (EID)	“Infectious diseases that are newly recognized in a population or have existed but are rapidly increasing in incidence or geographic range”	(McArthur, 2019)
Reservoir	“An ecological system in which an infectious agent survives indefinitely. It may or may not show signs of disease”	(Markotter et al., 2020)
Host	“The population of concern to the observer”	(Haydon et al., 2002)
Maintenance host population	Population larger than the critical community size (i.e. size under which the pathogen cannot be maintained in the community) in which the pathogen persists even in the complete absence of transmission from other hosts	(Haydon et al., 2002; Nugent, 2011)
Maintenance host community/host complex	“One or more epidemiologically connected populations or environments in which the pathogen can be permanently maintained”	(A. Fenton & Pedersen, 2005; Haydon et al.,

Terminology	Definition	References
	<p>“Any host complex in which disease persists indefinitely is a reservoir”</p> <p>“Host for which cross species transmission and inter-species transmission are high”</p>	2002; Nugent, 2011)
Bridge host	<p>“Non-maintenance host population able to transmit a pathogen from a maintenance host/complex to the target population, otherwise not or loosely connected to the maintenance complex”</p>	(Caron et al., 2015)
Intermediate host/secondary host	<p>A host that harbours the pathogen and transmits it to another host. In case of viral pathogens, this may accumulate mutation, recombination events or concentration to make it capable of transmission to other hosts.</p>	(Markotter et al., 2020)
Pathogen spillover	<p>« the pathogen typically reaches high prevalence in the reservoir, then spills over into the other host”</p>	(Daszak et al., 2000)

1.9 References Chapter 1

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Transition 1 : « Fantôme de nos nuits »

Le chapitre précédent nous a permis de synthétiser les connaissances actuelles de l'écologie et de la biologie de cet unique mammifère volant qu'est la chauve-souris. Malgré leur grande diversité, ces animaux restent mystérieux, encore peu étudiés et de nombreuses interrogations perdurent sur leur écologie. Les freins à leur étude demeurent leur rythme de vie nocturne, leur capacité de vol et la petite taille d'un grand nombre d'espèces de chauves-souris.

L'Afrique Centrale est le berceau de la deuxième plus importante forêt équatoriale au monde et abrite une diversité foisonnante et variée, dont celle des chiroptères. Cette région a également été à plusieurs reprises le foyer de plusieurs émergences zoonotiques d'importance provenant de la faune sauvage. Malgré ce contexte, les ouvrages de références actuels et la littérature scientifique sur les Chiroptères des pays d'Afrique Centrale comme la République du Congo sont déficients (ex : l'identification des espèces de chauves-souris insectivores africaines).

Entre 1878 et 2013, seules 17 études ont inventorié ou actualisé la liste des espèces présentes en République du Congo. Le **Chapitre 2** a pour vocation de contribuer à la connaissance de certaines espèces cavernicoles du Congo en se concentrant en particulier sur l'identification dans deux grottes de communautés de chauves-souris insectivores et sur leur écologie. Le résumé graphique (**Box 1**) illustre les principaux points importants de ce chapitre.

Transition 1 (English version): "Ghost of our nights"

The previous chapter has enabled us to summarise current knowledge of the ecology and biology of this unique flying mammal, the bat. Despite their great diversity, these animals remain mysterious, still little studied and many questions remain about their ecology. The obstacles to their study remain their nocturnal lifestyle, their ability to fly and the small size of many bat species.

Central Africa is home to the world's second-largest equatorial forest, and to a rich and varied diversity of species, including chiropterans. The region has also been the focus of several major zoonotic outbreaks in wildlife. Despite this background, current reference works and scientific literature on chiropterans in Central African countries such as the Republic of Congo are deficient (e.g. identification of African insectivorous bat species).

Between 1878 and 2013, only 17 studies have inventoried or updated the list of species present in the Republic of Congo. The aim of **Chapter 2** is to contribute to our knowledge of some of the cave-dwelling species in Congo, focusing in particular on the identification of communities of insectivorous bats in two caves and on their ecology. The graphical summary (**Box 1**) illustrates the main points of this chapter.

CHAPTER 2 – ARTICLE 1: CHARACTERIZATION OF INSECTIVOROUS BAT COMMUNITIES IN REPUBLIC OF CONGO USING MORPHOMETRIC AND ACOUSTIC DATA.

Based on the publication in prep. for *Acta Chiropterologica*:

Labadie, M., Morand, S., Niama, F. R., Caron, A., Nguilili, F., N’Kaya, T., Bourgarel, M., De Nys, H., 2024. Characterization of insectivorous bat communities in Republic of Congo using morphometric and acoustic data.

Box 1: Graphic abstract Chapter 2

Main objectives:

- To differentiate bats communities using Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) with morphological and acoustic data
- To describe species richness and reproductive status during 11 capture sessions

Methodology



11 capture sessions of bats for each cave using a Harp trap near the main entrance



692 bats captured



- Boundou cave : 353 individuals
- Mont Belo cave : 339 individuals

Collected data

- 9 to 10 morphometric measurements according to bat families ;
- Identification of bat family, sex and reproductive status ;
- acoustic calls recorded for 206 individuals to use as reference



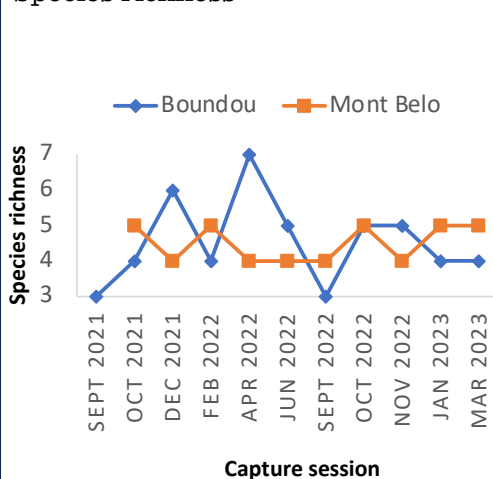
Focus on the top 4 most captured species :

- *Rhinolophus sp.* (N = 123)
- *Triadenops sp.* (N = 73)
- *Miniopterus sp.* (N = 152)
- *Hipposideros sp.* (N = 160)

PCA and LDA for morphometric and acoustic data

Species	Analysis	Morphometric data	Acoustic data
<i>Hipposideros sp.</i>	PCA	1 single group of individuals (sex and site included)	2 groups of individuals (sex and site included)
<i>Miniopterus sp.</i>	PCA/LDA	2 groups of individuals (sex and site included)	1 group of individuals (sex and site included)
<i>Rhinolophus sp.</i>	PCA/LDA	2 groups of individuals (sex and site included)	2 groups of individuals (sex and site included)
<i>Triadenops sp.</i>	PCA	1 single group of individuals (sex and site included)	2 groups of individuals by sex

Species richness



Reproductive status (year)

Species	Reproductive status	Boundou		Mont Belo	
<i>Hipposideros cf. caffer</i>		09, 10, 11, 12, 02, 04	03, 06	09, 10, 12, 01, 04	01, 02
<i>Miniopterus cf. minor</i>		09, 10, 03	01, 06	09, 10, 11, 06	01, 06
<i>Miniopterus cf. inflatus</i>		06	?	09, 10, 04, 06	?
<i>Rhinolophus cf. landeri-alcione</i>		?	?	09, 10, 02, 06	?
<i>Rhinolophus cf. denti</i>		09, 11	?	09, 11, 01	?
<i>Triadenops cf. afer</i>		09, 10, 11, 12, 01, 02, 04, 06	?		

: Pregnancy/lactation

: Copulation

Conclusion:

We identified the presence of 5 common species in the two caves: *Rhinolophus cf. landeri-alcione*, *Rhinolophus cf. denti*, *Hipposideros cf. caffer*, *Miniopterus cf. minor* and *Miniopterus cf. inflatus*.

The acoustic data suggest the potential presence of two cryptic species in *Hipposideros cf. caffer* (undetected by morphometric data). In addition, *Triadenops sp.* in Boundou cave showed sex differences through PCA of acoustic data

Characterization of insectivorous bat communities in Republic of Congo using morphometric and acoustic data.

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2.1 Abstract

Bats are the most abundant mammals in the world, and represent the second most numerous mammal species after rodents. They are well known for their crucial contribution to ecosystems' health (pollination, regulation of insect pests), but also as reservoir of zoonotic pathogens. However, our understanding of bats worldwide, and African bats in particular, remains patchy. Morphological and acoustic studies are needed to provide insights in bat diversity and distribution to promote their conservation while mitigating bat-associated health risks. Over a nineteen month study, we investigated bat communities in two separate caves in the Republic of Congo. We carried out eleven capture sessions at the entrance to the caves in order to collect morphometric and acoustic data. Principal component analysis (PCA) on morphometric data confirmed the presence of four bat genera (*Hipposideros*, *Miniopterus*, *Rhinolophus* and *Triaenops*) with six putative species. The use of linear discriminant analysis (LDA) on morphometric data validated these six putative bat species: *Hipposideros cf. caffer*, *Triaenops cf. afer*, *Miniopterus cf. minor*, *Miniopterus cf. inflatus*, *Rhinolophus cf. landeri-alcyone* and *Rhinolophus cf. denti*. In addition, PCA performed using acoustic data revealed the potential presence of two different species within the *Hipposideros cf. caffer* complex. Finally, PCA on acoustic data revealed sex difference in acoustic parameters in *Triaenops*.

Keywords: Principal component analysis, bat communities, acoustics, linear discriminant analysis

2.2 Introduction

In the context of current global changes and increasing health crises, understanding the ecology of our environment and its wildlife is becoming essential. Bats are the most abundant mammal in terms of biomass (Greenspoon et al., 2023), they are notorious for their role in the spread of zoonotic diseases (Cros, 2020; Dobson, 2005). Yet, they provide three key ecosystem roles (Ramírez-Fráncel et al., 2022) by (1) regulating insect pests and limiting damage to many crops, (2) enabling the spread and pollination of 549 plant species worldwide and (3) producing guano which serves as natural fertilizer. Over the last two decades, the number of identified bat species has increased by more than 30% (Mammal Diversity Database, 2023; Simmons & Cirranello, 2023; Wilson & Reeder, 2005). This achievement was possible thanks to taxonomic revision of the Chiroptera order (Solari et al., 2019; Taylor et al., 2018, 2022), the use of new capture techniques (such as the harp trap) (Tanshi & Kingston, 2021), the increasing number of bat surveys in remote locations (Moura & Jetz, 2021), and the progress in phylogenetics (Mayer et al., 2007) that led to the discovery of new species (Frick et al., 2020; Monadjem et al., 2016).

The order Chiroptera, which comprises 1,470 species, was traditionally divided into two main suborders commonly referred to as the megabats (Megachiroptera, Old World fruit eaters) and the microbats (Microchiroptera, echolocation bats). However, recent molecular studies have revealed a close relationship between the Pteropodidae and some microbats (Hutcheon & Kirsch, 2006). The order Chiroptera is now divided into two new suborders: the Yinpterochiroptera (also known as Pteropodiformes) and the Yangochiroptera (also known as Vespertilioniformes) (Hutcheon & Kirsch, 2006; Tsagkogeorga et al., 2013). Five of the six bat families within the Yinpterochiroptera sub-order are present in Africa, with the Pteropodidae, Rhinopomatidae, Megadermatidae, Rhinolophidae and Hipposideridae. The Yangochiroptera suborder comprises 13 distinct families, including Molossidae, Emballonuridae, Nycteridae and Miniopteridae (Monadjem et al., 2020; Teeling et al., 2005). In the Republic of Congo, bat inventories have been scarce with only 17 surveys performed between 1897 and 2013. The latest study by Bates et al. (2013) recorded the presence of 43 species. However, based on current knowledge from neighboring countries and ecoregions, Simmons and Ciranello (2023) estimated that 63 bat species belonging to 8 families should be present in the country. The

absence of a reference work on bat species present in Central Africa and in particular in the Republic of Congo underlines the importance of pursuing efforts to inventory and collect information.

Furthermore, certain morphometric measurements for several bat species are missing, or have only been recorded for a small number of individuals. The study of bat morphology not only enables rapid identification, but can also contribute to understand the evolution of morphological adaptation (such as the wing morphology in relation to the ecological niche used or foraging behavior) (Jeyaprabha et al., 2023; Luo et al., 2019; Miller-Butterworth et al., 2003). Morphological features helping distinguish bats at family level and sometimes up to species level are: body size, shape of nose, ears, tail, wings, eyes and dental formula (Monadjem et al., 2020). Some bat species, however, have similar morphological criteria due to their close phylogenetic relationship, which makes their identification difficult. Conversely, some bat species may also show intraspecific morphological variations depending on location (Vallo et al., 2018). Cryptic species can be differentiated using an integrative taxonomy that takes into account morphology, genetics and acoustics. Indeed, all insectivorous bats use acoustics to move and hunt. The acoustic call of a bat is specific to a genus and can be potentially distinguished down to species level by taking into account various parameters such as the sonotype (call shape) and acoustic frequency (Frequency maximum, Frequency minimum, duration, etc.) (Arias-Aguilar et al., 2018; Britzke et al., 2002; Parsons & Jones, 2000).

During eleven capture sessions over a period of nineteen months, we collected several morphometric, acoustics and genetic data on cave dwelling bats in two caves in the Republic of Congo. We described the bat communities at each site using multivariate analyses to differentiate genera and, when possible, species using relevant external morphometric data as well as acoustics data.

2.3 Materials and Method

2.3.1 Ethic statements

Capture and handling of bats was carried out with the permission from the Ministry of Forest Economy and Ethics Committee of the Ministry of Scientific Research and Technological Innovation in the Republic of Congo (N°212/MRSIT/IRSSA/CERSSA and N°687/MEF/CAB/DGEF-DFAP). All animals were handled carefully during morphometric measurements and sample collection following recommendations by Kunz and Parsons (2009). All individuals were released as soon as possible directly to their place of capture. If an animal was injured during capture, restraint or measurement, it was euthanized as quickly as possible. Pregnant or nursing females were handled as quickly as possible or released immediately, depending on their condition.

2.3.2 Study sites

We investigated insectivorous bat populations in two caves: Mont Belo Cave and Boundou Cave, in the Bouenza and Niari departments of the Republic of Congo respectively (**Fig. 11**). The Mont Belo cave (N site: GTCO 01) is surrounded by a small tertiary forest in a farming complex close to the small village of the same name. The cave has one main chamber and two secondary chambers. The Boundou cave (N site: GTCO 03), dug into the cavity of a rocky outcrop, is smaller, with one main chamber and another secondary cavity (not easily accessible). It is also surrounded by a patch of tertiary forest in a predominantly grassy savannah habitat with patches of forest. In the two studied caves, we observed the presence of between a hundred and a thousand individuals of insectivorous bats, depending on the season. Fruit-eating bats, on the other hand, were only slightly present, varying from zero individuals per month to a maximum of around thirty.

Figure 11: Map showing the location of the two caves in our study in the Republic of Congo. Mont Belo cave is in the Bouenza department and Boundou cave in the Niari department



2.3.3 Sampling and data acquisition

i. Capture session

During eleven different fieldwork sessions between September 2021 and March 2023, we installed a Harp trap (© Ecotone) near the main entrance of each cave between 5 pm and 10 pm. We were unable to capture bats at Mont Belo during the September 2021 session due to the absence of bats at that time. Their absence was potentially due to a fire in the surrounding crops that may have smoked out the cave, no doubt forcing the bats to flee the cave. In total, we captured 692 bats with 353 individuals at the Boundou cave (N site: GTCO 03) and 339 at Mont Belo cave (N site: GTCO 01) (**Fig. 11**). Each captured individual was photographed, sexed, and measured for its morphometric characteristics. We recorded also the reproductive status of each individual. Morphometric data for all individuals caught during our study are presented and available in **Appendix 3** (sheet 1).

ii. Preliminary identification of bat species

In the field, a preliminary identification was done up to the genus level or, if possible, to species level based on external morphological (e.g. shape of the nose, size of the eyes and body) trait using reference books (Bates et al., 2013; Kingdon, 2014; Monadjem et al., 2020).

iii. Morphometric data

For each captured bat, we measured the following morphological characters: total weight in grams (WT); forearm length in mm (FA); wingspan between wing tips in straight line (WS); length of head and body from tip to anus (HB); total length from tip of snout to posterior end of tail (TL); tail length from tip to anus (T); length of ear from notch to tip (E); the length from top of knee to base of ankle when both knee and ankle are bent (Tib) and length of hindfoot from heel to tip of longest claw (HF).

For bats recognized as belonging to *Rhinolophus*, *Triaenops* and *Hipposideros* genera we measured NL breadth (the maximum breadth of the noseleaf) and NL length (the length of the noseleaf) and for *Miniopterus* genus, we measured the length of tragus (usually from tip to junction of posterior margin with the ear (Tr) (**Appendix 4**). These additional morphometric measurements are specific to these genera. We used a dial caliper (© Ecotone) for the

measurement of FA, a tape measure for WS and HB, a ruler (© Ecotone) for T, E, Tr, Tib, HF, NL breadth and NL length and a Pesola balance of 100g (© Pesola - Ecotone) to weight bats. To minimize bias, measurements were taken by a single person and all lengths were in millimetres.

For the subsequent analyses, we removed pregnant females, females carrying a youngster, as well as all individuals with missing data. In total, our morphometric analyses were based on 508 individuals.

iv. Acoustics recording

For 129 individuals, acoustic calls were recorded with a portable recorder, Pettersson M500 – 354 USB (Pettersson Elektronik AB, Sweden), that was used when bats were either in the pouch (Zamora-Gutierrez et al., 2020) before being handled, or on release after manipulation for morphometric measurements as they flew away. To avoid recording calls of other bat species and to reproduce the conditions of a semi-enclosed environment (cave exit), we decided to use a new methodology. Recording reference sounds was therefore used for 30 additional individuals with at least one male and one female for each genus, except *Hipposideros sp.* (as only pregnant females were captured, we avoided to manipulate them). Individuals were released in early morning to fly freely in the room of a building, allowing the acoustic calls to be recorded using an SM4BAT recorder and its U1 microphone (Wildlife acoustics, Maynard, MA, USA).

v. Characterization of sonotype acoustics and acoustic parameters

Inside each reference audio file (N= 205 individuals with acoustic file), we selected a sequence of several calls of a single species with the best quality (without sound noise, without overlapping calls of several species, etc.). The acoustic parameters of the acoustic calls were estimated automatically on each reference acoustic file using Kaleidoscope 5.6.2 (Wildlife Acoustics, Maynard, MA, USA): Fpmean: mean frequency of the spectrum within the selection (multiple acoustic call); Fppeak: frequency which has the highest (Peak) energy within the selection; Fmax: average maximum frequency of call pulses; Fc: frequency of the point at the end of the body of the call pulse which is defined as the flattest part (lowest absolute slope) of the call; Fk: average knee frequency of calls; Dur (ms): average duration of call pulses within

the selection; Fmin: average minimum frequency of call pulses and Fmean: average frequency of echolocation pulses. If an individual had several acoustic reference files, the acoustic parameters were estimated on the average values. All acoustic parameters are given in the **Appendix 3** (sheet 2).

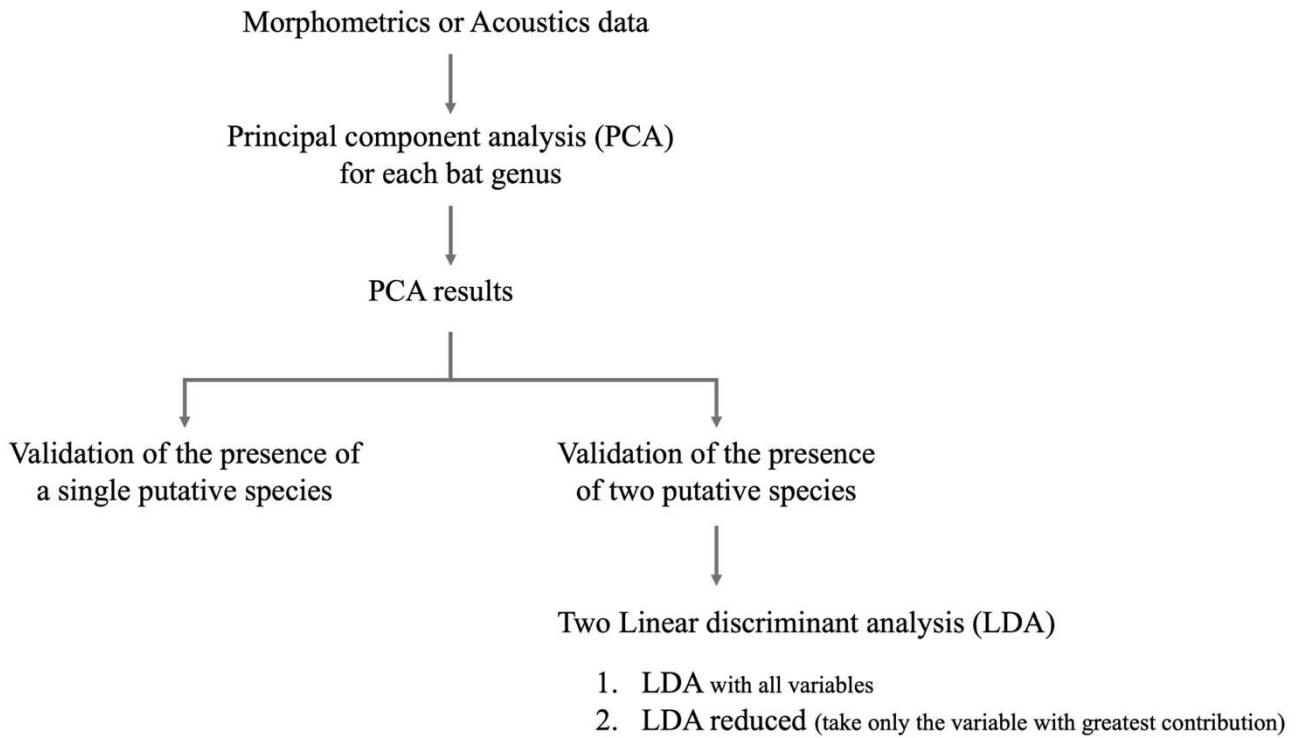
2.3.4 Statistical analysis

We applied principal component analysis (PCA) to identify the morphological traits which can be used to distinguish species and to confirm our presumed preliminary identification. To this end, we first standardized the morphometric data using the “log shape ratio” method to standardize all variables (Mosimann, 1970). This method makes it possible to transform the measurements into a series of ratios facilitating comparisons between individuals. Each individual measurement was divided by the mean total body size of the genus (TL), with the exception of the overall body size measurement, which was divided by the geometric mean of all measurements (Claude, 2013). A logarithmic transformation was then applied to all values to obtain the log shape ratio. These values were used as input data for the subsequent analyses. For acoustic values, no standardization of data was carried out in order to maintain variability.

We then performed PCAs using morphometric data for each genus to distinguish putative species. The PCAs enabled us to identify the morphological traits with the greatest weight for species identification, and to test the effects of study site (cave) and sex on morphometric measurements of the different genera. If the PCA confirmed the presence of distinct groups of species, we then carried out separate linear discriminant analyses (LDAs). A first LDA took into account all morphometric variables for each genus separately. A second LDA (LDA reduced) took only the morphometric variables with the greatest contribution to the PCAs performed on each genus. The same analysis procedure was repeated for the acoustic data. **Figure 12** summarizes the various stages of these analyses.

All analyses were performed using R software (R Core Team, 2023) using the following packages: ‘MASS’ (Venables et al., 2002), ‘FactoMineR’ (Lê et al., 2008), ‘cluster’ (Maechler et al., 2022) and ‘ggfortify’ (Tang et al., 2016).

Figure 12: Diagram of the statistical analysis process for morphometric and acoustic data in this study.



2.4 Results

2.4.1 Preliminary morphological identification

We identified six families of bats: *Pteropodidae*, *Emballuridae*, *Rhinolophidae*, *Miniopteridae*, *Rhinonycteridae* and *Hipposideridae* (**Appendix 5** and **Fig. 13**). In this study, we focused on the species with the greatest number of captures: *Rhinolophus spp.*, *Miniopterus spp.*, *Hipposideros sp.* and *Triaenops sp.*. The other three bat species, *Macronycteris gigas*. (n = 4), *Coleura afra* (n =1) and *Rousettus aegyptiacus* (n =1), were caught on very rare occasion during the nineteen month period of the study.

In both caves, we captured five species: *Miniopterus cf. minor* (N= 71), *Miniopterus cf. inflatus* (N=81), *Rhinolophus cf. landeri-alcyone* (N= 75), *Rhinolophus cf. denti* (N= 48) and *Hipposideros cf. caffer* (N=160). *Triaenops cf. afer* (N= 73) was found only in Boundou cave. The number of individuals per sex (males and females) and per site is shown in **Table 1**.

2.4.2 Species richness

Over the eleven capture sessions (six sessions for Boundou and five for Mont Belo), values of species richness were almost similar in the two caves (**Fig. 14**). In Boundou cave, the highest richness was recorded in April 2022 (seven species), followed by December 2021 (six species). Conversely, species richness was lowest during the two September sessions (Sept 2021, Sept 2022) (**Fig. 14**). For Mont Belo cave, four species were captured during five sessions and five species for the other five sessions (**Fig. 14**).

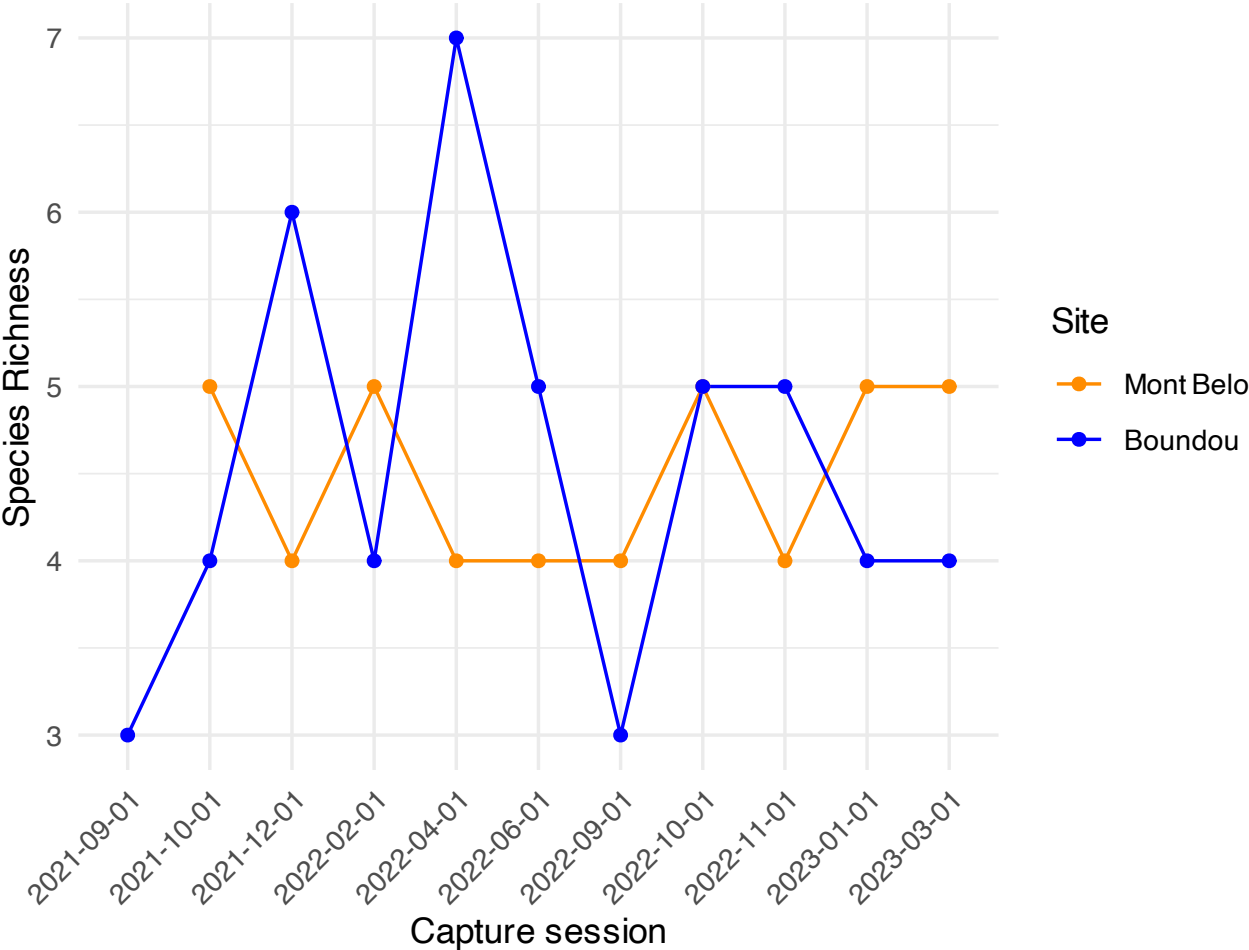
Figure 13: Bats Face book of some individuals of each genus captured and measured



Table 1: Total number of individuals captured, measured and identified at the level of preliminary identification of the species according to site (Mont Belo and Boundou) and sex.

Study sites	Mont Belo cave (GCSCO-01)		Boundou cave (GCSCO-03)		Total by species	Total by genus
	Sex					
Species	Male	Female	Male	Female		
<i>Rhinolophus cf. landeri- alcyone</i>	30	41	3	1	75	123
<i>Rhinolophus cf. denti</i>	10	3	6	29	48	
<i>Miniopterus cf. minor</i>	14	7	39	11	71	152
<i>Miniopterus cf. inflatus</i>	75	4	2	0	81	
<i>Hipposideros cf. caffer</i>	57	13	38	52	160	160
<i>Triaenops cf. afer</i>	0	0	40	33	73	73

Figure 14: Bat species richness according to capture sessions and caves (Boundou and Mont Belo). No capture sessions were carried out at Mont Belo in September 2021 due to the absence of bats.



2.4.3 Reproduction status

The reproductive status of species by site is shown in **Table 2**. Most species had a young-rearing period (pregnancy, parturition and lactation) during the long rainy season (October, November and December) and potentially a mating period during the long dry season (June,..). Some species, such as *Hipposideros spp.*, may have two breeding periods.



2.4.4 Morphometric data

i. PCA on individuals of all genera

The first principal component (PC1) axis represented 76.25 % of shape variation while the second represented 15.77% (**Appendix 6**). PCA based on eight main morphometric data confirmed the presence of the four genera identified in the field: *Rhinolophus*, *Hipposideros*, *Miniopterus* and *Triaenops* (**Appendix 6**).

ii. PCA on individuals of Triaenops genus

The PCA suggested the presence of a unique species on the basis of the morphometric traits used (**Fig. 15A**). All these individuals were trapped in Boundou cave (N site: GTCO 03) and were identified as African trident bat (*Triaenops cf. afer*). Site and sex had no effect on the morphometric variability of individuals. The first axis (PC1) of the PCA on site represented 99.5%, with a strong contribution from two morphometric parameters: weight (WT) and total length (TL). The PCA according to sex (**Fig 15B**) had a PC1 that explained 56.14% and a second component (PC2) that accounted for 31%. The three morphometric parameters with the greatest weight were wing size (WS), total body size (TL) and body length (excluding tail length) (HB) (**Fig. 15A and B**).

Table 2: Breeding and mating periods for different species groups in different caves (Boundou, Mont Belo). The pictogram  represents a period of juvenile rearing, including gestation, parturition and lactation. The pictogram  represents a period of male sexual activity. These data were collected on individuals of the various species caught during the eleven capture sessions. The question mark represents the absence of information on the reproductive status of the species.





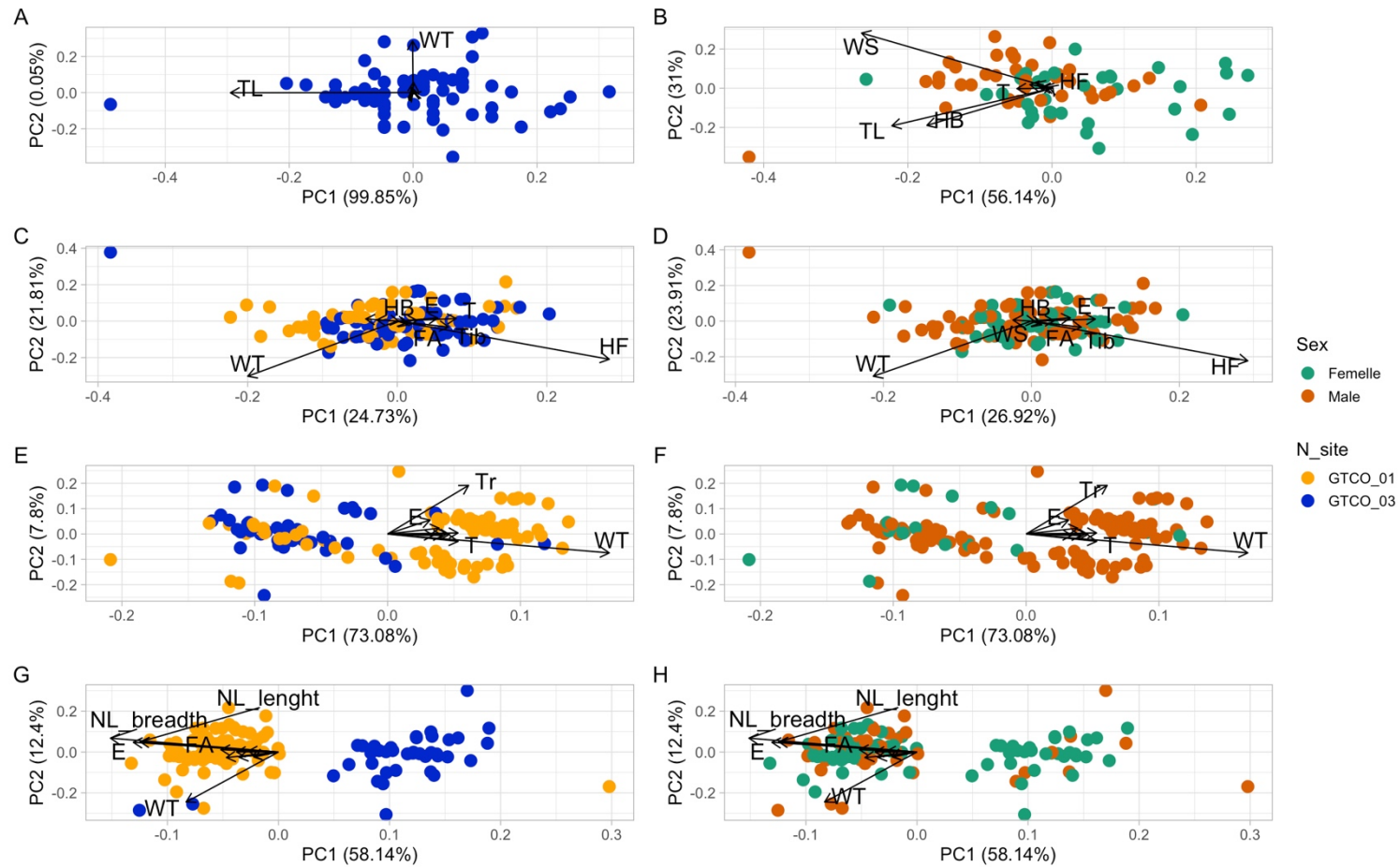
Sites and Reproductive status Species	Boundou		Mont Belo	
				
<i>Hipposideros cf. caffer</i>	Sept, Oct, Nov, Dec, Feb, Apr	Mar, Jun	Sept, Oct, Dec, Jan, Apr	Jan, Feb
<i>Miniopterus cf. minor</i>	Sept, Oct, Mar	Jan, Jun	Sept, Oct, Nov, Jun	Jan, Jun
<i>Miniopterus cf. inflatus</i>	Jun	?	Sept, Oct, Apr, Jun	?
<i>Rhinolophus cf. landeri-alcione</i>	?	?	Sept, Oct, Feb, Jun	?
<i>Rhinolophus cf. denti</i>	Oct, Nov, Jan	?	Oct, Nov, Jan	?
<i>Triaenops cf. afer</i>	Sept, Oct, Nov, Dec, Jan, Feb, Apr, Jun	?		

Figure 15: Principal Component Analysis (PCA) (PC1 vs PC2) based on morphometric measurement transformed into log shape ratio. For *Hipposideros*, *Triaenops* and *Rhinolophus* PCAs based on 10 morphometric data into log shape ratio (TL = total body length, T = tail length, WT = weight, WS = size of wing, HB = body size without tail, FA= forearm, HF =hindfoot, E = ear length, NL breadth = maximum breadth of the noseleaf and NL length = length of the noseleaf). For *Miniopterus* PCA based on nine morphometric data into log shape ratio (TL = total body length, T = tail length, WT = weight, WS = size of wing, HB = body size without tail, FA= forearm, HF =hindfoot, E = ear length and Tr = length of tragus). (A, B) For *Triaenops* depending on site and sex, (C, D) For *Hipposideros* depending on site and sex, (E, F) For *Miniopterus* depending on site and sex and (G, H) For *Rhinolophus* depending on site and sex.



iii. PCA on individuals of *Hipposideros* genus

The PCA for the genus *Hipposideros* showed that all individuals seemed to belong to the same putative species, regardless of capture site or sex. The weight of the PCA according to capture site or sex was almost identical for both PCAs (For site PCA: PC1 = 24.73% and PC2 = 21.8%; For sex PCA: PC1 = 26.92% and PC2 = 23.91%). In both cases, the morphometric variables with the greatest weight were individual weight (WT) and hindfoot (HF) (**Fig. 15C** and **15D**). In the field, all these individuals were identified as Sundevall's roundleaf bat (*Hipposideros cf. caffer*).

iv. PCA on individuals of *Miniopterus* genus

PCA suggested the presence of two putative species (**Fig. 15E** and **15F**). The differentiation of these two groups were more evident when using the raw data without logarithmic transformation of the aspect ratio (**Appendix 7**). These two distinct groups did not appear to differ when comparing capture site or sex (**Fig. 15E** and **15F**). In both cases, PCA had the same weight for capture site and sex (PC1 = 73.08% and PC2 = 7.8%), and the morphometric variables with the most weight were tragus (Tr) and total body weight (WT) (**Fig. 15E** and **15F**). In the field, these individuals were identified as *Miniopterus cf. minor* and *Miniopterus cf. inflatus*.

v. PCA on individuals on *Rhinolophus* genus

PCA showed the potential presence of two putative species in the genus *Rhinolophus* (**Fig. 15G** and **15H**). Capture site and sex had no effect on the morphometric variability (**Fig. 15G** and **15H**). The morphometric variables with the greatest weight were total body weight (WT), ear length (E), maximum width of the nose leaf (NL breadth) and length of the nose leaf (NL length) (**Fig. 15G** and **15H**). A priori, based on morphometric data in the field, two species were identified: *Rhinolophus cf. landeri-alcyone* and *Rhinolophus cf. denti*.

vi. Linear discriminant analysis on *Miniopterus*

Linear discriminant analysis with the nine morphometric variables (TL = total body length, T = tail length, WT = weight, WS = size of wing, HB = body size without tail, FA= forearm, HF =hindfoot, E = ear length and Tr = length of tragus) confirmed the presence of two *Miniopterus* morphological groups (**Table 3**). The identification of two putative species, *Miniopterus cf.*

minor and *Miniopterus cf. inflatus*, in the field was confirmed by the linear discriminant analysis (100% accuracy and no misclassified individuals) (**Table 3**). In contrast, a reduced discriminant analysis (LDA reduced, **Appendix 8**) performed on only two morphometric variables (WT and Tr), showed a decrease in the reliability of the predicted identification of putative species (95% identification of *M. cf. minor* and *M.cf. inflatus* (145/152).

Partition plot on *Miniopterus* morphometric values (on LDA) confirmed the differentiation of the two putative species (**Appendix 9**).

vii. *Linear discriminant analysis on Rhinolophus*

Within the genus *Rhinolophus*, linear discriminant analysis confirmed the presence of two species, previously identified in the field as *Rhinolophus cf. landeri-alcyone* and *Rhinolophus cf. denti* (**Table 4**).

Linear discriminant analysis showed a reliability of 90%, taking into account all morphometric criteria (TL = total body length, T = tail length, WT = weight, WS = size of wing, HB = body size without tail, FA= forearm, HF =hindfoot, E = ear length, NL breadth = maximum breadth of the noseleaf and NL length = length of the noseleaf) (**Table 4**). In the field, we classified the individuals of this genus as 75 *R. fumigatus* and 48 *R. landeri*. The LDA showed a reliability of 97% for *R. cf. landeri-alcyone* (2 misclassified individuals out of 75 identified as *R. cf. landeri-alcyone*) and a reliability of 79% for *R. cf. denti* (10 misclassified individuals out of 48 identified as *R. cf. denti*). The reliability of a reduced LDA, dropped to 88% (**Appendix 10**) with the use of four morphometric variable (WT, E, NL breadth and NL length). The use of a partition plot on *Rhinolophus* morphometric values confirmed the differentiation of the two species and helped identify misclassified individuals (**Appendix 11**).

Table 3: Results of LDA for *Miniopterus* with nine morphometric variables (TL = total body length, T = tail length, WT = weight, WS = size of wing, HB = body size without tail, FA= forearm, HF =hindfoot, E = ear length and Tr = length of tragus)

Species identified in the field	LDA	
	Putative species	
	<i>Miniopterus cf. inflatus</i>	<i>Miniopterus cf. minor</i>
<i>Miniopterus cf. inflatus</i>	81	0
<i>Miniopterus cf. minor</i>	0	71
Percent of correct identification on putative species	100%	100%
Mean percent of identification for LDA	100%	

Table 4: Results of LDA for *Rhinolophus* with ten morphometric variables (TL = total body length, T = tail length, WT = weight, WS = size of wing, HB = body size without tail, FA= forearm, HF =hindfoot, E = ear length, NL breadth = maximum breadth of the noseleaf and NL length = length of the noseleaf)

	LDA	
	Putative species	
Field identification species	<i>Rhinolophus cf. landeri-alcyone</i>	<i>Rhinolophus cf. denti</i>
<i>Rhinolophus cf. landeri-alcyone</i>	73	10
<i>Rhinolophus cf. denti</i>	2	38
Percent of correct identification on putative species	0.97%	0.79%
Mean percent of identification for LDA	0.90%	

2.4.5 Acoustics data

Details of the total number of individuals with a reference acoustic recording according to species, site (Mont Belo and Boundou) and sex are presented in **Table 5**.

*i. PCA on individuals of *Triaenops* genus*

The PCA confirmed the presence of a single species on the basis of the acoustics traits (**Fig. 16A** and **16B**). An effect of sex on the acoustic variability was observed for *Triaenops* individuals. For both PCA according to site and sex (**Fig. 16A** and **16B**) had a PC1 that explained 93.2% and a second component (PC2) that accounted for 4.8%. The five acoustics parameters with the greatest weight were Fmax, Fppeak, Fk, Fc and Fpmean (**Fig. 16A and 16B**).

*ii. PCA on individuals of *Hipposideros* genus*

The PCA on individuals of the genus *Hipposideros* showed that most individuals, with the exception of four individuals (GCSC0642, GCSC0286, GCSC0667 and GCSC0371), appeared to belong to the same putative species, regardless of capture site or sex. The weight of the PCA according to capture site or sex was identical for both PCAs (PCA: PC1 = 51.8% and PC2 = 43.3%). In both cases, the acoustic traits with the greatest weight were Fppeak, Fpmean, Fk, Fmax and Fc (**Fig. 16C and 16D**).

Figure 16: Principal Component Analysis (PCA) (PC1 vs PC2) based on acoustics traits. For *Hipposideros*, *Miniopterus* and *Triaenops* and *Rhinolophus* PCAs based on eight acoustic traits (Fpmean = mean frequency of the spectrum, Fppeak = frequency which has the highest (Peak) energy, Fmax = average maximum frequency of call pulses; Fc = frequency of the point at the end of the body of the call pulse which is defined as the flattest part (lowest absolute slope) of the call; Fk = average knee frequency of calls; Dur = average duration of call pulses in millisecond; Fmin = average minimum frequency of call pulses and Fmean = average frequency of echolocation pulses). (A, B) For *Triaenops* depending on site and sex, (C, D) For *Hipposideros* depending on site and sex, (E, F) For *Miniopterus* depending on site and sex and (G, H) For *Rhinolophus* depending on site and sex.

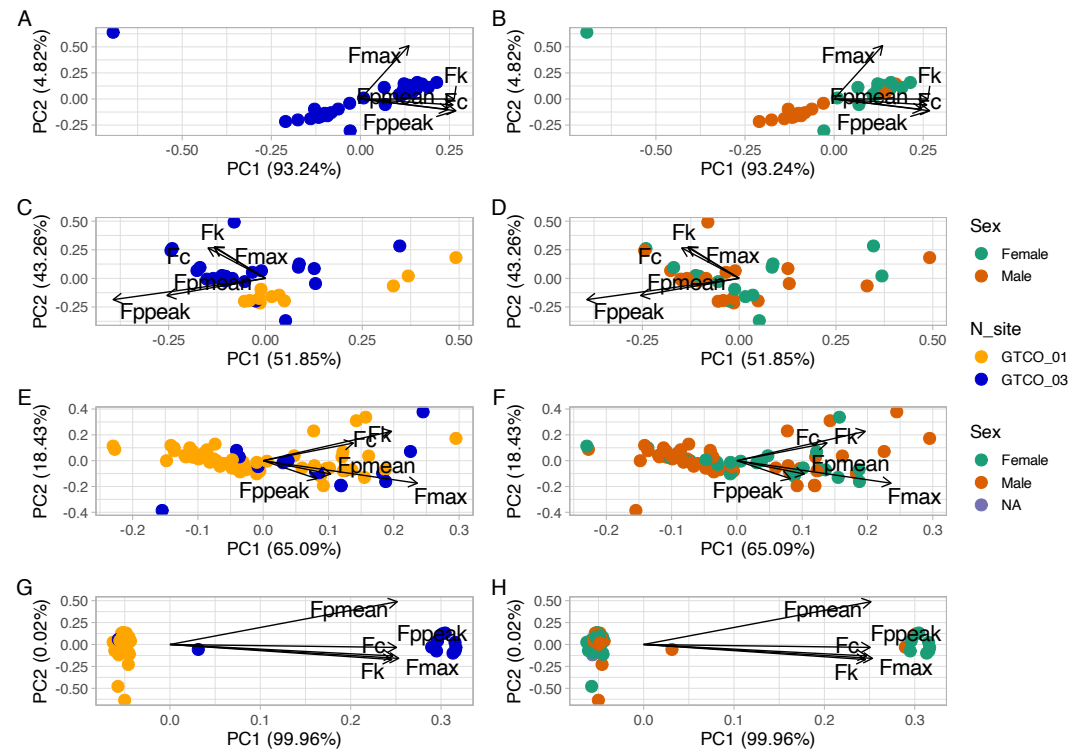


Table 5: Total number of individuals with a reference acoustic recording identified at the level of preliminary identification of the species according to site (Mont Belo and Boundou) and sex.

Study sites	Mont Belo cave (GCSCO-01)		Boundou cave (GCSCO-03)		Total by species	Total by genus
	Sex					
Species	Male	Female	Male	Female		
<i>Rhinolophus cf. landeri- alcyone</i>	15	32	1	1	49	64
<i>Rhinolophus cf. denti</i>	4	1	3	7	15	
<i>Miniopterus cf. minor</i>	10	11	8	5	44	87
<i>Miniopterus cf. inflatus</i>	31	10	2	0	43	
<i>Hipposideros cf. caffer</i>	8	4	12	10	34	34
<i>Triaenops cf. afer</i>	0	0	13	17	30	30

iii. *PCA on individuals of *Miniopterus* genus.*

Unlike the PCA on morphometric data, PCA on acoustic data did not show the presence of two putative species in the genus *Miniopterus* (Fig. 16E and 16F). No differences were observed when comparing capture site or sex (Fig. 16E and 16F). In both cases, the PCA had the same weight for capture site and sex (PC1 = 65% and PC2 = 18.4%). All the acoustic variables seem to have a high weight, with Fmax in first place (Fig. 16E and 16F).

iv. *PCA on individuals of *Rhinolophus* genus*

PCA on acoustic data suggested the presence of two putative species of *Rhinolophus* (Fig. 16G and 16H). Capture site and sex had no effect in explaining morphometric variability (Fig. 16G and 16H). The most significant acoustic variables were Fpmean, Fmax and Fppeak (Fig. 16G and 16H).

v. *Linear discriminant analysis on individuals of *Hipposideros**

Linear discriminant analysis confirmed the potential presence of two species in the *Hipposideros* complex, which were identified in the field as a single species *Hipposideros caffer*. Linear discriminant analysis showed 100% reliability, taking into account all acoustic criteria (Fpmean = average frequency of the spectrum, Fppeak = frequency with the highest energy (Peak), Fmax = average maximum frequency of the call pulses ; Fc = frequency of the point at the end of the body of the call pulse which is defined as the flattest part (lowest absolute slope) of the call; Fk = average knee frequency of calls; Dur = average duration of call pulses in milliseconds; Fmin = average minimum frequency of call pulses and Fmean = average frequency of echolocation pulses) (Table 6). In the field, we classified the 34 individuals recorded acoustically as a single species, *Hipposideros caffer*. The LDA with acoustic data seemed to show the presence of two groups in this complex, with 14 individuals in the *Hipposideros cf caffer* group and 20 individuals in the *Hipposideros cf. caffer 2* group (Table 6).

vi. *Linear discriminant analysis testing individuals of genus *Rhinolophus**

Linear discriminant analysis confirmed the presence of two species, with results identical to LDA with morphometric data (Table 7).

Table 6: Results of LDA for *Rhinolophus* with eight acoustic traits (Fpmean = mean frequency of the spectrum, Fppeak = frequency which has the highest (Peak) energy, Fmax = average maximum frequency of call pulses; Fc = frequency of the point at the end of the body of the call pulse which is defined as the flattest part (lowest absolute slope) of the call; Fk = average knee frequency of calls; Dur = average duration of call pulses in millisecond; Fmin = average minimum frequency of call pulses and Fmean = average frequency of echolocation pulses).

	LDA	
	Putative species	
Field identification species	<i>Rhinolophus cf. landeri-alcyone</i>	<i>Rhinolophus cf. denti</i>
<i>Rhinolophus cf. landeri-alcyone</i>	9	0
<i>Rhinolophus cf. denti</i>	6	49
Percent of correct identification on putative species	0.6%	100%
Mean percent of identification for LDA	0.90%	

Table 7: Results of LDA for *Hipposideros* with eight acoustic traits (Fpmean = mean frequency of the spectrum, Fppeak = frequency which has the highest (Peak) energy, Fmax = average maximum frequency of call pulses; Fc = frequency of the point at the end of the body of the call pulse which is defined as the flattest part (lowest absolute slope) of the call; Fk = average knee frequency of calls; Dur = average duration of call pulses in millisecond; Fmin = average minimum frequency of call pulses and Fmean = average frequency of echolocation pulses).

	LDA	
	Putative species	
Field identification species	<i>Hipposideros cf. caffer</i>	<i>Hipposideros cf. caffer 2</i>
<i>Hipposideros cf. caffer</i>	14	0
<i>Hipposideros cf. caffer 2</i>	0	20
Percent of correct identification on putative species	100%	100%
Mean percent of identification for LDA	100%	

2.5 Discussion

We confirmed the presence of four distinct genera, namely *Hipposideros*, *Rhinolophus*, *Triaenops* and *Miniopterus* using an integrative taxonomic approach. Individuals of the genus *Hipposideros* appeared to be the most common in both caves, followed by individuals of *Miniopterus*, *Rhinolophus* and *Triaenops*. Monadjem et al (2021) showed that these four genera can live together in the same cave, each exploiting a specific area, depending on the configuration of the cave and the microclimate (light, temperature and humidity) (Czenze et al., 2022).

We hypothesized the presence of *Hipposideros caffer* in both caves, and *Triaenops afer* in the Boundou cave, based on morphometric data and their geographic distribution (Bates et al., 2013; IUCN, 2023; Kingdon, 2014; Monadjem et al., 2020). PCA on morphometric data confirmed the presence of a single species of *Hipposideros* regardless of sex or site. On the other hand, the PCA and LDA on acoustic data showed that at least four *Hipposideros* individuals have different acoustic characteristics from the other individuals. We hypothesize that these individuals could be members of a cryptic species in the *Hipposideros cf. caffer complex*. Indeed, certain cryptic species cannot be identified without the use of acoustics and/or genetics (Baldwin et al., 2021; Vallo et al., 2011). To confirm this hypothesis, genetic analyses of our collected samples are necessary.

PCA on morphometric data on individuals of the genus *Triaenops*, suggested the presence of a single species. However, PCA on acoustic data showed segregation of individuals according to sex. This observation confirms the results of other studies showing variation in acoustic parameters between males and females in several species of insectivorous bats, including *Triaenops*.

For *Rhinolophus* and *Miniopterus*, PCAs on morphological data showed the presence of two distinct groups in each genus, irrespective of sex and site (**Fig. 15E, 15F, 15G and 15H**). For *Miniopterus*, individuals captured in the field were identified as *Miniopterus minor* and *Miniopterus inflatus*, based on morphology and geographic distribution (ACR, 2019; IUCN, 2023; Kingdon, 2014; Monadjem et al., 2020). The LDA confirmed the potential presence of these two putative species as well as the categorization of the bats in the field. Preliminary

genetic results for *Miniopterus* have shown the need for further taxonomic efforts. These species have not been referenced in the Republic of Congo, even though taxonomically close individuals have been referenced in neighbouring countries such as the Democratic Republic of Congo (Demos et al., 2019; Juste et al., 2007).

Concerning *Rhinolophus*, two putative species were identified: *Rhinolophus cf. landeri-alcyone* and *Rhinolophus cf. denti*. Further genetic studies need to be undertaken. These two species have not yet been recorded in the Republic of Congo. Confirmation of their presence at these two sites would increase their known geographical range and bolster the phylogenetic investigation of African species (Curran et al., 2022; Demos et al., 2019; IUCN, 2023). The PCAs and LDAs on morphometric and acoustic data suggested the existence of two distinct *Rhinolophus* species, but also highlighted the misclassification of certain individuals. LDA on *Rhinolophus cf. landeri-alcyone* appeared to be identified with an accuracy of 97%, unlike *Rhinolophus cf. denti*, whose accuracy was 79%. The field identification of the two *Rhinolophus* species was therefore not accurate.

This misclassification of individuals can be explained by (i) classification errors for species that are very similar morphologically, (ii) morphometric values that were outside the 'norm' for this species in the taxonomic literature and (iii) the collection of abnormal measurements.

African bats can be identified using typical morphological traits to differentiate bat families or certain genera and species. A few external traits might be sufficient for some taxa. For example, the Pteropodidae family is characterized by large eyes and snouted heads (i.e. Hammer-head bats (*Hypsignathus montrosus*)). The *Rhinolophus* genus is known for its horseshoe-shaped nose, and the *Nycteridae* family for its very large ears (ACR, 2019; Fenton & Simmons, 2020). However, many bat species need multiple and sometimes more invasive morphological measurements (e.g. cranial measurements) to be differentiated. Our results on *Rhinolophus* and *Miniopterus* highlighted the need to take several morphometric variables in order to improve the accuracy of species identification. *M. cf. inflatus* and *M. cf. minor* were also identified with high accuracy (100%) according to LDA results. The use of a reduced LDA (taking into account only two morphometric parameters) decreased this accuracy (95%). One solution to facilitate the identification of species with similar morphological traits (i.e. *R. cf. landeri-alcyone* and *R. cf. denti*) is the use of partition graphs. The development of these

graphs could assist in quick and accurate species identification thus limiting misclassifications (**Appendix 8, 10 and 11**).

In addition, the use of partition plots on fieldwork will reduce the number of morphometric measurements to be collected, and decrease the time needed to handle individuals, reducing stress. Finally, the integration of several types of data (morphometric and acoustic) should help to prevent misidentifications, but for a posteriori identification (and not in the field). In this study, the joint use of morphometric and acoustic data enabled us to refine our identification, especially for *Rhinolophus* and *Hipposideros*. On the other hand, morphometric data enabled us to separate the two putative species in *Miniopterus*, without using acoustic data.

In conclusion, this study highlights the importance of an integrative taxonomy for species identification at genus and species level. Our results showed the need to integrate all morphometric and acoustic measurements in order to identify species as accurately as possible. Taking all morphometric measurements is time-consuming, as it requires researchers to collect numerous measurements with precision and rigor. Accurate bat identification can be supported by genetics and acoustics but combining this with morphometric data remains essential for characterization of new species, bat evolution and distribution. We urge all researchers who handle bats for their research, particularly virologists, to take advantage of these rare opportunities to take essential data on morphometrics, acoustics and genetics. These data will improve the knowledge of bats in Africa and elsewhere, and, in particular, to complement epidemiological data together with ecological data.

2.6 Acknowledgments

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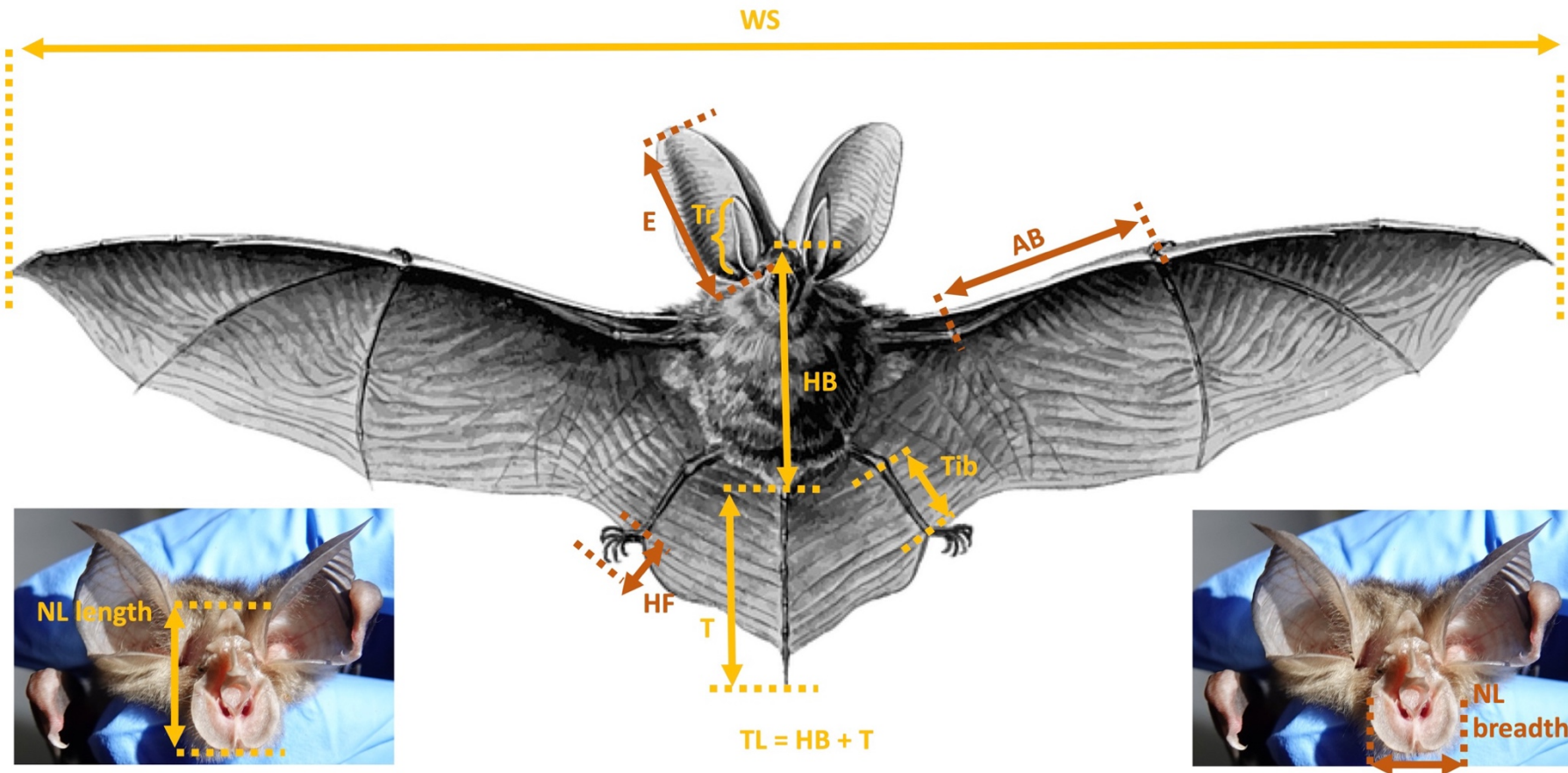
Finally, we would like to thank the local authorities in Dolisie and the local population for their help with this project.

2.7 Appendix Chapter 2

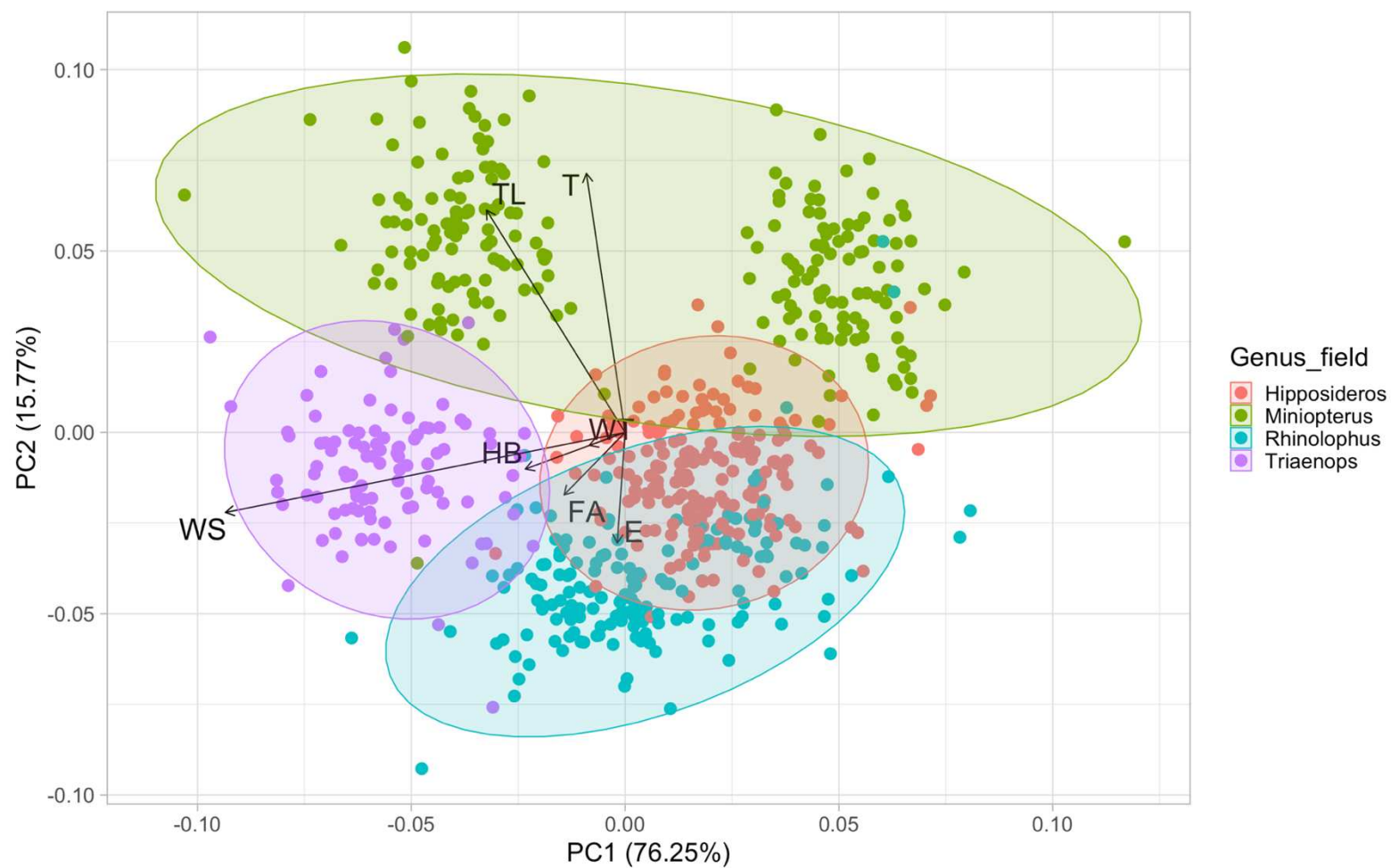
Appendix 3: Morphometric data of individual captured at Mont Belo cave and Boundou cave in republic of Congo

<https://dataverse.cirad.fr/privateurl.xhtml?token=f74428b3-7716-4fae-9bad-6a54ebae6263>

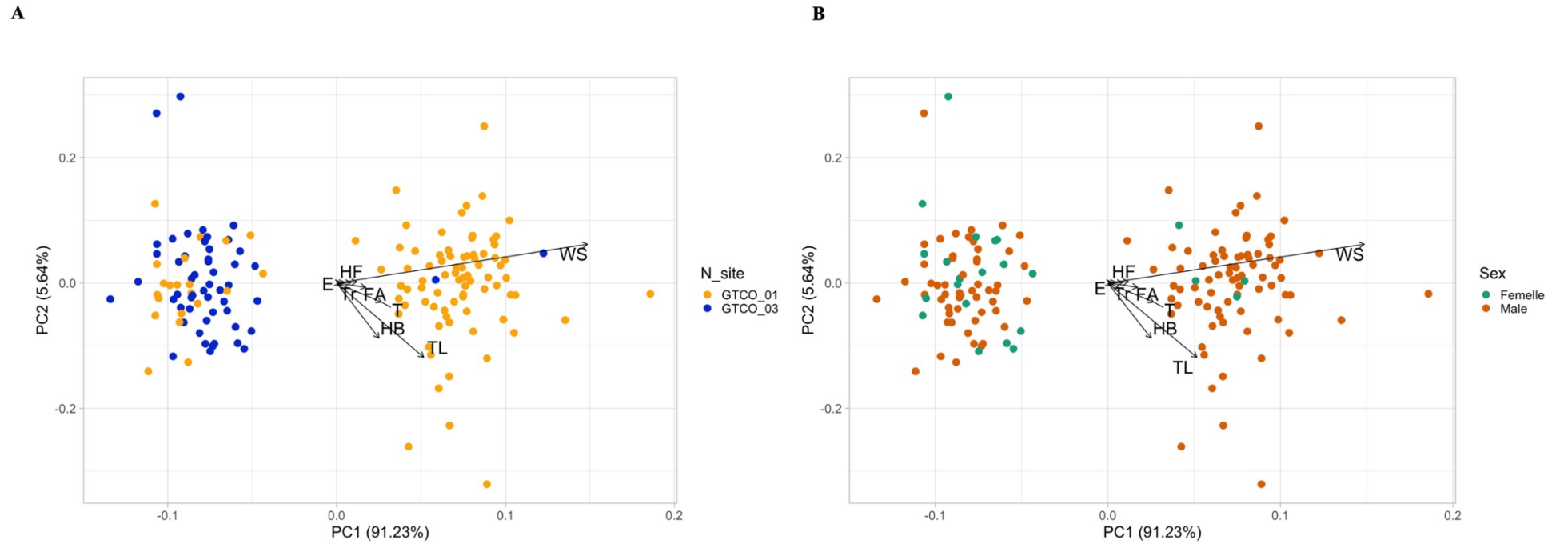
Appendix 4: Morphometric measurement diagram. For *Hipposideros*, *Triaenops* and *Rhinolophus* with 10 morphometric measurements (TL = total body length, T = tail length, WT = weight, WS = size of wing, HB = body size without tail, FA= forearm, HF =hindfoot, E = ear length and Tr = length of tragus).



Appendix 5: Principal Component Analysis (PCA) (PC1 vs PC2) based on eight linear morphometric measurements (TL = total body length, T = tail length, WT = weight, WS = size of wing, HB = body size without tail, FA= forearm, HF =hindfoot and E = ear length) for all bats individuals depending on four genera (*Hipposideros*, *Miniopterus*, *Rhinolophus* and *Triaenops*).



Appendix 6: Principal Component Analysis (PCA) (PC1 vs PC2) based on nine linear morphometric measurement (TL = total body length, T = tail length, WT = weight, WS = size of wing, HB = body size without tail, FA= forearm, HF =hindfoot, E = ear length and Tr = length of tragus) of *Miniopterus* genus depending on site (A) and sex (B).

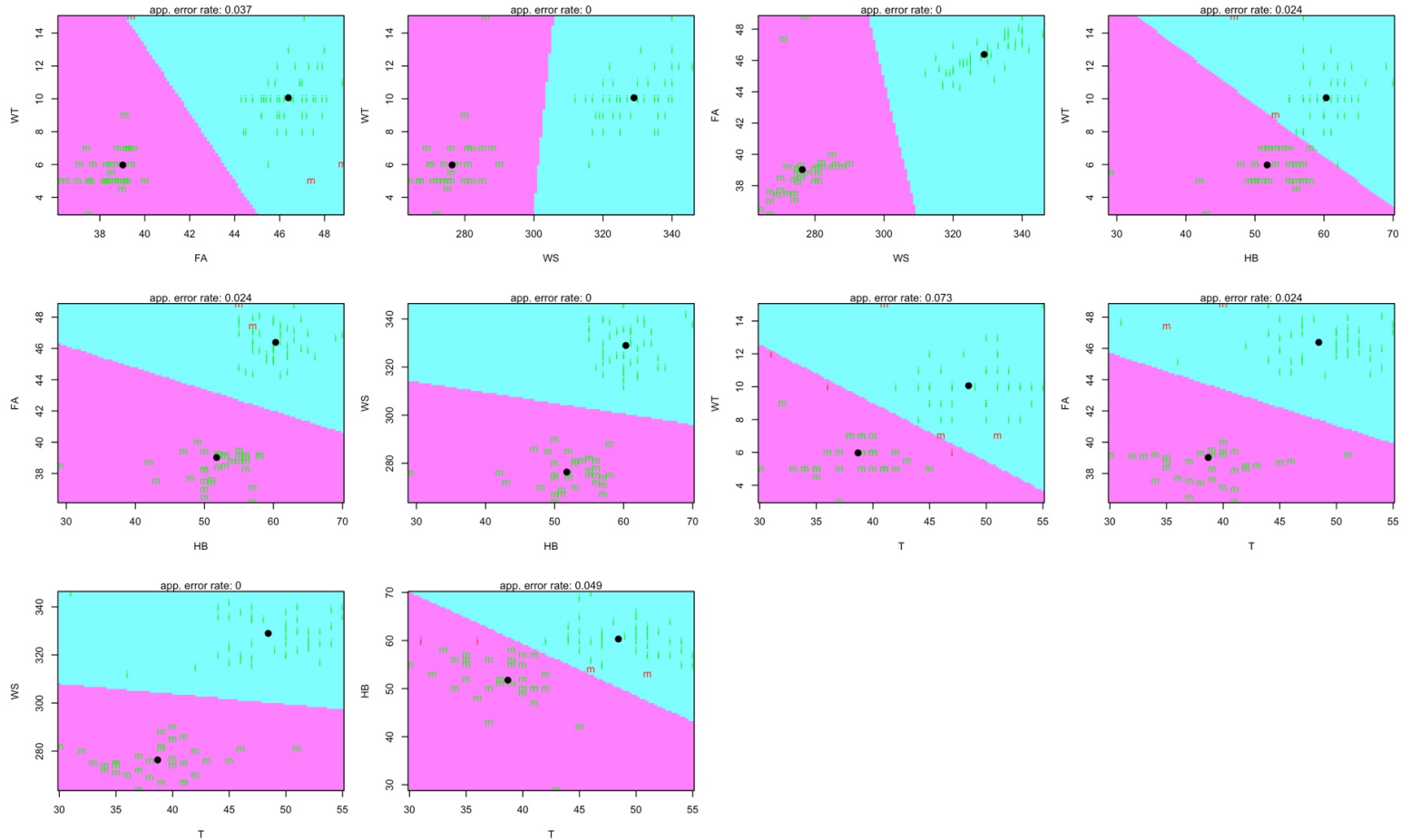


Appendix 7: Results of LDA reduced for *Miniopterus* performed on two morphometric variables (WT = weight, and Tr = length of tragus).

Predicted species	LDA reduced	
	Putative species	
	<i>Miniopterus cf. inflatus</i>	<i>Miniopterus cf. minor</i>
<i>Miniopterus cf. inflatus</i>	79	6
<i>Miniopterus cf. minor</i>	2	66
Percent of correct identification on putative species	0.97%	0.93%
Mean percent of identification for LDA	0.95%	

Appendix 8: Partition plot for *Miniopterus* with different morphometric data (TL = total length body, WT = weight, FA = forearm, HF = hindfoot, WS = size of wing and HB = body size without tail). The color differentiated the two presumed species with i for *M. cf. inflatus* and m for *M. cf. minor*.

Partition Plot

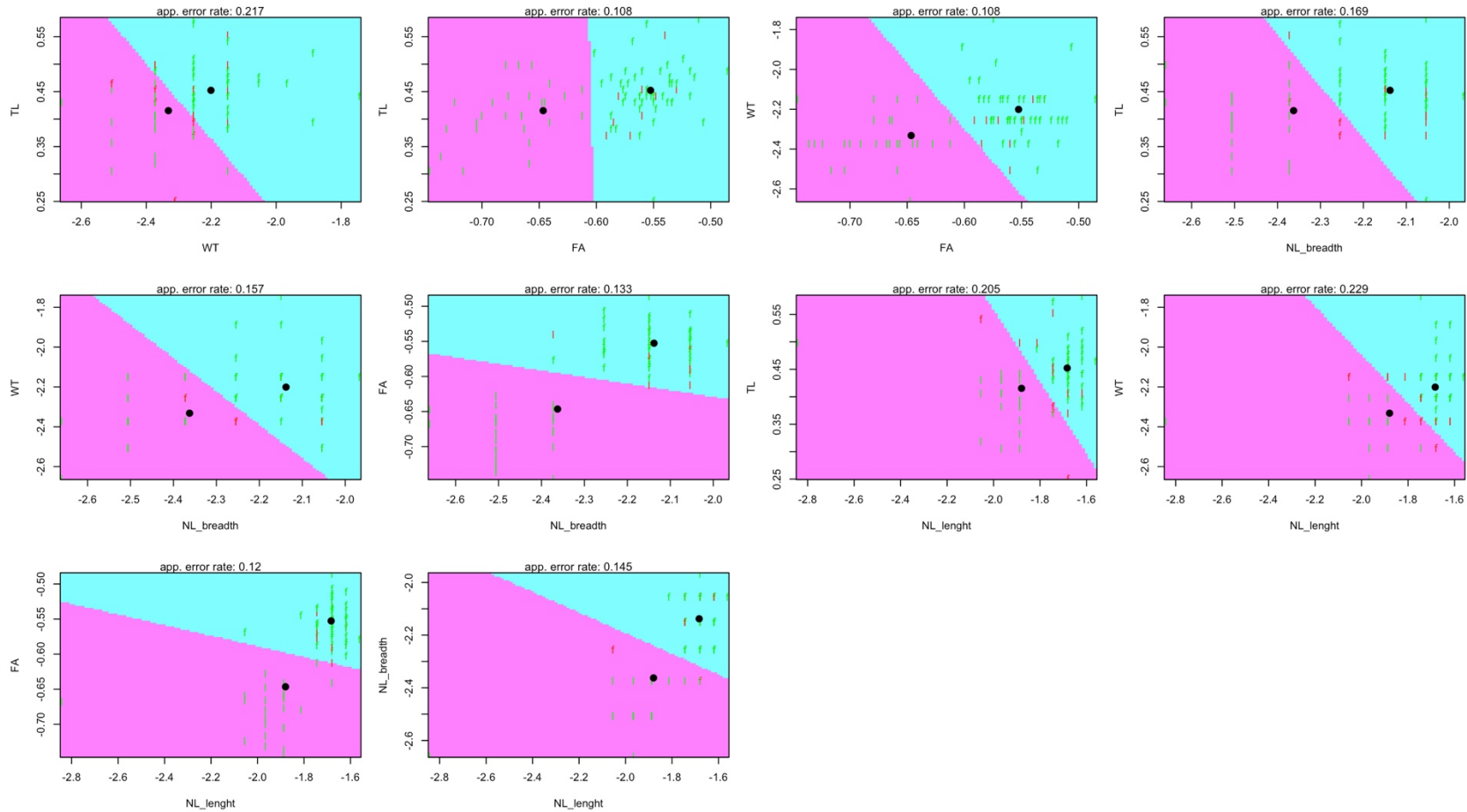


Appendix 9: Results of LDA reduced for *Rhinolophus* with the use of four morphometric variable (WT = weight, E = ear length, NL breadth = maximum breadth of the noseleaf and NL length = length of the noseleaf).

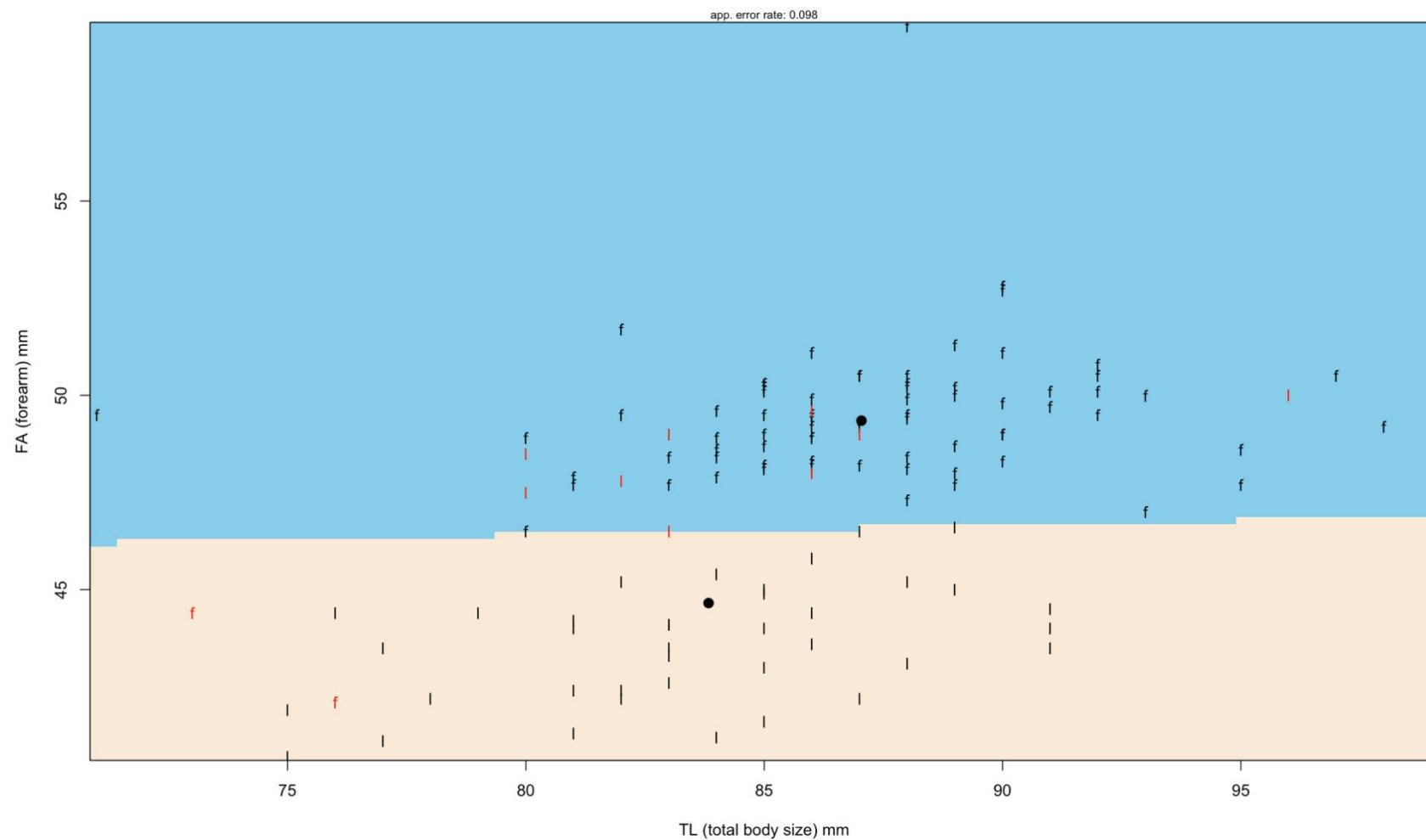
LDA reduced		
Putative species		
Predicted species	<i>Rhinolophus cf. landeri-alcyone</i>	<i>Rhinolophus cf. denti</i>
<i>Rhinolophus cf. landeri-alcyone</i>	72	10
<i>Rhinolophus cf. denti</i>	3	37
Percent of correct identification on putative species	0.96%	0.77%
Mean percent of identification for LDA	0.88%	

Appendix 10: Partition plot for *Rhinolophus* with five morphometric data (TL = total body size, WT = weight, FA = forearm, NL breadth = maximum breadth of the noseleaf and NL length = length of the noseleaf). The color differentiated the two presumed species with l for *R. cf. landeri-alcayne* and f for *R. cf. denti*.

Partition Plot



Appendix 11: Partitional plot for *Rhinolophus* between Total body size (TL) and forearm (FA) (f for *Rhinolophus cf. landeri-alcyone* and l for *Rhinolophus cf. denti*).



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Transition 2: L'acoustique, langage silencieux des chauves-souris, source d'une grande quantité d'information

Grâce à 11 sessions de captures dans les grottes de Boundou et de Mont Belo, nous avons identifié la présence d'un minimum de six espèces putatives de chauves-souris insectivores différentes réparties dans trois familles distinctes que sont les Rhinolophidae (2 espèces), les Miniopteridae (2 espèces), Hipposideridae (1 espèce) et Rhynonycteridae (1 espèce).

Toutes les chauves-souris insectivores utilisent « l'écho-perception » pour (i) se déplacer dans leur environnement (ii) localiser des proies dans l'espace et (iii) pour communiquer entre elles (cris sociaux). Or, la « signature acoustique » d'un individu, telle une « carte d'identité » permet de collecter de nombreuses informations par exemple sa famille, son espèce et dans certains cas son genre. Heureusement, la surveillance acoustique à l'aide d'un enregistreur pouvant capter ces « sons » s'est fortement démocratisée grâce à la miniaturisation des dispositifs et à la diminution de leur coût.

Ainsi, **le Volet II** de cette thèse s'appuie sur l'acoustique pour étudier la dynamique de ces espèces sur une échelle temporelle. Le **Chapitre 3** présente le protocole de surveillance acoustique passive mis en place pendant une durée de 19 mois dans les deux grottes de notre étude (Boundou et Mont Belo). Cette étude a aussi pour vocation de présenter les différentes étapes clefs de la méthodologie de traitement des fichiers acoustiques en s'appuyant sur Tadarida, un classificateur de sonotype mondial. Nous exposons les différentes étapes de son application sur nos données et les solutions employées pour limiter les « fausses identifications » en raison du chevauchement des sonotypes acoustiques de certaines espèces. Le graphique (**Box 2**) résume les principaux points importants de ce chapitre.

Le **Chapitre 4**, quant à lui, présente les modèles d'activités et la phénologie annuelle observés dans les deux grottes étudiées et pour chaque groupe acoustique (préalablement identifiés dans le chapitre 3). Le résumé graphique (**Box 3**) représente les principaux points de ce chapitre.

Transition 2 (English version): Acoustics, the silent language of bats, a source of rich information

Thanks to 11 capture sessions in the Boundou and Mont Belo caves, we have identified the presence of at least six different putative species of insectivorous bats in three distinct families: Rhinolophidae (2 species), Miniopteridae (2 species), Hipposideridae (1 species) and Rhynonycteridae (1 species).

All insectivorous bats use "echo perception" to (i) move around in their environment (ii) locate prey in space and (iii) communicate with each other (social calls). The "acoustic signature" of an individual, like an "identity card", can be used to gather a great deal of information, such as its family, species and, in some cases, gender. Fortunately, acoustic monitoring using a recorder capable of capturing these 'sounds' has become much more widespread thanks to the miniaturisation of devices and the reduction in their cost.

Part II of this thesis therefore uses acoustics to study the dynamics of these species over time. **Chapter 3** presents the passive acoustic monitoring protocol implemented over a period of 19 months in the two caves in our study (Boundou and Mont Belo). The aim of this study is also to present the various key stages in the methodology for processing acoustic files using Tadarida, a global sonotype classifier. We describe the various stages in its application to our data and the solutions used to limit 'false identifications' due to the overlap of the acoustic sonotypes of certain species. The graph (**Box 2**) summarises the main points of this chapter.

Chapter 4 presents the activity patterns and annual phenology observed in the two caves and for each acoustic group (previously identified in **Chapter 3**). The graphical summary (**Box 3**) represents the main points of this chapter.

PART II
USE OF A PASSIVE ACOUSTIC MONITORING PROTOCOL TO DESCRIBE
THE ACTIVITY OF COLONIES OF CAVE-DWELLING INSECTIVOROUS
BATS, BASED ON A SEMI-AUTOMATIC METHOD USING A SONOTYPE
CLASSIFIER

The bat is dun with wrinkled wings
Like fallow article,
And not a song pervades his lips,
Or none perceptible.

His small umbrella, quaintly halved,
Describing in the air
An arc alike inscrutable, —
Elate philosopher!

Deputed from what firmament
Of what astute abode,
Empowered with what malevolence
Auspiciously withheld.

To his adroit Creator
Ascribe no less the praise;
Beneficent, believe me,
His eccentricities.

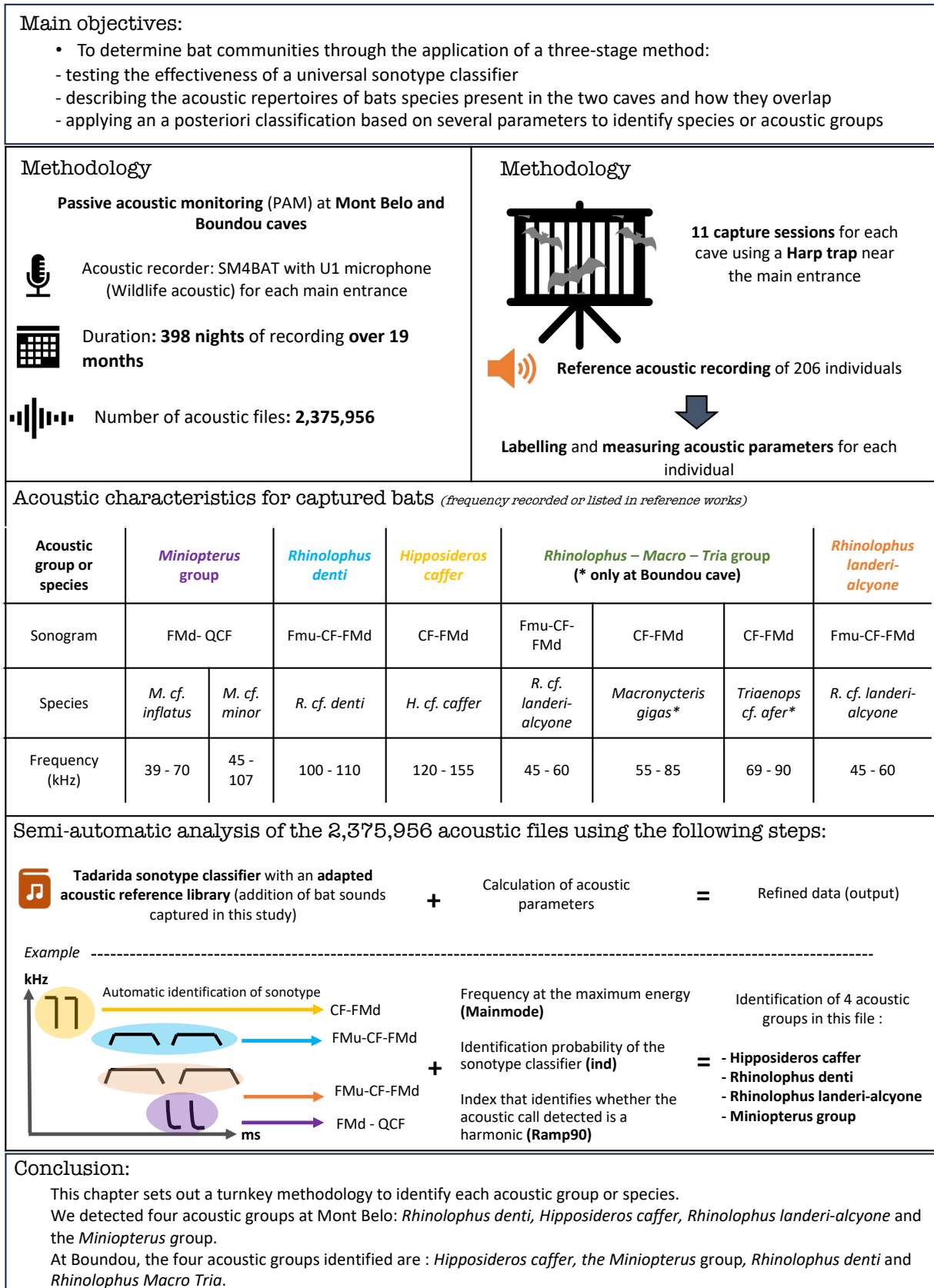
The bat, Emily Dickinson

CHAPTER 3 – ARTICLE 2: PASSIVE ACOUSTIC MONITORING OF CAVE-DWELLING BATS WITH A SONOTYPE CLASSIFIER

Based on the publication submit for *Bioacoustic*:

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Box 2: Graphic Abstract Chapter 3



Passive acoustic monitoring of cave-dwelling bats with a sonotype classifier

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3.1 Abstract

The democratisation and technological advancement of bioacoustics have opened up a multitude of research opportunities to study animals without disturbance, including insectivorous bats. The aim of this study was to make a quantitative survey of the communities of cave-dwelling bats using acoustics in an area with limited knowledge of their acoustic repertoire. Over a period of 19 months, we carried out 11 individual capture sessions to make a species inventory and to record reference sounds, and set up a passive acoustic monitoring protocol (PAM) at the exit of two caves in Congo for a total of 398 nights. We used the Tadarida automatic sonotype classifier to organize the acoustic vocalizations of the bats present in these two caves. Due to the acoustic overlap of several species, we grouped them into five distinct acoustic units using a posteriori classification based on four distinct parameters: call shape, acoustic frequency, a harmonics index and the identification probability. A random manual control stratified by sonotype showed an accuracy ranging from 82 to 98% depending on the group. This study is the very first local application of a bat sonotype classifier designed and developed to function all over the world. It confirms the possibility of undertaking quantitative assessments of bat communities with relatively minimal efforts, even in areas with limited knowledge of their acoustic repertoires.

Keywords: sonotype, bioacoustics, Congo, Chiroptera, automatic classifier, Tadarida

3.2 Introduction

The current biodiversity crisis triggered by the human encroachment on natural habitats threatens of extinction numerous wild species that require conservation attention. Bats are of particular concern as 15 % of bat species worldwide are classified as threatened by the IUCN (Ceballos et al., 2017; Frick et al., 2020; Tanalgo et al., 2023; Voigt & Kingston, 2016). In addition, 52.9% of bat species have unknown population trends and 17.7% of bat species are data deficient, which is far greater compared to other mammals and birds (Frick et al., 2020; IUCN, 2023). The lack of knowledge on bats is partly due to the fact that they are elusive and nocturnal that makes them difficult to monitor.

More than 46% of all threatened bat species use caves as roosts (IUCN, 2023) and this subterranean habitat supports some of the largest population of bats species in the world (Haest et al., 2021). Caves are a key habitat used by numerous bat species because they maintain a stable microclimate for roosting, offer protection against predators and weather during the day (Furey & Racey, 2016; Kunz et al., 2012). Moreover, caves are also used by bats for mating, raising their offspring, performing social interaction and hibernating (Kunz et al., 2012; Kunz & Fenton, 2005; McCracken & Wilkinson, 2000; Tvrtkovic, 2012). Yet, these key habitats, vital for bats, are under anthropic pressure as they are used for different purposes. In addition, their surrounding natural habitat is transformed into agricultural or other anthropogenic landscapes (Tanalgo et al., 2022).

The most common techniques used to study bats ecology include capture (i.e., harp traps and mist nets) with the possibility to mark them (CMR) (i.e., forearm ring or micropships) (Lobato-Bailón et al., 2023), direct visual counts at roosts (Gaisler, 1979; Krutzsch, 1955) and multimedia observations (i.e., photographic, video and thermal imaging) (Koger et al., 2023; Krivek et al., 2023; Robinson et al., 2023; Skalak et al., 2012). However, these methods are costly in terms of time and resources, difficult to standardize, not to mention they may cause stress, injury and mortality to bats. Over the last few decades, studies using passive acoustic monitoring (PAM) of ecosystems has become an increasingly popular method worldwide (Gibb et al., 2019; Sugai et al., 2020). However, in African countries, acoustic studies on bats are still scarce, despite their high diversity (Walters et al., 2013). Bats families, except the large majority of *Pteripodidae* (large frugivorous bats), primarily use echolocation for the purposes

of orientation and foraging (Neuweiler, 1989; Schnitzler et al., 2003). Acoustic offers therefore a different approach to answer various ecological questions, such as the diversity and abundance of species or communities, the spatial and/or temporal analysis of animal distribution and behaviour, including the impact of human activities on bat behaviour (Astaras et al., 2017; Campos-Cerqueira & Aide, 2016; Darras et al., 2018; Davis et al., 2017; Kalan et al., 2015; King et al., 2013; Lehnen et al., 2018; Lucas et al., 2015; Mcloughlin et al., 2019; Merchant et al., 2015; O'Farrell & Gannon, 1999; Petrusková et al., 2015, 2015; Pirodda et al., 2015; Stowell & Sueur, 2020; Wrege et al., 2017). PAM was also demonstrated to be very promising to monitor bat populations in caves (Kloepper et al., 2016; Revilla-Martín et al., 2020). It offers many perspectives such as understanding the annual phenology of the roost occupancy that is impossible to monitor by direct human observers (e.g., nocturnal behaviour, quantity of individual concerned). However, identifying acoustic calls to family and/or species level requires comparison with a reference database of bat calls. A number of techniques for creating reference libraries are currently being tested in the Northern Hemisphere, with the aim of minimizing stress on individuals and acquiring quality sounds for referencing a species. For example, Zamora-Gutierrez et al (2020) created a reference library of fifty percent of the bat species present in Mexico (e.g. 69 species) using five different methods to record them (hand release, zip lining, in bag, flight cage and flying from perch). But none of these methods has yet been validated as a reference protocol by the scientific community. Furthermore, it might be necessary to adapt these methods to the environment in which the recordings take place (clutter vs. open field), as bats adapt their echolocation to their environment (Jones & Holderied, 2007; Schaub & Schnitzler, 2007).

In addition, the use of PAMs limits disturbance to species while collecting a large amount of data over a long period, regardless of the environmental conditions or behavioural factors associated with the animals (Blumstein et al., 2011; Marques et al., 2013; Sugai et al., 2020). However, until recently, the time required for the analysis of the usually large amounts of data collected by PAM (acoustic files), was considerable. In recent decades, the development of semi-automated and non-automated classifiers have facilitated the analysis of bat acoustic data through the development of machine learning techniques (Borowiec et al., 2022; Kwok, 2019; Tabak et al., 2019; Thessen, 2016; Valletta et al., 2017). The scientific community has developed several classifiers using different approaches or methodologies to automate the

recognition of acoustic call features (Chen et al., 2020; López-Baucells et al., 2019; Yoh et al., 2022). Among those softwares, Tadarida (Bas et al., 2017), is a versatile program that can detect sound events and classify them. For geographic areas, such as Africa, which are not covered by a bat classifier at the species level, two sonotype classifiers (i.e., shape associated to frequency) have been developed to analyze the acoustic bat calls worldwide (Roemer et al., 2021; Yoh et al., 2022). In this study, we chose to use the sonotype classifier of Roemer et al. (2021), which has the advantage of having been already trained with a reference database of bat calls from all around the world. To our knowledge, no method about the local application of such a classifier has yet been published. Indeed, questions remain such as how the results of a sonotype classifier must be handled in the case of acoustic overlaps between species. The main objective of this study is to test whether it is possible to make a quantitative monitoring of the acoustic activity of bats using a universal sonotype classifier in an area where reference data are scarce and species classifiers are not available. To this end, we have developed an automatic classification method based on annual passive acoustic monitoring and applied our method to two caves in the Republic of Congo. We proceeded in three steps: (1) establish the list of species present in the two caves (2) describe the acoustic repertoire of these species and how they overlap, and (3) define how to apply the sonotype classifier, i.e., which species can be identified as one monospecific group using a posteriori classification based on several parameters.

3.3 Materials and methods

3.3.1 Ethic statements

A permit to capture and handle bats was validated by the Ministry of Forest Economy and the Ethics Committee of the Ministry of Scientific Research and Technological Innovation of the Republic of Congo (N°212/MRSIT/IRSSA/CERSSA and N°687/MEF/CAB/DGEF-DFAP). All animals were handled with care and as quickly as possible during morphometric measurements and sample collection, following the recommendations of Kunz and Parsons (2009). Fragile individuals, pregnant or nursing females were handled as quickly as possible or released immediately, depending on their condition. In the event of incapacitating injury, individuals were euthanized as quickly as possible to limit suffering, and their specimens preserved for taxonomic analysis (less than 0.1% of individuals captured during eleven sessions).

3.3.2 Study sites

Our study focuses on insectivorous bat populations in two caves located in the south of the Republic of Congo: Mont Belo Cave and Boundou Cave, in the Bouenza and Niari departments, about 50 km away from each other, near the town Dolisie, (**Fig. 11**).

In this region, there are four seasons: the short dry season spans from January to February, the short-rainy season from March to May, the long-dry season from June to August, and the long-rainy season from September to December (Samba et al., 1999). The landscape is mountainous and calcareous, favouring the presence of numerous caves and cavities. It is mainly composed of grassy savannah with patches of secondary forests and a patchwork of crops close to villages.

The Mont Belo cave (N site: GTCO 01) is surrounded by a small tertiary forest in a farming complex close to the small village of the same name. The cave has one main chamber and two secondary chambers. The Boundou cave (N site: GTCO 03), dug into the cavity of a rocky outcrop, is smaller, with one main chamber and another secondary cavity (not easily accessible by human) (**Appendix 12 and 13**). It is also surrounded by a patch of tertiary forest in a predominantly grassy savannah habitat with patches of forest. In the two caves studied, we observed the presence of between a hundred to a thousand individuals of insectivorous bats,

depending on the season. Fruit-eating bats, on the other hand, were only slightly present, varying from zero individuals per month to a maximum of around thirty.

Figure 17: Photograph of the two study caves and the microphones attached to an SM4 acoustic recorder, with (A) Boundou cave, (B) the microphone placed just in front of the main entrance to Mont Belo cave, (C) Boundou cave and (D) the microphone placed high up at the main entrance to Boundou cave. The yellow star represents the approximate location of the microphone.



3.3.3 Passive acoustic monitoring (PAM)

At each study site, an SM4BAT acoustic recorder with a U1 microphone (Wildlife acoustics, Maynard, MA, USA) was installed 5 m outside the cave. It was oriented towards the cave exit, to capture the bats' calls while they exit or enter the cave, and avoid recording calls while bats are inside (**Fig. 17**). The microphone was placed on a tree or pole at a minimum height of one meter to avoid ground noise and reduce "echo recordings". The microphone was deployed at a minimum distance of 1.5 m diameter from any obstacle that might obstruct the sound (vegetation, water) (Newson et al., 2015). The microphone cables were protected from insects and wildlife with PVC tubing, and small bags of desiccant were added to the battery compartment to minimize humidity in the case. Two SD memory cards with a minimum capacity of 128 GB were used to store the sound recordings. Over a period of 19 months (September 2021 to February 2023) at the two study sites, automatic recording was triggered 30 minutes before sunset and 30 minutes after sunrise. The SM4BATs were set up according to the French National Museum Natural History's fix points protocol for the Vigie-chiro project (Mariton et al., 2023). For sixteen of the nineteen months, we used batteries (four LR14 battery) to operate the recorders, for periods varying from 5 to 28 nights, depending on the month and the cave until the batteries were exhausted. The external battery (12V) was used for a period of three months (November 2021, December 2021 and January 2022). However, the abrupt shutdown of the recorders on several occasions, probably due to the poor quality of the batteries, resulted in a loss of data, necessitating a return to the four-battery system for the remaining 16 months. Every month, the batteries and SD cards were changed by the field team when they were present for other research activities or specifically by a referent.

3.3.4 Bat capture, morphometric and genetic data and reference sound recording

Over the 19-month period, 11 capture sessions (spread over several seasons) were carried out using a Harp trap (Ecotone, Poland) placed at the entrances of the two study sites, in order to identify the most common cave-dwelling species. Depending on field constraints, we were able to collect several types of data, such as morphometric data, genetic samples (wing punches or fecal sample) and/or a reference acoustic sound.

Species identification was carried out using the morphological criteria referenced for species supposedly found in the Republic of Congo (Bates et al., 2013; Kingdon, 2014; Monadjem et al., 2020). However, identification using morphological criteria was difficult due to the absence of an identification key for Congo species and the lack of information on many insectivorous species. Morphometric data for all individuals caught during our study are presented and available in **Appendix 3**.

For 173 individuals, acoustic calls were recorded with a portable recorder, the Pettersson M500 – 384 USB (Pettersson Elektronik AB, Sweden) used when bats were either in their pouch (Zamora-Gutierrez et al., 2020) before being handled, or on release after data collection as they flew away. However, 47 of these acoustic recordings were unusable due to poor recording quality or technical problems during recording. To avoid recording calls of other bat species and to reproduce the conditions of a semi-enclosed environment (cave exit), we decided to change the methodology. We thus performed reference recordings for the next 30 individuals (with minimum one male and one female for each genus except *Hipposideros sp* (as the females captured were pregnant, we preferred not to keep them for this potentially stressful stage) in the following way: individuals were placed in the room of a building early morning and allowed to fly freely, while acoustic calls were recorded using an SM4 recorder and its U1 microphone.

For each of these 159 acoustically recorded individuals, a genetic sample was collected (wing punch or feces) during handling.

In each reference file, ideally a sequence of several calls with a single species was selected. The acoustic parameters of the manually selected acoustic calls were measured automatically on each reference acoustic file using Kaleidoscope 5.6.2 (Wildlife Acoustics, Maynard, MA, USA): Fpmin: minimum signal frequency; Fpmax: maximum signal frequency; Fpmean: mean frequency of the spectrum within the selection (multiple acoustic call); Fppeak: frequency which has the highest (Peak) energy within the selection; Fmax: average maximum frequency of call pulses; Fc: frequency of the point at the end of the body of the call pulse which is defined as the flattest part (lowest absolute slope) of the call; Fk: average knee frequency of calls; Dur (ms): average duration of call pulses within the selection; Fmin: average minimum frequency of call pulses and Fmean: average frequency of echolocation pulses. We then calculated the









mean of each of the acoustic parameters with the standard deviation, grouped by species and sex.

3.3.5 Acoustic analyses

i. Automatic classifier and classifier training

The Tadarida classifier classifies bat calls into different sonotype categories (**Fig. 18**) based on a reference library. For a given acoustic recording containing several calls, the classifier groups the calls displaying the same sonotype (i.e., shape) and the same frequency at the maximum energy with a tolerance of 5 kHz. The classifier then calculates several parameters for each group of calls such as the mean frequency at the maximum energy, the mean call duration and the presence of harmonics. It also provides the probability of sonotype classification by the classifier (Ind), from 0 for low probability to 1 for high probability that the sonotype is correctly classified (Roemer et al., 2021). For more details on how the classifier works, see Bas et al. (2017 and Roemer et al. (2021). In an initial test of the classifier published in Roemer et al. (2021), we obtained a poor performance for the CF-FMd sonotype, which was displayed by several species in our study (*Hipposideros sp.*, *Triaenops sp.*, *Macronycteris sp.*). The CF-FMd sonotype was poorly represented in the database used to train the classifier (i.e., 5,346 calls labelled for the CF-FMd sonotype while the mean number of calls labelled per sonotype was 39,067). Fortunately, the classifier offers a degree of flexibility, and reference sounds can be added. With Tadarida L (Bas et al., 2017), we manually labelled a total of 145 acoustic files (reference files, see previous section, or cave recorder files) with 695 calls with different sonotype shapes, 50% of which belonged to the CF-FMd category. These files were then added to the classifier database and a new classifier was trained. For the training of this new classifier, the parameter SubSamp was set to 0.02 to put more weight on rare sonotypes, and GradientSamp was left at -0.1. This classifier is available by following the link in the **Appendix 14**.

Figure 18: Description of bat sonotypes. FM, Frequency modulated; CF, Constant frequency; QCF, Quasi-constant frequency; d, downward; u, upward. See Figure 1 for the definition of prefix, main element and suffix. From Roemer et al. (2021).

Sonotype	Prefix	Main element	Suffix	Sonogram	Example species
FMd-QCF	Downward FM or none	QCF	–		<i>Pipistrellus pipistrellus</i> , <i>Lasurus borealis</i>
FMu-QCF	Upward FM	QCF	–		<i>Promops centralis</i>
QCF-FMd	–	QCF	Downward FM or none		<i>Peropteryx macrotis</i> , <i>Molossus molossus</i>
CF-FMd	–	CF	Downward FM		<i>Hiposideros commersoni</i> , <i>Noctilio leporinus</i>
FMu-QCF-FMd	Upward FM	QCF	Downward FM		<i>Cormura brevirostris</i>
FMu-CF-FMd	Upward FM	CF	Downward FM		<i>Rhinolophus ferrumequinum</i> , <i>Pteronotus cf. parnellii</i>
FMd	–	Downward FM	–		<i>Myotis nattereri</i> , <i>Carollia perspicillata</i>
CF-FMd-CF	CF	Downward FM	CF		<i>Pteronotus personatus</i>

3.3.6 Acoustic species identification and accuracy check

We fragmented the acoustic files collected into files of 5s maximum using Kaleidoscope light software (Wildlife acoustics, Maynard, MA, USA). We used the adapted Tadarida classifier to identify the sonotypes present in each acoustic file collected from the two caves in our study. The classifier returns a table in which each line corresponds to a group of calls with similar shape and frequency. Each group is associated with a sonotype, a summary of the acoustic parameters, and a probability of identification (Ind). In order to check the accuracy of the classifier and identify the probability of identification threshold under which the classifier had an unsatisfying performance (e.g., sonotype misclassification), we carried out a manual check of 5,458 files, spread over two caves and two different months (September and October 2021).

3.3.7 Classification a posteriori

Due to the presence of several bat species with similar call characteristics, the results of the sonotype classifier needed to be refined with a posteriori classification. The goal was to try and separate species, or else place them in acoustic groups. We compared the collected recordings with our reference recordings (see dedicated section above) and the literature to identify the various key parameters needed to achieve greater accuracy in automatic identification. For this purpose, we selected the MainMode used by Tadarida to build groups of calls based on their frequency at the maximum energy (see above), the identification probability of the sonotype classifier (Ind) and Ramp90 (an index that identifies whether the acoustic call detected is a harmonic or a fundamental frequency). If the Ramp90 index is below zero, it is a fundamental frequency; an index close to zero is a fundamental frequency with a harmonic and a number greater than zero is a harmonic. Furthermore, in order to take into account the potential variations in acoustic frequency due to the environmental and methodological conditions of our study, we decided to define the acoustic frequency interval of our acoustic species based on two different data sources. For each acoustic interval of the species group, we used: i) the acoustic data from the literature and ii) the minimum and maximum of the reference acoustic data we measured using Kaleidoscope (Fppeak, Fpmin, Fpmax for all the species groups with the addition of Fmax for the *Miniopterus* species).

3.4 Results

3.4.1 Species identification

A total of 680 individuals were captured with harp traps over 11 sessions. In Mont Belo and Boundou caves, we regularly captured three different bat genera: *Miniopterus*, *Hipposideros* and *Rhinolophus*. In addition, in Boundou cave, we regularly captured species of *Triaenops* genus and occasionally two other genera of insectivorous bats, *Macronycteris* and *Coleura*.

Using morphometric and acoustic data from captured bats, we identified the presence of five species in both caves: *Miniopterus cf. inflatus*, *Miniopterus cf. minor*, *Rhinolophus landeri-alcyone*, *Rhinolophus cf. denti* and *Hipposideros cf. caffer*. In the Boundou cave, we also identified *Triaenops cf. afer*, *Macronycteris gigas* and *Coleura afra*. At Boundou, *Coleura afra* was only caught once during the April 2022 session and *Macronycteris gigas* was caught three times out of the 11 sessions (December 2021, April 2022, November 2022). Details for each species and the distribution of sexes according to capture sessions are presented in **Appendix 15**. Morphometric species identification is detailed in. in another paper (submitted)

3.4.2 Acoustics characteristics of the species communities

Of the 680 individuals captured, 159 (23.5%) were recorded for reference purposes, and their main acoustic characteristics are presented in **Table 8**. The acoustic parameters found in the literature are reviewed in **Table 9**. The overview of both our reference recordings and the literature allowed us to establish the following characteristics for the eight species present in our study caves. Among *Rhinolophus* (FMu-CF-FMd, see **Fig. 19**), the two different species have distinct frequency ranges: *R. cf. landeri-alcyone* with a frequency (FMu-CF-FMd or other mammals) ranging from 45 to 62 kHz and *R. denti* with a frequency (FMu-CF-FMd or other mammals) between 95 and 110 kHz (**Fig. 19**). For *Miniopterus* (FMd-QCF, see **Fig 19**), the two species have frequency ranges between 39 and 107 kHz (**Table 8** and **9, Fig. 19**). At Mont Belo, *Hipposideros caffer* (CF-FMd, **Fig 18**) has an acoustic frequency ranging from 120 to 155 kHz and is not in overlap with other species. But at the Boundou cave, three FMu-CF-FMd species have overlapping frequencies: *Rhinolophus landeri-alcyone* (between 45 - 62 kHz), *Triaenops sp.* (between 69 and 87 kHz) and *Macronycteris gigas* (between 51 and 56 kHz) (**Table 8** and **9, Fig. 19**).

Table 8: Mean (\pm standard deviation) echolocation parameters of 159 bats caught in two caves (BD =Boundou and MB = Mont Belo) depending on sex (F = female and M = male) in Republic of Congo. N = number of bats in sample; Fpmin : estimate of minimum signal frequency; Fpmax : estimate of the maximum signal frequency; Fpmean : mean frequency of the spectrum within the selection; Fppeak : frequency which has the highest (Peak) energy within the selection; Fmax : average maximum frequency of call pulses; Fc : the point at the end of the body of the call pulse which is defined as the flattest part (lowest absolute slope) of the call; Fk : average knee frequency of calls; Fmin : average minimum frequency of call pulses, Fmean : average frequency of echolocation pulses and Dur (ms) : average duration of call pulses within the selection.

Species	Cave	Sex	N	Fpmin (kHz)	Fpmax (kHz)	Fpmean (kHz)	Fppeak (kHz)	Fmax (kHz)	Fc (kHz)	Fk (kHz)	Fmin (kHz)	Fmean (kHz)	Dur (ms)
<i>Hipposideros cf. caffer</i>	BD	F	7	141 \pm 4.2	138.3 \pm 13.2	133.2 \pm 11.6	135.7 \pm 15	NA	73.3 \pm 9.7	72.7 \pm 9.4	NA	73 \pm 9.7	4.3 \pm 1.1
	BD	M	8	136.1 \pm 15.1	149 \pm 7.8	139.6 \pm 5.7	142.8 \pm 9.1	NA	79.7 \pm 9.2	79.7 \pm 9.4	NA	79.7 \pm 9.3	4.2 \pm 0.7
	MB	F	3	133.5 \pm 0	145.2 \pm 8.8	134.9 \pm 2.1	109.7 \pm 47.2	NA	55.3 \pm 0.2	39.5 \pm 27.8	NA	56.6 \pm 0.1	6.2 \pm 2.5
	MB	M	5	126.3 \pm 23.4	147.7 \pm 3.6	132.3 \pm 20.7	134.5 \pm 21.7	NA	52.8 \pm 3.9	53 \pm 3.4	NA	52.5 \pm 4.3	5.2 \pm 3.6
<i>Miniopterus cf. inflatus</i>	BD	M	1	NA	55.5 \pm 0	54.2 \pm 0	51.2 \pm 0	105.3 \pm 0	98.8 \pm 0	104 \pm 0	44 \pm 0	69.2 \pm 0	2.2 \pm 0
	MB	F	7	39.6 \pm 3.3	69.6 \pm 11.1	54.6 \pm 6.2	47.6 \pm 2.7	82 \pm 17.8	53.5 \pm 16.6	56.2 \pm 16.2	44.6 \pm 2.9	58.7 \pm 9	4.1 \pm 0.2
	MB	M	24	37.3 \pm	62.5 \pm	52.5 \pm 7.7	48.1 \pm	77.1 \pm 14.5	47.3 \pm	51 \pm 8.2	44.9 \pm	55.4 \pm	2.8 \pm 0.5

Species	Cave	Sex	N	Fpmin (kHz)	Fpmax (kHz)	Fpmean (kHz)	Fppeak (kHz)	Fmax (kHz)	Fc (kHz)	Fk (kHz)	Fmin (kHz)	Fmean (kHz)	Dur (ms)
				8.6	9.9		7.5		8.4		5.2	7.6	
<i>Miniopterus cf. minor</i>	BD	F	5	53.2 ± 1	76.2 ± 6.7	67.9 ± 5.8	64.8 ± 6	107 ± 12.9	60 ± 3.2	64.4 ± 5.7	58 ± 3.1	75 ± 6	2.4 ± 0.4
	BD	M	10	52.5 ± 2.1	78.1 ± 5	68.9 ± 4.9	68.7 ± 13.1	97.9 ± 21.9	60.3 ± 6.9	58.5 ± 21.5	57.2 ± 2.7	73 ± 7.3	2.9 ± 0.8
	MB	F	10	46 ± 3.8	71.1 ± 11.9	62.7 ± 8.8	56.7 ± 5	93 ± 15.1	55 ± 6.5	61.3 ± 8	51.7 ± 4.6	66 ± 9	2.8 ± 0.8
	MB	M	8	47.2 ± 3.2	72.9 ± 7.6	66.8 ± 7.3	61.1 ± 4	96.4 ± 20.3	59 ± 6.6	68.6 ± 19.3	NA	69.7 ± 8.7	2.6 ± 0.5
<i>Rhinolophus cf. denti</i>	BD	F	6	88.2 ± 8.6	110.5 ± 3.9	101.5 ± 1.2	101.7 ± 1.2	NA	101.2 ± 1.3	100.6 ± 1.4	NA	101 ± 1.4	32 ± 17.5
	BD	M	1	85.5 ± 0	103.5 ± 0	99.3 ± 0	99.3 ± 0	NA	98.4 ± 0	99.2 ± 0	NA	99.4 ± 0	18.5 ± 0
<i>Rhinolophus cf. landeri-alcyone</i>	BD	F	1	49.5 ± 0	58.5 ± 0	54.3 ± 0	54.3 ± 0	NA	54.4 ± 0	54.2 ± 0	NA	54.3 ± 0	9.2 ± 0
	MB	F	29	46.4 ± 4.6	60.6 ± 1.9	54.7 ± 0.7	55 ± 0.6	NA	55 ± 0.5	54.7 ± 0.6	NA	54.8 ± 0.6	23 ± 10.2
	MB	M	14	45 ± 4.8	61.9 ± 2.2	54.4 ± 2.7	55.4 ± 0.5	NA	55.3 ± 0.6	55.3 ± 0.4	NA	55.1 ± 0.5	19.5 ± 8.9

Species	Cave	Sex	N	Fpmin (kHz)	Fpmax (kHz)	Fpmean (kHz)	Fppeak (kHz)	Fmax (kHz)	Fc (kHz)	Fk (kHz)	Fmin (kHz)	Fmean (kHz)	Dur (ms)
<i>Triaenops afer</i>	BD	F	9	79.12 ± 1.9	87.7 ± 5.3	80.4 ± 3.2	82.2 ± 1.6	NA	81.7 ± 1.4	82.1 ± 1.9	NA	81.1 ± 1.5	6.2 ± 2
	BD	M	10	69.3 ± 7.1	82 ± 2.9	75.1 ± 2.9	75.7 ± 2.6	NA	75.6 ± 2.5	75.5 ± 2.9	NA	75.2 ± 2.5	7.5 ± 1.7
<i>Macronycteris gigas</i>	BD	F	1	51 ± 0	NA	54.2 ± 0	54.3 ± 0	NA	54 ± 0	54.8 ± 0	NA	54.3 ± 0	12.4 ± 0

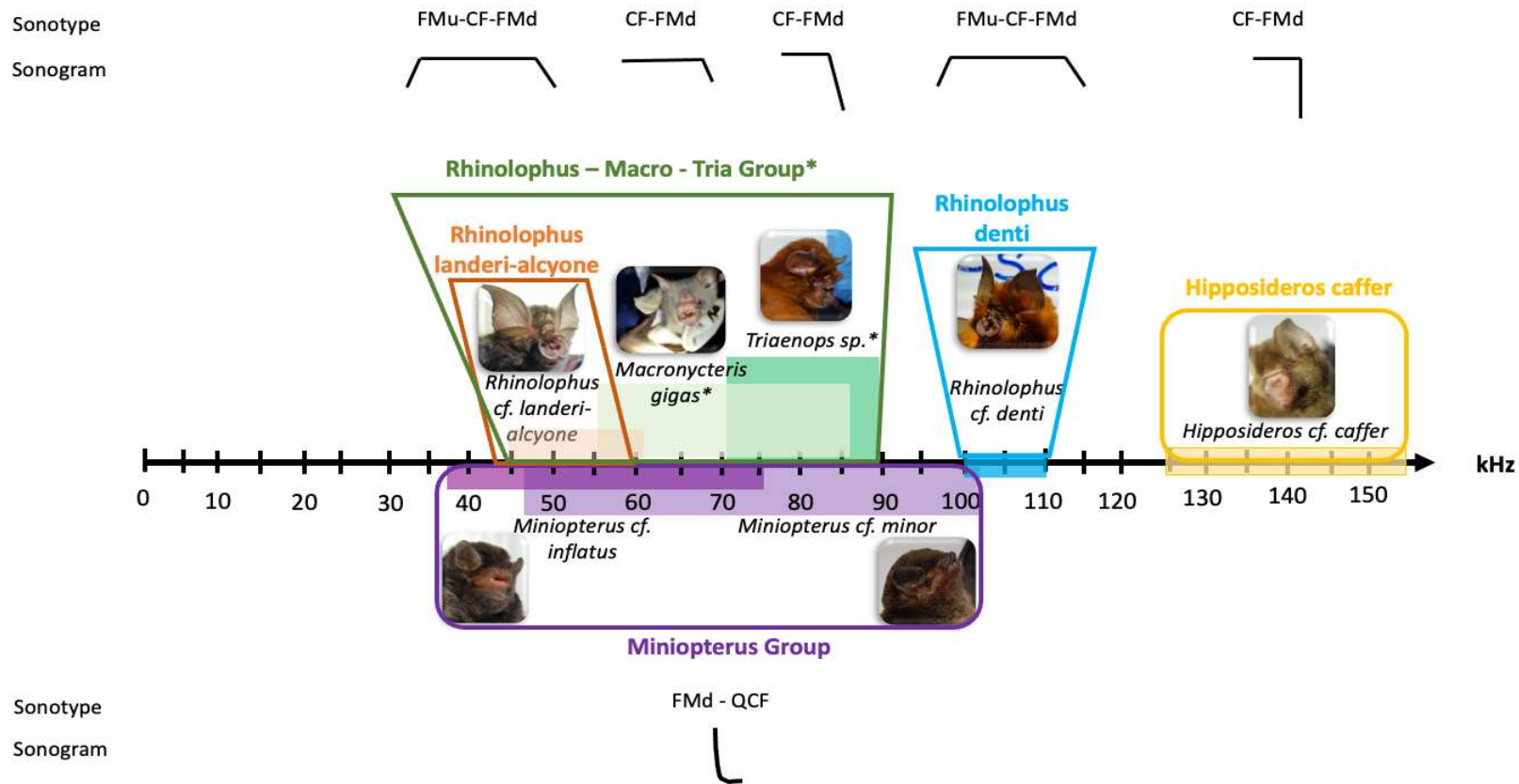
Table 9: Review of the acoustic frequency range of bat species identified in the two study caves using available reference works or studies. Fmin = average minimum frequency of call pulses; Fmax = average maximum frequency of call pulses; Fc = the point at the end of the body of the call pulse which is defined as the flattest part (lowest absolute slope) of the call; Fk = average knee frequency of calls and Dur (ms) = average duration of call pulses within the selection.

Species	Sex	Fmin (kHz)	Fmax (kHz)	Fc (kHz)	Fk (kHz)	Dur (ms)	Comments	References
<i>Hipposideros caffer</i>	Both	121.2 – 139	143.8 – 159	130 – 145.9	141.4 - 156	4.5 – 10.6	The fundamental harmonic may be present on the spectrogram at ~ 71 kHz	(Brinkley et al., 2021; Kingdon, 2014; Moir et al., 2021; Monadjem et al., 2017, 2020; Taylor et al., 2005; Webala et al., 2019; Wright, 2009)
<i>Miniopterus inflatus</i>	Both	47.2	58.1	47.4	50	3.2		(Kingdon, 2014; Monadjem et al., 2020)
<i>Miniopterus minor</i>	-	-	-	-	-	-	Data deficient-f	
<i>Rhinolophus landeri</i>	Both	73 - 100	102 - 110	101 - 122	108 - 109	21 - 67		(Monadjem et al., 2020; Tanshi et al., 2019; Taylor et al., 2005)
<i>Rhinolophus denti</i>	Both	-	-	110 - 111	-	23.4		(Jacobs et al., 2008, 2016; Monadjem et al., 2020)
<i>Rhinolophus alcyone</i>	Both	53.6	54.4	50 (45*) -	53.7	29 – 67	*Perhaps misidentified by	(Adams & Kwiecinski, 2018;

Species	Sex	Fmin (kHz)	Fmax (kHz)	Fc (kHz)	Fk (kHz)	Dur (ms)	Comments	References
				59			Pye and Roberts (1970) if we refer to the commentary by Monadjem et al. (2020)	Brinkley et al., 2021; Monadjem et al., 2020; Pye & Roberts, 1970; Weier et al., 2020)
<i>Triaenops afer</i>	F	-	-	82 - 88	77	7.6 – 12.2	Sexual differences in echolocation call frequency	(Happold & Happold, 2013; Kaipf et al., 2015; Monadjem et al., 2020; Webala et al., 2019)
	M	-	-	70 - 78	77	7.6 – 12.2		
<i>Triaenops persicus</i> or <i>Triaenops p. majusculus</i>	Unspecified	-	-	75 – 80.5	-	-	Perhaps sexual differences in echolocation call frequency, as in other <i>Triaenops sp.</i>	(Monadjem et al., 2020; Pye & Roberts, 1970)
		-	-	85.5 - 89	-	-		
<i>Coleura afra</i>	Both	29.5 – 35.4	30.7 – 38.4	-	30 – 36.4	2.9 – 13		(Kingdon, 2014; Monadjem et al., 2020; Taylor et al., 2005)
<i>Macronycteris</i>	F	-	-	53	-	10.4 –	Sexual differences in	(Tanshi et al., 2019; Webala

Species	Sex	Fmin (kHz)	Fmax (kHz)	Fc (kHz)	Fk (kHz)	Dur (ms)	Comments	References
<i>gigas</i>						19.2	echolocation call frequency	et al., 2019)
	M	-	-	53 - 54	-	10.4 – 19.2		

Figure 19: Acoustic characteristics for the different bat species captured at the two study sites and categorization into different acoustic groups or species. Detail of sonotype, sonogram and acoustic frequency recorded or listed in reference works for each bat genus and species captured in the two caves (species with * are present only at Boundou). In the Boundou cave, there are four acoustic groups (*Rhinolophus – Macro – Tria* group; *Rhinolophus denti*; *Hipposideros caffer* and *Miniopterus* group) and in the Mont Belo cave there are four acoustic groups (*Rhinolophus landeri-alcyone*; *Rhinolophus denti*; *Hipposideros caffer* and *Miniopterus* group).



* Only at Boundou cave

3.4.3 Acoustic dataset obtained from the passive monitoring

We obtained a total of 2,375,956 wav files collected with an average of 60,927 files per month collected in Boundou cave and 64,123 files per month in Mont Belo cave. A total of 398 nights were collected, with 74.6 percent (297 nights) collected simultaneously in both caves. Details for each month are shown in the **Appendix 16**.

3.4.4 Automatic classification of acoustic recordings

Due to the overlap of certain calls for *Rhinolophus - Macronycteris – Triaenops* at Boundou or for the two *Miniopterus* species in both caves, we decided to group the seven species into five different groups: (1) *R. landeri-alcyone*, (2) *R. denti*, (3) *Miniopterus* group (*M. cf minor/ M. cf. inflatus*), (4) *Hipposideros caffer* (*H. cf. caffer*) and (5) *Rhinolophus – Macro – Tria* group (*R. landeri-alcyone /M. gigas/Triaenops sp.*) (only present in Boundou cave). **Figure 19** shows the sonotypes and frequency ranges of species from both caves. The acoustic characteristics of each individual are presented in **Appendix 17**.

In Mont Belo cave, we manually randomly checked 1043 acoustic files from September 2021 (i.e., 1.5% of all files collected that month in this cave) and 1,051 files in October 2021 (1.7% of all files). For Boundou cave, we checked 2,133 acoustic files from September 2021 (2.7% of the total number of files collected) and 1,234 files in October 2021 (1.7% of the total number of files). In total, we verified the accuracy of Tadarida using a posteriori classification on 5,458 files. The **Table 10** and **11** shows the parameters chosen for each group at each study site, enabling a group to be identified with over 95% accuracy. For instance, in the two caves, for the *Miniopterus* group, we used a filter with the FMd-QCF sonotype, a Mainmode ranging from 39 to 107 kHz, a confidence probability index greater than 0.15 for Mont Belo (and 0.20 for Boundou) and a harmonic index (Ramp90) less than zero. For *R. denti*, we could not find parameters allowing an identification over 95 % accuracy, and attained only 82% accuracy (see **Tables 10** and **11**).

During peak periods of simultaneous emergence of numerous individuals of several species, we observed a time and frequency overlap of acoustic calls. This type of scenario was quite frequent, especially during the peak emergence times for the various species (6.30-7.30pm) or during periods when there was a large abundance of individuals in the caves (the main rainy

season: November, December or during mating periods, i.e. in July for *Rhinolophus*). This overlap could lead to a misidentification of the sonotype by the classifier or to a mismeasurement of the frequencies. Concrete examples of these scenarios are shown in **Table 12**.

3.4.5 Identification of other species

Acoustic recordings also enabled us to detect the regular presence of at least one other species in the Boundou cave: *Coleura afra*. The acoustics characteristics of this species detected by the Tadarida classifier are either i) a FMd-QCF sonotype with an acoustic frequency between 36 - 65 kHz and a harmonic index between 1 and 8 or ii) a CF-FMd sonotype with a frequency of 49 - 75 kHz and a harmonic index greater than 1. As mentioned above, this species was only captured once during the April 2022 capture. However, the acoustic characteristics of this species (see sonogram **Fig. 20**) were identified by the classifier during the 19 months of acoustic monitoring.

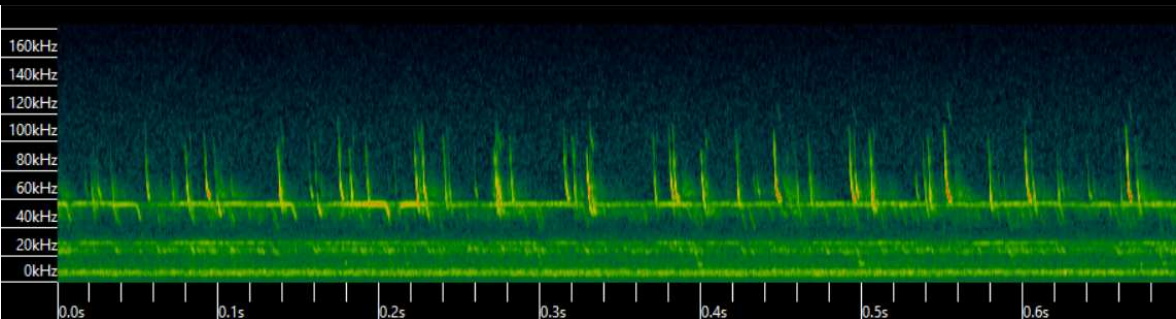
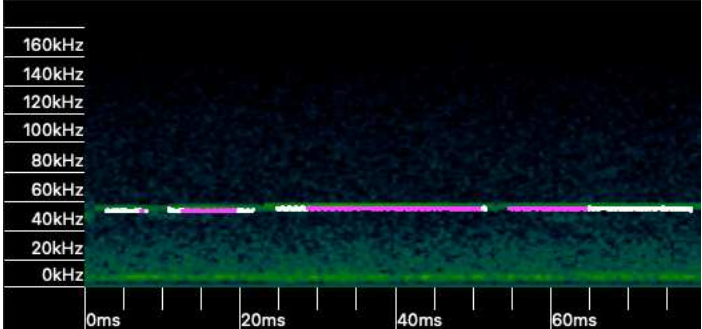
Table 10: A posteriori classification of the different species or groups of bats in the Mont Belo cave using the different parameters and manual verification of these files.

Species or Group name	Sites and period	Parameter used for a posteriori classification	Number of files checked manually	Number of files that do not correspond to the corresponding bat guild
<i>Miniopterus</i> Group	Mont Belo Cave – Sept. 2021	Sonotype: FMd-QCF Frequency (Mainmode): 39 – 107 kHz Harmonic index (Ramp90) ≤ 1 ID Probability ≥ 0.15	361	0
	Mont Belo Cave -Oct. 2021	Sonotype: QCF-FMd Frequency (Mainmode): 55 – 62 kHz Harmonic index (Ramp90) ≤ 0 ID Probability ≥ 0.2	152	0
<i>Rhinolophus landeri-alcyone</i>	Mont Belo Cave – Sept. 2021	Sonotype: FMu-CF-FMd Frequency (Mainmode): 45 – 62 kHz Harmonic index (Ramp90) ≤ 0	428	1
	Mont Belo Cave -Oct. 2021	Sonotype: Other mammals Frequency (Mainmode): 45 – 62 kHz Harmonic index (Ramp90) ≤ 0	224	0
<i>Rhinolophus denti</i>	Mont Belo Cave - September 2021	Sonotype: FMu-CF-FMd Frequency (Mainmode): 95 – 110 kHz Harmonic index (Ramp90) < 0 ID Probability ≥ 0.35	28	5
	Mont Belo Cave -October 2021	Sonotype: Other mammals Frequency (Mainmode): 95 – 110 kHz Harmonic index (Ramp90) ≤ 0	3	0
<i>Hipposideros caffer</i>	Mont Belo Cave - September 2021	Sonotype: CF-FMd Frequency (Mainmode): 120 – 155 kHz Harmonic index (Ramp90) ≤ 0	167	0
	Mont Belo Cave -October 2021		138	12

Table 11: A posteriori classification of the different species or groups of bats in Boundou cave using the different parameters and manual verification of these files.

Species or Group name	Sites and period	Parameter used for a posteriori classification	Number of files checked manually	Number of files that do not correspond to the corresponding bat guild
<i>Miniopterus</i> Group	Boundou cave - September 2021	Sonotype: FMd-QCF Frequency (Mainmode): 39 – 107 kHz Harmonic index (Ramp90) ≤ 1 ID Probability ≥ 0.2	183	2
	Boundou cave - October 2021	Sonotype: QCF-FMd Frequency (Mainmode): 55 – 62 kHz Harmonic index (Ramp90) ≤ 0 ID Probability ≥ 0.2	197	6
<i>Rhinolophus Macro-Tria</i> Group	Boundou cave - September 2021	Sonotype: FMu-CF-FMd Frequency (Mainmode): 45 – 85 kHz Harmonic index (Ramp90) ≤ 0	248	0
	Boundou cave - October 2021	Sonotype: CF-FMd Frequency (Mainmode): 45 – 89 kHz Harmonic index (Ramp90) ≤ 0 Sonotype: Other mammals Frequency (Mainmode): 45 – 85 kHz Harmonic index (Ramp90) ≤ 0	366	0
<i>Rhinolophus denti</i>	Boundou cave - September 2021	Sonotype: FMu-CF-FMd Frequency (Mainmode): 95 – 110 kHz Harmonic index (Ramp90) < 0 ID Probability ≥ 0.35	179	2
	Boundou cave - October 2021	Sonotype: Other mammals Frequency (Mainmode): 95 – 110 kHz Harmonic index (Ramp90) ≤ 0	113	1
<i>Hipposideros caffer</i>	Boundou cave - September 2021	Sonotype: CF-FMd Frequency (Mainmode): 120 – 155 kHz Harmonic index (Ramp90) ≤ 0	291	11
	Boundou cave - October 2021	Sonotype: FMd Frequency (Mainmode): 120 – 155 kHz Harmonic index (Ramp90) ≤ 0.1 ID Probability ≥ 0.2	127	0

Table 12: Example of errors detected in the Tadarida classifier

Example of a Sonogram	Description of the problem identified	Example of consequences in the Tadarida classification
	<p>Presence of several sonotypes at the same time, some of which intersect or overlap.</p>	<p>One out of the two sonotypes is identified and the mainmode is the mean of both sonotypes</p>
	<p>Acoustic call of a Rhinolophus bat of poor quality because only the constant frequency (Fc) is present.</p>	<p>The Tadarida classifier did detect the calls, but they were categorised as other mammal instead of FMu – CF - FMD.</p>

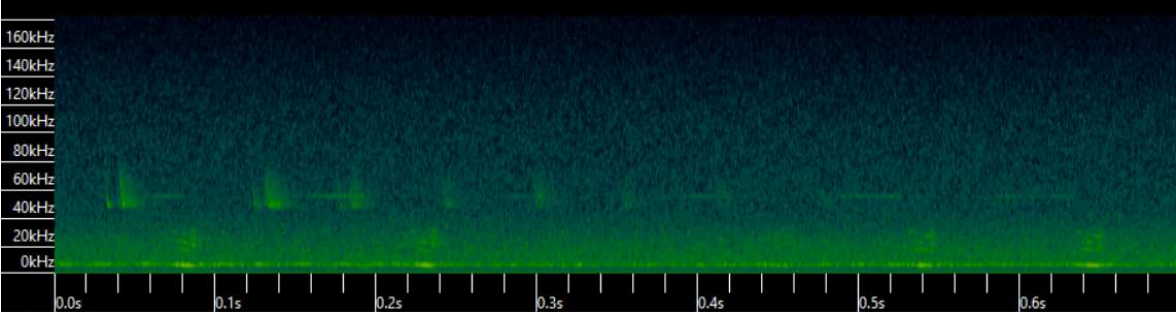
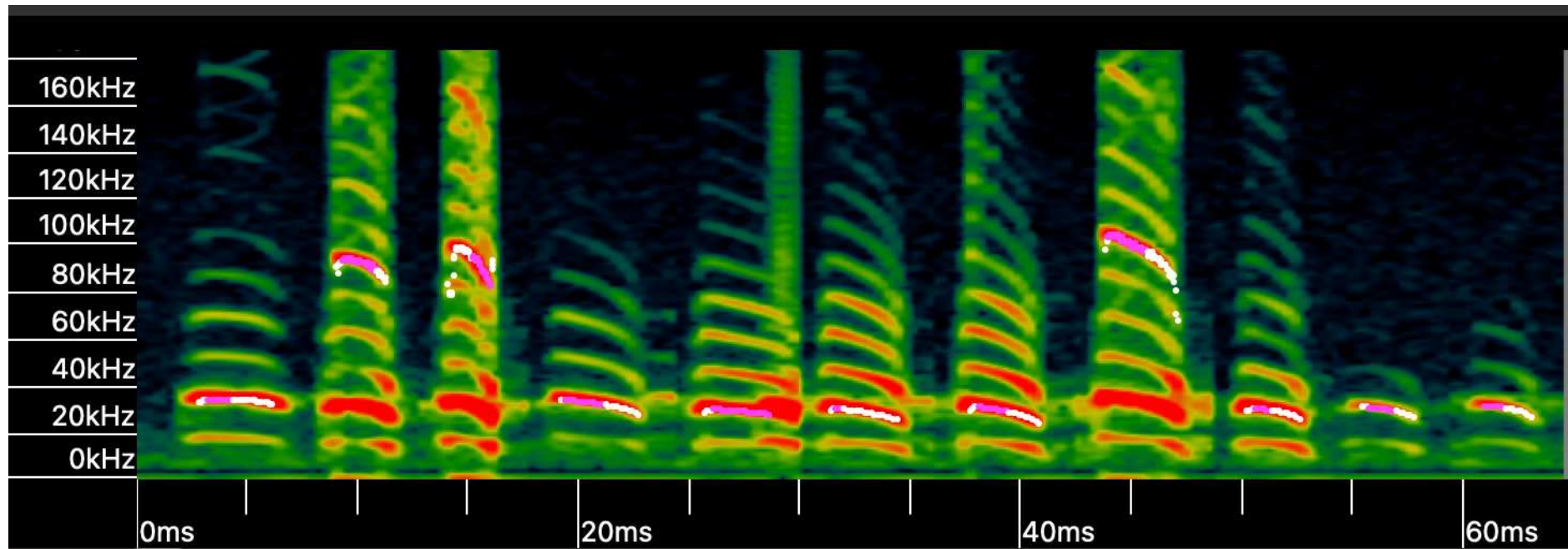
Example of a Sonogram	Description of the problem identified	Example of consequences in the Tadarida classification
	<p>Some recorded acoustic calls may be of poor quality (e.g. incomplete sonotype).</p>	<p>The Tadarida classifier is able to detect them, but their categorization in the different sonotypes may be erroneous or complex due to the absence of part of the acoustic call.</p>

Figure 20: Acoustic call of a bat of the Emballonuridae family (probably *Coleura afra*) recorded by the Boundou acoustic recorder (20 September 2021 at 23:05). Visualized with Kaleidoscope (© Wildlife acoustics).



3.5 Discussion

This study is the first in Africa and one of the first worldwide to apply a sonotype classifier in a country without any species classifier. In addition, this semi-automatic method with *a posteriori* classification makes it possible to analyse a large amount of data while overcoming most of the problems of overlapping acoustic characteristics of different species.

With the exception of Europe and North America, acoustic studies on bats are still scarce, especially in tropical countries despite their high bat diversity (Walters et al., 2013). This study improves the acoustic knowledge of cave-dwelling bats in Central Africa thanks to the recording of 145 reference sequences. These data will highly facilitate future acoustic studies in Central Africa, and will strengthen the database to identify bats in Africa.

This semi-automatic method can help researchers quickly identify the bat species present in a habitat while testing ecological hypotheses. In order to implement this method, we had to solve two challenges: recording acoustic reference calls and limiting the number of errors in the automatic classifier for bat species. To tackle the first challenge, we did not dispose of any morphological identification key for local bat species, and no acoustic reference data from a recent complete inventory of the species present in the Republic of Congo. In addition, the scientific community has yet to reach a consensus on a reference acoustic recording method that would allow interference-free sound quality while considering individual well-being. To overcome this problem, we made reference recordings using three different methods (free flight in a large room during the day, recording during the release of the individual and in the pouch) in order to maximize the quality of the acoustic recordings. In our study, the best reference acoustic recording quality (without the presence of other bats and the absence of ancillary noise) was achieved using the method of recording bats during flight in a large room. The advantage of this method was that it replicated the conditions of acoustic calls in a semi-open environment that bat experience when emerging from caves, while limiting the presence of other acoustic calls. In addition, it avoided the potential variations in calls that can be observed when recording in hand or in a pouch, especially for the Rhinolophidae and Hipposideridae families.

The second challenge was to limit the number of errors in the automatic classifier for bat species. Even with manual identification, it was still difficult to classify certain species using acoustics only. For example, it was difficult to differentiate the two *Miniopterus* species using the automatic classifier because of overlaps in acoustic characteristics. This problem has also been highlighted in other studies involving other families of Vespertilionidae (Russo & Voigt, 2016; Zamora-Gutierrez et al., 2016). We decided to use two processes to limit identification errors. Firstly, the data was analyzed according to acoustic species or groups when there was a doubt. This notion of acoustic group is closely related to the notion of acoustic guild that was defined by Denzinger and Schnitzler (2013) such as “a group of bat species with similar acoustic characteristics and wing morphology adapted to a dominant foraging behavior”. In our case, the two *Miniopterus* species could belong to the same guild. The second process used to limit identification errors was *a posteriori* classification in order to bring the acoustic groups together and thus limit classification errors. We then carried out a multi-criteria sorting for each of these species or groups, defining the *a priori* acoustic criteria and checking the effect of their introduction *a posteriori*. For example, for *Hipposideros cf. caffer*, we initially sorted by sonotype type and acoustic frequency only. However, this did not prevent us from obtaining *Rhinolophus* acoustic calls in this selection. We therefore refined our selection by excluding harmonics to obtain a better result. We obtained accuracies similar to those found in other studies (>80%), although our results varied between acoustic groups (Britzke et al., 2013; Chen et al., 2020; López-Baucells et al., 2019; MacSwiney G et al., 2008; Yoh et al., 2022; Zamora-Gutierrez et al., 2016). This back-and-forth between raw data, *a priori* and *a posteriori* classification enabled us to obtain an identification performance of over 98% for most species or acoustic groups. This process is time demanding, even though it saves time once the *a posteriori* classification criterion are defined, and this should be considered when applied to other studies.

We were unable to identify all bat species down to species level. However, the use of sonotypes may be sufficient to answer ecological questions such as activity patterns or habitat use, especially if the group can be considered as a guild. In future studies, the *Tadarida* sonotype classifier could be improved (i.e., distinction between CF-FMd / FMu-CF-FMd) by increasing the species reference database (e.g., reference sounds with different noises and

species or greater variation in reference sound quality). Finally, it would be interesting to complete the Tadarida outputs with additional acoustic criteria such as the duration of each acoustic call, which can help to differentiate species (e. g. *Rhinolophus*).

Tadarida is an open-source software package associated to a reference acoustic database of sonotypes of many bat families from around the world, which can be enhanced with additional data, as we did in this study, contributing to more open, accessible and reproducible research (Hampton et al., 2013). Even though the sonotypes classifiers are open, the reference database used to build the classifier is unfortunately not freely available to the research community. Yoh et al. (2022) published both their classifier and reference database, but they are up to now limited to restricted geographical areas. Tadarida, compared to the classifier of Yoh et al. (2022), offers an additional functionality that was not exploited in this study, as it can also detect bat buzzes, identify other animal species (birds, insects, etc.) and human noises. Therefore, Tadarida offers flexibility for a wide variety of studies.

Our study was focused on cave roosts. Acoustic recordings at populated bat roosts is a challenge in itself because, during peak bat emergence periods, several hundreds of individuals may emerge from the cave at the same time. Overlapping acoustic calls from different species interfered with the automatic identification of sonotypes by Tadarida and distorted the measurement of frequencies, both used to separate species from one another. Nonetheless, this problem was limited to periods of high species abundance (e.g., breeding season or peak emergence periods common to all species). In spite of this, the use of passive acoustic recording protocols at roost exits makes it possible to collect valuable data with the potential to study activity patterns, presence and absence of certain species, or the impact of human disturbance while limiting disturbance to bat populations. These disturbances can have a major impact on bat colonies, particularly on the roosts where females give birth. In addition, the detection by the classifier of the regular presence of *Coleura afra* in the Boundou cave confirms the importance of combining acoustics and capture. Acoustics is more sensitive and allows for detection of rare or elusive species (Appel et al., 2021; Carvalho et al., 2023; Silva & Bernard, 2017).

3.6 Conclusion

We presents a turnkey method to support researchers studying the acoustic communities of species in a geographical area where species classifiers do not exist. It can be applied and replicated worldwide by adapting the method to the species complex present in future study areas. The widespread use and automation of acoustic monitoring methods will help to improve ecological knowledge of chiropterans and contribute to their conservation.

3.7 Acknowledgments

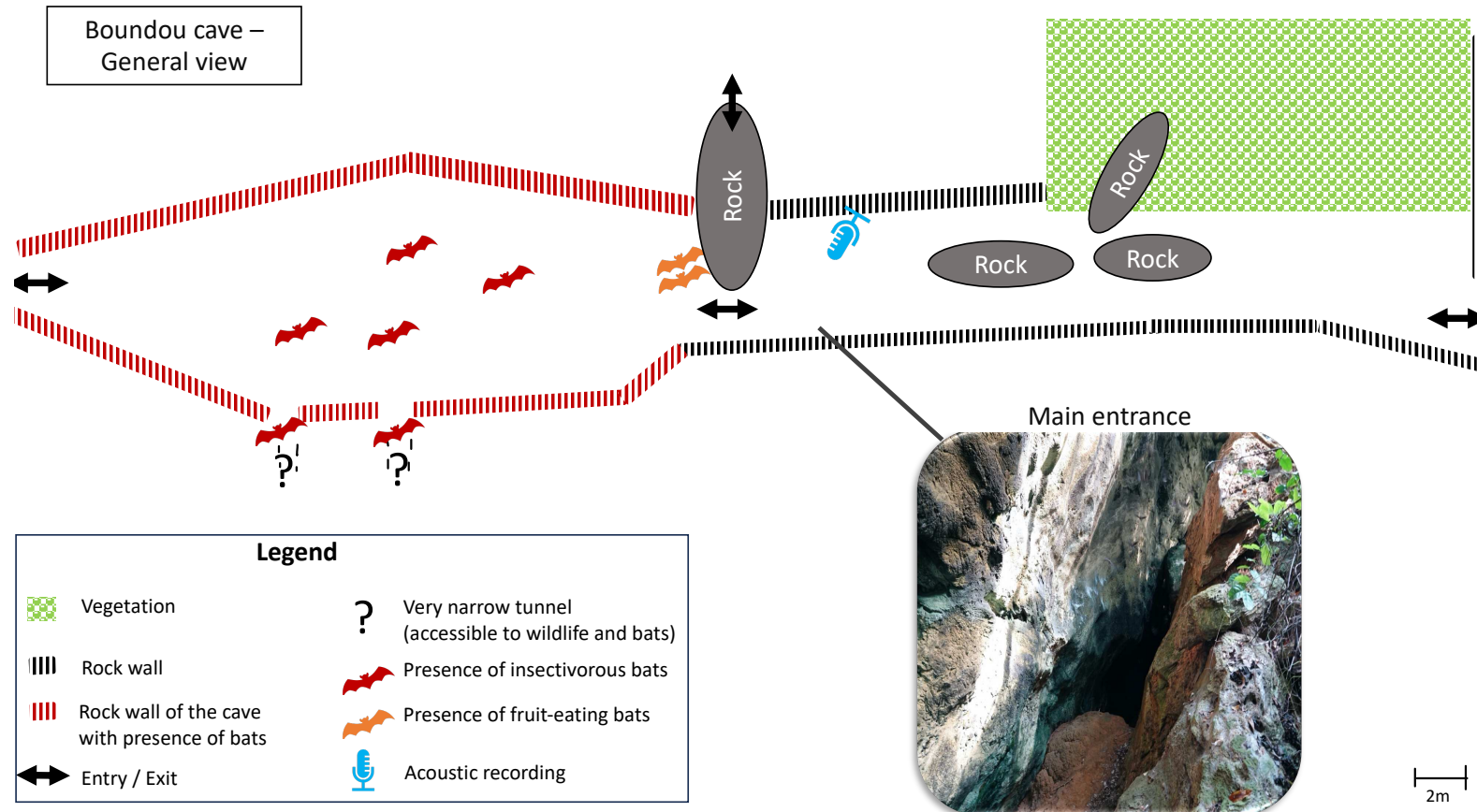
Many thanks to Yves Bas, researcher at the CESCO laboratory of the French Museum of Natural History for his help and advice in setting up this methodology. We would like to thank our main Congolese partners: the “Laboratoire National de Santé Publique de Brazzaville” (LNSP), the “Direction Générale de l'Élevage” (DGE) and “Ministère de l'économie forestière” for their support in this project, their help with the fieldwork and the applications for the capture and ethics permits. Finally, many thanks to the local communities who accepted the project and to the invaluable help of the field team, including our sorcerer, in collecting the data (C. Bazola, N. Nguimbi, R. Dimoukissi and R. Nguimbi).

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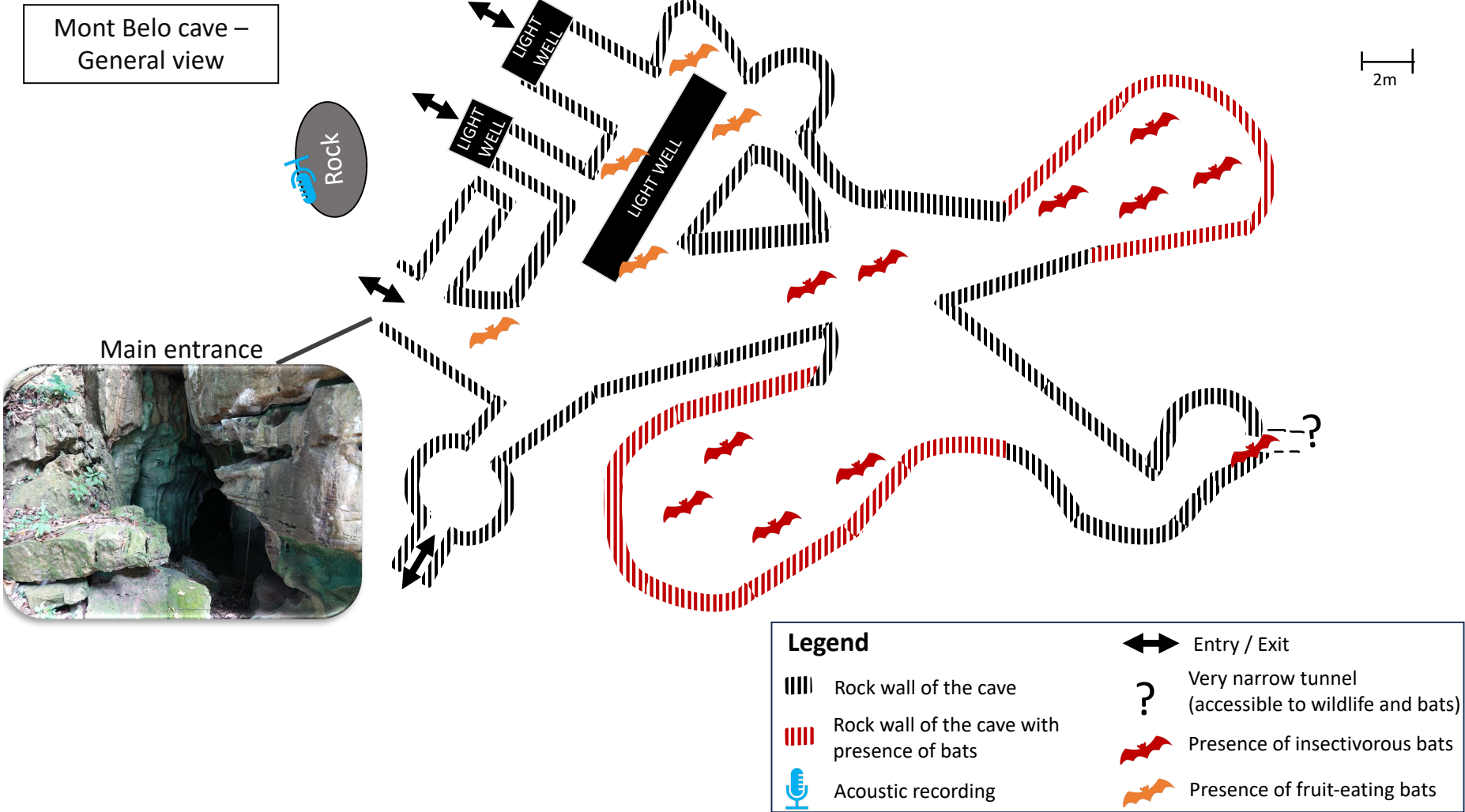
This study was funded by EBOSURSY Project “Capacity building and surveillance for viral haemorrhagic fevers”, project number FOOD/2016/379-660. LM was funded by a doctoral grant from the same project, the Laboratoire National de Santé Publique de Brazzaville and Cirad.

3.9 Appendix - Chapter 3

Appendix 12: Diagram of Boundou cave topography with location of acoustic recorder and bats.



Appendix 13: Diagram of Mont Belo cave topography with location of acoustic recorder and bats.



Appendix 14: Download link for the classifier database used in this study.

<https://dataverse.cirad.fr/privateurl.xhtml?token=7699d39e-d0de-4b01-9e09-fccb214d730>

Appendix 15: Presence of various insectivorous species in the two study caves (Mont Belo and Boundou). *No capture at Mont Belo cave due to the presence of a slash-and-burn farming in the area around the cave. ♀ ♂: capture of males and females; ♀: capture of females only, ♂: capture of males only, grey box: absence of this species in the captures.

Sessions of capture	Sites	<i>Triaenops</i> sp.	<i>Hipposideros</i> cf. <i>caffer</i>	<i>Rhinolophus</i> cf. <i>denti</i>	<i>Rhinolophus</i> cf. <i>landeri_alcyone</i>	<i>Miniopterus</i> cf. <i>minor</i>	<i>Miniopterus</i> cf. <i>inflatus</i>	<i>Macronycteris</i> sp.	<i>Coleura</i> <i>afra</i>
18 September 2021	Boundou cave	♀ ♂	♀ ♂			♀ ♂			
	Mont Belo cave	_*	_*	_*	_*	_*	_*	_*	_*
18 – 19 October 2021	Boundou cave	♀ ♂	♀ ♂	♀		♀ ♂			
	Mont Belo cave		♂	♀ ♂	♀ ♂	♂	♀ ♂		
3 – 4 December 2021	Boundou cave	♀ ♂	♀ ♂	♂	♂	♂		♂	
	Mont Belo cave		♀ ♂		♀ ♂	♂	♂		
5 – 6 February	Boundou cave	♀ ♂	♀ ♂		♂	♀ ♂			

Sessions of capture	Sites	<i>Triaenops</i> <i>sp.</i>	<i>Hipposideros</i> <i>cf. caffer</i>	<i>Rhinolophus</i> <i>cf. denti</i>	<i>Rhinolophus</i> <i>cf.</i> <i>landeri_alcyone</i>	<i>Miniopterus</i> <i>cf.</i> <i>minor</i>	<i>Miniopterus</i> <i>cf. inflatus</i>	<i>Macronycteris</i> <i>sp.</i>	<i>Coleura</i> <i>afra</i>
2022	Mont Belo cave		♀ ♂	♂	♀ ♂	♀	♂		
3 – 4 April 2022	Boundou cave	♀ ♂	♀ ♂	♀		♀ ♂	♂	♂	♂
	Mont Belo cave		♀ ♂	♂	♀ ♂		♀ ♂		
8 – 9 June 2022	Boundou cave	♀ ♂	♀ ♂			♂	♀ ♂		
	Mont Belo cave		♀ ♂		♀ ♂	♀ ♂	♀ ♂		
12 – 13 September 2022	Boundou cave	♀ ♂	♀ ♂			♀ ♂			
	Mont Belo cave		♂	♀ ♂	♀ ♂	♀ ♂			
19 – 22 October 2022	Boundou cave	♀	♀ ♂	♀	♀	♀ ♂			
	Mont Belo		♀ ♂	♂	♀ ♂	♂	♀ ♂		

Sessions of capture	Sites	<i>Triaenops</i> <i>sp.</i>	<i>Hipposideros</i> <i>cf. caffer</i>	<i>Rhinolophus</i> <i>cf. denti</i>	<i>Rhinolophus</i> <i>cf. landeri_alcyone</i>	<i>Miniopterus</i> <i>cf. minor</i>	<i>Miniopterus</i> <i>cf. inflatus</i>	<i>Macronycteris</i> <i>sp.</i>	<i>Coleura</i> <i>afra</i>
	cave								
29 – 30 November 2022	Boundou cave	♀ ♂	♀ ♂	♀ ♂	♀			♀	
	Mont Belo cave		♂	♀	♀ ♂	♀	♂		
5 – 6 January 2023	Boundou cave	♀ ♂	♀ ♂	♀		♀ ♂			
	Mont Belo cave		♀ ♂	♀	♀ ♂	♀	♂		
7 – 8 March 2023	Boundou cave	♀ ♂	♀ ♂	♀		♀ ♂			
	Mont Belo cave		♀ ♂	♂	♀ ♂	♀ ♂	♂		

Appendix 16: Number of raw acoustic files collected over the 19-month period at the two study sites (Boundou cave and Mont Belo cave)

Month and year	Boundou cave (number of files and period of collect)	Mont Belo cave (number of files and period of collect)	Comments
September 2021	78 554 files (from 20/09 to 19/10)	68 962 files (from 19/09 to 6/10)	
October 2021	71 901 (From 19/10 to 10/11)	66 420 files (From 20/10 to 06/11)	
November 2021	12 222 (from 11/11 to 14/11)	26 007 files (from 11/11 to 19/11)	Battery problem for both recorders
December 2021	15 354 files (From 5/12 to 7/12)	58 381 files (From 5/12 to 19/12)	Battery problem for both recorders
January 2022	39 128 files (From 10/01 to 17/01)	389 files (From 5/01 to 5/01)	Battery problem for both recorders
February 2022	90 352 files (From 7/02 to 2/03)	80 044 files (From 7/02 to 2/03)	
March 2022	36 987 files (From 2/03 to 9/03)	30 954 files (From 2/03 to 10/03)	
April 2022	98 654 files (From 6/04 to 8/05)	55 315 files (From 6/04 to 8/05)	
May 2022	108 758 files (From 8/05 to 8/06)	105 300 files (From 8/05 to 8/06)	
June 2022	94 552 files (From 9/06 to 2/07)	89 080 files (From 10/06 to 3/07)	
July 2022	102 927 files (From 3/07 to 29/07)	72 890 files (From 3/07 to 13/08)	
August 2022	85 502 files (From 13/08 to 2/09)	75 754 files (From 13/08 to 3/09)	
September 2022	74 995 files (From 12/09 to 7/10)	128 880 files (From 14/09 to 13/10)	
October 2022	60 076 files (From 21/10 to 5/11)	85 781 files (From 22/10 to 15/11)	
November 2022	23 233 files (From 30/11 to 8/12)	59 527 files (From 29/11 to 18/12)	
December 2022	49 949 files (From 21/12 to 2/01)	61 355 files (From 18/12 to 5/01)	

January 2023	42 850 files (From 6/01 to 10/01)	39 357 files (From 5/01 to 15/01)	
February 2023	25 941 files (From 5/02 to 17/02)	75 895 files (From 5/02 to 26/02)	
March 2023	45 674 files (From 27/02 to 9/03)	38 056 files (From 26/02 to 9/03)	Only few day in march 2023

Total	1 157 609 files	1 218 347 files	2 375 956 files
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Appendix 17: *Acoustic data for species references captured and recorded*

<https://dataverse.cirad.fr/privateurl.xhtml?token=2059b6fe-6183-4eac-bf79-c15ce686fd99>

3.10 References Chapter 3

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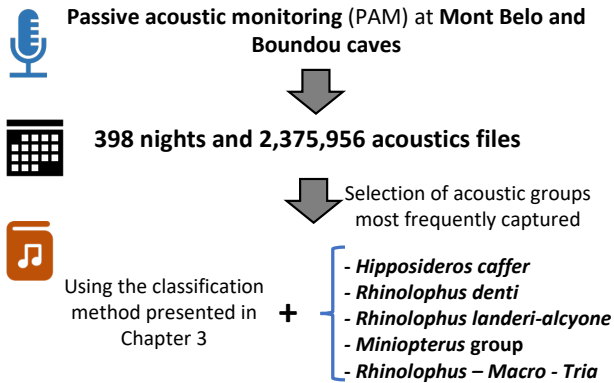
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Box 3: Graphic abstract Chapter 4

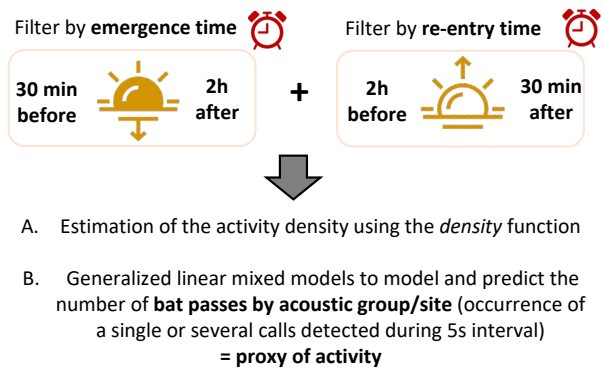
Main objectives:

- Presentation of a new method for studying the nocturnal activity and annual phenology of the five acoustic groups identified in our study sites.
- Description of this activity in relation to the reproductive stages of the acoustic groups.

Methodology

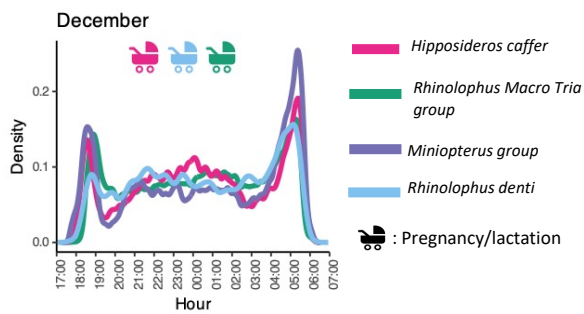


Bats counting techniques



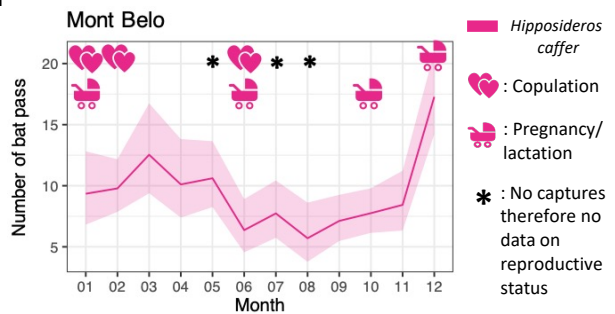
Distribution of activity during night

Tri- or quadri-modal activity at the end of the long rainy season (Oct, Nov, Dec) - short dry season (Jan, Feb) (e.g Boundou below)



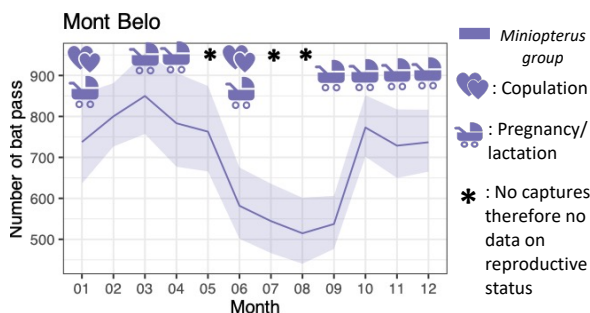
Prediction of emergence activity

Example at **Mont Belo** with the ***Hipposideros caffer***



Prediction of emergence activity

Example at **Mont Belo** with ***Miniopterus group***



Purpose of this activity

- **Tri – or quadri modal activity** : lactating female going back and forth at night
- **High activity from October to March**: (i) rearing of young by all acoustic groups and (ii) independence of young bats
- **Significant drop in activity from June to August**: potential mating period and low insect availability compared to the rainy season.

Conclusion:

- The high activity is linked to the lactation period and the independence of young bats.
- The breeding activity of insectivorous bat species is synchronized with periods of high resource availability.
- This method can be applied to data-deficient bat communities worldwide to quantify cave bat activity

**CHAPTER 4 – ARTICLE 3:
LISTEN, WHO IS IT? A NOVEL METHOD FOR STUDYING THE NIGHTLY
AND ANNUAL PHENOLOGIES OF CAVE BAT COMMUNITIES IN DATA
DEFICIENT AREAS OF THE WORLD**

Based on the publication in prep. for *Wildlife research*:

Labadie, M., Morand, S., Caron, A., De Nys, H., Niama, F. R., Nguilili, F., N’Kaya, T., Bourgarel, M., Bas, Y., Roemer, C., 2024. Listen, who is it? A novel method for studying the nightly and annual phenologies of cave bat communities in data deficient areas of the world

Listen, who is it? A novel method for studying the nightly and annual phenologies of cave bat communities in data deficient areas of the world.

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4.1 Abstract

We present a new method for studying the nocturnal and annual phenologies of cave bat communities in data deficient regions. In two caves (Mont Belo and Boundou) in the Republic of Congo, which are home to several species of insectivorous bats throughout the year, we set up an acoustic monitoring protocol (PAM) over a period of 19 months. In both caves, we collected a total of 2,375,956 acoustic files. We detected the presence of five distinct bat groups or species. Our results showed bimodal activity throughout the year, except during the long rainy season, when tri- or quadri-modal activity was detected. Activity distributed throughout the night is related to the period of juvenile rearing (gestation, parturition and lactation). For all acoustic groups, we also observed high activity from October to March (long rainy season, short dry season and short rainy season) and low activity during the long dry season (June, July, August). This high activity is linked to the lactation period, the independence of young bats and potentially the mating period. Finally, the reproductive activity of insectivorous bat species is synchronized with the period of high resource availability. The use of this technique can provide essential information to estimate insectivorous bat populations in habitats that have been poorly sampled using this passive acoustic monitoring protocol.

Keyword: PAM, Tadarida, activity pattern, abundance, bats phenology, Congo

4.2 Introduction

Human activities are currently triggering a massive loss of biodiversity, with rates of species decline or extinction unprecedented for several millennia (Barnosky et al., 2011; Ceballos et al., 2015). However, the scale of this crisis is probably still concealed by the lack of data on the populations of many species around the world (Hughes, 2017). Chiropterans are one of these taxa, as information on their populations is still poor despite the many threats they face (Furey & Racey, 2016; Mickleburgh et al., 2002).

Of the 1,470 recognised bat species worldwide, 34% are cave-dwelling species (IUCN, 2023; Simmons & Cirranello, 2023), living in caves or natural or man-made underground cavities (e.g. mines or tunnels). These habitats are crucial for bats, which use them to reproduce, raise their young, rest on a daily or temporary basis and hibernate (Barros et al., 2020; Keeley & Tuttle, 1999; Kunz, 1982; Kunz et al., 2003; Lewis, 1995; Mering & Chambers, 2014; Ormsbee et al., 2007; Russo & Ancillotto, 2015; Struebig et al., 2009; The Protection of Bat Roost Guidelines Subcommittee et al., 1992). In tropical environments, most cave-dwelling species use caves as permanent roosts and several species can cohabit together by hundreds or thousands (Kunz, 1982; Monadjem et al., 2020). However, despite their ecological importance, these fragile environments are threatened and still poorly protected on a global scale (Balogh et al., 2020; Medellin et al., 2017; Neuhuber et al., 2020). They are exposed to resources extraction (minerals, guano, etc.) or recreational activities (caving, tourism, etc.) (Okonkwo et al., 2017; Simons, 1998). However, there is little information about bat populations and the animal community they interact with in these habitats, especially in African countries.

In order to meet its three primary needs (feeding, reproduction or rest), an organism will adopt activity patterns located in spatial and temporal dimensions (Alcock, 2013; Appel et al., 2019; Jones & Rydell, 1994). For example, at the end of gestation, female bats will reduce their foraging rate due to the increase in their body weight (Rydell, 1993). During the parturition and lactation periods, female bats may reduce their flight distance in search for food while increasing their

activity due to the energy costs associated with milk production. (Cumming & Bernard, 1997; Henry et al., 2002; Kurta et al., 1989; Rydell, 1993). The temporal phenology of the activity of a species is also an indicator of its function in the ecosystem (i.e. niche sharing (Schoener, 1971), predator-prey dynamics (Flynn & Wolkovich, 2018; Forrest & Miller-Rushing, 2010; Tambling et al., 2015; Violle et al., 2017). Any alteration in this phenology could indicate an adaptation to an environmental change (Wolkovich et al., 2014).

Historically, bat communities have been studied using both invasive and non-invasive methods. Invasive methods such as bat capture remain often necessary to make an exhaustive species inventory, verify individual reproductive status and collect biometric data or samples (Appel et al., 2021; Barnhart & Gillam, 2014; Flaquer et al., 2007). Direct observation of individuals in their roost (e.g., cave, tree) or when they emerge to forage have also provided information on their behavior, abundance and movements (Thomas & West, 1989). Visual counts are sometimes difficult because of the large number of bats or the difficulty accessing the roosting sites. In addition, human observations are time-consuming and bring observer bias (Forsyth et al., 2006; Koger et al., 2023; Westcott & McKeown, 2004). The development of new technologies have made it possible to set up precise counts of individuals in caves using digital image analysis, thermal cameras (Azmy et al., 2012; Sabol & Hudson, 1995) or bioacoustics (Sugai et al., 2020; Whiting et al., 2021).

Passive acoustic monitoring (PAM) has a number of advantages: it is non-invasive, applicable in all environments (even the most remote ones), can collect a large amount of information such as abundance proxies, and the analysis can be automated (Hill et al., 2019). This technique exploits the physiological characteristic of more than 70% of the world's bats (Browning et al., 2017; Gibb et al., 2019) (except Pteropodidae) which use echolocation to move and forage. Recording these sounds, which are often specific to a species, makes it possible to make a quantitative monitoring of a bat species and/or communities. In recent years, several protocols, including that of Revilla-Martin et al (2020), have demonstrated the reliability of this technique compared to other methods of counting bat populations as they leave caves.

Our goal was to describe the activity patterns of communities of cave-dwelling bats using a novel method (counting bat pass during emergence and entry period) in bioacoustics and discuss these patterns in the light of their biological status assessed with capture data. Our study was implemented in an area in which no species classifier was developed, and thus we tested a new method using a sonotype classifier (Labadie et al, in prep). Firstly, we described the nightly phenology of the five acoustic groups identified in these caves and compare it across the different months of the year. Secondly, we looked at their annual phenology for each site. We assumed that nightly and annual phenologies are linked to bat life-cycle. Thus, we expected acoustic groups to be more active during periods when the species are breeding and rearing their young (Catto et al., 1995; Dietz & Kalko, 2006).

4.3 Materials and Methods

4.3.1 Study area

Our study took place in the Republic of Congo, in a highly karstic region favouring the presence of numerous caves and/or cavities of various sizes in a rolling habitat of herbaceous savannah with more or less important patches of tropical forest.

Boundou and Mont Belo caves, are located in the Bouenza and Niari departments, about 50 km apart. Both caves are close to Dolisie, one of the three most important towns in the Republic of Congo on the southbound Brazzaville - Pointe Noire route. In this region, the seasons are divided into four: the short dry season (January to February), the short rainy season (March to May), the long dry season (June to mid-September) and the long rainy season (mid-September to December).

The Mont Belo cave has several chambers (a main chamber and two secondary chambers). The Boundou cave, on the other hand, is a smaller cavity integrated into a rocky complex, with a main chamber and an annex chamber that is very difficult for humans to access, and houses a small internal lake.

Details of the configuration of the two caves in this study are presented in **Fig. 21** and **Fig. 22**.

4.3.2 Ethic statements

The protocols presented below have all been validated by the Ethics Committee of the Ministry of Scientific Research and Technological Innovation and by the Ministry of Forest Economy of the Republic of Congo (N°212/MRSIT/IRSSA/CERSSA and N°687/MEF/CAB/DGEF-DFAP). This work was also carried out with the agreement and participation of the authorities in the Niari and Bouenza departments, as well as the communities and landowners close to our study sites.

4.3.3 Capturing bats

During the 19 months of data collection, eleven capture sessions (**Appendix 3**) using a harp trap were set up at the main entrance of each cave, in order to identify the different bat species.

During these capture sessions, a wide range of information was collected, including the time of

capture, the family and species identified in the field, sex, age and reproductive status. At the beginning of the study, in order to confirm the genera and species of bats present in the caves, we collected genetic samples, acoustic recordings and specimens for reference purposes.

4.3.4 Passive acoustic monitoring (PAM)

At each main entrance of the two caves, at a maximum distance of five meters, we placed an SM4BAT acoustic recorder with a U1 microphone (Wildlife acoustics, Maynard, MA, USA). Each microphone was placed on a pole or tree and oriented to best record the acoustic calls of bats emerging and re-entering through the main entrance. These recorders ran continuously several weeks per month for a period of 19 months (September 2021 to first day of March 2023). The SM4BATs were set up according to the stationary points protocol of Vigie-chiro, a citizen science programme for the acoustic monitoring of bat populations in France (French Museum of Natural History, see (Mariton et al., 2023), starting 30 minutes before sunset and 30 minutes after sunrise. **Figures 21** and **22** show the location of the acoustic recorder in relation to the configuration of the study site.

Figure 21: General diagram of the configuration of the Boundou cave, the location of the camera trap and the bat roosting sites.

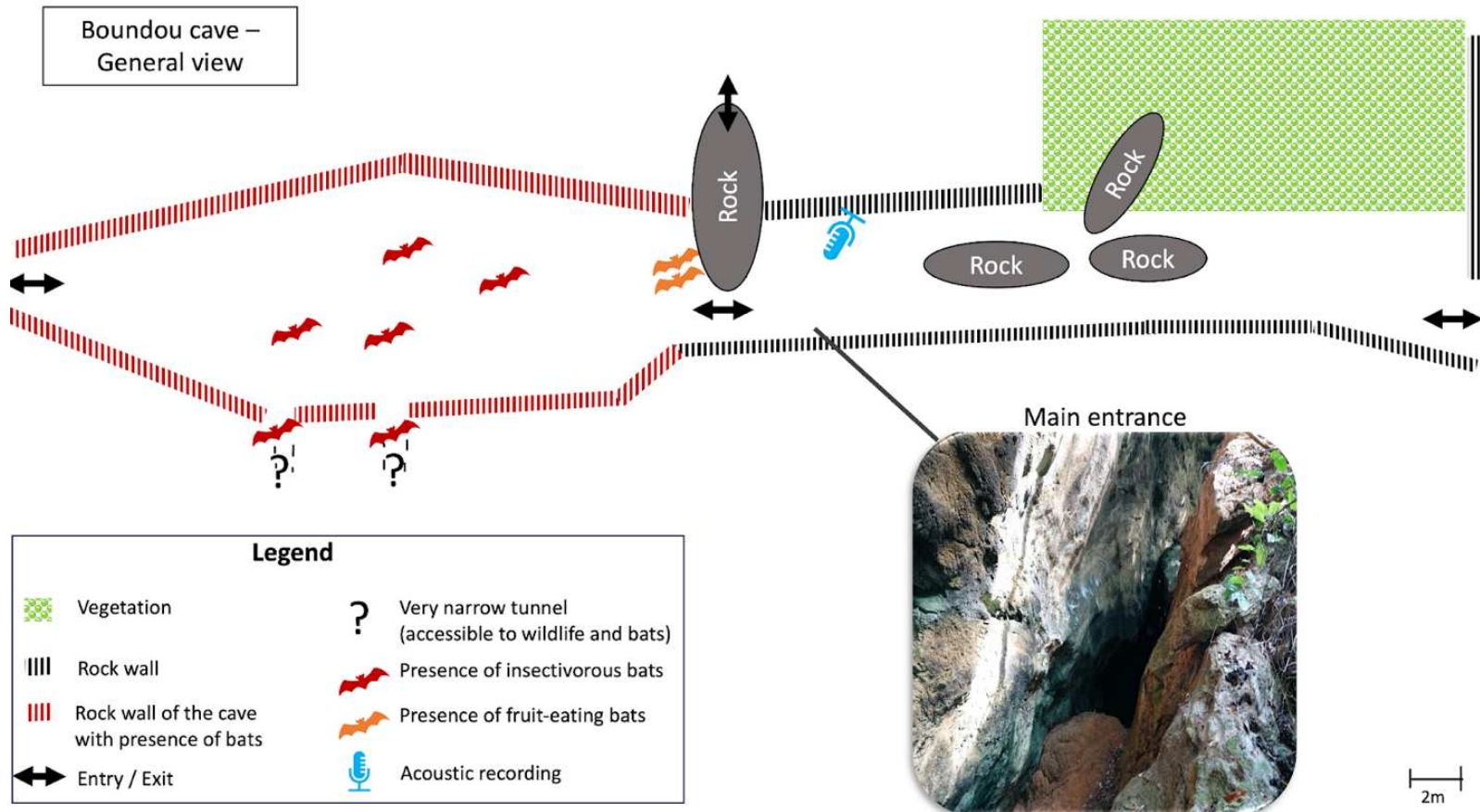
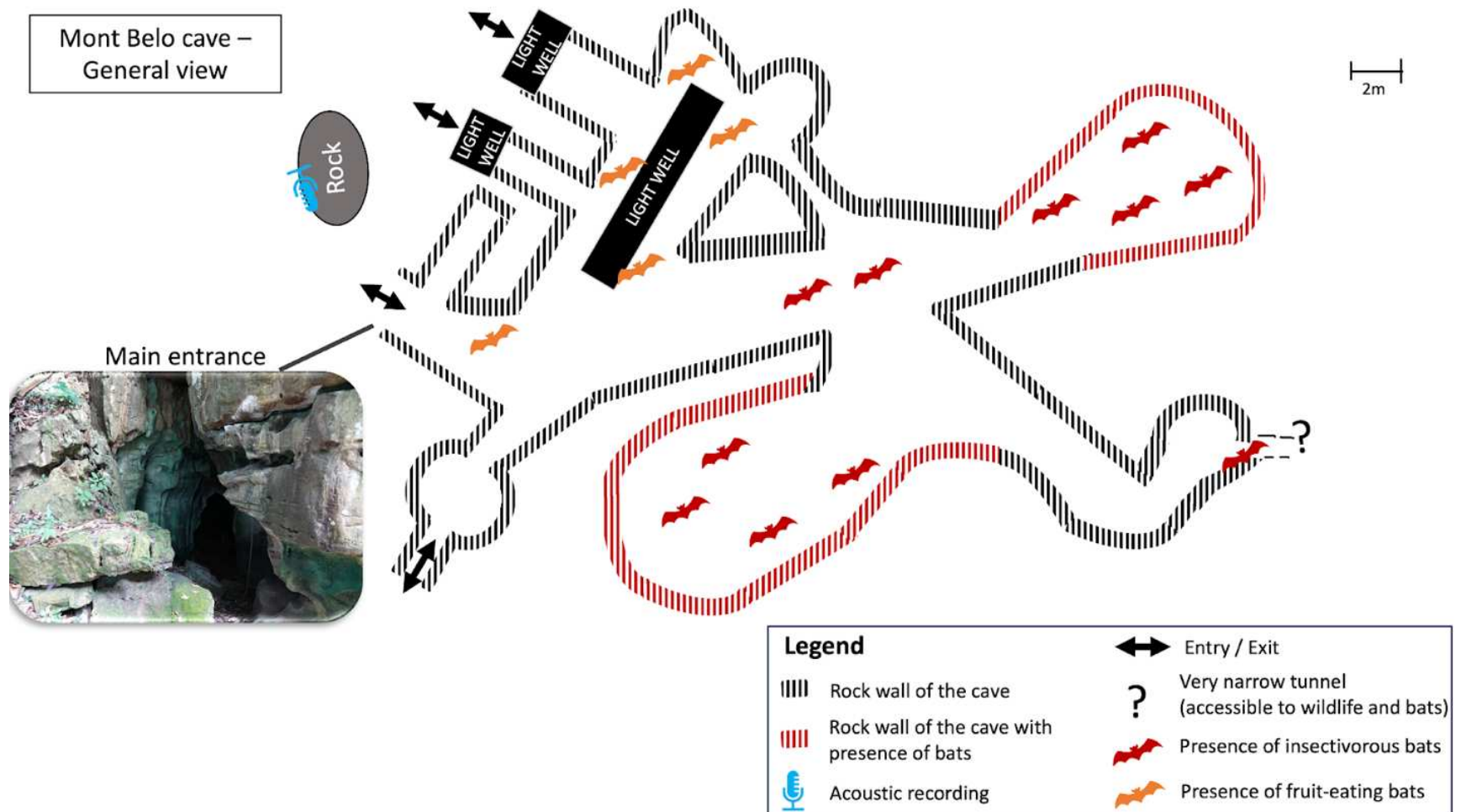


Figure 22: General diagram of the configuration of the Mont Belo cave, the location of the camera trap and the bat roosting sites.



4.3.5 Processing acoustic data

Recordings were cut in durations of five seconds maximum. In order to process all the acoustic files collected during this study in the two caves, we used the automatic sonotype classifier published in Roemer et al., (2021) and modified in Labadie et al. (in prep) to identify the presence of different sonotypes inside each file. The classifier returns a table in which each line corresponds to a group of calls with similar call shape and frequency, associated with information about their acoustic parameters (e.g. frequency, duration). Because of an overlap in the shape and frequency of certain acoustic calls from bat species of the same family, we decided to group the species at each study site using a posteriori classification (detail for the different acoustic groups in Labadie et al., in prep). The Boundou cave has four distinct groups: (1) the *Miniopterus* group (*M. cf. minor*/*M. cf. inflatus*), (2) *Hipposideros caffer* (*Hipposideros cf. caffer*), (3) *Rhinolophus denti* (*Rhinolophus cf. denti*) and (4) the *Rhinolophus* - Macro - Tria group (*R. cf. landeri-alcyone* /*M. gigas*/*Triaenops cf. afer*). The Mont Belo cave also has four distinct groups: (1) the *Miniopterus* group (*M. cf. minor*/*M. cf. inflatus*), (2) *Hipposideros caffer* (*Hipposideros cf. caffer*), (3) *Rhinolophus denti* (*Rhinolophus cf. denti*) and (4) *Rhinolophus landeri-alcyone* (*Rhinolophus cf. landeri-alcyone*). For more details on the automatic classification and on a posteriori sorting of acoustic groups, please respectively refer to Roemer et al. (2021) and Labadie et al. (in prep.).

4.3.6 Statistical analysis

Post-sorting of the Tadarida output and all the statistical analyses presented below were carried out using R software v. 4.3.1 (R Core Team, 2023). The graphics were produced using ggplot2 (Wickham, 2016) or ggradar (Bion, 2023).

i. Bat counting techniques and acoustics analyses

Bats may emerge from and enter a cave at any time during the night; nonetheless, most of them emerge after sunset and enter before sunrise (Erkert, 1982). In order to assess the activity of the five groups of bats during emergence and their entry at each cave during the 19 months, we decided to standardise our methodology based on the protocol of Mariton et al. (2023). As in other acoustic studies (Millon et al., 2015), we defined the activity of the different groups of bats as the number of *bat passes*. A bat pass is the occurrence of a single or several calls detected during a five seconds interval.

As our study took place in the tropics and in two caves that are geographically close, the characterisation of the diel activity of groups of bats can be based on a time scale (on sunrise and sunset). To begin with, we defined the time of sunset and sunrise based on the geographical coordinates and using the *getSunlightTimes* function in the *suncalc* package (Thieurmél et al., 2022). We then quantified the number of bat passes, over two specific time periods: (a) the period of emergence, from 30 minutes before sunset to two hours after sunset, and (b) the period of entry into the cave, from two hours before sunrise to 30 minutes after sunrise. This calculation was carried out for each species, each site and each day on which a complete night was recorded by the SM4BATs.

ii. Distribution of activity during the night

To calculate the distribution of the activity of acoustic groups of bats throughout the night, we estimated the activity density using the *density* function in the R package. This calculation was done for the twelve months of the year (some months were sampled repetitively during different years) for each species and each study site.

iii. Monthly phenology of the emergence and the entry at the roost

We used Generalised Linear Mixed Models (GLMM) to model the number of bat passes (proxy of activity) during their emergence and entry as a function of the month of the year in each cave and for each acoustic group.

We added month and year as a random effect (1|month_year). In total, we have eight different models (see **Appendix 18**). We used the glmmTMB package (Brooks et al., 2017) to build our models. We used a lognormal distribution and checked the conditions and diagnostics using the DHARMA package (QQ plot residuals, Residuals vs predicted, dispersion, zero-inflation) (Hartig & Hartig, 2022). We used a Bonferroni correction to limit the type I error rate (false positive) when interpreting generalised linear models (Cabin & Mitchell, 2000).

4.4 Results

4.4.1 Passive acoustic monitoring

In total, during nineteen months, we collected 2,375,956 acoustic files (five seconds duration) with 48.7% coming from Boundou and 51.3% from Mont Belo. The acoustic groups identified at each cave are shown in **Figure 23 (Appendix 19, raw data for each group)**.

4.4.2 Nightly phenology

In both caves, for the five acoustic groups (*Hipposideros caffer*, *Miniopterus* group, *Rhinolophus denti*, *Rhinolophus landeri-alcyone*, *Rhinolophus Macro Tria* group), the patterns of emergence and entry activity in the caves were generally bi-modal. More precisely, the first peak of activity was at the beginning of the night around 6pm (after sunset) and another peak between 3am and 4am, depending on the species (**Fig. 24** and **Fig. 25**).

In December, bat activity was more evenly distributed throughout the night than during the other months for all five acoustic groups and in both caves (**Fig. 24** and **Fig. 25**). In addition, the Boundou cave activity was more spread out during January and February (**Fig. 24**). In the Mont Belo cave, activity was also more spread out throughout the night in November and July (**Fig. 25**).

4.4.3 Annual phenology

Because analyses using emergence and entry activity produced similar annual phenologies, we decided to comment our results for the emergence activity only (**Appendix 20, 21, 22** and **23**). The activity at emergence varied according to the acoustic group and the month (**Appendix 20** and **21**). For *Rhinolophus denti*, their low activity during emergence in Mont Belo cave meant that the model had no results (the model had too little data to run) (**Appendix 22** and **23**).

Figure 23: Acoustic characteristics for the different bat species captured at the two study sites and categorization into different acoustic groups or species. Detail of sonotype, sonogram and acoustic frequency recorded or listed in reference works for each bat genus and species captured in the two caves (species with * are present only at Boundou). In the Boundou cave, there are four acoustic groups (*Rhinolophus – Macro – Tria* group; *Rhinolophus denti*; *Hipposideros caffer* and *Miniopterus* group) and in the Mont Belo cave there are four acoustic groups (*Rhinolophus landeri-alcylene*; *Rhinolophus denti*; *Hipposideros caffer* and *Miniopterus* group).

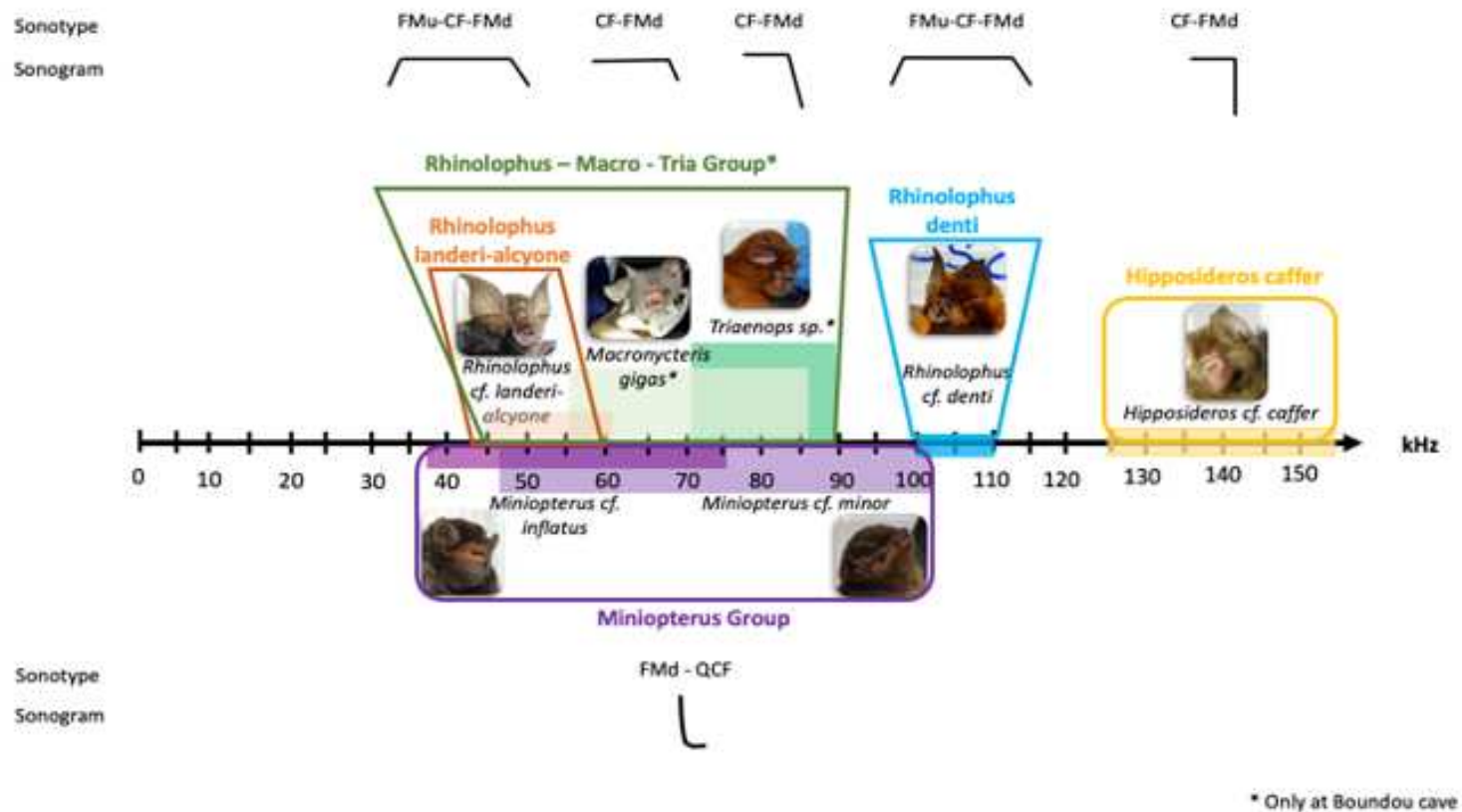


Figure 24: Number of bat passes for each acoustic group according to month at Boundou cave. The reproductive status of the species or acoustic group is indicated by two pictograms with the color of the acoustics groups: 🍼 young rearing (lactation, pregnant) and 💜 : mating period/male activity. The months of May, July and August, which have an *, are month when no captures were made, hence the absence of data on the reproductive status of the acoustic group.

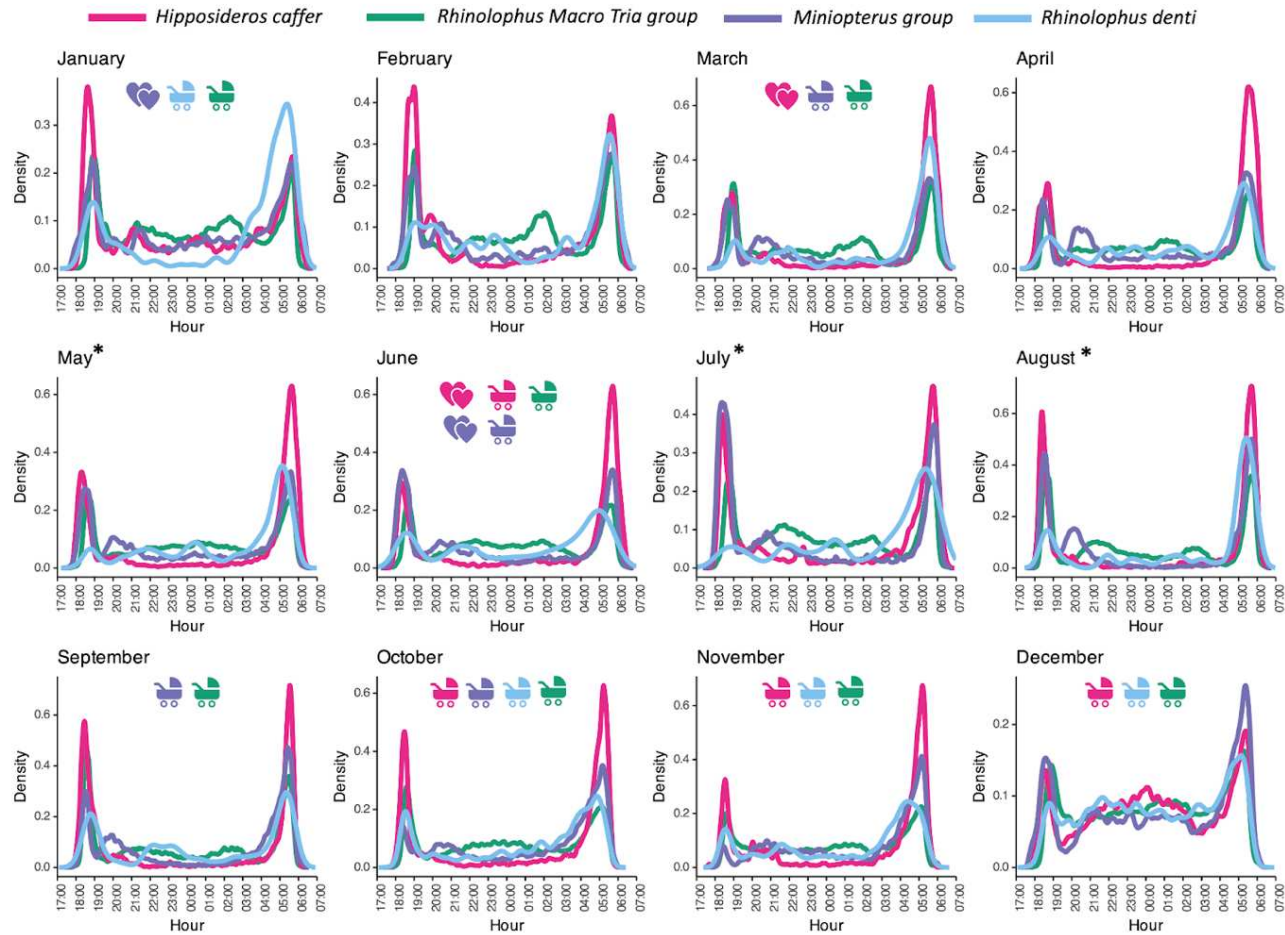
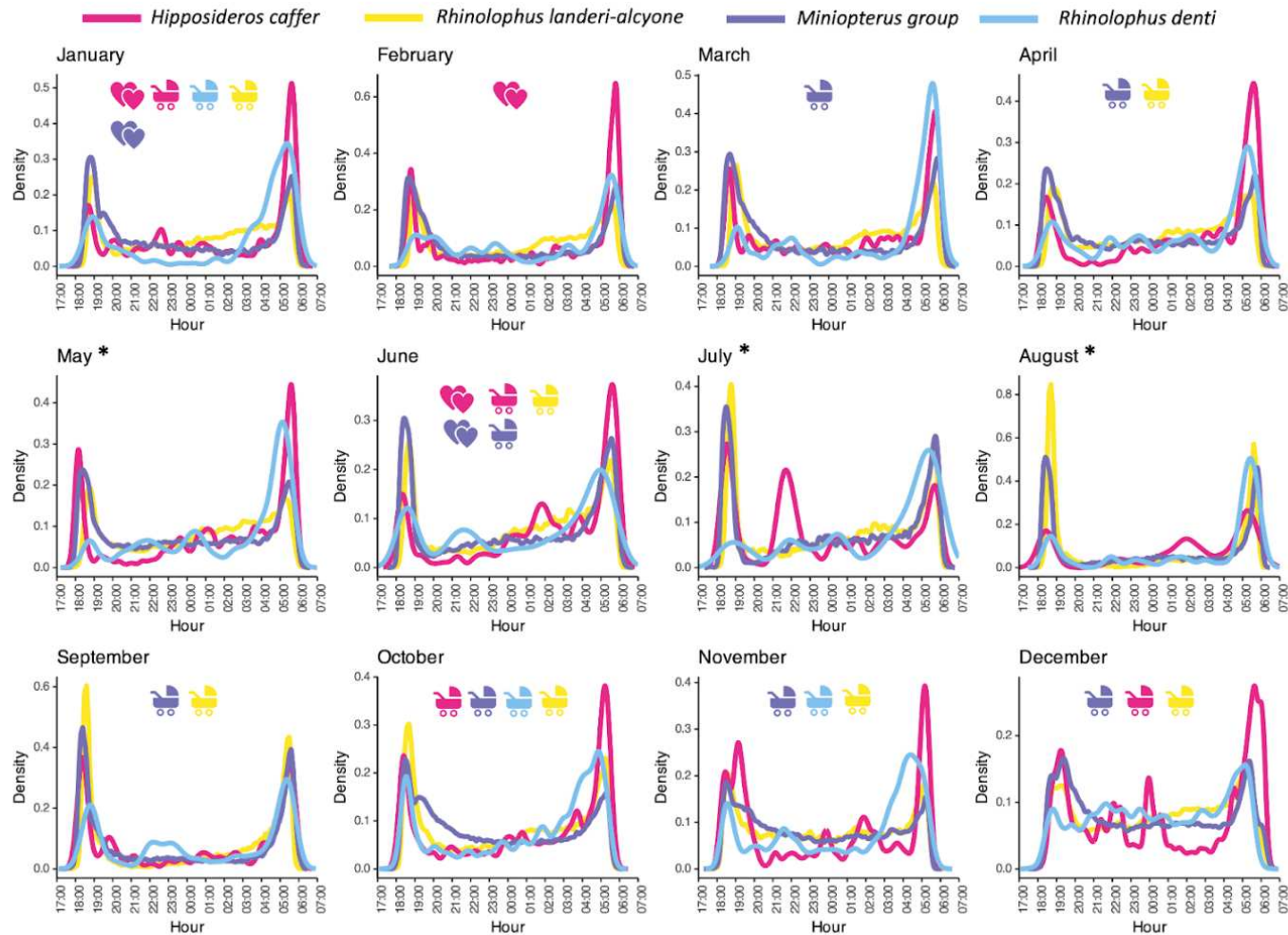


Figure 25: Number of bat passes for each acoustic group according to month at Mont Belo cave. The reproductive status of the species or acoustic group is indicated by two pictograms with the color of the acoustics groups: 🛒 young rearing (lactation, pregnant) and 💕 : mating period/male activity. The months of May, July and August, which have an *, are month when no captures were made, hence the absence of data on the reproductive status of the acoustic group.



For the *Miniopterus* group, in both caves, predicted activity (including emergence and entry) showed an increase in activity during the short dry season (Jan, Feb; of which January is a copulation period) and the short rainy season (March, April, May; with March a period for rearing young). For this group, we observed a decline in activity during the long dry season (June, July, August; June was a period for copulation and rearing young, depending on the individual) and the long rainy season (September, October, November, December; period for rearing young) (**Figure 26 and Appendix 24**).

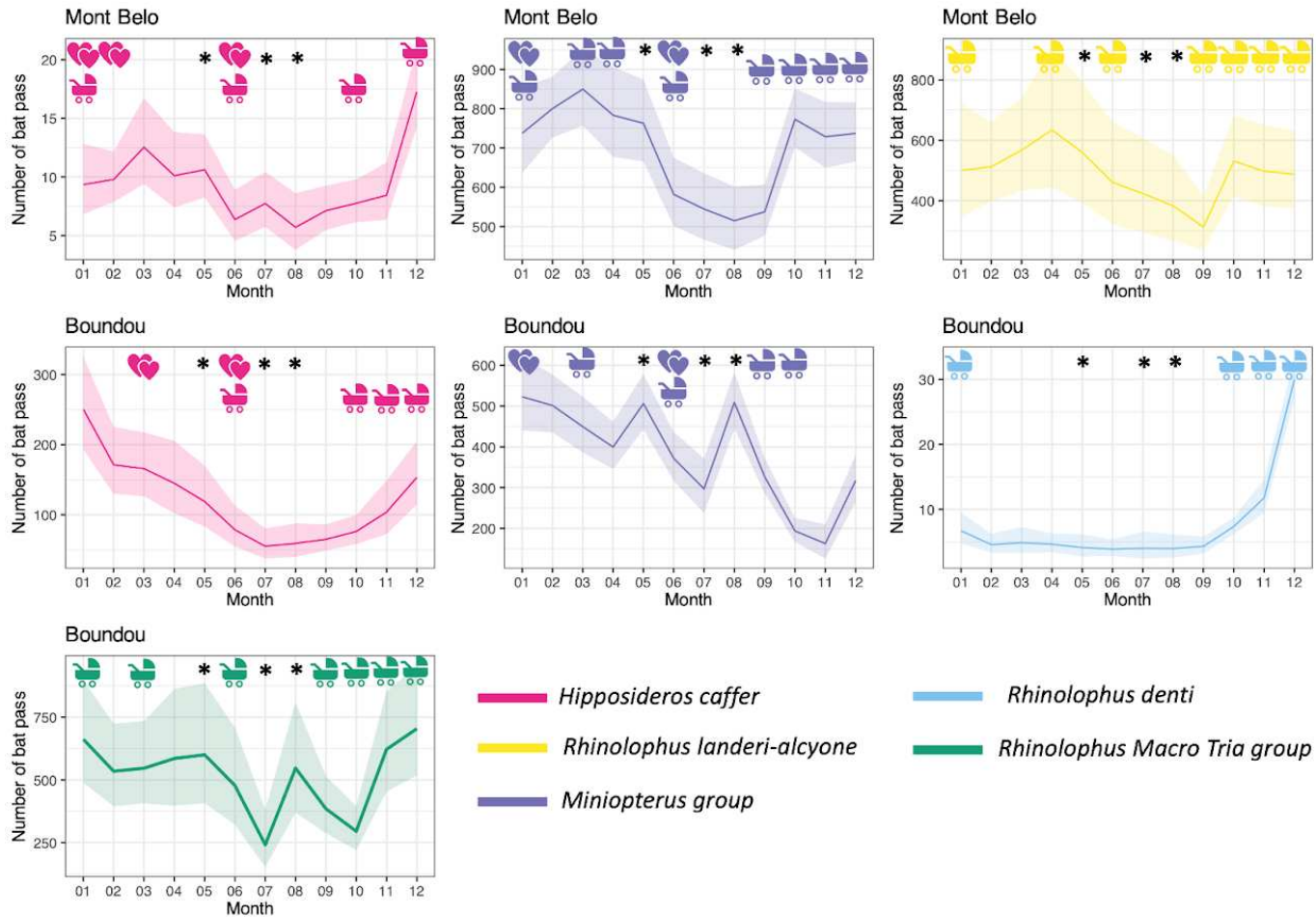
For *Hipposideros caffer*, in both caves we observed an increase in activity during the short dry season (January, February) and the short rainy season (March, April, May). In this group, these two periods are linked to the mating activity by certain individuals and the rearing of young. Secondly, *Hipposideros caffer* showed a sharp drop in predicted activity during the long dry season, which stabilized during the long rainy season (end of the year and period of juvenile rearing) (**Figure 26 and Appendix 24**).

For Mont Belo cave, the predicted activity for *Rhinolophus landeri-alcyone* showed an increase in activity during the short dry season and the short rainy season. Then, during the long dry season, we observed a sharp drop in activity, which rose again during the long rainy season (the period when females are pregnant, give birth and nurse their young) (**Figure 26 and Appendix 24**).

For Boundou cave, the *Rhinolophus denti* showed a strong increase in predicted activity during the long rainy season and the start of the short dry season (end of the year and start of the new one; period of rearing young), and low activity during two seasons: the short rainy season and the long dry season (no individuals captured with individuals rearing young or mating) (**Figure 26 and Appendix 24**).

Finally, in Boundou cave, we observed a predictive activity for the *Rhinolophus Macro Tria* group that increased during the short dry and rainy seasons (Jan, Feb, Mar, Apr) and decreased during the long dry and rainy seasons (**Figure 26 and Appendix 24**).

Figure 26: Prediction of acoustic group emergence activity with 95% confidence interval as a function of months and study sites (Boundou and Mont Belo cave). The *Rhinolophus denti* group at Mont Belo has not been represented here due to lack of data. The reproductive status of the species or acoustic group is indicated by two pictograms with the color of the acoustics groups: 🛒 young rearing (lactation, pregnant) and 💜: mating period/male activity. The months of May, July and August, which have an *, are month when no captures were made, hence the absence of data on the reproductive status of the acoustic group.



4.5 Discussion

This study is the first to use passive acoustic monitoring at the entrances of caves in a country without a classifier designed for the local species, to quantify the activity (proxy of abundance, (Revilla-Martín et al., 2020) of five acoustic groups of bats and to describe their nightly and annual phenologies. Our results characterized the variation in activity of acoustic groups in relation to season and linked to their breeding status.

4.5.1 Description of nightly and annual phenologies

With a few exceptions, bats emerged from their daytime roosts at dusk to fly to feeding sites (Bullock et al., 1987). The time of this nocturnal emergence was correlated with the time of sunset (Erkert, 1982). Unlike northern countries, Central African countries such as Congo have little variation in sunrise and sunset throughout the year (no more than half an hour) (WorldData, 2024). Furthermore, in many bat colonies, bat emergence is characterised by "burst activity", where several bats emerge in rapid succession, interspersed with periods during which few bats emerge (Swift, 1980). Jones et Rydell (1994) that bats exit from the roost to forage for most of the night before returning to rest at the end of the night to rest. Our study confirmed these results by showing the presence of strong bi-modal activity (a period of emergence and a period of entry into the cave) for most months in both caves. Thus, when they are absent from the site, we could hypothesise that a large number of individuals go hunting before returning to the cave. The other individuals may move to another resting place or return later.

Our study showed that the activity of the different species of insectivorous bats in these two caves was mainly bi-modal throughout the year, with the exception of December and January. These two months correspond to a period during which females suckle their young or finish raising them. This hypothesis is based on field observations and the ecology of these species. We therefore hypothesise that this activity spread throughout the night corresponded to females coming back and forth to the cave to alternatively suckle their young and forage. These results are in accordance with several studies which have shown that the relationship between activity

patterns in bat foraging habitats is influenced by the reproductive status of the individuals (Catto et al., 1995; Dietz & Kalko, 2006; Maier, 1992; Swift, 1980, 1997). In addition, the study by Henry et al (2002) showed that *Myotis lucifugus* decreased its flight distance by 35% and its home range by 51% between its gestation and lactation periods. In this study, lactating females returned to the roost between 1 and 2 times per night, imposing a distance constraint on foraging activity and observing an increase in roost activity 3 hours after the initial emergence time.

During the main rainy season (October, November, December), bat activity showed a sharp increase in terms of numbers of individuals. This sharp increase in activity can be explained by the formation of maternity colonies by the females for the period when they are rearing their young. (gestation, parturition and lactation, represented by a stroller in the figures) which forces them to change their behavior. During the short dry season (Jan, Feb; just after the main rainy season) and during the short rainy season (Mar, Apr, May), we observed a strong peak in activity in all of the acoustic groups in our study. This high activity may indicate (i) the beginning of juvenile independence and (ii) the beginning of a reproductive cycle for certain species such as *Hipposideros caffer* or the *Miniopterus* group.

The time when youngs reach their independence can vary according to bat species. For example, young *Hipposideros caffer* become independent between 3 and 3.5 months after birth (Wright, 2009). However, the young are not carried by their mother throughout this period, and may begin to fly 1 month after birth, staying in the roost with other individuals of the same generation (Wright, 2009). In fact, the two reproduction periods of the insectivorous species in our study could be explained by two hypotheses. The first is that the two reproductive cycles identified during the captures confirm the presence of two groups of species (as is the case for the *Miniopterus* group and the *Rhino Macro Tria* group). Or, we could have the presence of a cryptic complex (as for the *Hipposideros*) (Wright, 2009).

Finally, for most of the species present, our results showed relatively low activity during the long dry season (June, July, August) compared with the other seasons. We hypothesize that since this is not the period when young are reared, activity decreases drastically and/or that bat species do

not congregate in large numbers in these habitats during this period, which is not very favourable in terms of resources (Barclay & Kurta, 2007; Patriquin & Ratcliffe, 2016; Patterson et al., 2007; Postawa & Gas, 2009). The most favourable period in terms of resources is the long rainy season (Oct, Nov, Dec) and the short rainy season (March, April, May) for insectivorous bats (Fenton and Thomas, 1980). These wet periods favour the presence of a great diversity and abundance of insects (Jenber & Wili, 2021; Wolda, 1988). Indeed, species tend to synchronize their period of reproduction with periods of high resource availability (Cumming & Bernard, 1997). In mammals, including bats, this synchronization may be all the more important because of the high energy expenditure by females when suckling their young (Racey & Entwistle, 2000). In addition, the aggregation of individuals can be an advantage, as females cooperate with each other to raise their young. This also enables individuals, including young, to group together to share their body heat, thus reducing the cost of thermoregulation (Boyles et al., 2008; Carter & Wilkinson, 2013; Willis & Brigham, 2007).

Scattered activity was observed throughout the night during the long dry season (June, July and August). Unfortunately, we did not capture in July and August, and could not assess the reproductive status of the individuals. However, the observation of a gestation period from late September to early December in the females of all the acoustic groups would tend to place the copulation period towards the end of summer during the long dry season (June, July). This hypothesis is fairly consistent with other studies of bat copulation behaviour in other countries (Günther et al., 2016). For example, according to several studies concerning *Hipposideros caffer*, the copulation period occurs at the end of the first rainy season (June-July) and calving in the middle of the second rainy season (October-November) (Bernard, 1982; Brosset, 1968).

The only exception to this drop in activity was the *Minioptera* group in Boundou cave, which was very active in August (long dry season). The females of this species may use the cave to gather in a maternity colony before giving birth in September and October (obs. per ML). This would support Cumming and Bernard (1997) who mentioned that many bat species give birth to their young about a month before the peak in resource availability.

4.5.2 Variation in roost use by month and species

Our study showed a difference in species use of the two caves over the time scale; for example, *Rhinolophus denti* seemed to use both caves only for rearing young, not for mating. Conversely, *Hipposideros caffer* used both caves for mating and rearing young. We can note that for *Hipposideros caffer*, the mating period seemed to vary from cave to cave (January, February, June in Mont Belo cave and March and June in Boundou cave). This variation in breeding months could potentially be explained by the presence of two cryptic species of *Hipposideros* inside this study (Labadie et al., in prep.).

4.6 Conclusion

We have demonstrated the high variability of activity in five acoustic groups of cave-dwelling insectivorous bats. These variations were strongly linked to the season and/or the biological cycle of these species. This information is relevant to understand the mechanisms by which cave bat colonies may merge and fission. Furthermore, this study also provided valuable information on the activity of insectivorous species in Congo. In addition, this innovative acoustic method makes it possible to estimate the abundance of bat populations, and can be applied at low cost in regions where data on these species is lacking. Studies on the population movements of cave-dwelling species, taking into account the spatio-temporal aspect, are essential to help quantify potential population changes that can be caused by human disturbance or by current climate change. Our method confirms the usefulness of passive acoustic monitoring to access the dynamics and activity of insectivorous bats populations while keeping disturbance to a minimum.

4.7 Acknowledgments

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I would also like to extend my warmest thanks to Léa Mariton for her support and advice on the R scripts for the density of bat activity over time.

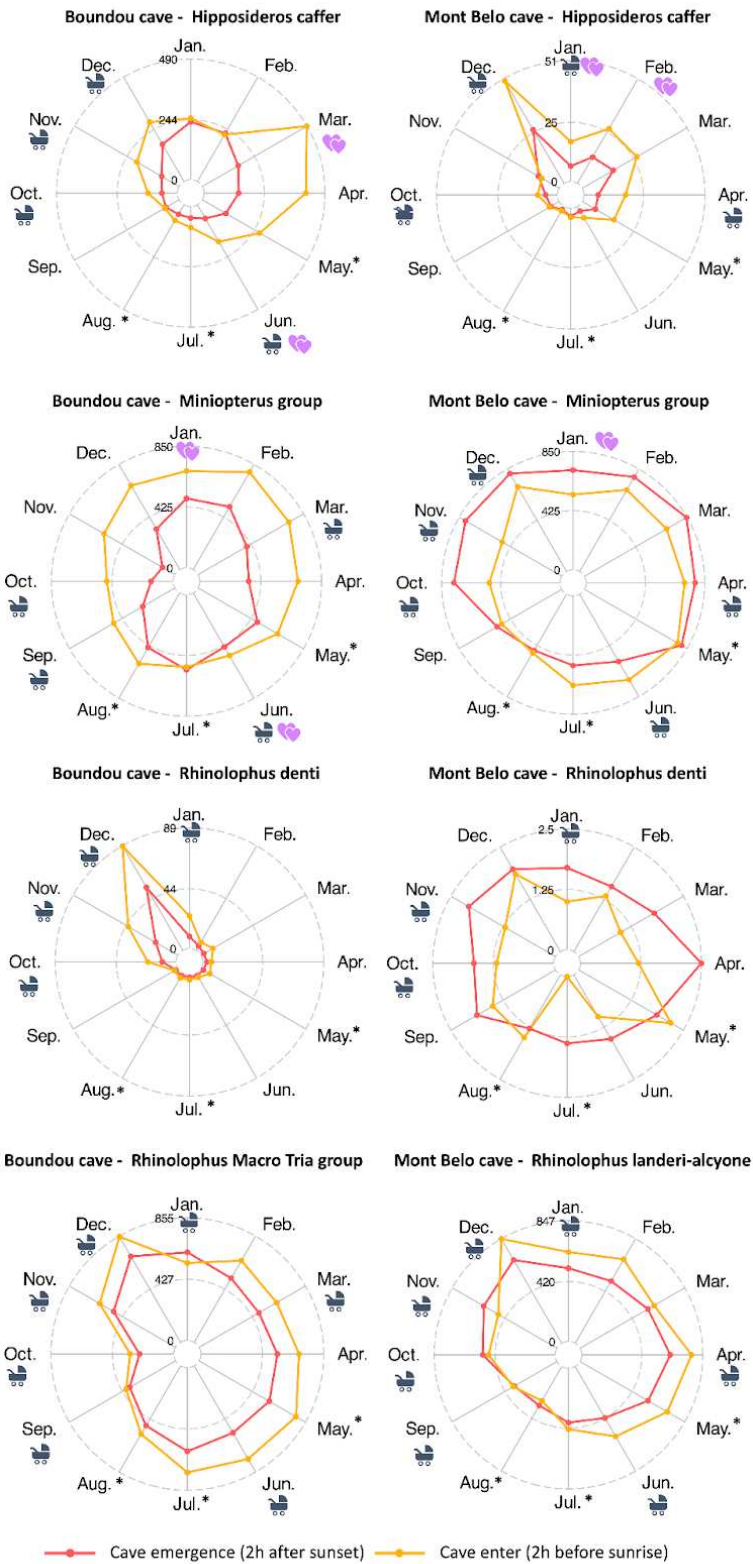
Many thanks to our Congolese partners: the “Laboratoire National de Santé Publique de Brazzaville” (LNSP), the “Direction Générale de l'Élevage” (DGE) and “Ministère de l'économie forestière” for their support in this project, their help with the fieldwork and the applications for the capture and ethics permits. Thanks also to the bat field team who helped collect data: F. Nguilili, C. Bazola, N. Nguimbi (our wizard), R. Dimoukissi and R. Nguimbi. Finally, we would like to thank the local authorities in Dolisie and the local population for their help with this project.

4.8 Appendix – Chapter 4

Appendix 18: Summary table of eight emergence models for each cave (Boundou and Mont Belo) and each acoustics group tested in this study.

Model name	Description
Model <i>Hipposideros caffer</i> emergence Boundou	Nb_batpass ~ Month + (1 month-year)
Model <i>Miniopterus</i> group emergence Boundou	Nb_batpass ~ Month + (1 month-year)
Model <i>Rhinolophus denti</i> emergence Boundou	Nb_batpass ~ Month + (1 month-year)
Model <i>Rhinolophus landeri-alcyone</i> emergence Boundou	Nb_batpass ~ Month + (1 month-year)
Model <i>Hipposideros caffer</i> emergence Mont Belo	Nb_batpass ~ Month + (1 month-year)
Model <i>Miniopterus</i> group emergence Mont Belo	Nb_batpass ~ Month + (1 month-year)
Model <i>Rhinolophus denti</i> re-enter	Nb_batpass ~ Month + (1 month-year)
Model <i>Rhinolophus Macro Tria</i> emergence	Nb_batpass ~ Month + (1 month-year)

Appendix 19: Number of bat passes for each acoustic group according to month and site (Boundou and Mont Belo). The reproductive status of the species or acoustic group is indicated by two pictograms:  young rearing (lactation, pregnant) and  : mating period/male activity. The months of May, July and August, which have an *, are month when no captures were made, hence the absence of data on the reproductive status of the acoustic group.



Appendix 20: Generalized linear mixed (GLM) with Bonferroni correction describing the relationship between activity and month for the four acoustic groups in the Boundou cave during the emergence time of bats.

Predictors	Hipposideros caffer emergence Boundou			Minoipterus group emergence Boundou			Rhinolophus denti emergence Boundou			Rhinolophus Macro Tria group emergence Boundou		
	Estimates	CI	p	Estimates	CI	p	Estimates	CI	p	Estimates	CI	p
Intercept Jan	250.45 ^{***}	192.77 – 325.39	<0.001	522.93 ^{***}	440.28 – 621.08	<0.001	6.71 ^{***}	4.73 – 9.51	<0.001	661.78 ^{***}	488.20 – 897.08	<0.001
Feb	0.68	0.47 – 1.00	0.593	0.96	0.77 – 1.19	1.000	0.68	0.43 – 1.06	1.000	0.81	0.53 – 1.24	1.000
Mar	0.66	0.45 – 0.96	0.382	0.86	0.69 – 1.08	1.000	0.72	0.43 – 1.21	1.000	0.83	0.54 – 1.26	1.000
Apr	0.58	0.37 – 0.89	0.166	0.76	0.61 – 0.95	0.191	0.69	0.44 – 1.07	1.000	0.88	0.54 – 1.45	1.000
May	0.47 [*]	0.30 – 0.74	0.012	0.97	0.78 – 1.20	1.000	0.61	0.36 – 1.03	0.750	0.91	0.55 – 1.48	1.000
Jun	0.31 ^{***}	0.20 – 0.49	<0.001	0.71 [*]	0.57 – 0.89	0.038	0.58	0.37 – 0.91	0.203	0.72	0.44 – 1.19	1.000
Jul	0.22 ^{***}	0.14 – 0.35	<0.001	0.57 ^{***}	0.43 – 0.74	<0.001	0.60	0.33 – 1.08	1.000	0.37 ^{**}	0.21 – 0.63	0.003
Aug	0.24 ^{***}	0.15 – 0.38	<0.001	0.97	0.79 – 1.21	1.000	0.59	0.34 – 1.01	0.671	0.83	0.50 – 1.36	1.000
Sep	0.26 ^{***}	0.18 – 0.38	<0.001	0.62 ^{***}	0.51 – 0.77	<0.001	0.64	0.41 – 0.99	0.557	0.58	0.38 – 0.89	0.137
Oct	0.30 ^{***}	0.21 – 0.44	<0.001	0.37 ^{***}	0.30 – 0.46	<0.001	1.10	0.76 – 1.58	1.000	0.45 ^{**}	0.29 – 0.68	0.002
Nov	0.41 ^{**}	0.27 – 0.64	0.001	0.31 ^{***}	0.23 – 0.42	<0.001	1.75	1.19 – 2.59	0.056	0.94	0.61 – 1.46	1.000
Dec	0.61	0.41 – 0.90	0.166	0.61 ^{***}	0.48 – 0.77	0.001	4.47 ^{***}	3.13 – 6.38	<0.001	1.06	0.69 – 1.64	1.000
Random Effects												
σ^2	58.54			192.82			7.76			191.71		
τ_{00}	0.03 _{month2}			0.00 _{month2}			0.00 _{month2}			0.04 _{month2}		
ICC	0.00									0.00		
N	19 _{month2}			19 _{month2}			19 _{month2}			19 _{month2}		
Observations	275			275			182			275		
Marginal R ² / Conditional R ²	0.003 / 0.004			0.001 / NA			0.047 / NA			0.001 / 0.001		
AICc	2867.068			3566.270			900.772			3695.560		

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

Appendix 21: Generalized linear mixed (GLM) with Bonferroni correction describing the relationship between activity and month for the four acoustic groups in the Mont Belo cave during the emergence time of bats.

Predictors	Hipposideros caffer emergence Mont Belo			Miniopterus group emergence Mont Belo			Rhino landeri-alcione emergence Mont Belo			Rhinolophus denti emergence Mont Belo		
	Estimates	CI	p	Estimates	CI	p	Estimates	CI	p	Estimates	CI	p
Intercept Jan	9.35 ***	6.82 – 12.82	<0.001	737.51 ***	636.15 – 855.01	<0.001	499.73 ***	346.52 – 720.68	<0.001	1.00	NaN – NaN	NaN
Feb	1.05	0.74 – 1.47	1.000	1.08	0.91 – 1.29	1.000	1.03	0.66 – 1.60	1.000			
Mar	1.34	0.91 – 1.98	1.000	1.15	0.96 – 1.39	1.000	1.14	0.72 – 1.79	1.000			
Apr	1.08	0.72 – 1.63	1.000	1.06	0.86 – 1.31	1.000	1.27	0.76 – 2.11	1.000			
May	1.14	0.79 – 1.64	1.000	1.03	0.85 – 1.26	1.000	1.12	0.68 – 1.86	1.000			
Jun	0.68	0.44 – 1.05	0.958	0.79	0.64 – 0.97	0.316	0.92	0.55 – 1.54	1.000			
Jul	0.83	0.55 – 1.24	1.000	0.74	0.60 – 0.91	0.064	0.85	0.51 – 1.42	1.000			
Aug	0.61	0.37 – 1.00	0.588	0.70 *	0.56 – 0.86	0.012	0.77	0.46 – 1.28	1.000			
Sep	0.76	0.52 – 1.11	1.000	0.73 *	0.60 – 0.88	0.013	0.63	0.40 – 0.99	0.542	1.00	NaN – NaN	NaN
Oct	0.83	0.58 – 1.18	1.000	1.05	0.88 – 1.25	1.000	1.06	0.68 – 1.65	1.000	1.00	NaN – NaN	NaN
Nov	0.90	0.61 – 1.33	1.000	0.99	0.82 – 1.19	1.000	1.00	0.63 – 1.57	1.000			
Dec	1.85 **	1.34 – 2.56	0.003	1.00	0.84 – 1.19	1.000	0.98	0.62 – 1.53	1.000			
Random Effects												
σ^2	13.88			152.17			169.52			0.00		
τ_{00}	0.00 _{month2}			0.00 _{month2}			0.03 _{month2}			0.00 _{month2}		
ICC				0.00			0.00					
N	18 _{month2}			18 _{month2}			18 _{month2}			4 _{month2}		
Observations	271			293			293			4		
Marginal R ² / Conditional R ²	0.007 / NA			0.000 / 0.000			0.000 / 0.000			0.000 / NA		
AICc	1644.111			3796.908			3859.854			NA		

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

Appendix 22: Generalized linear mixed (GLM) with Bonferroni correction describing the relationship between activity and month for the four acoustic groups in the Boundou cave during the enter time of bats.

<i>Predictors</i>	Hipposideros caffer enter Boundou			Miniopterus group enter Boundou			Rhinolophus denti enter Boundou			Rhinolophus Macro Tria group enter Boundou		
	<i>Estimates</i>	<i>CI</i>	<i>p</i>	<i>Estimates</i>	<i>CI</i>	<i>p</i>	<i>Estimates</i>	<i>CI</i>	<i>p</i>	<i>Estimates</i>	<i>CI</i>	<i>p</i>
Intercept Jan	249.41 ***	156.62 – 397.20	<0.001	463.67 ***	314.74 – 683.07	<0.001	17.20 ***	11.53 – 25.65	<0.001	439.89 ***	289.90 – 667.50	<0.001
Feb	1.07	0.59 – 1.96	1.000	1.71	1.07 – 2.74	0.290	0.43 *	0.25 – 0.73	0.020	1.65	0.96 – 2.85	0.865
Mar	1.57	0.87 – 2.85	1.000	1.63	1.02 – 2.60	0.497	0.55	0.33 – 0.91	0.242	1.54	0.89 – 2.67	1.000
Apr	1.16	0.58 – 2.31	1.000	1.53	0.91 – 2.57	1.000	0.37 **	0.21 – 0.64	0.005	1.70	0.90 – 3.20	1.000
May	1.18	0.60 – 2.34	1.000	1.45	0.86 – 2.44	1.000	0.41 *	0.24 – 0.72	0.021	1.86	0.99 – 3.50	0.656
Jun	0.75	0.38 – 1.49	1.000	0.76	0.44 – 1.31	1.000	0.29 ***	0.16 – 0.50	<0.001	1.26	0.66 – 2.40	1.000
Jul	0.48	0.24 – 0.96	0.457	0.89	0.52 – 1.52	1.000	0.31 ***	0.17 – 0.55	0.001	1.50	0.80 – 2.84	1.000
Aug	0.46	0.22 – 0.93	0.377	1.36	0.81 – 2.30	1.000	0.36 **	0.20 – 0.65	0.007	1.40	0.74 – 2.65	1.000
Sep	0.49	0.27 – 0.89	0.229	1.17	0.74 – 1.87	1.000	0.29 ***	0.17 – 0.50	<0.001	1.02	0.59 – 1.76	1.000
Oct	0.67	0.37 – 1.21	1.000	1.19	0.75 – 1.89	1.000	0.48 *	0.30 – 0.77	0.028	0.83	0.48 – 1.44	1.000
Nov	0.69	0.37 – 1.31	1.000	1.12	0.68 – 1.83	1.000	1.15	0.72 – 1.82	1.000	1.39	0.79 – 2.44	1.000
Dec	1.00	0.54 – 1.84	1.000	1.38	0.85 – 2.23	1.000	2.80 ***	1.73 – 4.54	<0.001	2.01	1.16 – 3.47	0.154
Random Effects												
σ^2	155.58			202.81			9.54			229.51		
τ_{00}	0.06 _{month2}			0.03 _{month2}			0.02 _{month2}			0.06 _{month2}		
ICC	0.00			0.00			0.00			0.00		
N	19 _{month2}			19 _{month2}			19 _{month2}			19 _{month2}		
Observations	276			277			242			277		
Marginal R ² / Conditional R ²	0.001 / 0.001			0.000 / 0.000			0.042 / 0.044			0.000 / 0.001		
AICc	3301.278			3769.686			1366.644			3790.904		

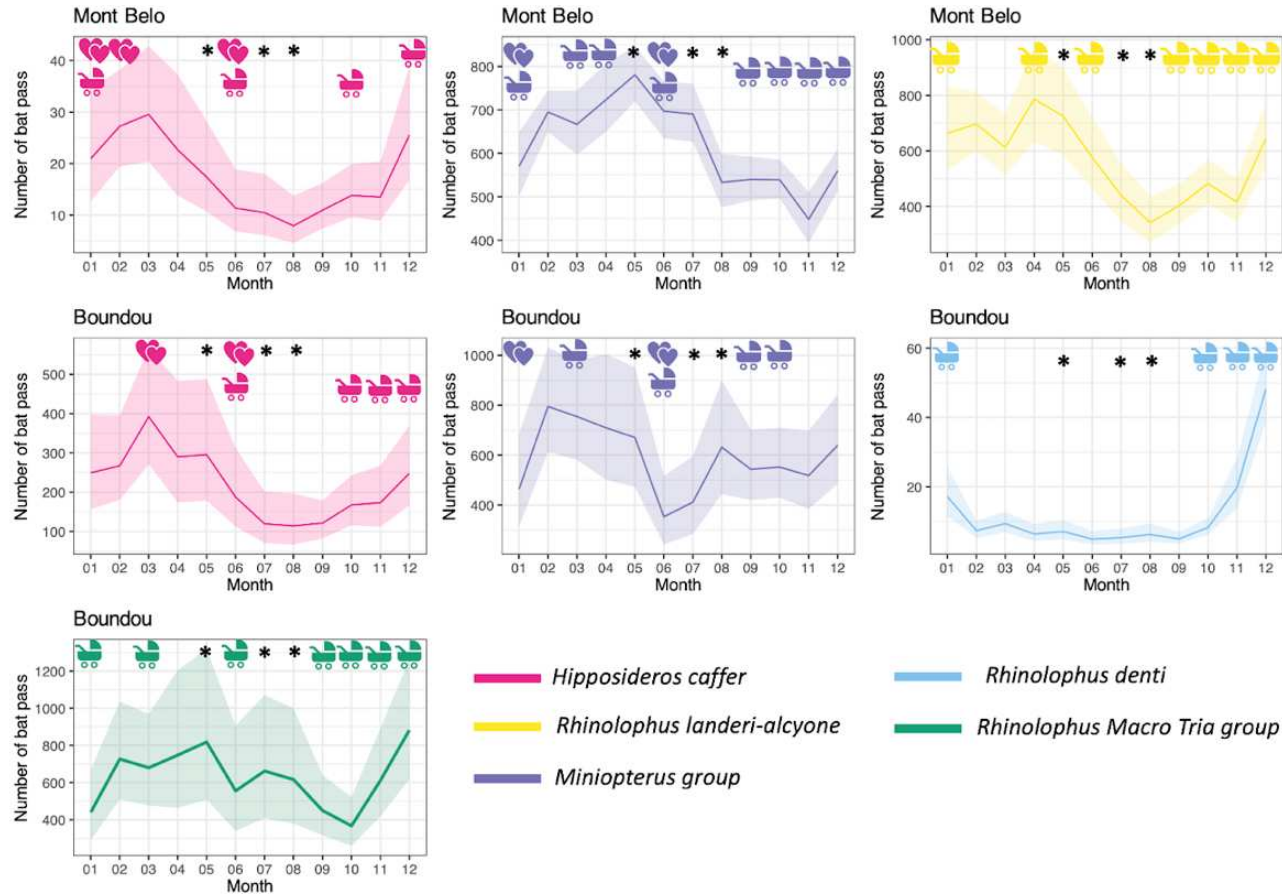
* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

Appendix 23: Generalized linear mixed (GLM) with Bonferroni correction describing the relationship between activity and month for the four acoustic groups in the Mont Belo cave during the enter time of bats.

Predictors	Hipposideros caffer enter Mont Belo			Miniopterus group enter Mont Belo			Rhino landeri-alcione enter Mont Belo			Rhinolophus denti enter Mont Belo		
	Estimates	CI	p	Estimates	CI	p	Estimates	CI	p	Estimates	CI	p
Intercept Jan	20.98 ***	12.75 – 34.52	<0.001	570.35 ***	501.54 – 648.60	<0.001	663.65 ***	530.64 – 830.00	<0.001	1.17	0.47 – 2.93	1.000
Feb	1.30	0.72 – 2.33	1.000	1.22	1.05 – 1.41	0.086	1.05	0.80 – 1.38	1.000			
Mar	1.41	0.77 – 2.58	1.000	1.17	0.99 – 1.38	0.822	0.92	0.69 – 1.23	1.000			
Apr	1.08	0.54 – 2.15	1.000	1.27	1.07 – 1.50	0.063	1.19	0.87 – 1.62	1.000			
May	0.83	0.42 – 1.63	1.000	1.37 ***	1.18 – 1.59	0.001	1.09	0.80 – 1.49	1.000	1.00	0.32 – 3.13	1.000
Jun	0.54	0.27 – 1.08	0.988	1.22	1.04 – 1.43	0.153	0.87	0.64 – 1.19	1.000			
Jul	0.50	0.24 – 1.03	0.705	1.21	1.03 – 1.42	0.229	0.66	0.48 – 0.92	0.155			
Aug	0.38	0.18 – 0.78	0.103	0.94	0.79 – 1.11	1.000	0.52 ***	0.37 – 0.72	0.001			
Sep	0.52	0.28 – 0.96	0.453	0.95	0.81 – 1.11	1.000	0.61 **	0.46 – 0.81	0.007			
Oct	0.66	0.36 – 1.20	1.000	0.94	0.81 – 1.10	1.000	0.73	0.55 – 0.96	0.279			
Nov	0.64	0.34 – 1.20	1.000	0.79	0.66 – 0.94	0.110	0.63 *	0.47 – 0.84	0.021			
Dec	1.22	0.66 – 2.25	1.000	0.98	0.84 – 1.14	1.000	0.97	0.73 – 1.29	1.000	1.55	0.53 – 4.57	1.000
Random Effects												
σ^2	22.89			166.33			157.22					
τ_{00}	0.04 _{month2}			0.00 _{month2}			0.01 _{month2}					
ICC	0.00						0.00					
N	18 _{month2}			18 _{month2}			18 _{month2}			4 _{month2}		
Observations	279			294			294			4		
Marginal R ² / Conditional R ²	0.007 / 0.009			0.000 / NA			0.000 / 0.000			NA		
AICc	1995.258			3840.501			3818.869			-12.700		

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

Appendix 24: Prediction of the activity of acoustic group entries with 95% confidence interval as a function of months and study sites (Boundou and Mont Belo cave). The *Rhinolophus denti* group at Mont Belo has not been represented here due to lack of data. The reproductive status of the species or acoustic group is indicated by two pictograms with the color of the acoustics groups: 🛒 young rearing (lactation, pregnant) and 💕: mating period/male activity. The months of May, July and August, which have an *, are month when no captures were made, hence the absence of data on the reproductive status of the acoustic group.



4.9 Reference – Chapter 3

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Transition 3: Ma chère amie chauves-souris, avec qui vis-tu ?

Qui sont tes amies et tes ennemies ?

Les précédents chapitres de cette thèse, nous ont permis de décrire les communautés de chauves-souris et leur activité dans les deux grottes étudiées. Nos résultats mettent en avant la forte utilisation de nos sites comme site de maternité. Nous avons aussi identifiés la grande saison des pluies comme une période clef d'agrégations des individus et d'élevage des jeunes en raison de la forte disponibilité en ressources.

Or, certains cas de transmission de maladies zoonotiques seraient liés à la période d'élevage des jeunes chauves-souris et les interactions qu'elles ont pu avoir avec des humains lors de cette période spécifique (ex : épidémie de Marburg causée par les interactions entre des humains et des *Rousettus aegyptiacus*, dans certains cas lors de la période d'élevage des jeunes). Ainsi, de nombreux efforts de recherche se sont concentrés sur les facteurs à l'origine de la transmission de ces pathogènes à cette période spécifique. Malgré ces efforts, peu d'études se concentrent sur l'écologie des interfaces sur une échelle temporelle dans des habitats pouvant favoriser les interactions chauves-souris – faune – humains.

Le troisième volet de cette thèse a pour vocation de se concentrer sur la niche écologique que représentent deux grottes abritant des colonies de chauves-souris cavernicoles en République du Congo et les interfaces entre ces colonies, la faune et l'homme. En s'appuyant sur un protocole de pièges photographiques, le **Chapitre 5** de cette thèse décrit les taxons et espèces exploitant l'intérieur et l'extérieur de la niche écologique « grotte extérieure / intérieure ». Cette étude détaille également la richesse et la diversité en espèce, les comportements et les interactions observés dans ces habitats entre les chauves-souris – la faune et les humains. Le **Chapitre 6**, quant à lui, se concentre sur la description du comportement opportuniste de prédation des genettes dans la grotte de Boundou en s'appuyant sur des vidéos récoltées lors de cette étude. Les **Box 5** et **Box 6** résument les points importants de ces chapitres.

Transition 3 (English version): My dear bat friend, who do you live with? Who are your friends and who are your enemies?

The previous chapters of this thesis enabled us to describe the bat communities and their activity in the two caves studied. Our results highlight the high use of our study sites as maternity sites. We also identified the main rainy season as a key period for the aggregation of individuals and the rearing of young because of the high availability of resources.

Some cases of zoonotic disease transmission have been linked to the rearing period of young bats and the interactions they may have had with humans during this specific period (e.g. the Marburg epidemic caused by interactions between humans and *Rousettus aegyptiacus*, in some cases during the rearing period). Many research efforts have therefore focused on the factors behind the transmission of these pathogens during this specific period. Despite these efforts, few studies have focused on the ecology of interfaces on a temporal scale in habitats that may favour bat-wildlife-human interactions.

The third part of this thesis aims at focusing on the ecological niche represented by two caves housing colonies of cave bats in the Republic of Congo and the interfaces between these colonies, wildlife and humans. Using a photographic trap protocol, **Chapter 5** of this thesis describes the taxa and species exploiting the interior and exterior of the 'exterior/interior cave' ecological niche. This study also details the species richness and diversity, behaviours and interactions observed in these habitats between bats - fauna and humans. **Chapter 6** focuses on the description of the opportunistic predation behaviour of genets in the Boundou cave, based on videos collected during this study. **Box 5** and **Box 6** summarise the main points of these chapters.

PART III
**SHARING ECOLOGICAL NICHES AND THE INTERFACE BETWEEN CAVE-
DWELLING INSECTIVOROUS BATS, WILDLIFE AND HUMANS**

“As in the cavern of some rifted den,
Where flock nocturnal bats, and birds obscene;
Cluster’d they hang, till at some sudden shock
They move, and murmurs run through all the rock ! “

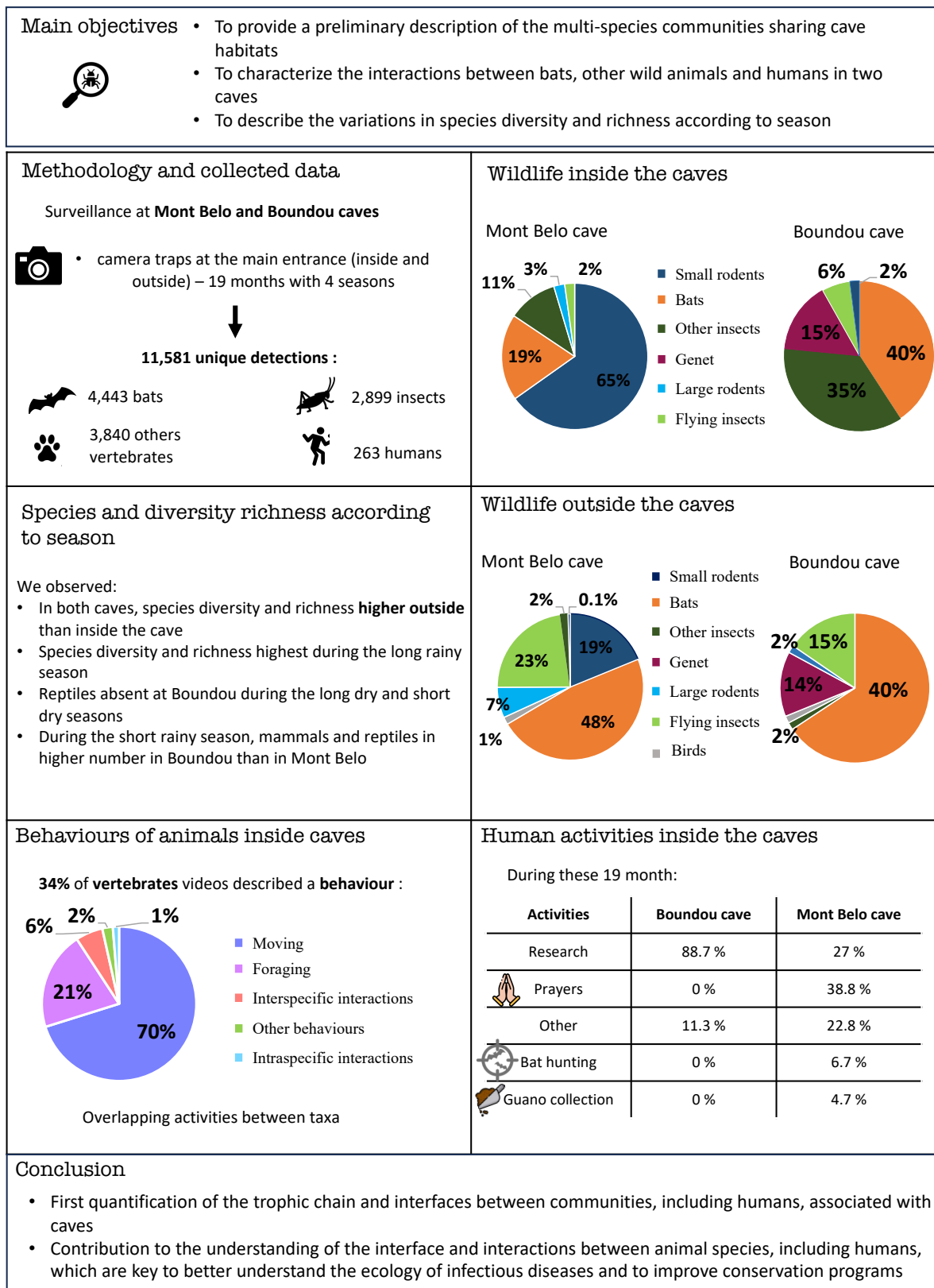
Homer, Odyssey. Book XXIV

CHAPTER 5 – ARTICLE 4: HABITAT SHARING AND INTERSPECIES INTERACTIONS IN CAVES USED BY BATS IN THE REPUBLIC OF CONGO

Based on the publication accepted for *PeerJ*:

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Box 4: Graphic abstract Chapter 5



Habitat sharing and interspecies interactions in caves used by bats in the Republic of Congo

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5.1 Abstract

Bats play key roles in ecosystem functions and provide services to human populations. In order to protect bat populations and mitigate the risks associated with pathogen spillover, we need to study the interfaces between bats, wildlife and humans. Caves are key habitats for many bat species, which use them as roosting and breeding sites. Caves, bats and their guano also attract many other animals along trophic chains, which might favor direct or indirect interspecies interactions. Two caves hosting colonies of insectivorous bats have been investigated in the Republic of Congo to characterize habitat sharing and interactions between bats, humans and animals. We set up a camera-trap monitoring protocol during nineteen months at the entrance of and inside each cave. Our results demonstrate the richness and complexity of the species interactions around and within these caves depending on season. We identified and/or quantified mainly rodents, but also numerous species of insects, birds, reptiles and carnivores using the caves. We investigated the temporal variation in the use of caves and the potential interactions between humans, wild animals and bat colonies. Our study provides some of the first quantified insights of the trophic chains and interfaces within communities, including humans, associated with caves. This study contributes to the understanding of the interface and interactions between animal species, including humans, which are key to understand better the ecology of infectious diseases and improve conservation programs.

5.2 Introduction

Bats are associated with emerging infectious diseases, yet they are also key species in ecosystems. They provide important ecological functions helping to pollinate and disperse the seeds of over 549 plant species, and regulating arthropod and insect pest populations, thereby limiting economic losses for farmers worldwide (Kunz et al. 2011; Ramírez-Fráncel et al. 2022; Ghanem and Voigt 2012; Castillo-Figueroa 2020). Bats are threatened by numerous human activities (destruction of their habitat, pesticides, hunting) (Almeida et al. 2021; Furey and Racey 2016), and they are also victims of bad reputation due to their role in the transmission of zoonotic pathogens (Afelt et al. 2018; Banerjee et al. 2019; Calisher et al. 2006; López-Baucells, Rocha, and Fernández-Llamazares 2018; MacFarlane and Rocha 2020). Therefore, it is important to understand bat ecology and their interactions with other wildlife and humans in order to better assess potential health risk to human populations and advance efforts in bat conservation.

Caves offer very specific light, humidity and temperature conditions that create ecological niches that benefit the many cave-dwelling species of animals, plants and micro-organisms (Gabriel and Northup 2013; Kosznik-Kwaśnicka et al. 2022; Kováč 2018; Tomczyk-Żak and Zielenkiewicz 2016; Pacheco et al. 2020). The presence of caves in an ecosystem therefore influences local biodiversity through their contribution of specialized cave-dwelling species, but also attracts non-cavernicolous species through the feeding, foraging or hunting opportunities they provide.

Caves are key habitats for many bat species, which use them as resting and refuge places as well as breeding and parturition sites (Barros, Bernard, and Ferreira 2020; Ormsbee, Kiser, and Perlmeter 2007; Struebig et al. 2009; Meierhofer et al. 2024). Caves can host a high diversity of bat species. In addition, some bat species can gather in caves by hundreds or thousands (Tanalgo et al., 2022). This habitat can also be important for many other animal species (e.g., insects, birds) that use them for different purposes (e.g., refuges, breeding, foraging). In a cave populated by bats, many direct and indirect interactions can occur between, them other animals (wild or domestic) and humans (Furey and Racey 2016; McCracken 1989). Wild animals take advantage of the presence of bat colonies as a food source, with numerous examples of predation by birds, small mammals or snakes, but also insects, e.g., centipedes (Mallick, Hossain, and Raut 2021;

Mas, López-Baucells, and Arrizabalaga 2015; Molinari et al. 2005; Ridley 1898; Scrimgeour, Beath, and Swanney 2012; Tanalgo et al. 2019). Domestic pets, cats and dogs can also be major predators of these bat colonies (Costa-Pinto 2020; Merz et al. 2022; Oedin et al. 2021). In addition, predation by some of these domestic animals, such as cats, could facilitate pathogen transmissions to human populations (Salinas-Ramos et al., 2021).

From time immemorial, humans have been using caves as places of refuge and/or worship (Bonsall and Tolan-Smith 1997; Moyes 2012; Straus 1979). More recently, humans have been exploiting these habitats to extract minerals and guano, or as tourist attractions (Okonkwo, Afoma, and Martha 2017; Simons 1998). Recent studies have suggested an increased risk of zoonotic transmission in highly disturbed environments characterized by a rise in the frequency and the intensity of wildlife-humans interactions (Afelt et al. 2018; Allen et al. 2017; Becker et al. 2018; Johnson et al. 2020; Plowright et al. 2021; Rulli et al. 2017; Wilkinson et al. 2018).

Indeed, events involving the transmission of pathogenic micro-organisms (fungi, viruses, bacteria or parasites) between cave bats and animals, including human, have been recognized (Agustin et al. 2019; Federici et al. 2022; Jurado et al. 2010; Karunarathna et al. 2023). Studying the animal-human interface to identify interactions and potential pathogen transmission routes is essential (Caron et al. 2021; de Garine-Wichatitsky et al. 2021).

However, studies on bat-animal interfaces (including humans) are still rare. The emergence of many zoonotic pathogens from bats has highlighted the need to study the interfaces between bats and humans, as well as the interactions that bats may have with other wild or domestic animals that could create potential transmission routes to humans. This is not only important for the field of disease ecology, but will also help to understand bat ecology, including the importance of bats in the trophic chain, and promote bat conservation.

In the Republic of Congo, a camera trapping protocol was designed and set up in two caves hosting bat colonies to characterize the interfaces between cave bats, other animals and humans. Firstly, we described the communities exploiting the inside and outside of the cave using a species richness index. We hypothesized that each caves constitute different microhabitats and are

therefore occupied or used differently. Inside the cave, cave-dwelling species should be the most represented. Outside the cave, we hypothesised the presence of species exploiting the cave interior and as well as other species not entering the cave. Secondly, the daily overlap in activity patterns between non-bat species and bats was characterized in order to identify times of day conducive to contact, competition or predation. We hypothesized that species with strong interactions will have overlapping activities. Finally, we investigated whether the non-bat species richness and diversity of taxa varied over time. We hypothesized that seasonal variations may have an impact on species richness in both caves due to variations in food resources.

5.3 Materials & Methods

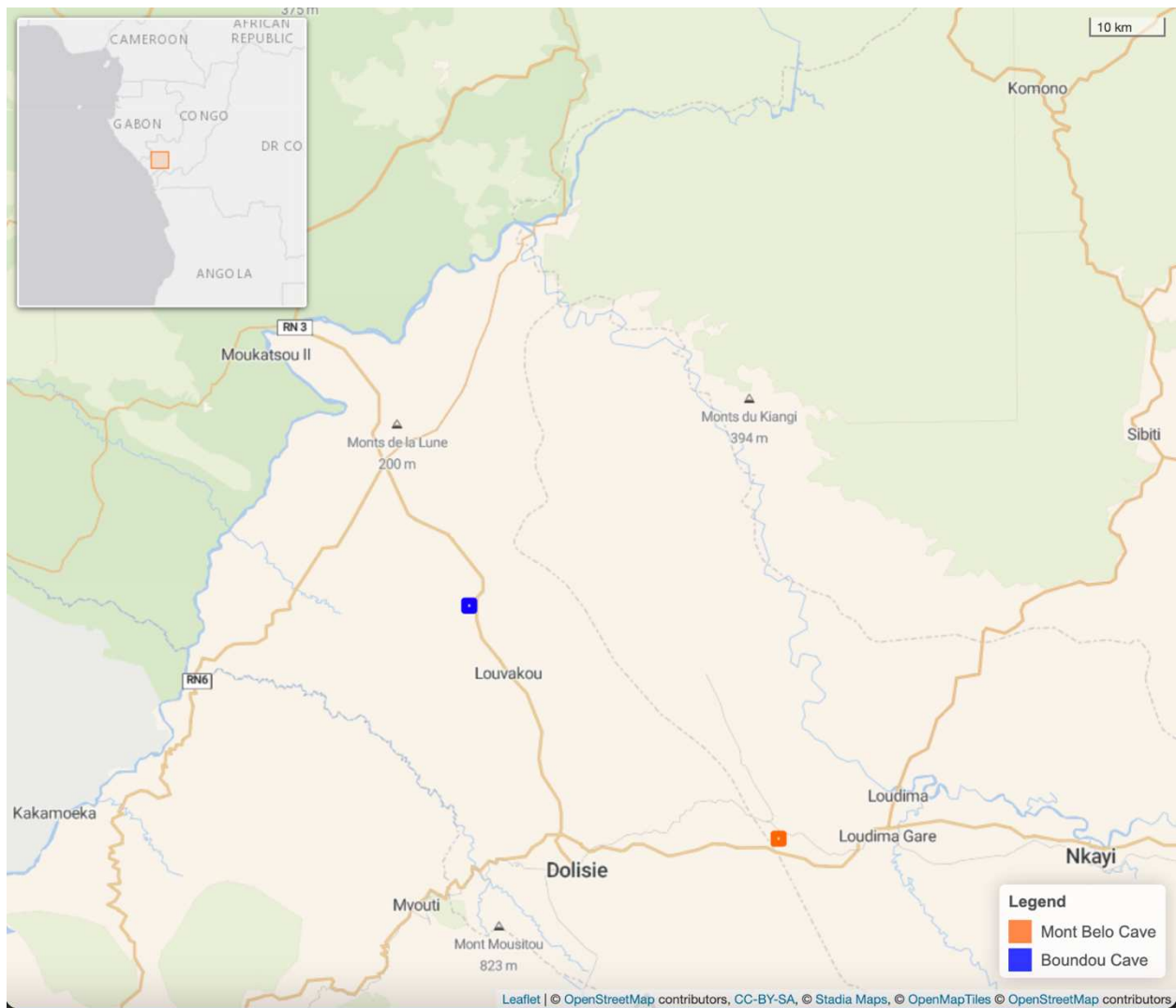
5.3.1 Ethic statements

All protocol was carried out with the permission from the Ministry of Forest Economy and Ethics Committee of the Ministry of Scientific Research and Technological Innovation in the Republic of Congo (N°212/MRSIT/IRSSA/CERSSA and N°687/MEF/CAB/DGEF-DFAP).

5.3.2 Study Area

Our study took place between 2021 to 2023 in two different caves situated in the Niari and Bouenza Department, about 50 km away from each other, near the town of Dolisie, in the South of the Republic of Congo (**Fig. 27**). This region is subject to four seasons: the short dry season (January and February), the short-rainy season (March to May), the long-dry season (June to August), and the long-rainy season (September to December) (Samba, Maloba Makanga, and Mbayi 1999). The landscape is mountainous and calcareous, favoring the presence of numerous caves and cavities. It is mainly composed of grassy savannah with patches of secondary forests and a patchwork of crops close to villages.

Figure 27: Map showing the location of the two caves in our study in the Republic of Congo. Mont Belo cave is inside the Bouenza department and Boundou cave inside the Niari department. (Map Background Stadia Outdoors).



5.3.2 Study sites

We selected the two caves on the basis of the following criteria: (1) annual presence of colonies of insectivorous and potentially frugivorous bats in the caves, (2) one site close to a rural settlement and the other less subject to human disturbance, (3) caves with few entrances/exits to facilitate surveillance by camera traps, and (4) sites that are easily accessible at any time of year, including the wet season.

The first cave, Mont Belo (MB), consists of several chambers with a main entrance. The cave is surrounded by a small patch of secondary forest, followed after by large variety of food crops (peanuts, cassava, tomatoes, etc.) located at almost five km from the village (see **Appendix 25** for a general diagram of the cave's configuration). Mont Belo cave attracts many pilgrims and members of the local population for religious rites. The second cave, named Boundou (BD), is a tunnel-shaped cave in the rock face, with a main entrance and a small exit at the end on the other side (see **Appendix 26** for a general diagram of the cave's configuration). This cave is also surrounded by a small secondary forest surrounded by a large grassy savannah. The nearest village is more than five km away, and human activity is much less than MB. Boundou cave, despite its sacred nature, is rarely visited by the local population, and to our knowledge there are no regular religious activities or pilgrimages. Both caves are considered sacred by the local population.

Catches of bats in the two caves identified the presence of several bat genera. Boundou cave hosts at least six genera of insectivorous bats (*Rhinolophus sp.*, *Coleura sp.*, *Triaenops sp.*, *Macronycteris sp.*, *Miniopterus sp.*, *Hipposideros sp.*) and one genus of fruit bats (*Roussettus sp.*). Conversely, only four bat genera (fruit-eating: *Roussettus sp.* and insectivores: *Rhinolophus sp.*, *Miniopterus sp.*, *Hipposideros sp.*) were detected in the Mont Belo cave. Both caves are maternity colonies for insectivorous bat species (see **Appendix 25** and **26** for location - presence of bat colonies in both caves). The abundance of these species varies according to the season and the species' life cycle. Fuller details of these colonies will be given in another publication (Labadie et al., in prep.).

5.3.3 Camera trap data collection

The camera traps survey was conducted between September 2021 and March 2023. We used nine Moultrie M50 cameras (Moultrie, Birmingham, USA) deployed inside and outside the two caves (see **Appendices 25** and **26** for the location of each camera trap in the two caves). We installed at least one camera trap in front of the main entrance of the cave (i.e. “outside”), another inside the cave in the main chamber, and additional cameras at the other entrances/exits which serve as passages for wild animals. In both caves, we did not cover the aerial exits (exit wells) which lead to the top of the caves, due to technical and safety constraints.

In total, four camera traps were installed in Mont Belo cave (including one inside and three entrances – outside camera) and five camera traps in Boundou cave (including one inside and four entrances – outside camera) over nineteen months. The cameras were programmed to trigger automatically (high-sensitivity detector) by taking a picture followed immediately by a 30s video, and to re-trigger after a five-minute delay. This configuration enabled us to limit repeated triggering by the same individual and to visualize their behavior through a video. The nineteen month study period was hampered by technical problems with certain camera traps (camera shutdown due to technical problems, presence of insects or dirt obstructing the camera lens, over exposure). During December 2021 and January 2022, the cameras inside the caves (one of the two cameras at Mont Belo and the single camera inside Boundou) experienced technical problems, resulting in unusable data (black images and videos). Other cameras suffered from other technical problems and were replaced and re-started as soon as the problem was detected. During our field activities (approximately every two months, defined in this article as research activities), we restarted the cameras after our visit with new batteries and SD cards. For the other months when we were not present, a local guide was trained to come and restart the cameras at the beginning of the month (between the 2nd and 10th of the month) with new batteries and SD cards. In total, the camera traps at both sites operated for 171 months (19 months x 9 cameras = 171 minus 12 months of malfunction = 159 months).

5.3.4 Data preparation

All videos and images were integrated into Timelapse (Greenberg 2023; Greenberg and Godin 2015) to extract metadata and standardize the extraction of data from photos and videos. We used Megadetector (Beery, Morris, and Yang 2019; Fennell, Beirne, and Burton 2022), an artificial intelligence tool, to make an initial screening of visual material regarding the detection of animals. This software only works on photos, and indicates animal presence with a blue square, and human presence with a red square. Megadetector results can be integrated into Timelapse. We defined one event of “detection” of a specific species or animal category as the presence of this species or animal category on a photo. One detection can be the presence of several individuals at the same time, for example a detection of two humans at the same time on an image.

A comparison of detections between Megadetector and manual analysis of the photos by an observer was carried out on all photos recorded over the first 6 months of the study. Megadetector proved its effectiveness in detecting all presences (except insects and bats), despite a few detection errors (false detection on a landscape element, absence of detection for fast-moving bats). Hence, we decided to use Megadetector to reduce the time of treatment of the last thirteen months. The videos linked to the pictures on which animals or humans were detected by Megadetector were viewed manually in order to verify the number of individuals and to describe the behavior of animals. When the number of individuals of a species or animal category varied between the photo and corresponding video, we retained the highest count of individuals for our analysis. As Megadetector can only be used on photos and not on videos, and is not parameterized to specifically identify bats and insects, we estimated that the detection of these two specific taxa was under-represented. Moreover, counting the number of insects and bats on video is feasible but complicated and time consuming. To avoid bias and to standardize our analysis, we decided to note the presence of insects and bats, without counting the number of individuals.

5.3.4 Species identification

Species on pictures and videos were identified using zoological books (Kingdon et al. 2013; Sinclair and Ryan 2003), or with the help of experts. If identification at species level was not possible due to poor image or video quality, identification was made to class or order level. As rodents were difficult to identify to species level, we classified them into three categories: small rodents (< 60 cm without tail), large rodents (> 60 cm without tail) and porcupines. We also grouped birds according to their size: small (from 2 to 20 cm in length) or medium (from 21 to 60 cm in length), and raptors (owl and hawk species). To simplify the presentation of our results, we also categorized insects into two groups: (1) flying insects, which include midges, butterflies and bees, and (2) other crawling insects (cave beetles, crickets) and arachnids (spiders).

Our results are presented using common names for species or the categories mentioned above.

5.3.5 Categorizing human activities

During the course of our study, we consulted local communities, including, landowners, village chiefs and local guides (non-standardized informal interviews) on several subjects of interest to our study, such as cave use and wildlife species consumed by the local population. They informed us that Mont Belo cave was used for prayer rites, while no human activities were reported in Boundou cave. Local people acknowledged performing bat hunting (mainly large frugivorous bats) and guano harvesting but rarely in the two studied caves. Local communities have also pointed out that they consume other species of wildlife (bushmeat), including porcupines, rats and genets. Due to the high level of human activity at Mont Belo, it was decided with the local community to cover the cameras if necessary to avoid disturbing religious practices. In some cases, presence of a human was followed by the recording of a "black photo" for a certain period of time, which could indicate the presence of prayer activity. We viewed the 30s videos in order to identify and categorize the human behaviors detected in the two caves. We defined five distinct categories: (1) guano collection inside the caves, (2) hunting activity on bats present in the caves (we saw nets being laid inside the cave), (3) praying activity or religious rites, (4) our research activities such as changing cameras, capturing bats or collecting guano and (5) others,

for all undefined activities (e.g., people who apparently just visited the cave), encompassing activities that could not be clearly categorized.

5.3.6 Categorizing animal species behaviors

The behavior of an animal is classified into five different categories: (1) moving, (2) foraging (an animal appearing to look for something to eat in the cave), excluding the action of hunting, (3) interspecific interaction (i.e. hunting, feeding on another animal), (4) other behavior (i.e. defecating, grooming, sitting, observing) and (5) intraspecific interaction (i.e. chasing each other, fighting or mating). We did not categorize the behavior of insects, bats or unidentified animals or when the videos were of poor quality.

5.3.7 Statistics analysis

i. Selection of data

We identified detection events as independent if the detected species or animal category on simultaneous pictures or videos was different for each camera trap and if the detection interval between the previous and next detection was greater than or equal to 30 minutes (O'Brien, Kinnaird, and Wibisono 2003; Sollmann 2018). All analyses were performed using the number of independent detections except for the graphs on animal and human behavior, where we used the exact number of individuals detected per independent event.

ii. Analysis

All figures were produced using `ggplot2` (Wickham 2016), `patchwork` (Pedersen 2023) and `cowplot` (Wilke 2020) packages implemented in R software (R Core Team 2023).

We presented the results as a function of the number of camera days, i.e. the number of detections divided by the sum of the number of days each camera was in operation per site, multiplied by one hundred (Rovero et al. 2013; Yasuda 2004).

To quantify the temporal activities between the species or categories and the extent of temporal overlap, we used the `camtrapR` package (Niedballa et al. 2016). The "activityOverlap" function was used to estimate the kernel density (non-parametric method) for study location (Mont Belo or Boundou cave, inside or outside), which calculates the probability density function of a detection distribution (Meredith and Ridout 2014). The overlap coefficient (D_{hat1}) can vary between zero and one (no overlap = 0 and total overlap = 1) (Linkie and Ridout 2011).

Three species richness indices with standard error were calculated for each study site and location (inside or outside the cave) using the `vegan` package (Oksanen et al. 2022). The first index, the Chao estimator, calculates an estimate of the total number of species, taking into account "rare" species. The second index, Jackknife, gives an overview of potential bias and variability by systematically removing samples, and the third index, bootstrap, assesses uncertainty by simulating sampling while providing a confidence interval and standard error (Chiu et al. 2014; O'Hara 2005; Smith and Belle 1984). In addition, a species diversity index (Shannon index) was calculated for each study location.

We used a non-parametric Kruskal-Wallis test coupled with a Dunn's test (post hoc test with Bonferroni) to test the effect of the four seasons and study location (Mont Belo or Boundou cave and inside or outside) on the number of detections for each taxonomic class (Dinno, 2015; Tomczak & Tomczak, 2014).

We also tested the existence of associations between the different species (with the most detections in each study site) using a Pearson's product-moment correlation. Our data being tied, we were unable to use a non-parametric Spearman's test.

5.4 Results

5.4.1 Dataset

In total, we collected 88,231 observations (including photos, videos and duplicate of one detection of different species) over a nineteen month period, of which 45,670 (51.8 %) came from Boundou and 42,561 (48.2 %) from Mont Belo. We detected the presence of animals or humans in 24% of observations (21,123 observations) with 12,001 (56.8 %) detections from Boundou cave and 9,122 (43.2 %) detections from Mont Belo cave. Of these 21,123 detections, 8,836 (41.8 %) were bat detections, 8,218 (38.9 %) were detections of other taxa, including humans and 4,069 (19.3 %) were insect detections. Following selection of independent detections (>30 min interval), the dataset contained 11,581 detections, including 4,443 bats, 2,899 insects, 263 humans and 3,840 detections of others vertebrates (**Appendix 27**). The number of days of functional cameras varied between month, with an average of 16.4 days per month for camera traps at Boundou cave and 11.5 days for camera traps at Mont Belo cave (**Fig. 27** and **Appendix 28**). Cameras inside the caves generally performed better than those outside (14.5 days vs 12.2 days) (**Appendix 28**).

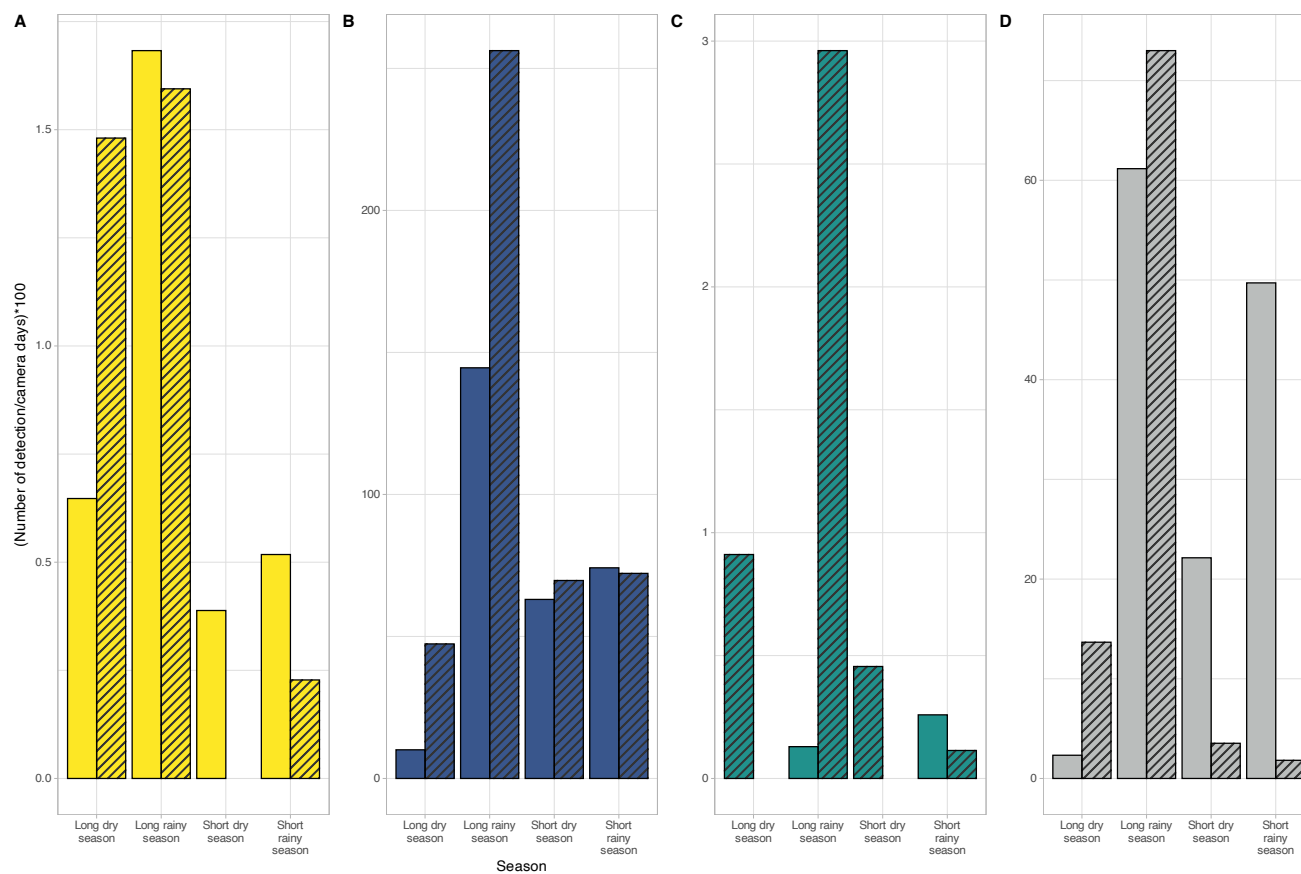
5.4.2 Presence of vertebrates and invertebrates in the two caves

In both caves, we mostly observed the presence of mammals (72.7 % of all detections) including humans (2.3% of all mammalian detection), followed by insects (25 % of all detections), birds and reptiles (**Fig. 29A, 29B, 29C** and **29D**). Species richness and diversity were higher outside for both caves compared to inside (**Table 13**). In the two sites, the accumulation curve for species richness showed that the number of species detected inside the caves reached an asymptote in contrast to the species detected outside the caves (**Fig. 30**). All taxonomic classes showed some variation in the number of detections accross the different seasons and the two study sites (**Fig. 29A, 29B, 29C** and **29D**), but none was significant (p value > 0.05; **Table 14**). We observed greater species richness and diversity outside both caves compared to inside (**Table 15**).

Figure 28: Number of days the camera traps were in operation during the study. A total of nine cameras for all sites, with five for Boundou cave (including one inside the cave) and four for Mont Belo cave (including 2 inside the cave).



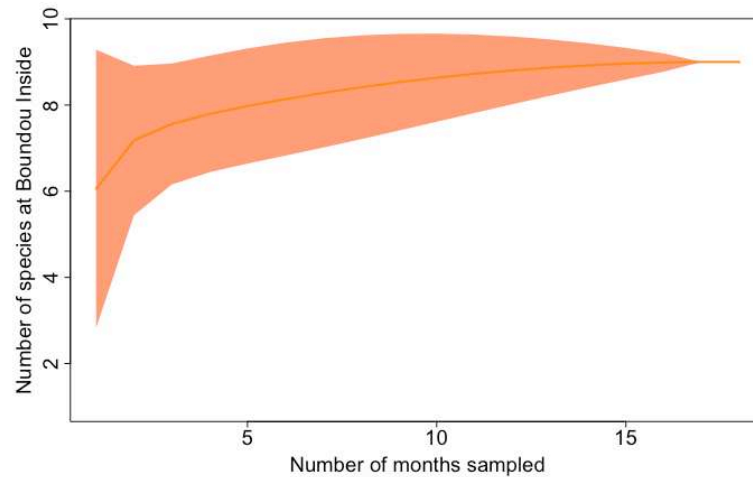
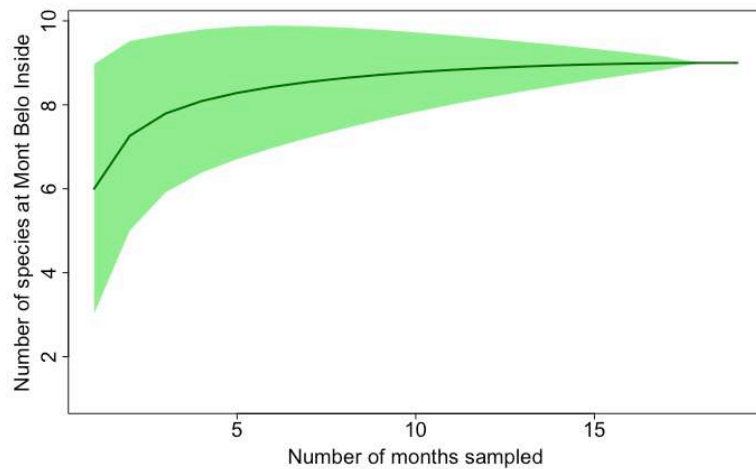
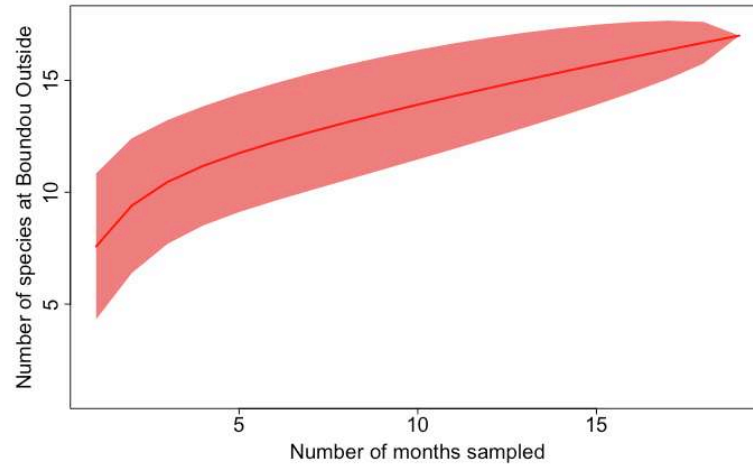
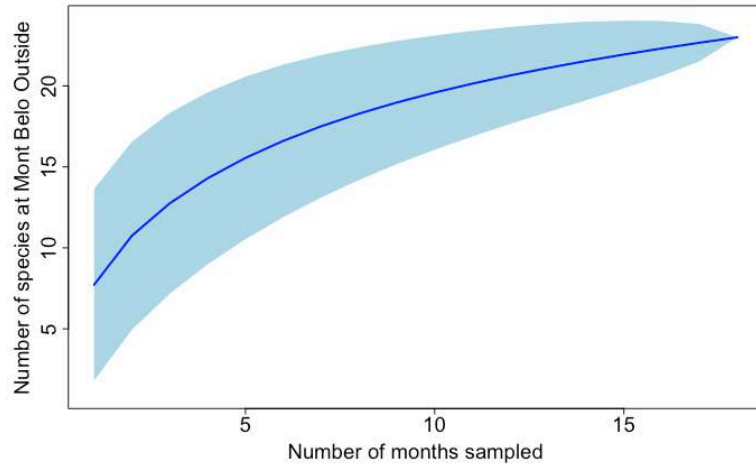
1 **Figure 29:** Number of detections per camera day multiplied by 100 according to seasons and study sites (Boundou and Mont Belo cave) and
 2 grouped by taxonomic class: (A) for the bird class, (B) for the mammal class (C) for the reptile class and (D) for the insect class. (N total = 11 581
 3 detections with 8,422 mammals; 2,899 insects; 79 birds and 45 reptiles)



4

Sites □ Boundou ▨ Mont Belo Class ■ Birds ■ Insects ■ Mammals ■ Reptiles

- 5 **Figure 30:** Accumulation curves of species richness depending on study location (Mont Belo outside and inside, Boundou outside and outside)
6 with confidence interval at 95 %.



7

Table 13: Estimated species richness (Bootstrap method with standard error, se) and species diversity richness (Shannon method with standard error, se) in relation to study location (Mont Belo and Boundou cave, inside and outside).

Study location	Total Species	Bootstrap \pm se	Shannon \pm se
Mont Belo Outside	23	25.6 \pm 1.6	4 \pm 0.1
Boundou Outside	17	19.3 \pm 1.2	3.9 \pm 0.1
Mont Belo Inside	9	9.1 \pm 0.3	3.8 \pm 0.1
Boundou Inside	9	9.2 \pm 0.4	3.7 \pm 0.1

Table 14: Results of Kruskal-Wallis (non-parametric test) and Dunn's test (post-hoc test) of classes identified between study location (Mont Belo and Boundou cave, inside and outside) and seasons (long rainy season (LRS), short rainy season (SRS), long dry season (LDS) and short dry season (SDS)).

Class	Study location	p value	LDS - LRS	LDS - SDS	LRS - SDS	LDS - SRS	LRS - SRS	SDS - SRS
Insects	Mont Belo Inside	0.39	0.6	1	1	1	1	1
	Mont Belo Outside	0.39	1	1	1	0.6	1	1
	Boundou Inside	0.39	0.6	1	1	1	1	1
	Boundou Outside	0.39	0.6	1	1	1	1	1
Mammals	Mont Belo Inside	0.39	0.6	1	1	1	1	1
	Mont Belo Outside	0.39	0.6	1	1	1	1	1
	Boundou Inside	0.39	0.6	1	1	1	1	1
	Boundou Outside	0.39	0.6	1	1	1	1	1
Birds	Mont Belo Inside	-	-	-	-	-	-	-
	Mont Belo Outside	0.39	1	1	1	0.6	1	1
	Boundou Inside	-	-	-	-	-	-	-
	Boundou Outside	0.39	1	1	1	0.6	1	1
Reptiles	Mont Belo Inside	0.39	1	1	1	1	0.6	1
	Mont Belo Outside	0.39	1	1	1	0.9	0.9	1
	Boundou Inside	0.39	0.6	1	1	1	1	1
	Boundou Outside	0.39	1	1	0.9	1	1	0.9

Table 15: Species richness index (Bootstrap) and species diversity index (Shannon) in relation to season (Inside and Outside both cave) with standard error (se).

Cave location	Long rainy season	Short dry season	Short rainy season	Long dry season
Species richness index: Bootstrap \pm se				
Mont Belo Inside	8 \pm 0	9 \pm 0	6 \pm 0	9 \pm 0
Boundou Inside	9 \pm 0	8 \pm 0	8 \pm 0	6 \pm 0
Mont Belo Outside	20 \pm 0	11 \pm 0	12 \pm 0	16 \pm 0
Boundou Outside	13 \pm 0	10 \pm 0	12 \pm 0	9 \pm 0
Species diversity index: Shannon \pm se				
Mont Belo Inside	1.3 \pm 0.3	0.9 \pm 0.4	0.8 \pm 0.4	1.1 \pm 0.4
Boundou Inside	1.4 \pm 0.3	1.4 \pm 0.4	1.3 \pm 0.4	1.2 \pm 0.4
Mont Belo Outside	1.7 \pm 0.3	1.5 \pm 0.3	1.7 \pm 0.3	2.1 \pm 0.2
Boundou Outside	1.3 \pm 0.3	1.1 \pm 0.4	1.2 \pm 0.3	1.5 \pm 0.3

In both caves (inside and outside), species and diversity richness were highest during the long rainy season, followed by the long dry season and the two short seasons (wet and dry) (**Table 15**). The long rainy season was the season with the highest number of detections of any class. During the long and short rainy seasons, Boundou cave recorded a higher number of bird detections than Mont Belo cave (**Fig. 29A**). During the short rainy season, mammals and reptiles were in higher numbers in Boundou cave than in Mont Belo cave (**Fig. 29B** and **29C**). Insects were detected in greater numbers in Boundou cave during the short dry and short rainy seasons, but the opposite trend was observed for the other two seasons, long dry and long rainy seasons (**Fig. 29D**) in Mont Belo. During the short dry season, birds were not detected at Mont Belo (**Fig. 29A**). Reptiles were absent at Boundou during the long dry and short dry seasons (**Fig. 29C**).

More specifically, inside the Mont Belo cave, we mostly detected small rodents (62.2 % of detection inside MB), followed by bats (18.3 % of detection inside MB), other insects - arachnids (crickets, spiders) (10.5 % of detection inside MB), large rodents (2.3 % of detection inside MB) and flying insects (2.1 % of detection inside MB) (**Fig. 31**). Inside Boundou cave, bats were most often detected (40 % of detection inside BD), followed by other insects - arachnids (cave beetles, crickets) (35 % of detection inside BD), genet (14.3 % of detection inside BD) and flying insects (5.9 % of detection inside BD) (**Fig. 31**). In Boundou cave, the genet, identified as the rusty-spotted genet (*Genetta maculata*) was one of the most frequently observed species both inside and outside the cave (**Fig. 31** and **31**). However, at Mont Belo cave, the genet, identified as the servaline genet (*Genetta servalina*) was not observed inside the cave, but only six times outside this cave (**Fig 31** and **31**).

Outside both caves, we identified the presence of species already detected inside the caves such as small rodents, genets, other insects - arachnids, large rodents, but also the presence of other species such as birds of different sizes (raptors), monkeys, pangolins and Nile monitors (**Fig. 31**). We also had a high level of detection of flying insects outside compared to inside the caves (14.4 % of detection inside BD and 17.7 % of detection inside MB) (**Fig 31** and **31**). The presence of humans was detected inside and outside both caves (**Fig 31** and **31**).

Figure 31: Number of vertebrate species per camera days multiplied by 100 according to study sites (Boundou and Mont Belo cave) and position of camera (inside or outside) (A) Inside Boundou and Mont Belo cave and (B) Outside Boundou and Mont Belo cave. Note the different scale on number of detections by camera days between A and B.

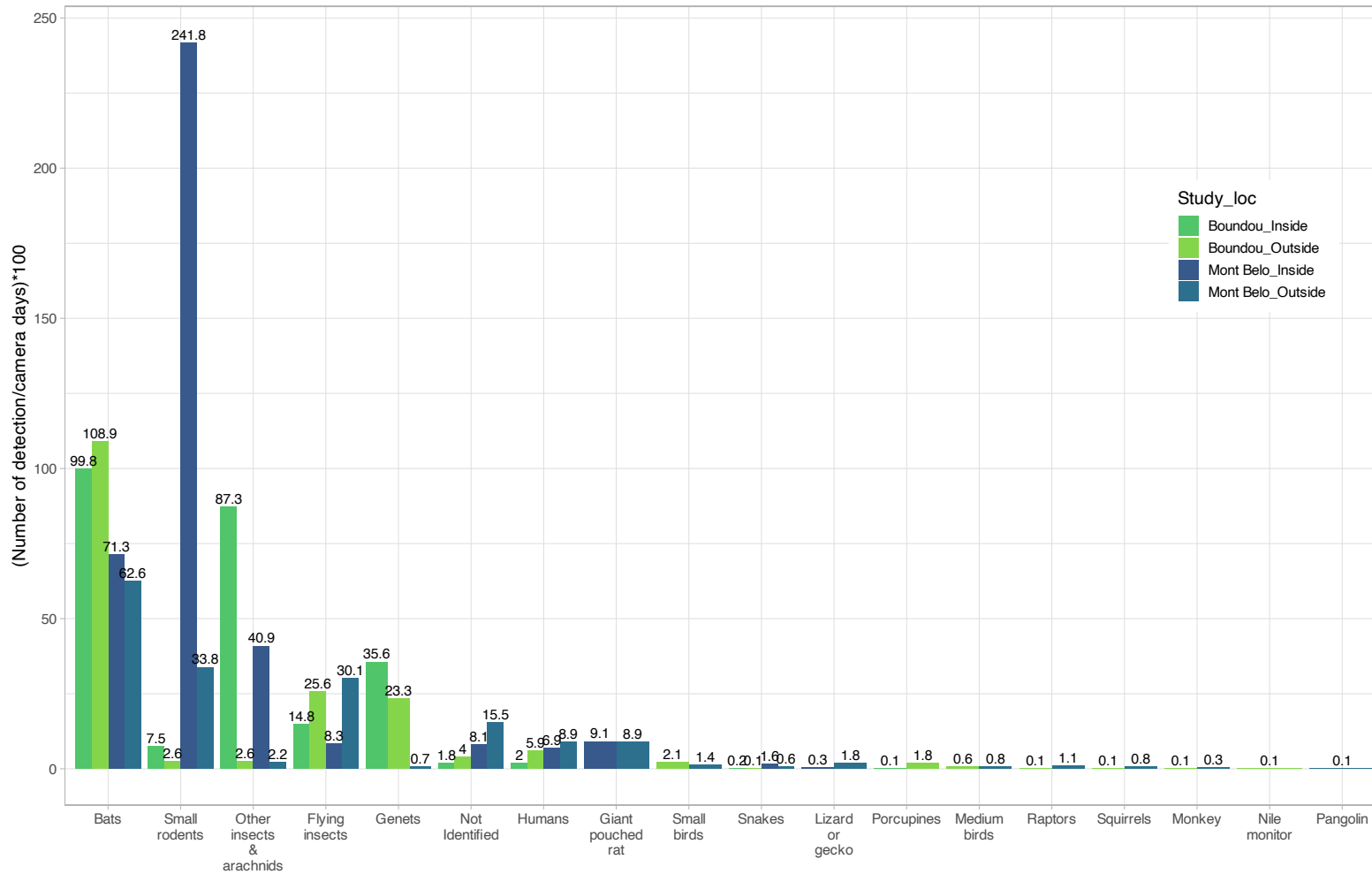


Figure 32: Characterization of vertebrate behavior (excluding humans, insects and bats) inside Mont Belo cave and Boundou cave

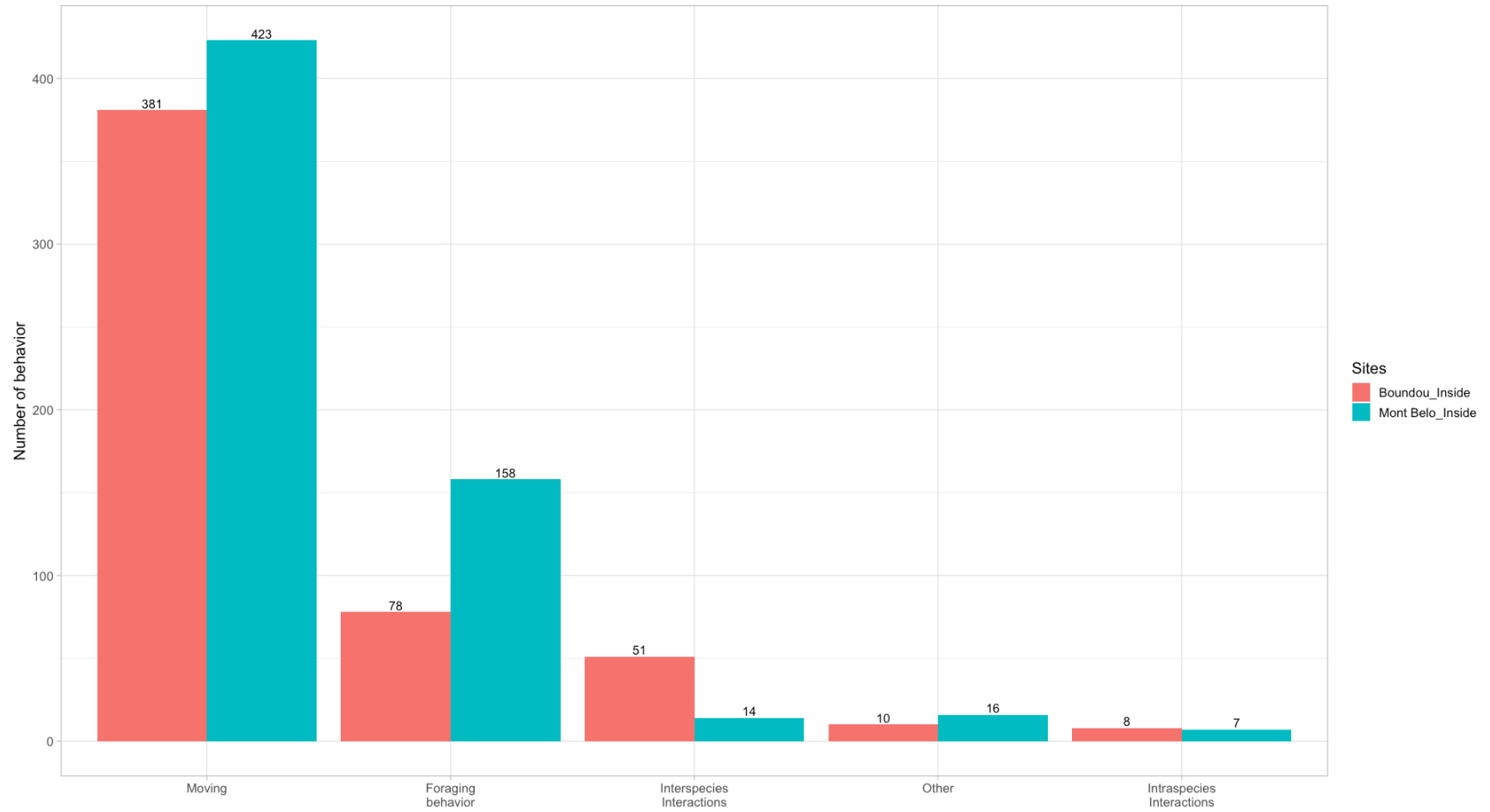


Figure 33: Description of human activities in (A) Mont Belo cave and (B) Boundou cave. Note the different scale on number of detections by camera days between A and B.



In Mont Belo cave, we observed a significant association between bats and flying insects ($p < 0.01$) as well as between small rodents and other insects - arachnids ($p < 0.01$) (**Table 16**). In Boundou cave, we observed a significant association between bats and both types of insects (flying insects: $p < 0.01$ and other insects - arachnids: $p < 0.01$), and between both types of insects ($p < 0.01$) (**Table 17**).

Appendix 29 provides a detailed list of all the identified animals and number of individuals per site. Some examples of images recorded for different species are also shown in **Appendix 30**.

5.4.3 Animal behavior inside the two caves - viewing video recordings

For 34% of the detected vertebrates (with the exclusion of bats, insects, humans and unidentified animals; $N = 7,742$ detections), usable videos (i.e. enabling the action to be seen clearly, $N = 500$ detections) were analyzed to characterize their main behavior. For vertebrates counted in both caves ($N = 1146$), we observed in descending order: moving behavior (70.1 % of all behavior observed), followed by foraging behavior (20.6 %), interspecific interactions (5.7 %), other behaviors (2.2 %) and intraspecific interactions (1.3 %) (**Fig. 32**).

Some pictures of giant pouched rat showed one or more individuals that appear to have swollen jowls (at least five different observations) (**Appendix 31**), which may suggest the use of the cave as a food storage site by this species. In the case of small rodents, we observed a few videos showing predatory behavior on the insects present in the cave.

The rusty-spotted genet regularly visited the inside of the Boundou cave and showed strong foraging and predation behavior, and potential scavenging behavior inside the cave towards bats, rodents and insects. The servaline genet, on the other hand, was not recorded entering the Boundou cave.

Concerning birds, we observed the presence of the African wood owl (*Strix woodfordii*) at the entrance of the Mont Belo cave on ten occasions over a nineteen month. Each time, videos showed that the individuals positioned themselves on a rocky promontory, close to the cave entrance, at around 18:00 to 19:00, a period of high bat flying activities at the entrance of the cave. In one of these videos, we were able to observe the hunting behavior of this owl as it dived towards the bats flying out from Mont Belo cave. Detail of the various interactions and potential

contact between the different species, including humans, in the two caves in this study (inside and outside) are shown in **Table 18**.

5.4.4 Human activities in the two caves

We observed more human activities in Mont Belo cave (n= 59.2 % of the number of humans detected) than in Boundou cave (n=40.8 % of the number of humans detected). In Mont Belo cave, we detected in descending order: prayer activities (n = 38.8 %), research activities (27 %), other activities (i.e., undefined human activities) (22.8 %), bat hunting (6.7 %) and guano collection (4.7 %) (**Fig 33A**). The human presence at Boundou cave was observed for only two categories: our research activities (88.7 %) followed by other undefined activities (11.4 %) (**Fig. 33B**).

5.4.5 Activity patterns of vertebrates of interest

We focused our analyses on the pattern of daily activity for vertebrates with the highest detection rates (> 950 detections at both sites), with the exception of humans.

Genet exhibited a bimodal pattern of activities which peaked mainly at sunset (18:00) and to a lesser extent at night until sunrise (between 03:00 and 06:00) (**Fig. 34A**). Bats and flying insects also showed a bimodal activity pattern, with a main peak of activities at sunset (around 18:00) and another peak before sunrise (between 04:00 and 06:00) (**Fig. 34A and 34B**). Small rodents showed a main peak of activities at sunset (around 18:00) and their activities remained fairly constant throughout the night, decreasing at sunrise (**Fig. 34C and 34D**). The highest activity overlap coefficient was observed between small rodents and other insects - arachnids (ground insects) (Dhat1= 0.86), followed by genet and bats (Dhat1= 0.84), small rodents and bats (Dhat1= 0.75) and flying insects and bats (Dhat1= 0.71) (**Fig. 34A, 34B, 34C and 34D**). Details of activity at each survey location for the most frequently detected taxa are presented in **Appendices 32 and 33**. The daily pattern of human activity was observed to be unimodal, almost exclusively during the day (**Fig. 35**). Only prayer activities inside the cave showed activity with several peaks,

including one at night (**Fig. 35D** and **Fig. 35E**). **Table 19** summarizes the overlapping activities of the most frequently detected species (wildlife and humans).

Figure 34: Density of individuals in all study sites depending on time with (A) activity overlap between genet and bats, (B) activity overlap between flying insects and bats, (C) activity overlap between other insects and small rodents and (D) activity overlap between bats and small rodents.

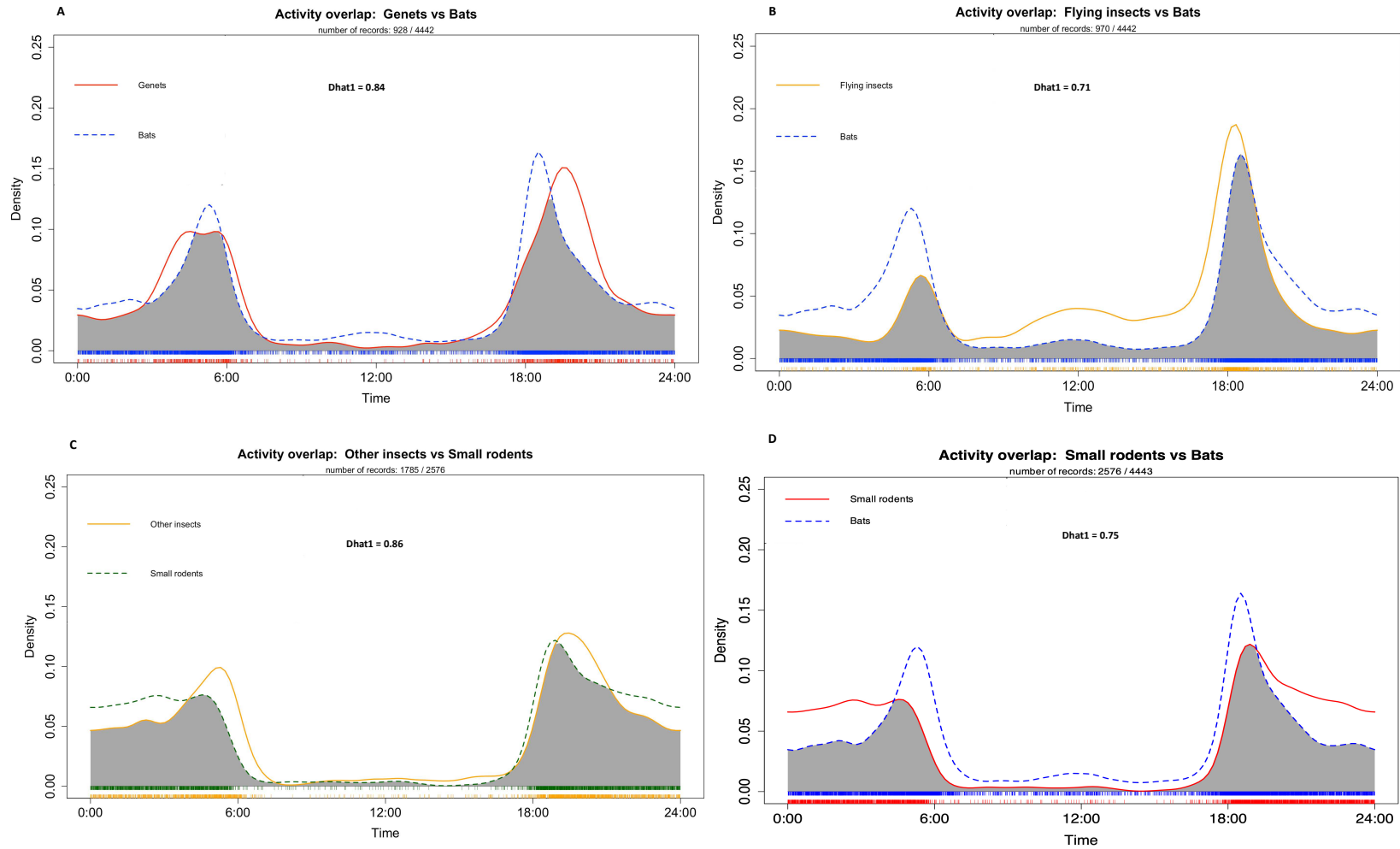


Figure 35: Daily activity pattern of humans according to activities outside or inside the Mont Belo cave. (A, B) Hunting activities outside and inside the cave, (C) Guano collection activities outside the cave, (D, E) Prayer activities outside and inside the cave, and (F, G) other activities outside and inside cave.

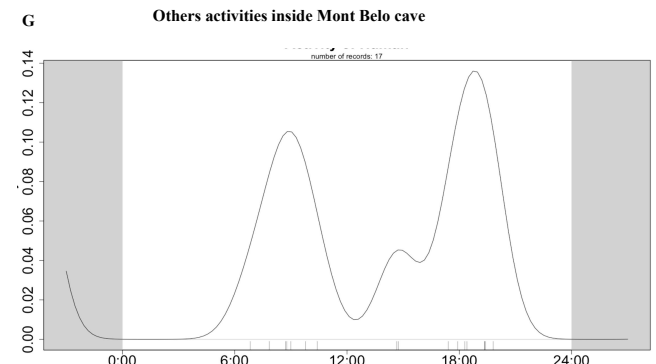
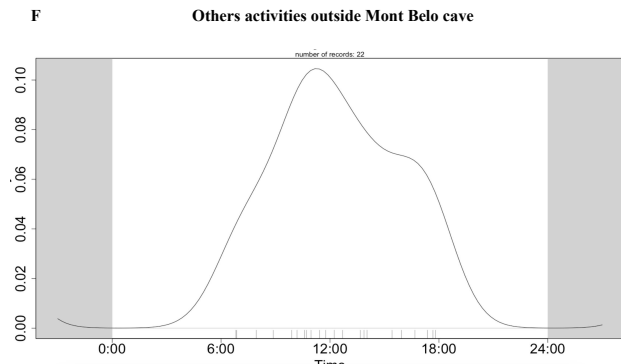
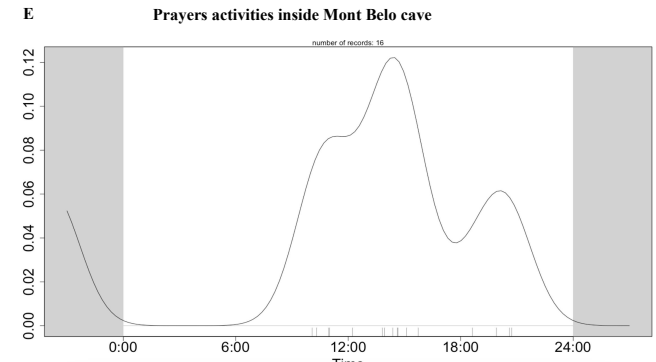
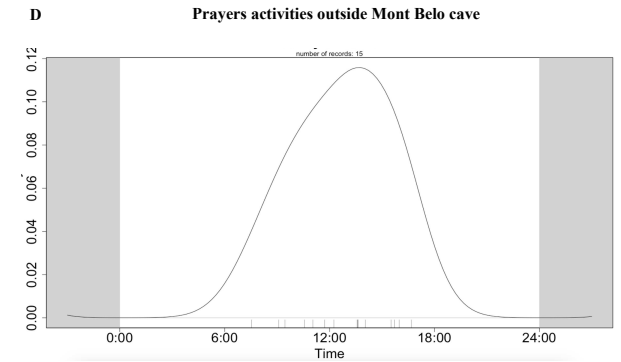
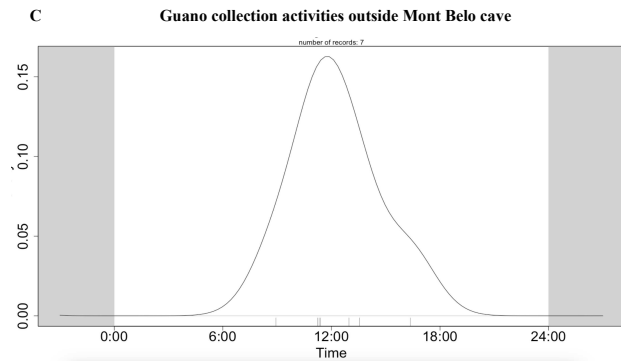
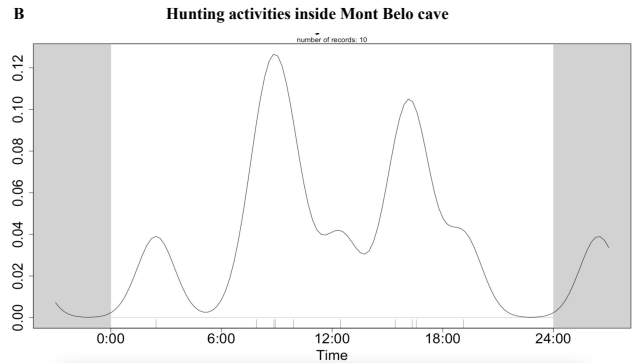
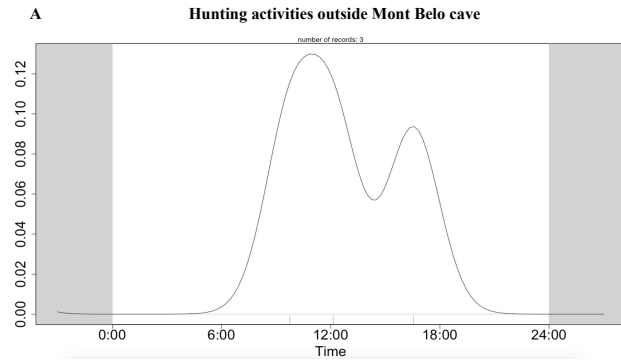


Table 16: Association between detections of several animal categories (insects, bats, rodents) for Mont Belo cave. Significant associations were tested using Pearson’s non parametric test (degree of freedom and significant P-value on the lower triangular table and correlation value with 95 percent confidence interval on the upper triangular table).

Species	Other insects	Flying insects	Bats	Small rodents	Giant pouched rat
Other insects	-	0.12 (-0.36 – 0.56)	0.30 (-0.17- 0.67)	0.64 (0.26 – 0.85)	0.06 (-0.42 – 0.51)
Flying insects	16, > 0.05	-	0.95 (0.87 – 0.98)	-0.01 (-0.46 – 0.44)	-0.05 (-0.52 – 0.43)
Bats	17, > 0.05	17, <0.01***	-	0.22 (-0.25 – 0.61)	0.01 (-0.44 – 0.46)
Small rodents	17, <0.01***	17, > 0.05	17, > 0.05	-	0.08 (-0.39 – 0.51)
Giant pouched rat	16, > 0.05	15, > 0.05	17, > 0.05	17, > 0.05	-

Table 17: Non parametric test of Pearson for Boundou cave depending on the species of interest, month and year of study. Degree of freedom and significant P-value on the lower triangular table and correlation value with 95 percent confidence interval on the upper triangular table.

Species	Other insects	Flying insects	Bats	Small rodents	Genets
Other insects	-	0.77 (0.48 – 0.90)	0.88 (0.72 – 0.95)	0.28 (-0.19 – 0.65)	0.33 (-0.14 – 0.68)
Flying insects	17, < 0.01 ***	-	0.92 (0.81 – 0.97)	0.001 (-0.45 – -0.45)	0.29 (-0.18 – 0.66)
Bats	17, < 0.01 ***	17, < 0.01 ***	-	0.22 (-0.26 – 0.61)	0.43 (-0.02 – 0.74)
Small rodents	17, > 0.05	17, > 0.05	17, > 0.05	-	-0.01 (-0.46 – -0.44)
Genets	17, > 0.05	17, > 0.05	17, < 0.06	17, > 0.05	-

Table 18: Summary of observed contacts, interactions, competitions and foraging behavior in the two caves (including inside and outside) between fauna and humans.

Species	Other insects & arachnids	Flying insects	Bats	Small rodents	Genets	Giant pouched rat	Human	Owl
Other insects & arachnids	Interaction Competition	-	Contact potential	Foraging	Foraging	Contact potential	Contact potential	-
Flying insects	Contact potential	Interaction Competition	Foraging Contact potential	Contact potential	-	Contact potential	Contact potential	-
Bats	Contact potential	Foraging	Interaction Competition	Contact potential	Foraging	Contact potential	Contact potential	Foraging
Small rodents	Foraging	-	Contact potential	Competition Interaction	Foraging	Contact potential Interaction	Contact potential	-
Genets	Foraging	Contact potential	Foraging	Foraging	Interaction Competition	-	-	-
Giant pouched rat	Contact potential	-	Contact potential	Interaction	-	Interaction Competition	-	-
Human	Contact potential	Contact potential	Contact potential	Contact potential	-	-	Interaction Competition	-
Owl	-	-	Foraging	-	-	-	-	-

Table 19: Summary of the activities of the various species and humans in the two caves.

Species	Mont Belo				Boundou			
	Time				Time			
	0 – 6h	6h – 12h	12h – 18h	18h – 24h	0 – 6h	6h – 12h	12h – 18h	18h – 24h
Other insects & arachnids	X			X	X			X
Flying insects	X		X	X	X		X	X
Bats	X			X	X			X
Small rodents	X	X		X				
Genets	X			X	X	X		X
Giant pouched rat	X			X				
Human		X	X	X		X	X	
Owl				X				

5.5 Discussion

This study provides novel information on the use of the complex habitats of caves (Fenolio et al. 2006; Gnaspini 2012; Simon 2012) by animal species, including humans, on inter- species interactions (contacts and feeding activities) and seasonal variations of species richness and diversity.

It is, to our knowledge, the first comprehensive study of cave communities characterizing the interactions between bats and other animals, including humans.

This study contributes to the understanding of the interface and interactions between animal species, including humans (Caron et al. 2021; de Garine-Wichatitsky et al. 2021). These results are important in the acquisition of knowledge on the ecology of infectious diseases, while improving our understanding of animal behaviour in these karst habitats. These results may help to improve bat conservation programs (predicting the impact of environmental/biodiversity changes, etc.).

5.5.1 Species richness and diversity inside/outside caves

This study demonstrates this complexity by describing the presence, of these three major taxonomic classes inside caves (mammals, insects and reptiles) and the addition of a fourth class, birds, outside cave. We detected greater species diversity and richness outside of both caves compared to inside, particularly during the long rainy season and the long dry season. The accumulation curves obtained from outdoor cameras showed that the nineteen months of collection were not sufficient to detect all the species present in the environment. These results could be explained by two factors. First, each species has its own specific ecology (Beaver, 1979), and caves are a particular habitat. Thus, some species do not use caves in their life cycle (feeding, roosting) and live outside. Interestingly, we observed that certain animals, such as a primate, behaved "fearfully" towards these gaping holes in the rock (observation from a video of this study). Second, the absence of an asymptote for the species accumulation curve for outdoor cameras could be explained by the fact that our protocol focused on the passages of animals near

the caves. However, as with all camera trap protocols, various parameters can have an impact on species detection, such as height and orientation of cameras, field conditions (vegetation, light, etc.) and the difficulty of detecting certain species (e.g. snakes, tree-dwelling animals) (Trolliet, 2014; Burton et al., 2015).

We also detected a greater species diversity and richness outside both cave and a significant effect of season on the presence of the different taxonomic classes. Mammals and reptiles are more frequently detected in the Boundou cave than in Mont Belo during the short rainy season. Insects were detected in greater numbers in the Boundou cave during the short dry season and the short rainy season, but the opposite trend was observed for the other two seasons (the long dry season and the long rainy season) in the Mont Belo cave. During the short dry season, birds were not detected at Mont Belo, and reptiles were absent from Boundou cave during the two dry seasons (the long dry season and the short dry season).

This difference in richness, diversity and presence of taxa according to cave and season between the two caves in our study could be explained by two factors. Firstly, the unique configuration of each cave (shape of the entrance, number of chambers, humidity, temperature, species present), the type of external habitat (forest, savannah, presence of crops) and other factors (accessibility, proximity to inhabited areas, use of the cave by the local population) will create unique characteristics that will influence the microhabitat present in and around the cave (Gabriel and Northup 2013; Gnaspini 2012; Kosznik-Kwaśnicka et al. 2022).

Secondly, seasonal variations have an impact on the availability of resources in a habitat and on the behaviour of individuals (movement, habitat use) (Aarts et al., 2013; Cumming & Bernard, 1997; Osman, 1978; Tonkin et al., 2017). Despite the limitations of our protocol, which prevented us from counting with accuracy the quantity of insects and bats present in the caves. We can hypothesise that during wet seasons, bats tend to be more numerous due to the synchronisation of the rearing period with the period of high resource availability (Arlettaz et al. 2001; Nurul-Ain, Rosli, and Kingston 2017; Paksuz, Özkan, and Postawa 2008). Their high presence may favour the presence of predators (e.g. snakes feeding on the young) or animals exploiting the high guano

presence during this period (insects exploiting the litter) (Di Russo et al., 1994; Jurado et al., 2010; Nitzu, 2021; Tlapaya-Romero et al., 2021, 2021).

Our results showed an overlap between bat activity and flying insects inside the study caves (**Fig. 34B**). We also observed an association of bats with flying insects in Mont Belo cave, and with both types of insects in Boundou cave. These results may reflect an increased presence/activity of insects in the habitat at specific time such as dusk. Our result suggests a common period of their activities in both types of insects (flying and otherwise) and the microhabitat of cave (Gnaspini, 2000; Royzenblat, 2023). Nonetheless, we cannot ignore the link between the detection of a mammal (potentially a bat) and the observation of insects once the camera was triggered with our methodology (using Megadetector and its poor performance in detecting bats and insects) (Leorna, 2022).

5.5.2 Species identified and behavior

Four major groups of mammals are significantly represented: bats, rodents (small and big), genets and humans. As both caves are home to bat colonies, our camera traps detected a high level of bat presence and activity. The periods of flying activity of bats corresponded with emergence at dusk and then return to the cave at dawn.

The high density of bats in a given area can favor the predatory behavior of certain species such as snakes, various carnivorous mammals and birds (Ridley 1898; Tanalgo et al. 2019). For example, at the entrance of Mont Belo cave, we observed the presence of the African wood owl on ten occasions. This species was most probably preying on bats exiting in the cave, as it has been observed flying to hunt (Kemp and Calburn 1987; Looney, 1972; Sieradzki, 2020).

The frequent detection of rodents (i.e more than 20 detections in one night), especially inside Mont Belo cave, could be explained by the presence of food resources (i.e insects) and the use of the cave by individuals as a refuge or for reproduction. Inside Mont Belo cave, we observed a high presence of several insect species that seemed to exploit the bat guano such as crickets and cave beetles. Several studies (Gnaspini 2012; Sakoui et al. 2020) have shown the great diversity of insects that exploit bat guano litter. These insects could also be a food resource for some of

the small rodents. We made this assumption after several events of hunting behavior by rodents (N= 18) hunting insects were observed on video recordings. Moreover, we identified a species of small insectivorous rodent in the cave after setting a few rodent traps in the Mont Belo cave (pers. obs. ML).

In addition, the daily overlap activities of small rodents and other insects - arachnids also showed significant relationship (**Fig. 34C**). In Mont Belo cave, we also detected a significant association between small rodents and other insects - arachnids (**Table 15**). However, our camera trap protocol allowed us to measure only the presence of insects inside and outside the caves through the frequency of detection. To obtain an accurate measure of insect abundance according to the season, it would be necessary to set up an insect collection protocol.

We identified a giant pouched rat (*Cricetomys emini*) which seems to use Mont Belo cave as a food storage site. This result is in accordance with existing literature. This species is known to store its food in specific locations (Skinner and Chimimba 2005; Tosso et al. 2018). The daily activity of giant pouched rat was not the same as the other rodents. It visited the cave regularly but showed irregular activity patterns (**Appendix 32 - 33**), which could also indicate food storage behaviour in the cave.

Other frequently detected mammals were the rusty-spotted genet in Boundou cave (both inside and outside the cave) and the servaline genet outside Mont Belo cave. Our results showed that rusty-spotted genets regularly visited the inside of Boundou cave in search of food by preying on bats, rodents or insects, or by scavenging on bats (Labadie et al., under submission). We also detected a strong overlap in daily activity between genets and bats. However, we did not detect any significant association between small rodents and genets. These results seem to validate the hypothesis of an opportunistic foraging behavior by genets in Boundou cave. The specific topography of the Boundou cave (**Appendix 26**) could also accentuate and favor bat hunting success.

5.5.3 Human activities

Our results showed that human activities in the two caves varied greatly between Mont Belo and Boundou. In Mont Belo cave, the camera traps detected a high level of prayer activities over a period of nineteen months, while Boundou cave was little or not used by the local population, as notified by the communities. In the Mont Belo cave, we also detected other human activities, including bat hunting and guano harvesting. At Mont Belo, local people collect bat guano as natural fertilizer for crops, which is a common practice in many countries around the world (Sakoui et al. 2020). Hunting activity was only detected during two different months (March 2022 and January 2023). The hunting intensity could be low due to the sacred nature of the cave and the almost exclusive presence of insectivorous bat species. One of the bat-hunting periods favoured by the hunters was January, which is a period of high fruit abundance for fruit-eating species, and the end of juvenile rearing for insectivorous bats. In our study area, close to the two caves, we were able to observe quite significant hunting pressure on colonies of frugivorous cave bats.

5.5.4 Implication for disease ecology and for conservation

Disease transmission from reservoir hosts to humans depends on multiple factors including: (1) the distribution and density of the reservoir species, (2) the dynamics of the pathogen in the reservoir host, (3) the exposure of the human to the pathogen and (4) some internal factors of the person in contact (Ermonval and Morand, 2023; Plowright, 2017; Taylor et al. 2001).

In Central Africa, caves are teeming with mammals and insects creating a specific microbiome both inside and outside the cave (Furey & Racey, 2016). The presence of bats and their guano provides attractive food resources for other species in the cave. The daily emergence of bats attracts opportunistic predators such as birds of prey, genets or reptiles outside the cave. Our results characterized intra-specific interactions and potential factors involved in the trophic chain comprising main bat-consuming species, inside and outside cave, but also some other species exploiting the guano. This work may help to investigate the potential mechanisms of micro-

organisms transmission between different species; some of which might act as bridge hosts between species known to carry numerous known pathogens (bats and rodents) and other at risk-species (humans, predators). This type of information is invaluable in the field of health ecology, especially if we want to understand the mechanisms and risks of inter- and intra-species transmission of zoonotic pathogens (Borremans et al., 2019; Cross et al., 2019; Ellwanger & Chies, 2021). Behavioral data can also help identify key factors in species conservation by: (i) studying population dynamics and dispersal, (ii) identifying priority habitats according to their use (feeding area, reproduction) and (iii) protecting habitats - areas of high connectivity (Berger-Tal et al., 2016; Caro, 2016; Greggor et al., 2016) .

This study contributes to the science of the interface and interactions between animal species, including humans, which is essential for understanding infectious disease ecology and improving conservation programmes.

5.5.5 Limitation of our study

We faced several limitations due to field constraints. Due to the rapid movement of the animals, the quality of the videos and the environment (poor light and extreme humidity), it was difficult to identify the rodents down to species level (Burns et al. 2017). One way of solving this problem would be to use cameras inside rodent traps as suggested by Gracanin et al. (2019). We used size categorization for rodents. However, the size of individuals may vary according to the features of the camera field in which the individual was detected (near a rock, close to the camera or towards the back of the photo), its position in the photo or video (facing or from behind) and its behavior when the camera was triggered (e.g., running, sniffing or resting). Cameras placed inside caves also tended to operate for shorter periods due to the numbers of triggers caused by the movement of bats in the cave, as well as the presence of many insects. This problem may have limited the detection of some animals at certain periods. We also had to contend with a number of technical malfunctions due to the specificities of the cave environment (camera shutdowns, poor-quality photos and videos) which resulted in data loss. These various limitations have highlighted the improvements that could be made to this protocol to help increase data quality. This protocol could be improved by coupling it with additional protocols, such as collecting faeces

from certain animals (rodents, genets, owls) in order to analyze their diet and confirm some of our hypotheses.

5.6 Conclusions

This study is the first one to our knowledge that characterizes the interactions between bats, wild animals and humans in two caves in central Africa. Our results enabled us to provide a preliminary description of the multi-species communities sharing cave habitats and pave the way for further and optimized similar studies based on our experience.

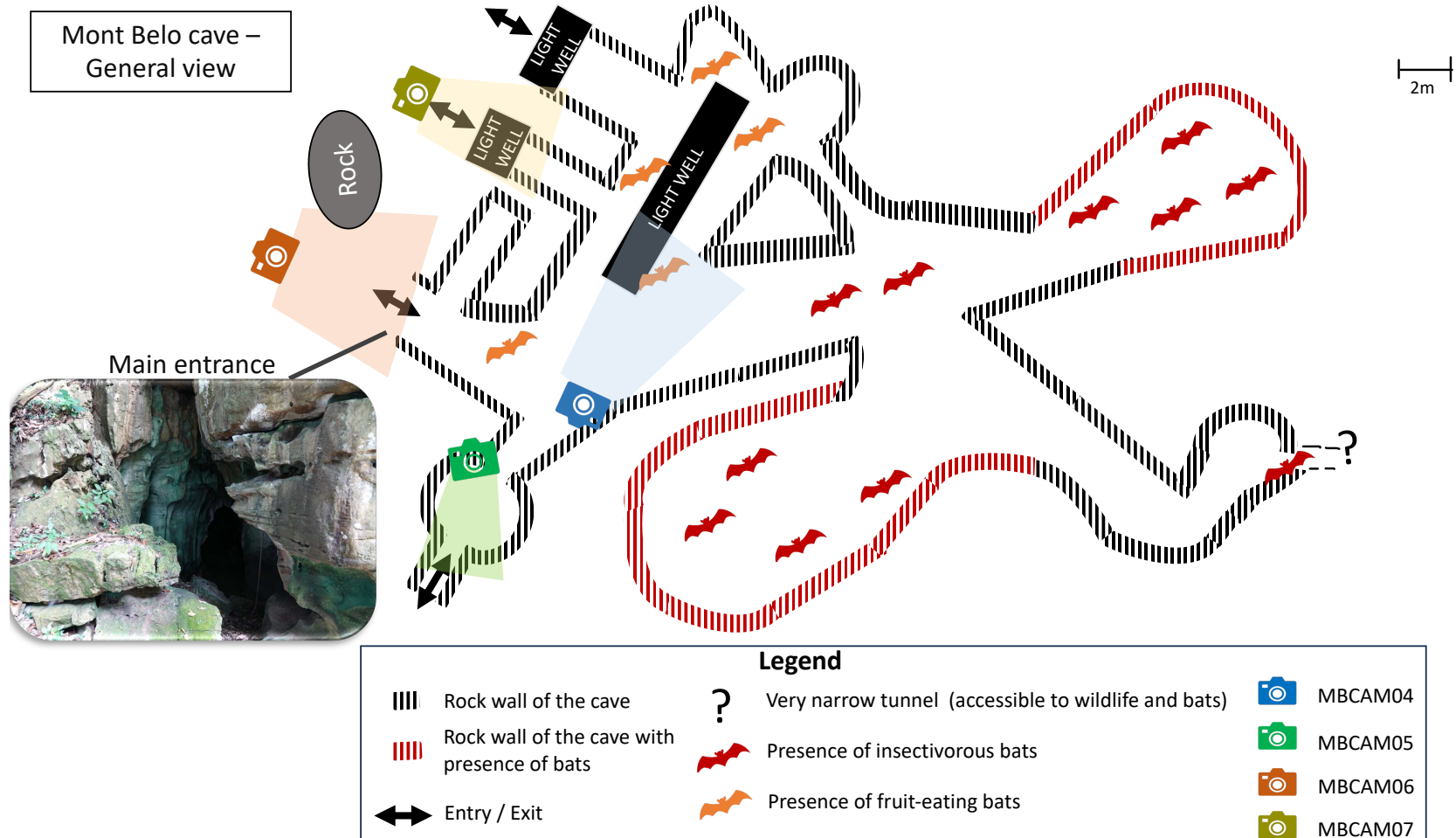
A better understanding of mechanisms of pathogen spillover from bats to humans is needed to mitigate risk transmission together with improved bat conservation and the ecosystem services bats provide. This work advocates for more research at the wild/domestic/human interface in order to cope with health hazards and promote coexistence between wildlife and humans.

5.7 Acknowledgements

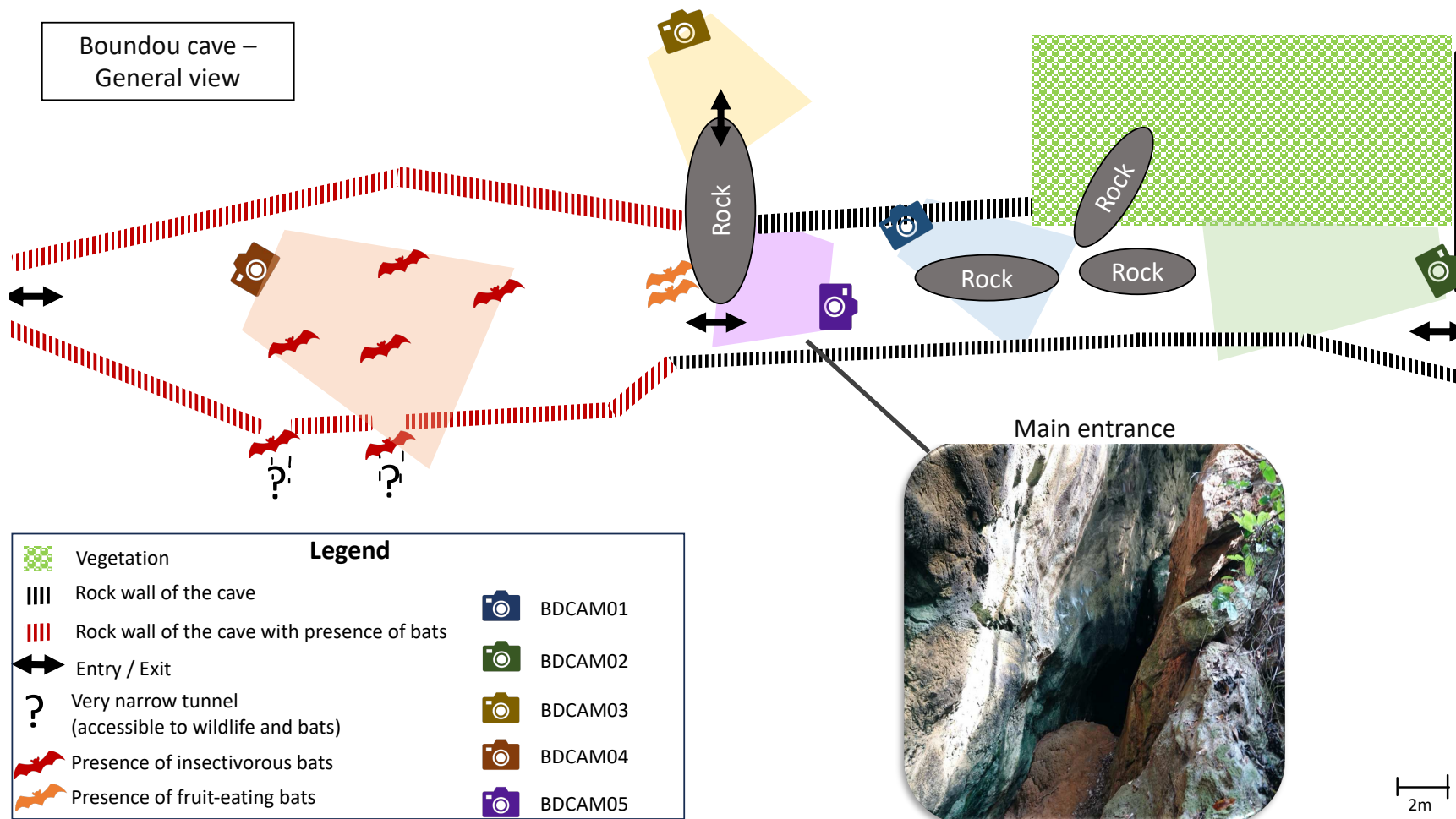
We are very grateful to James Hogg, Olivier Hamerlynck and Zév Hamerlynck who identified the bird species and to Emmanuel Do Linh San for confirming the genet species. We would also like to thank the local authorities in Dolisie and the Congolese partners for their help during this project. Finally, we would like to thank the field team (F. Nguilili, C. Bazola, N. Nguimbi, R. Dimoukissi and R. Nguimbi) and the local communities who accepted the project.

5.8 Appendix Chapter 5

Appendix 25: General diagram of the configuration of the Mont Belo cave, the location of the camera trap and the bat roosting sites.



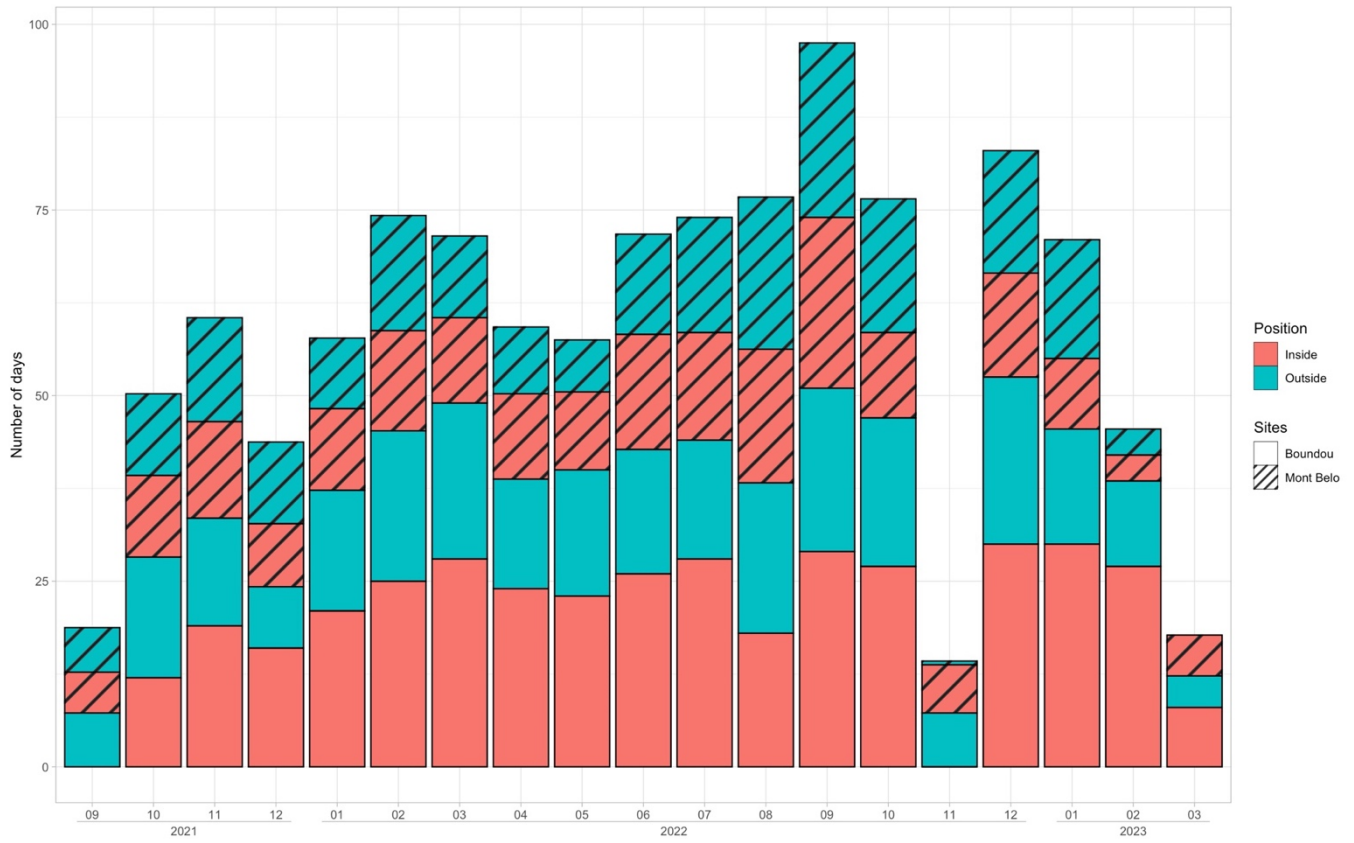
Appendix 26: General diagram of the configuration of the Boundou cave, the location of the camera trap and the bat roosting sites.



Appendix 27: Raw data of detection

<https://dataverse.cirad.fr/privateurl.xhtml?token=a3f476f6-1bfa-4a0b-b88b-46f90909b262>

Appendix 28: Number of days the camera traps were in operation during the study. A total of nine cameras for all sites, with five for Boundou cave (including one inside the cave) and four for Mont Belo cave (including 2 inside the cave).

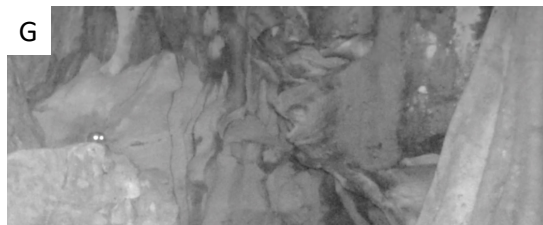
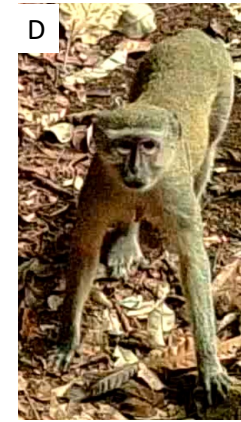
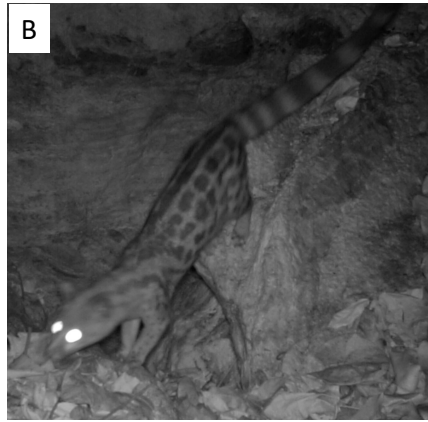


Appendix 29: List of all detected species and scientific name if available (N = 11,581 detections for a total of 11,767 animals)

Common names	Scientific Name	Group	Study site and position	Number of detection	Number of animals
African harrier hawk	<i>Polyboroides typus</i>	Raptors	Boundou Outside	1	1
African wood owl	<i>Strix woodfordii</i>	Raptors	Mont Belo Outside	10	10
Ants	NA	Other insects	Mont Belo Outside	1	1
Bats	NA	Bats	Boundou Inside	1562	1562
Bats	NA	Bats	Boundou Outside	1705	1705
Bats	NA	Bats	Mont Belo Inside	626	626
Bats	NA	Bats	Mont Belo Outside	550	550
Large rodents	<i>Cricetomys emini</i>	Giant pouched rat	Mont Belo Inside	79	79
Large rodents	<i>Cricetomys emini</i>	Giant pouched rat	Mont Belo Outside	78	78
Large rodents	NA	Large rodents	Mont Belo Inside	1	1
Black-bellied seedcracker	<i>Pyrenestes ostrinus</i>	Small birds	Mont Belo Outside	1	1
Butterflies	NA	Flying insects	Boundou Outside	10	10
Butterflies	NA	Flying insects	Mont Belo Outside	13	13
Cave beetles	NA	Other insects	Boundou Inside	1334	1334
Cave beetles	NA	Other insects	Boundou Outside	15	15
Cave beetles	NA	Other insects	Mont Belo Inside	79	79
Genets	<i>Genetta maculata</i>	Genets	Boundou Inside	557	561
Genets	<i>Genetta maculata</i>	Genets	Boundou Outside	364	377
Genets	<i>Genetta servalina</i>	Genets	Mont Belo Outside	6	6
Collared sunbird	<i>Hedydipna collaris</i>	Small birds	Mont Belo Outside	2	2
Crickets	NA	Other insects	Boundou Inside	15	15
Crickets	NA	Other insects	Boundou Outside	10	10
Crickets	NA	Other insects	Mont Belo Inside	253	253
Crickets	NA	Other insects	Mont Belo Outside	4	4
Dove and pigeon	NA	Medium birds	Boundou Outside	10	14
Dove and pigeon	NA	Medium birds	Mont Belo Outside	2	2
Flies	NA	Flying insects	Boundou Inside	232	232
Flies	NA	Flying insects	Boundou Outside	388	388
Flies	NA	Flying insects	Mont Belo Inside	73	73
Flies	NA	Flying insects	Mont Belo Outside	251	251
Giant pangolin	<i>Smutsia gigantea</i>	Pangolin	Mont Belo Outside	1	1
Humans	NA	Humans	Boundou Inside	31	40
Humans	NA	Humans	Boundou Outside	93	98
Humans	NA	Humans	Mont Belo Inside	61	80
Humans	NA	Humans	Mont Belo Outside	78	114
Icterine greenbul	<i>Phyllastrephus icterinus</i>	Medium birds	Mont Belo Outside	1	1
Lesser striped swallow	<i>Cercropsis abyssinica</i>	Small birds	Mont Belo Outside	8	8
Lizard or gecko	NA	Lizard or gecko	Mont Belo Inside	3	3

Common names	Scientific Name	Group	Study site and position	Number of detection	Number of animals
Lizard or gecko	NA	Lizard or gecko	Mont Belo Outside	16	16
Monkey	Chlorocebus cynosuuros	Monkey	Boundou Outside	1	1
Monkey	Chlorocebus cynosuuros	Monkey	Mont Belo Outside	3	3
NI	NA	Not Identified	Boundou Inside	28	29
NI	NA	Not Identified	Boundou Outside	62	71
NI	NA	Not Identified	Mont Belo Inside	71	78
NI	NA	Not Identified	Mont Belo Outside	136	152
Nile monitor	Varanus niloticus	Nile monitor	Boundou Outside	2	2
Palm thrushes	NA	Medium birds	Mont Belo Outside	2	2
Passerine birds	Malimbus sp	Small birds	Boundou Outside	1	1
Pied crow	Corvus albus	Medium birds	Mont Belo Outside	1	1
Porcupines	Atherurus africanus	Porcupines	Boundou Inside	2	2
Porcupines	Atherurus africanus	Porcupines	Boundou Outside	28	29
Scaly spurfowl	Pternistis squamatus	Medium birds	Mont Belo Outside	1	1
Small rodents	NA	Small rodents	Boundou Inside	117	119
Small rodents	NA	Small rodents	Boundou Outside	39	39
Small rodents	NA	Small rodents	Mont Belo Inside	2123	2170
Small rodents	NA	Small rodents	Mont Belo Outside	297	310
Snake	NA	Snakes	Boundou Inside	3	3
Snake	NA	Snakes	Boundou Outside	1	1
Snake	NA	Snakes	Mont Belo Inside	14	14
Snake	NA	Snakes	Mont Belo Outside	5	5
Snowy-crowned robin-chat	Cossypha niveicapilla	Small birds	Boundou Outside	32	32
Snowy-crowned robin-chat	Cossypha niveicapilla	Small birds	Mont Belo Outside	1	1
Spider	NA	Other insects	Boundou Inside	18	18
Spider	NA	Other insects	Boundou Outside	15	15
Spider	NA	Other insects	Mont Belo Inside	27	27
Spider	NA	Other insects	Mont Belo Outside	14	14
Squirrels	NA	Squirrels	Boundou Outside	1	1
Squirrels	NA	Squirrels	Mont Belo Outside	7	7
Typical striped grass mouse	Lemniscomys striatus	Small rodents	Boundou Outside	2	2
Wasp	NA	Flying insects	Boundou Outside	3	3

- 1 **Appendix 30:** Facebook of species with camera traps taken during the nineteen months of study. (A) *Genetta servalina*, (B) *Genetta maculata*, (C)
- 2 *Atherurus africanus*, (D) *Chlorocebus cynosuros*, (E) small rodents, (F) *Cricetomys emini*, (G) *Strix woodfordii*, (H) *Cossypha niveicapilla* and (I)
- 3 *Varanus niloticus*.



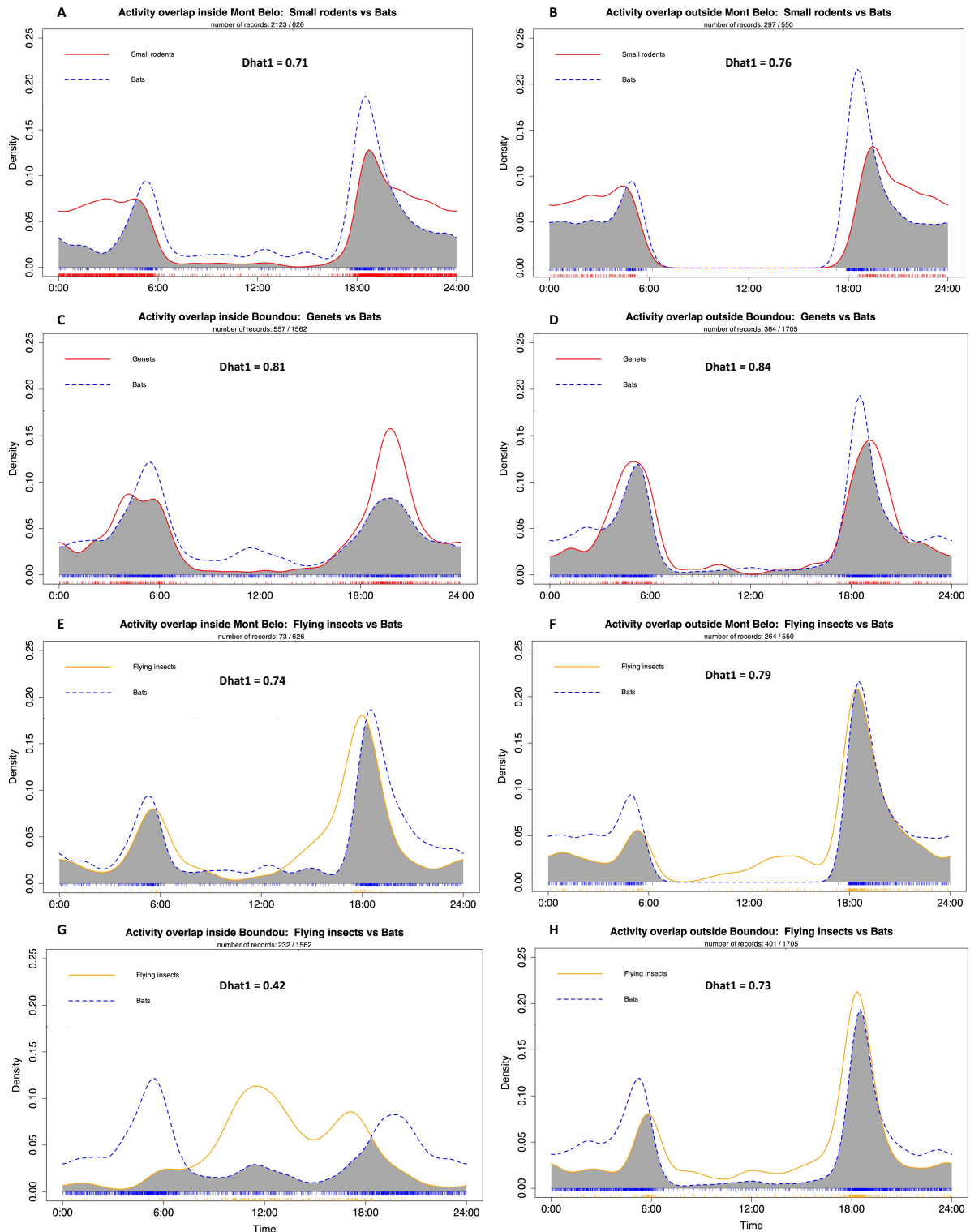
4

5 **Appendix 31:** Picture of one individual of Giant pouched rat (*Cricetomys emini*) with swollen jowls

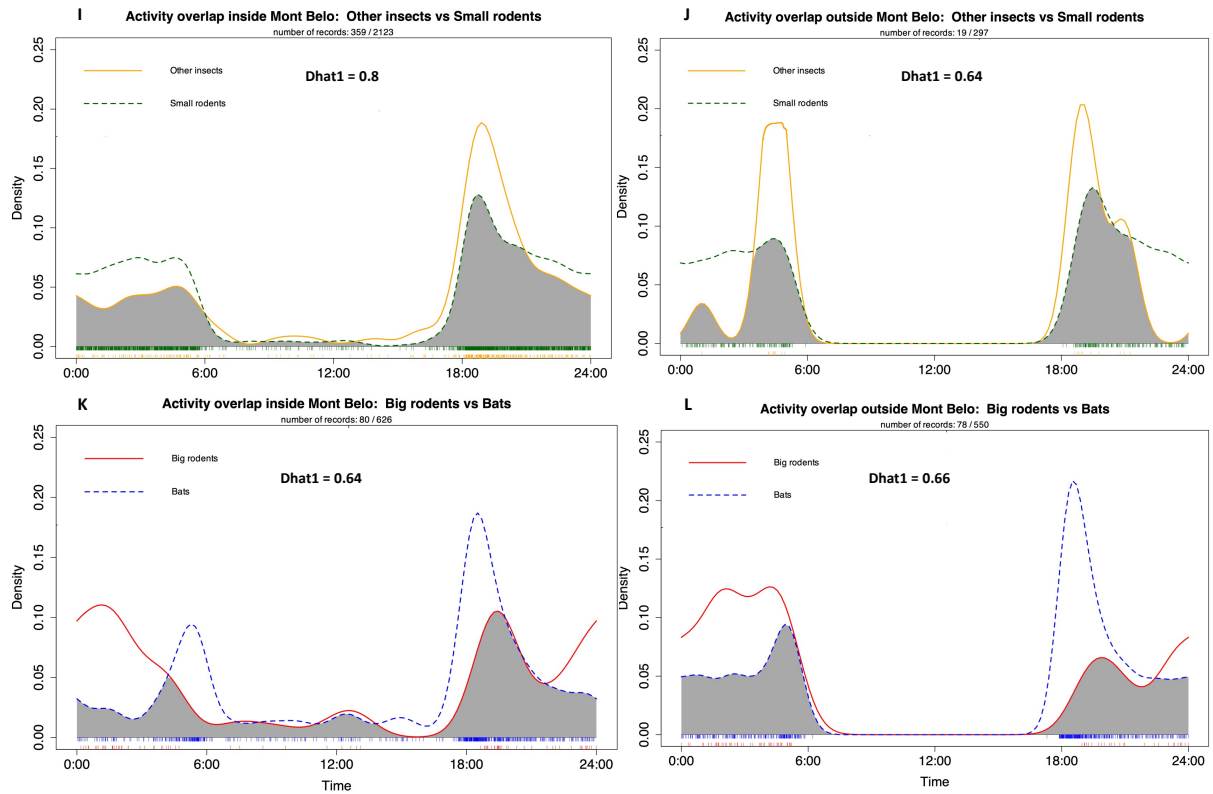


6

Appendix 32: Daily activity pattern of species according to activities outside or inside Mont Belo or Boundou cave. (A, B) overlap activities outside and inside Mont Belo cave for small rodents vs bats, (C, D) overlap activities outside and inside Boundou cave for genets vs bats, (E, F) overlap activities outside and inside Mont Belo cave for flying insects vs bats, and (G, H) overlap activities outside and inside Boundou cave for flying insects vs bats.



Appendix 33: Daily activity pattern of species according to activities outside or inside Mont Belo cave. (A, B) overlap activities outside and inside Mont Belo cave for other insects and arachnid vs small rodents and (C, D) overlap activities outside and inside Mont Belo cave for giant pouched rodent (big rodent) vs bats.



5.9 References Chapter 5

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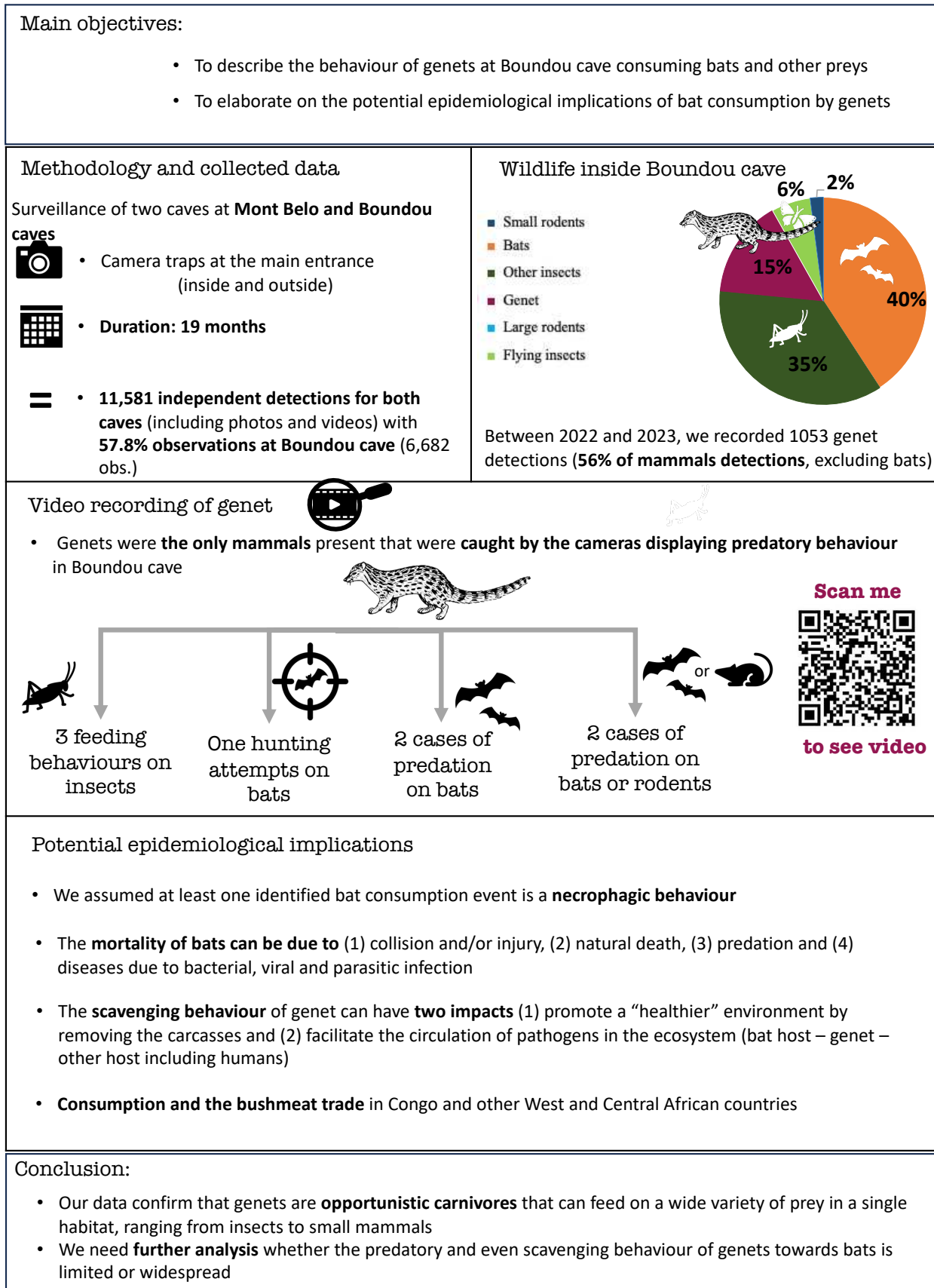
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**CHAPTER 6 – ARTICLE 5: VIDEO-RECORDING RUSTY-SPOTTED
GENETS CONSUMING BATS AND OTHER PREY IN A CAVE:
BEHAVIORS OBSERVED AND ECO-EPIDEMIOLOGICAL
CONSIDERATIONS**

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Box 5: Graphic abstract Chapter 6



Video-recording rusty-spotted genets consuming bats and other prey in a cave: behaviors observed and eco-epidemiological considerations

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Open Research statement

Upon acceptance videos will be available via the following link:

<https://dataverse.cirad.fr/privateurl.xhtml?token=7859fbc4-e507-4e63-9348-0ce6d4182cde>

6.1 Abstract

Following the implementation of a camera-trap monitoring protocol of interactions between cave bats and wildlife in the Republic of Congo, we identified sustained rusty-spotted genet (*Genetta maculata*) activity in Boundou cave. This cave, consisting of a single chamber, is home to a colony of several species of insectivorous bats throughout the year. Between 2022 and 2023, we recorded four events of bat or rodent consumption, one hunting attempt on bats and three feeding behaviors on insects. We describe and discuss the various behaviors and briefly elaborate on the potential epidemiological implications of bat consumption. To the best of our knowledge, our videos are the first to depict the consumption of bats by rusty-spotted genets.

Keywords : Camera traps, *Genetta maculata*, foraging behavior, predation, scavenger, trophic chain.

6.2 Introduction

With more than 1470 known species (Simmons & Cirranello, 2023), bats (Chiroptera) are the mammalian order represented globally by the largest number of individuals (Greenspoon et al., 2023). They are present in a wide range of habitats and exhibit a great diversity in their foraging and refuge behaviors, providing numerous ecosystem services (Kunz 2013). They are also an essential element in the food chain, serving as food for many predators (Kasso & Balakrishnan 2013), including humans (Mickleburgh et al., 2009). During their resting period or when they emerge from their roosts, bats are predated by snakes (Price, 2004; Ridley, 1898), birds from different families (Tanalgo et al., 2019), centipedes (Molinari et al., 2005), as well as wild and domestic mammals (Costa-Pinto, 2020; Mallick et al., 2021; Oedin et al., 2021; Scrimgeour et al., 2012).

The Viverridae (order Carnivora) is a family of medium-sized, feliform carnivorous mammals comprising at least 34 different species distributed across three continents (southern Europe, Africa and Asia), divided into 14 distinct genera including genets, civets and binturongs (Do Linh San, 2022; Jennings & Veron, 2009). Mainly known as nocturnal and terrestrial and/or arboreal hunters, they have a varied diet ranging from small vertebrates and invertebrates to fruits, seeds and other plant materials (Jennings & Veron, 2009; Kingdon & Hoffmann 2013). In Africa, there are between 14 and 17 recognized species of genets (Gaubert, 2013; Gaubert et al., 2005), with possibly up to four species living in the Republic of Congo (see Appendix ES1 in Do Linh San et al., 2013). The rusty-spotted genet or Central African large-spotted genet (*Genetta maculata*; note that the species epithet *maculata* is no longer valid according to the International Commission on Zoological Nomenclature, and thus should only be used as a provisional naming; ICZN, 2007) is present in a wide range of countries in Africa (Angelici et al., 2016), including the Republic of Congo, where it is likely one of the most common genet species. It is known to be mainly carnivorous (small mammals, insects, gastropods, fish, amphibians, small birds and eggs), but it can supplement its diet with fruits, seeds and berries (Angelici & Gaubert, 2013; Roux et al., 2016).

Some genet species are known to consume bats on a more or less regular basis as part of their diet, such as the Cape genet (*Genetta tigrina*) (Azzaroli & Simonetta, 1966) and the common genet (*Genetta genetta*) (Carvalho et al., 2016; Fuller et al., 1990; Mas et al., 2015;

Riols & Nadal, 2018). Although bat remains have been found in genet feces or stomachs, their direct consumption has not been well documented and predation events have rarely been observed. To our knowledge, only two studies, both in South Africa, mention cases of predation by *Genetta maculata* on bats. Carpenter (1970) described a rusty-spotted genet repeatedly consuming bats (average of six individuals per night) and Rautenbach (1978) mentioned the presence of a bat in the food bowl of an individual captured for an inventory.

In Africa and Asia, Viverridae are hunted for food (Brookes et al., 2022; Gaubert et al., 2015; Gossé et al., 2022) and to create clothing from their skins, or are captured alive to serve as exotic pets (Angelici et al., 2016; Carvalho et al., 2016; Widdows et al., 2016). Scientists suspect that at least one of these practices may be behind the outbreak of a new form of severe viral pneumonia (SARS) in 2002 due to interaction with masked palm civet (*Paguma larvata*) (Shi & Hu, 2008; Wang & Eaton, 2007; Xu et al., 2004; Yu et al., 2003). These predatory species can indeed contribute to the transmission of viruses to humans as bridge hosts by coming into contact with reservoir species such as bats (Borremans et al., 2019; Caron et al., 2015, 2018).

Camera monitoring has become a widespread technique, notably to detect the presence and determine the occupancy of wildlife species in focal regions and habitats (Ahumada et al., 2013; Fleming et al., 2014; Kucera & Barrett, 2011). However, the use of video recording can also provide precious and possibly novel information about interspecies interactions, including the feeding behavior of some predators and the interactions with their prey (Currylow et al. 2022; Yin et al. 2023).

Here, following a camera-trap monitoring program of cave-dwelling bats in the Republic of Congo, we report and describe, to our knowledge, the first recorded visual observations of rusty-spotted genets consuming bats (active predation and potential scavenging inside and outside caves). We also present observations of rodent and insect consumption by genets. We describe and discuss the various behaviors in relation to the current state of knowledge on the feeding ecology of *Genetta* species and briefly elaborate on the potential impact of these feeding behaviors on pathogen circulation pathways.

6.3 Study area and methods

In September 2021, in the framework of a EBOSURSY project investigating the non-human animal and human interface and the associated risks of viral emergence, we started a monitoring program with camera traps to study the interactions between bat colonies and wildlife at two separate cave sites in the Republic of Congo (**Fig. 36**). Mont Belo cave (4.1669° S; 12.9239° E), located in the department of Niari has one main chamber and two secondary chambers. Around Mont Belo cave, the habitat consists mainly of food crops and grassy savannah. Boundou cave (3.8916° S; 12.5573° E), located in the department of Bouenza consists of a main chamber and a secondary chamber which is not easily accessible by people due to a very narrow passage close to the ground. The habitat around Boundou cave is dominated by grassy savannah with patches of secondary forest. There is little human presence near the cave, with the first village 5 km away. In both caves, the presence of at least five species of bats has been recorded using harp traps at different times of the year (*Miniopterus* sp., *Rhinolophus* sp., *Hipposideros* sp. and *Roussettus aegyptiacus*) (ML, pers. obs., 2021–2023).

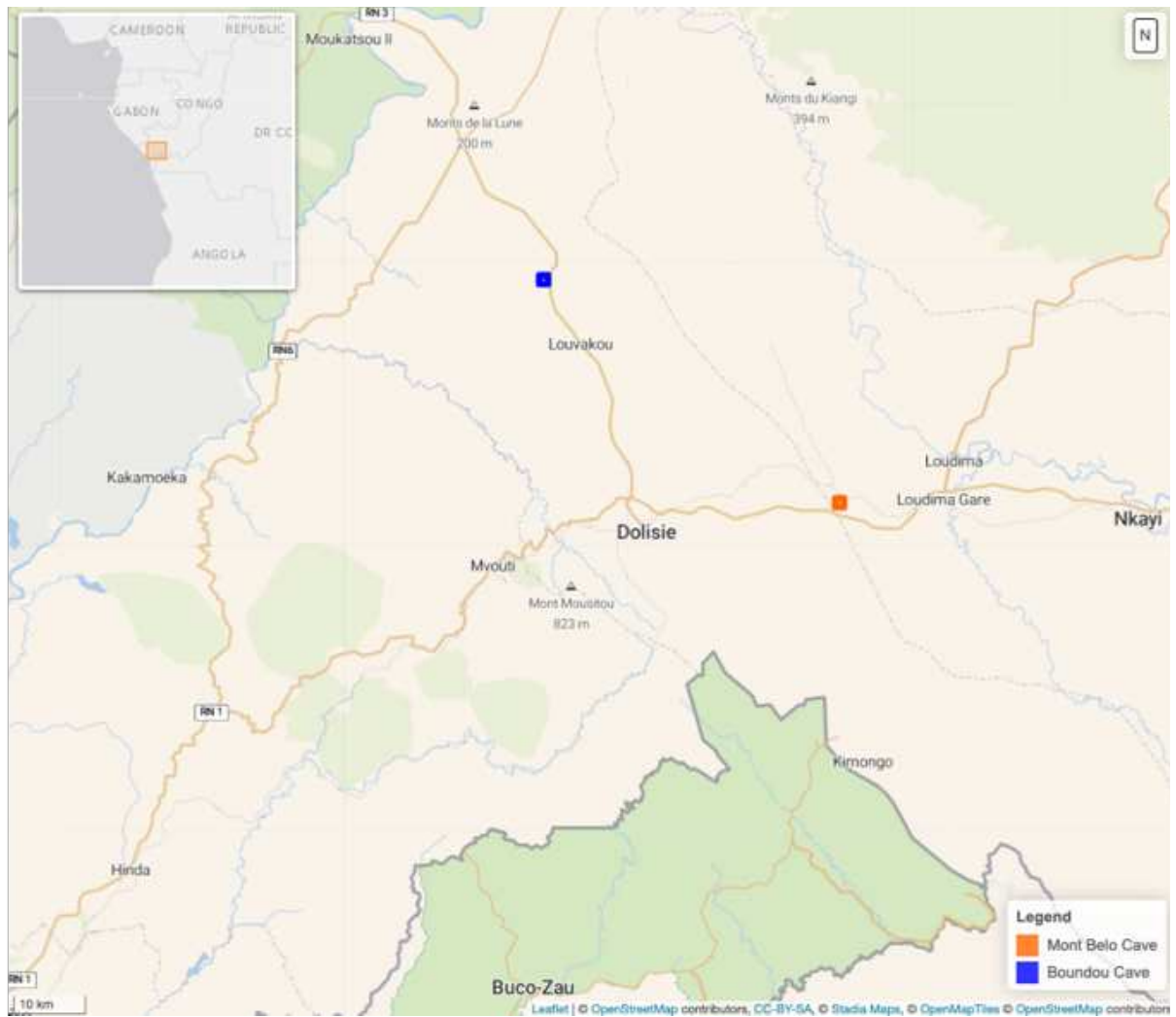
In total, nine Moultrie M50 cameras (Moultrie, Birmingham, USA) (five at Boundou cave and four at Mont Belo cave) were placed to cover the entrance of each cave. We also placed at least one camera inside the main chamber to observe the behavior of wildlife inside the caves housing the bat colonies. From October 2021 to March 2023, we recorded wildlife activities at each cave for an average of 18 days every month. Cameras were set to take a photo and a 30 s video after each trigger. Camera sensitivity was set to maximum and a delay of 5 min between two detections was selected.

The detection of genet presence in pictures was initially carried out using the Megadetector software (Beery et al., 2019; Fennell et al., 2022) and all the corresponding videos were viewed by only one of the authors (ML). Following this first viewing, recordings showing feeding behaviors were extracted and analyzed. The interpretation of genet behavior on the videos of interest was carried out independently by two observers including an expert in small carnivores (ML and EDLS).

Three species of genets have so far been reported in the Republic of Congo, namely the rusty-spotted genet, servaline genet (*Genetta servalina*) and king genet (*Genetta poensis*), while the presence of crested genet (*Genetta cristata*) is uncertain (see Appendix ES1 in Do

Linh San et al., 2013). Of these, only rusty-spotted and servaline genets were detected at the study site. Both species lack the presence of an erectile mid-dorsal crest and display a broad intraspecific variation in coat coloration and spot and tail ring patterns (EDLS, pers. obs.). However, the former can generally be visually differentiated from the latter by a combination of morphological features: the presence of a limited number of large dark or rusty blotches (of round or square shape) in the first two dorsal rows; legs that are generally pale in colour; a lower number of pale rings on the tail; and the tail tip is dark and appears elongated (Gaubert et al., 2008). For videos with poor quality or that were very dark, we used the software Vegas Pro (Magix Software GmbH, Berlin, Germany) to increase quality and/or brightness.

Figure 36: Study site in the Republic of Congo with Mont Belo and Boundou caves located about 50 km from each other.



6.4 Results

In total, over a period of two years, in Boundou cave, we recorded 1053 genet detections (corresponding to 56% of mammal detections, excluding bats), with 634 inside the cave and 419 at the cave entry. The genets were the only mammals present that were caught by the cameras displaying predatory behavior in Boundou cave. In contrast, at Mont Belo cave, we detected the presence of genets only seven times outside the cave, and no genet was ever filmed in the cave. The species detected at Mont Belo has been identified as the servaline genet (recorded by video moving outside the cave) and the species present at Boundou as the rusty-spotted genet.

Feeding behaviors of genets were observed in Boundou cave only. Inside that cave, we recorded two cases of predation on bats, two others where it was not possible to distinguish between bats and rodents (due to the poor quality of the video and the angle), one attempt of hunting behavior on bats and three feeding events on insects. **Table 20** lists the corresponding video files and summarizes the behaviors observed. All these events took place at night between 03:00 and 06:00 am.

The first video (Video S1) shows an adult genet with its back facing the camera, eating a relatively large prey that is light in color. The individual could potentially be eating a rodent or a bat (see Video S1). The second video (Video S2) again shows a genet with its back facing the camera, actively searching around a rock and retrieving a prey with its mouth. The genet then turns towards the camera to eat the animal (most probably a bat, given the shape).

The unequivocal consumption of bats by rusty-spotted genets was observed during two different months (June and September 2022) inside Boundou cave (see Videos S3 and S4). The third video starts by showing an adult genet holding a bat (**Figure 37**) that appears dead in its mouth. It then puts the bat back on the ground for a few seconds, before taking it back into its mouth and turning its back to the camera to consume the bat (see Video S3). The fourth consumption event shows the genet initially walking (with its back facing the camera) and then finding a bat lying on the ground (see Video S4). The animal then moves 1–2 m away from the initial site to eat it (see Video S4).

Video S5 shows a hunting attempt on bats by a genet outside Boundou cave. The event takes place on January 26, 2021 at 06:03 am, the time when bats enter the cave to rest before sunrise. We observe a genet sitting on a rock at the cave's main entrance, right in

the middle of one of the passageways used by the bats to enter or leave the cave (the cave entrance is in the right-hand frame of the video, Video S5). On several occasions, the genet seems to attempt to catch bats flying close to it (Video S5).

Active predation on insects also took place on three different occasions (see Videos S6, S7 and S8). In the first video (Video S6), a genet is rummaging in the leaf litter outside Boundou cave when it suddenly catches a ground insect before consuming it. The individual then moves around, while continuing to sniff the leaf litter. In video S7, the genet appears to be searching for something on the cave floor (guano litter) before suddenly moving and catching a ground insect which is quickly consumed before moving elsewhere in the cave. The last video (see Video S8, very dark) shows a genet coming rapidly into the camera's field of view, looking for something close to the ground before suddenly moving and quickly catching an insect with its two front legs. After eating it, the individual continues to move inside the cave in search of new prey (see Video S8).

Table 20: Summary of videos from this study, metadata, observed behaviors and additional comments

Video evidence	Video ID and location	Date and time	Behavior	Other comments
Video S1	4842 – Inside Boundou Cave	4 June, 2022 at 05:45 pm	Consumption of a bat or rodent	Ignore the date in the video, the camera had the wrong date.
Video S2	8270 – Inside Boundou Cave	27 January, 2023 at 03:44 am	Consumption of a bat or rodent	
Video S3	5058 – Inside Boundou Cave	19 June, 2022 at 05:26 am	Consumption of a bat	Not clear whether the bat was killed or found dead.
Video S4	6118 – Inside Boundou Cave	7 September, 2022 at 03:55 am	Consumption of a bat	Not clear whether the bat was killed or found dead.
Video S5	2845 – Outside Boundou Cave	26 January, 2021 at 06:03 am	Hunting behavior towards bats flying around the genet	The rock where the genet sits is located at the entrance of the cave.
Video S6	4819 – Outside Boundou Cave	20 May, 2022 at 03:11 am	Predatory behavior on ground insects in the leaf litter outside the cave	Several bats flying around, but genet not paying attention.
Video S7	8144 – Inside Boundou Cave	17 January, 2023 at 04:35 am	Predatory behavior on ground insects in the guano litter inside the cave	High abundance of beetles on the ground. Several bats flying around, but genet not paying attention.
Video S8	8378 – Inside Boundou Cave	3 February, 2023 at 03:15 am	Predatory behavior on insects in the guano litter inside the cave	Very dark video.

Figure 37: Putative scavenging behavior of a rusty-spotted genet (*Genetta maculata*) on a bat inside Boundou cave in the Republic of Congo (screenshot of Video S3).



6.5 Discussion

Our study is the first to record sequences of feeding behaviors of genets—namely rusty-spotted genets—on bats through predation and/or scavenging in Africa. We also recorded rodent and insect consumption inside and outside the cave. During each event filmed by the cameras, genets were active during the night period between 03:00 am and 06:00 am. These results are in line with the biology of the species presented by Fuller et al. (1990), with 70% of their activity taking place between 07:00 pm and 07:00 am. It is important to stress that we only detected a few feeding behaviors on bats, rodents and insects. However, it is quite possible that their frequency is underestimated due to camera angle (only 65% of the cave interior was covered by the camera), video quality and detection distance (notably for inside Mont Belo cave, where no genet was detected).

Genets are known to frequently include arthropods in their diet (Kingdon and Hoffmann, 2013; Skinner and Chimimba, 2005; Virgós et al., 1999) and three videos in this study prove this behavior (see Videos S6–S8). However, we observed limited insect consumption despite their constant presence year-round in the cave guano. We can hypothesize that the insect species present at the time were not priority targets or that the genet individuals that came into the cave had an opportunity of catching more nutritious prey such as bats or rodents. Our observations support Palmeirim and Rodrigues (1991), who reported that common genets (*Genetta genetta*) in South Portugal regularly hunt cave-dwelling bats, taking advantage of their roosting behavior.

Our data on the presence of genets in Boundou cave show that they were very active throughout the seasons, with at least two behaviors involving bat consumption and up to two cases of rodent consumption (see Videos S1–S4). In addition, one video showed the hunting behavior of a genet at the entrance of the cave, precisely in the bottleneck where the bats pass to exit and re-enter the cave (see Video S5). The configuration of Boundou cave has three specific features that may both favor and facilitate the consumption of bats by genets: (1) rocky promontories that allow bats to reach significant heights, hence providing opportunities to hunt bats; (2) a narrow rocky neck that allows genets to hunt bats flying through that narrow space; and (3) a high concentration of bats per square meter at certain periods of the year, which forces some individuals to sleep very low on the walls and provides a regular and predictable amount of carcasses, as some bats naturally die

while roosting. On the contrary, Mont Belo cave has very high ceilings and no rocky promontories allowing access to the bat colony, which could be one of the reasons why we didn't observe any genets inside the cave. At Mont Belo, as the servaline genet has not been observed using the cave for feeding, we can hypothesise that it does not seek food in this ecological niche.

One of our videos shows a genet potentially killing a bat on the ground (see Video S4). Normally, cave bats have no reason to be on the cave floor. The majority of bat species spend most of their lives perched upside down, clinging to surfaces using their hindlimbs, which have a digital tendon locking mechanism that does not require any energy expenditure (Quinn & Baumel, 1993; Schutt, 1993). The most likely hypotheses for the presence of a bat on the ground would be due to an injury, the weakness of the individual or an inexperienced young bat that has fallen.

We assumed that at least one identified bat consumption event (see Video S3) is a necrophagic behavior, as we had no prior record of genet hunting activity in the cave for the day of the event. The scavenging behavior of genets on dead bats in the cave raises a number of questions. The mortality of bats in their natural environment can be due to several factors such as (1) collision and/or injury, (2) natural death, (3) predation and (4) diseases due to bacterial, viral and parasitic infection (Gillette & Kimbrough, 1970; Mühldorfer et al., 2011; O'Shea et al., 2016; Wilson, 2015). Knowledge of mortality events and their frequency in cave bat colonies is incomplete. Recent studies have focused on the mortality rate of bats following the introduction of white-nose syndrome fungus into colonies (Hoyt et al., 2021; Langwig et al., 2017), but not on the mortality rate in healthy colonies. It is therefore difficult to estimate mortality rate in the caves in our study, even if the observation of bat carcasses in caves is quite common (MB, pers. obs.).

The scavenging behavior of genets can have two potential impacts on pathogen circulation in the ecosystem. Firstly, by consuming dead animals, many scavengers promote a "healthier" environment by removing the carcasses and thus, limit the spread of potential diseases, especially in communities that live in enclosed spaces (Vicente & VerCauteren 2019). Secondly, it might facilitate the circulation of pathogens in the ecosystem. Indeed, some bat species are known or suspected to be maintenance hosts for pathogens, some of which are a risk for human populations (Brierley et al., 2016; Han et al., 2015; Smith & Wang, 2013). Scavenging could create a pathway for pathogen circulation between the bat

host, to a bridge host, the genet, which then can continue the chain of transmission towards other hosts, including humans. The latter step could be relevant, as genet consumption and the bushmeat trade in Congo and other West and Central African countries are sustained (Angelici et al., 1999; Gaubert et al., 2015; Lindsey et al., 2013). Given that the secretive and nocturnal lifestyle of bats limits direct contacts between bats and humans, the role of potential bridge hosts must be taken into consideration in the ecology of bat-borne diseases. So far bridge hosts have been demonstrated for Hendra virus and the role of horses; the initial emergence of Nipah virus and the role of the pig farm; some outbreaks of Ebola and the role of hunted or scavenged non-human primates; and the emergence of SARS-coronavirus and the presence of masked palm civets (*Paguma larvata*) in wet markets (Murray et al., 1995; Pulliam et al., 2011; Sanchez et al., 1999; Walsh et al., 2007; Wong et al., 2007). More recently, the bridge host role of masked palm civets has also been suspected in the emergence of SARS-COV-2 (Worobey et al., 2022).

In conclusion, our data confirm that rusty-spotted genets are opportunistic carnivores that can feed on a wide variety of prey in a single habitat, ranging from insects to small mammals. In Boundou cave, the wide availability and accessibility of bats seems to favor opportunistic predation behavior on bats. To evaluate whether genets' predatory and even scavenging behavior towards bats is limited or widespread, future studies could similarly make use of camera trap monitoring in other caves and countries, and/or collect genet feces from individuals living in the vicinity of these caves for diet analysis.

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Transition 4 : A la recherche des virus ?

Dans les volets précédents, nous avons caractérisé les communautés de chauves-souris présentes dans la grotte de Boundou et de Mont Belo tout en détaillant leur activité en fonction des saisons. Ensuite, nous avons concentré nos recherches sur les interfaces et les interactions entre ces communautés de chauves-souris et les animaux, dont les hommes. L'acquisition de ces connaissances a pour objectif d'étudier ces mammifères volants à la lumière des données de richesse virale identifiées grâce à la collecte de leur guano. Malheureusement, plusieurs contraintes ont entravé la progression de l'analyse de ces échantillons.

De ce fait, ce volet présente et introduit l'unique **Chapitre 7** qui a pour vocation d'identifier les déterminants écologiques expliquant la richesse des familles virales chez les espèces de chauves-souris africaines en s'appuyant sur des données existantes, extraites de la littérature. Les principaux points importants sont résumés dans la **Box 6**.

Transition 4: Looking for the virus?

In the previous sections, we characterised the bat communities present in the Boundou and Mont Belo caves, while detailing their activity according to the seasons. We then focused our research on the interfaces and interactions between these bat communities and animals, including humans. The aim of acquiring this knowledge was to study those flying mammals in the light of the viral richness data identified through the collection of their bat guano. Unfortunately, several constraints hindered the progress of the analysis of these samples.

As a result, this section presents and introduces the only **Chapter 7**, which aims at identifying the ecological determinants explaining the richness of viral families in African bat species, based on existing data extracted from the literature. The main points are summarised in **Box 6**.

VOLET IV: VIRAL RICHNESS OF AFRICAN SPECIES AND SAMPLING BIAS

Sleek, faery creature,
Strange freak of Nature
That through the twilight comes and goes,
Could we the mystery
Of thy life's history
Resolve, and learn what no man knows,
From what weird forces,
What hidden sources,
Thy winged soul sprang into being
Then might we clearly
Divine more nearly
The world that lies beyond our seeing.

Quaint, mimic angel!
Thy new evangel
Disclose, and share it now with me,
While through the gloaming
Thus lightly roaming,
Thou flittest round this old oak tree;
Tell me what Ages,
What Cosmic stages,
Evolved thy Spirit in the Past;
The far stars glisten,—
Speak, for I listen;
Teach me the Wisdom that thou hast.

Nay, spectral flitter,
Where glow-worms glitter,
Thou art more silent than the sphinx;
Through eras ended
Thou hast descended
Down from the sphere of 'missing links',—

Like pterodactyl
Thy race runs back till
The distance foils our dazed sight,
To prehistoric,
Rude, allegoric,
Brute offspring of the Infinite.

The Past hath vanished,
From memory banished,
What of the Future canst thou tell?
In words aesthetic,
Sage and prophetic,
Our doubting and our fears dispel;
When life is over
Shall Darkness cover
Thy twilight wanderings with the Night,—
Or from Death's portal
Wilt thou immortal
Speed forth into the realm of light?

Mute mystic rover!
Could we discover
Thy wisdom though thou answer'st not,
There is no human,
Or man or woman,
But hath the knowledge thou hast got;
We know we know not!
The gods bestow not
On thee a wider, clearer view;—
Thou art surrounded,
On all sides bounded,
By thine own ignorance,—adieu!


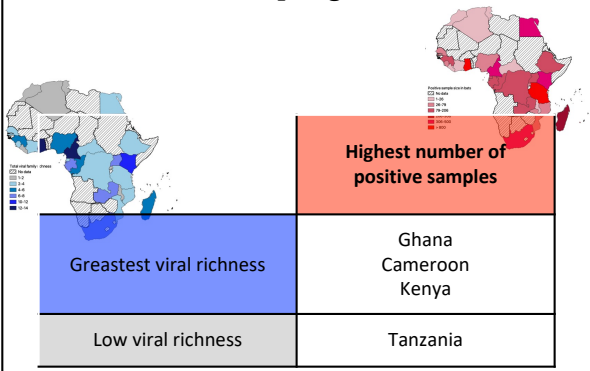

Samuel Waddington

CHAPTER 7 – BIAS IN VIRUS DISCOVERY: VIRAL RICHNESS IN AFRICAN BATS.

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Box 6: Graphic abstract Chapter 7

<p>Main objectives:</p> <p>Using existing data, we investigated and discussed:</p> <ol style="list-style-type: none"> (1) viral richness as a function of sampling size and African countries (2) the number of positive samples detected on a precise time scale (before and after epidemics) (3) ecological factors that explain viral family richness, in 62 African bat species using a PGLS (4) life history traits of bats species that may affect sample size using a structural equation modelling (SEM) 					
<p>Methodology and collect data</p> <p>Using data from:</p> <ol style="list-style-type: none"> (A) DBatVir database (Zhou et al., 2020) (B) PREDICT project (PREDICT Consortium., 2021) <p style="text-align: center;">  6,245 samples of bats </p> <p>Observation divided in 3 sub-groups:</p> <ol style="list-style-type: none"> i) 102 species (individual with species identification) ii) 86 species (spe. iden. + more than one ind.) iii) 62 species (spe. iden. + at least 5 ind.) 	<p>Viral richness - sampling size and locations</p>  <table border="1" style="margin-left: auto; margin-right: auto;"> <tr> <td style="background-color: #f08080; color: white; text-align: center;">Highest number of positive samples</td> </tr> <tr> <td style="text-align: center;">Ghana Cameroon Kenya</td> </tr> <tr> <td style="background-color: #d3d3d3; text-align: center;">Low viral richness</td> </tr> <tr> <td style="text-align: center;">Tanzania</td> </tr> </table>	Highest number of positive samples	Ghana Cameroon Kenya	Low viral richness	Tanzania
Highest number of positive samples					
Ghana Cameroon Kenya					
Low viral richness					
Tanzania					
<p>Detection positive sample on bats</p> <p>Focus on Coronavirus:</p> <ul style="list-style-type: none"> • Following MERS in 2012, gradual increase in positive coronavirus detection (peak in 2018). • Sawtooth variation between 2019 and 2022 <p>Focus on Filovirus:</p> <ul style="list-style-type: none"> • Peak of positive sample are greater during/after a specific event (e. g. sharp increase detection of filovirus sample in Pteropodidae) 	<p>Determinant of viral family richness - PGLS</p> <ul style="list-style-type: none"> • A higher viral family richness was associated with a larger sample size (PGLS 62 species: $P < 0.05$) • The migratory or nomadic behaviour of bats was closed to have a significant effect on the viral family richness detected (PGLS 62 species: $P = 0.05$) • On a larger scale (greater number of species, PGLS sub files 102 and 86 species, Appendix 6) factors such as the distribution area, average size of roosting colony and migration behaviour also have an effect on viral family richness 				
<p>Factor influencing bat sampling effort</p> <ul style="list-style-type: none"> • Distribution area and size of colony showed positive significant effects on bat sample size <p style="text-align: center;">  </p> <ul style="list-style-type: none"> • A bat species living in a large colony with a large distribution range had more chance to be sampled extensively 	<p>Discussion</p> <ul style="list-style-type: none"> • The current research strategy seems to maintain blind spots by focusing on knowledge-oriented hypotheses and opportunistic sampling focusing on the most accessible bat populations • This strategy has some logic but should be pursued or changed acknowledging the bias identified here • We suggest that future studies exploring viral richness in bats should take into account this bias. The sampling strategy – which bat species, which sampling method, in which region and in which habitat – must be systematized according to the objectives of the study and the research needs in the field 				
<p>Conclusion:</p> <ul style="list-style-type: none"> • Viral family richness mainly detected by sample size and marginal effect of bat migration behaviour • A sampling bias was observed in virus screening that depended on average size of roosting colony and bat distribution, with a phylogenetic effect. 					

Bias in virus discovery: viral richness in African bats.

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7.1 Abstract

1. Bats have been one of the most epidemiologically investigated taxon for the past 20 years due to multiple bat-borne disease outbreaks. Investigating viral richness in bats remains important to prevent and improve surveillance of emerging zoonotic diseases.
2. Our understanding of viral richness depends on the data obtained from host sampling. We investigated the ecological factors that explain viral richness, here viral family richness, in 62 African bat species using a comparative analysis based on existing data. We explored the life history traits of bats species that may affect sample size using a structural equation modelling (SEM). We also present the viral richness in different African countries as a function of sampling size and African countries. Finally, we look at the number of positive samples detected on a precise time scale (before and after epidemics) for two families of viruses that are heavily sampled in Africa: filoviruses and coronaviruses.
3. We found that viral family richness was mainly explained by sample size. We also observed a marginal effect of bat migration behaviour. A sampling bias was observed in virus screening that depended on average size of roosting colony and bat distribution, with a phylogenetic effect.
4. Synthesis and applications: The current research strategy seems to maintain blind spots by focusing on knowledge-oriented hypotheses and opportunistic sampling focusing on the most accessible bat populations. This strategy has some logic but should be pursued or changed acknowledging the bias identified here. We suggest that future studies exploring viral richness in bats should take into account this bias. The sampling strategy – which bat species, which sampling method, in which region and in which habitat – must be systematized according to the objectives of the study and the research needs in the field. Future epidemics and pandemics originating from bats can be prevented by understanding better the diversity of bats and their ecology, their viral richness and the diversity of interfaces between bats and other animals, including humans.

Keywords: African bats, comparative analysis, life traits, sampling bias, virus diversity

7.2 Introduction

Over the past few decades, human societies have faced a dramatic increase in the incidence of emerging infectious diseases (EIDs) originating from wildlife as the recent global SARS-CoV2 pandemic showcased (Smith et al., 2014). Despite research efforts, many questions remain about the risk factors associated with the spread of EID (Carlson et al., 2021; Jones et al., 2008). However, certain factors appear to play a major role in the spread, persistence and intensity of EIDs, such as (i) the taxonomy and characteristics of the pathogen (e.g. biochemical, evolutionary potential - mutation rate), (ii) the range of hosts it can affect, (iii) and the spatio-temporal structure (host life cycle, host ecology, spatial structure) and (iv) perturbation and socio-economic factors (habitat change and fragmentation, biodiversity loss and climate change) (Baker et al., 2022; Bolzoni et al., 2007; Carlson et al., 2021; Chauhan et al., 2020; Cleaveland et al., 2001; Dobigny & Morand, 2022; Holmes & Drummond, 2007; Plowright et al., 2008; Pulliam & Dushoff, 2009; Roberts et al., 2021; Webb et al., 2007; Woolhouse & Gowtage-Sequeria, 2005).

Another factor with a key impact on preventing zoonotic spillovers is understanding viral richness (i.e., number of viral families detected in each bat species) in wildlife is essential to monitor, prevent and control the emergence of zoonotic diseases with epidemic and pandemic risks (Wolfe et al., 2005). However, our current knowledge of the diversity of viruses in wildlife is patchy. The various constraints on wildlife sampling such as costs, time and access have resulted in studies being concentrated on certain families of zoonotic viruses and with a limited capacity to account for the spatiotemporal dimensions of pathogen dynamics in host populations (Lipkin, 2013). Finally, studies on the viral diversity of different taxa are biased because of the technique used to detect viruses, the sampling location, the species sampled and/or the sampling period (Weber et al., 2023; Wille et al., 2021).

All animals in the animal kingdom host viruses, but viral richness is not uniform across species or orders. Recent studies by Olival et al. (2017) and Shaw et al. (2020) have shown that a greater total number of viruses are present in bats, rodents, primates and ungulates than in any other mammalian order.

Among mammals, bats are the second most diverse taxon after rodents, with more than 1,470 species (Simmons & Cirranello, 2023) spread all over the globe (except the Arctic and

Antarctic). Sociable species, often gregarious and sympatric, the vast majority of which live in colonies of a few individuals up to millions in certain seasons (Markotter et al., 2020; Monadjem et al., 2020). It is the most abundant mammalian taxon (Greenspoon et al., 2023), and known to harbour a high diversity of zoonotic viruses (Luis et al., 2013) with more than 60 zoonotic viruses detected or isolated from blood, excreta, or organs (Beena & Saikumar, 2019). Several bat species are known to be a reservoir of viruses infecting humans, domestic and other wild mammals such as coronaviruses, marburgviruses, henipaviruses and lyssaviruses (Han et al., 2015) and the role of bats in the ecology of other viruses is still unclear (e.g., ebolaviruses, Caron et al. 2019). The importance of bats in EID dynamics is likely related to their life history traits (e.g., long life, flight), social behaviours, and their specific innate immunology derived from a long coevolution with their viruses (Brook & Dobson, 2015; Letko et al., 2020; Wang et al., 2011). In addition, numerous studies have investigated the ecological characteristics that may be associated with viral richness in this taxon, such as gregariousness, migrations, roosting sites, their reproductive status or feeding behaviour (Brook & Dobson, 2015; Gay et al., 2014; Luis et al., 2013, 2015; Maganga et al., 2014; Turmelle & Olival, 2009; Wang et al., 2011) (see **Appendix 34** for more details).

The COVID-19 pandemic has emphasized the importance of a multidisciplinary approach to the management of EIDs and the interest in integrated approaches to health such as the "One Health" (OHHLEP et al., 2022). In order to prevent the spillover of zoonotic viruses and the emergence of epidemics, it is also essential to understand the mechanisms and factors that affect the richness and dynamics of viruses detected in a reservoir population threatening species of interests such as domestic animals or humans. In this study, we focused on the African continent, with its rich conserved wildlife diversity, its extensive natural habitats remaining and growing human populations, it is a hotspot for EIDs of zoonotic origin (Chauhan et al., 2020).

The aim of this study was to assess the determinants of viral richness in African bat species based on existing data. We hypothesized that viral richness in bats is driven by life history traits and environmental factors. However, sampling effort could potentially bias knowledge about bat viral richness. Firstly, we explored the spatial distribution of bat sampling as a function of bat species diversity in Africa. Secondly, we examined the total richness of viral families and

sample sizes for the African countries sampled. Third, we observed the number of positive samples detected for filoviruses and coronaviruses on a specific time scale (before/after outbreaks) in order to detect greater sampling effort. Fourthly, we carried out a comparative analysis of viral richness in bat individuals and species, taking into account the phylogeny of the species considered. Finally, we specifically explored the factors that may have biased the sampling effort.

7.3 Material and method

7.3.1 Identification of the main determinants

We performed a non-extensive literature review of the comparative studies on bat viral diversity and/or richness. The main factors studied and their potential effects on bat viral diversity and/or richness were extracted and presented in **Appendix 34**. We explored search engines such as Google scholar and Scopus for articles that tested factors (determinants) having an effect on viral diversity and/or richness.

7.3.2 Virological data

Data on viruses were obtained from the DBatVir database (Zhou et al., 2020), which allowed us to gather bat species and virus identification in Africa (corresponding to 118 bibliographic references). A second source of information was the PREDICT project (PREDICT Consortium., 2021). Virus detection techniques and the samples collected vary from one publication to another. Depending on the samples collected (e.g., faeces, saliva, blood), the most commonly used techniques for virus detection are antibodies, DNA or RNA testing (Cassedy et al., 2021). These observations were divided into three sub-groups: a first one of 102 species restricted to individual bats having a species identification (**Appendix 35**); a second sub-group of 86 species restricted to bats with species identification and each represented by more than one individual; and a final sub-group of 62 species restricted to bats with species identification and with at least five individuals. Viral family richness was defined as the number of unique viral families in a bat species. In this study, we decided not to present viral diversity. Many studies did not necessarily present the total number of samples analyzed or the negative samples. We have therefore concentrated our analyses on the positive samples.

7.3.3 Ecological data

For each bat species, we extracted the following ecological information from the bibliography (GBIF, 2023; Happold & Happold, 2013; IUCN, 2023; Kleiman et al., 2003; Monadjem et al., 2020, 2021; Zikal, 2020): (1) type of roost (closed or opened), (2) known average size of roosting colonies on three classes (small size with 1 to 15 individuals; medium size with up to 1000 individuals and large size with 1000 individuals to millions of individuals) (Maganga et al.,

2014), (3) aggregation behaviour in the roost in cluster (i.e. the individuals will group together and may even stick to each other) in roosts (Kunz, 2013)) (Yes, No, not known with NA), (4) migration behaviour (Yes for migrating or nomadic individuals, No for non-migrating individuals, NA for species with no information) and (5) IUCN status (Least concern, Near threatened, Endangered, Data deficient, Vulnerable) (Appendix 2). To simplify the analyses, the data was transformed into a numerical factor. We computed the geographical distribution area of each bat species using the maps provided by the IUCN Red List of Threatened Species. The shapefiles of bat geographical distribution were imported in QGIS-LTR freeware (QGIS Development Team, 2023) allowing the aggregation of the polygons of the geographical distributions to compute the distribution area of each species in square kilometres (km²).

7.3.4 Sample size

We grouped the individuals by bat species and calculated the sample size by summing up the total number of virus positive entries into datasets for each bat species.

7.3.5 Bat phylogeny

Phylogenetic trees for each sub-category of our dataset (102 species, 86 species and 62 species) (**Appendix 36**) were created using the “taxize” package (Chamberlain & Szöcs, 2013) implemented in R (R Core Team, 2022) and the ITIS database (ITIS, 2022). The phylogenetic tree is shown in **Appendix 36**.

7.3.6 Map creation

The maps were created with R software using the following packages: `rworldmap` (South, 2011), `tmap` (Tennekes, 2018), `ggspatial` (Dunnington, 2022), `sp` (Bivand et al., 2013), `sf` (Pebesma & Bivand, 2023) and QGIS.

7.3.7 Graphical representation of Filoviruses and Coronavirus detection depending of bat family over time

We have counted the number of viruses in the coronavirus and filovirus families (all detection techniques combined) according to publication years and bat families of interest. We chose two families of viruses known to be intensely studied in Africa. The filovirus family because of the multiple epidemics that have repeatedly affected African human populations, and coronaviruses because of the attention they have received since the emergence of MERS and SARS viruses. We selected bat families known to be related to filovirus (i.e., Molossidae, Pteropodidae) (Koch et al., 2020) and coronavirus (i.e., Hipposideridae, Pteropodidae and Rhinolophidae) (Banerjee et al., 2019).

7.3.8 Statistics Analysis

i. Determinants of viral richness at bat species level

Testing for phylogenetic dependence is important, as traits between related species may be similar due to common ancestry and should not be considered independent in comparative analyses (Felsenstein, 1985). We tested the phylogenetic independence, the phylogenetic signal of species and the phylogenetic correlation of explanatory variables using the following packages: “`phylosignal`” (Keck et al., 2016), “`caper`” (Orme et al., 2018) and “`phytools`” (Revell, 2012) implemented in R.

We checked the distribution of variables using “`fitdistrplus`” package (Delignette-Muller & Dutang, 2015). If necessary, in order to obtain a normal distribution of the residues, the richness of the viral family was log transformed. Stepwise regression was used for its performance when dealing with high-dimensional data containing multiple predictor variables. With the exception of four determinants (longevity, season, age and sex; which was

not mentioned for each study), all the other factors presented in **Table 21** (also detailed in the ecological variables) are tested in the global model. This global model aims to explain viral family richness using a generalized linear model. We selected the most adapted model using stepwise model selection technique and the function “stepAIC” of “Mass” package (Venables & Ripley, 2002). Following the selection of the most parsimonious models, using the AIC criteria (Burnham et al., 2011) and BIC criteria (Schwarz, 1978), we built a phylogenetic generalized linear model (PGLS) using the package “caper” by verifying beforehand that the conditions of applications were well respected. PGLS is a modification of generalized least squares that uses knowledge of phylogenetic relationships to produce an estimate of the expected covariance in the interspecies data (Symonds & Blomberg, 2014). This method was used for the three sub-groups of the dataset (102, 86 and 62 bat species).

Table 21: Comparison of models explaining viral family richness in African bats using phylogenetic generalized linear modelling (PGLS). The initial model included the following variables: sample size (number of hosts sampled for each species); roost type (closed or open); average size of roosting colonies (small, medium or large); IUCN status (Least concern, Near threatened, Vulnerable, Data deficient); distribution area (geographical distribution size); migratory or nomadic (yes or no); aggregation cluster while roosting (yes or no) and bat diet type (insectivore or frugivore). Models are ranked from the most to the least supported according to corrected Akaike information criteria (AIC).

Model	Dependent variables	Best models	AIC	BIC
	Viral family richness	Sample size + Roost type + Migratory nomadic + Distribution area	56.75	63.756
		Sample size + Roost type + Aggregation clusters while roosting + Migratory nomadic + Distribution area	58.244	66.651
		Sample size + Roost type + Aggregation clusters while roosting + Migratory nomadic + Distribution area + Bat diet type	60.188	69.997

ii. Explanation of the sampling effort with different ecological parameters

In order to understand the factors which influence the sampling effort, we performed a structural equation modelling (SEM) using the piecewiseSEM package (Lefcheck, 2016) implemented in R. The SEM combines measurement models (e.g., reliability) with structural models (e.g., regression), thus testing a potential causal chain between sample size and the different factors investigated. In our case, this statistical method allows the study of relationships between sample size and distribution area, IUCN status, roosting behaviour, average size of roosting colony, migration behaviour and taxonomy (eigenvalue_MD).

7.4 Results

7.4.1 Potential determinants of viral richness and/or diversity in bats

The main biological, ecological and environmental traits investigated in comparative studies on bat viral diversity and/or richness were extracted from 24 references (**Appendix 34**). We have deliberately decided not to present the articles in review format and to focus on the major scientific studies that have tested these traits on viral diversity in a comparative manner. Not all the factors presented in **Appendix 34** are necessarily tested in our models in the second part of the analyses due to lack of data.

7.4.2 Selection of dataset

Our dataset contained 6,245 positive samples for 102 bats species out of the 331 bat species listed in Africa by IUCN. Depending on the sample size used (i.e., > 1 , ≥ 5 , ≥ 10 or ≥ 30), we observe a decrease in the number of species. We decided to select a sample with more than 5 individuals per species with 62 bats species in order to test the factors on a representative sample (**Appendix 37**). Our results below focus on the dataset of 62 species, 86 species and 102 species (**Appendix 35** presents the 102 species).

7.4.3 Sampling locations, viral richness and sampling size.

If we disregard the absence of sampling sites for South Africa and Madagascar, we can see that virus-positive samples are mainly found in areas with the highest diversity of bat species (**Fig. 38**). The African countries with the highest number of positive samples (Ghana, Cameroon, Kenya) have the greatest viral richness, with the exception of Tanzania, which has a low viral

richness in bats for a very large sample size. (**Fig. 38**). We also observe that positive samples for viruses of interest mostly occur in areas with the greatest diversity of bat species (**Fig. 38 and Fig. 39, Appendix 38**).

Figure 38: Gradient of species diversity of bats in Africa. The black dots represent positive sampling sites of bats. Data from South Africa and Madagascar are not shown as they were not precisely geolocated.

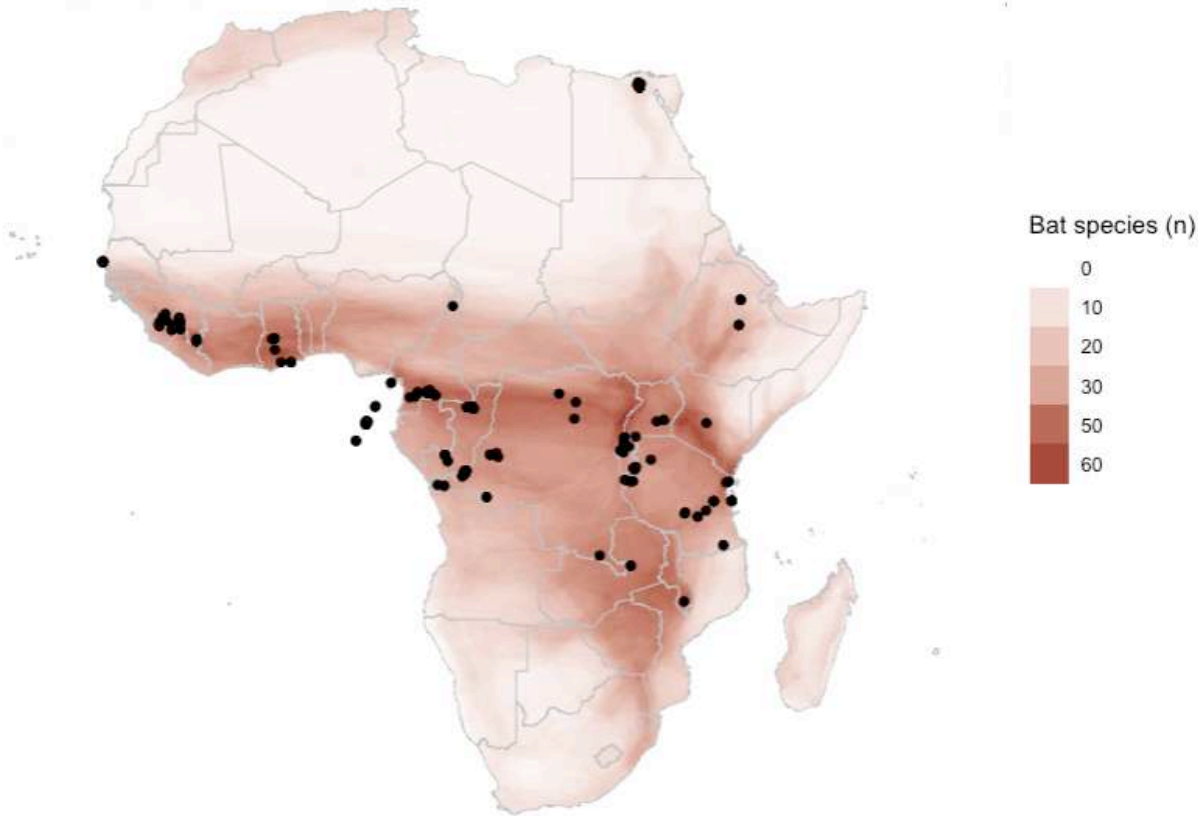
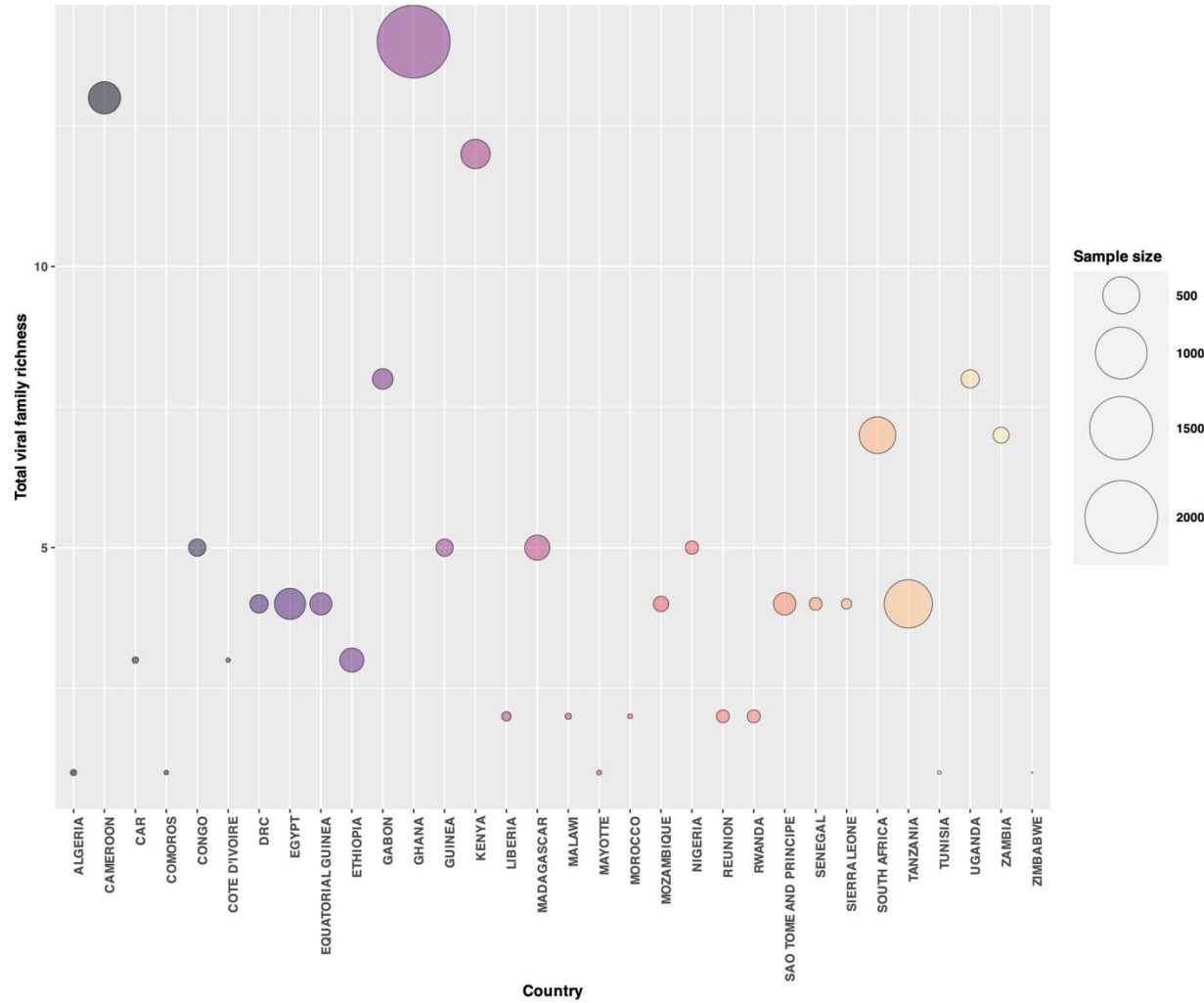


Figure 39: Positive sample size and total viral family richness in datasets of 62 species by African countries (DRC = Democratic Republic of Congo and CAR = Central Africa Republic). The positive sample size is expressed by the size of the dot. All countries not mentioned had no sample.



7.4.4 Coronaviruses and filoviruses by bat families of interest over time

Figure 40 shows that detection peaks of positive samples are greater during or after a particular event. For example, during the Ebola epidemic in West Africa (2014), there was a sharp increase in the detection of positive filovirus samples in the Pteropodidae family (**Fig. 40A**). In 2015, after Marí Saéz et al. (2015) indicated a potential link between filoviruses and the Molossidae family, there was a sharp increase in the detection of positive Molossidae samples in the following publications (**Fig. 40A**).

For coronaviruses, following the emergence of MERS in 2012, there has been a gradual increase in positive coronavirus detections in publications (**Fig. 40B**). The peak of positive coronavirus detections in bat samples occurred in 2018 (**Fig. 40B**). Then, during the COVID-19 pandemic starting in late 2019, a sharp drop in publications of detections of coronavirus-positive samples was followed in 2020 by a rise in detections (**Fig. 40B**). For the years 2021 and 2022, we observe a decrease in detections of positive samples on bats, probably due to the lag between sampling and publications in recent years (**Fig. 40B**).

7.4.5 Determinant of viral family richness

For the 62-species dataset, the three most parsimonious models, according to the AIC and BIC criteria, were presented in **Table 21**. A higher viral family richness was associated with a larger sample size (PGLS 62 species: $P < 0.05$) (**Table 22**). The migratory or nomadic behaviour of bats was closed to have a significant effect on the viral family richness detected (PGLS 62 species: $P = 0.05$) (**Table 22**). On a larger scale (greater number of species, PGLS sub files 102 and 86 species, **Appendix 39**) factors such as the distribution area, average size of roosting colony and migration behaviour have also an effect on viral family richness.

Table 22: Factors explaining viral family richness for the best PGLS models of 62 species of African bats (* significant differences).

Model	Dependant variable	Independent factor	Estimate	Std error	t value	Pr (> t)	F, R ² (P)
Sample size + Roost type + Migratory nomadic + Distribution area							
	Viral family richness	Sample size	<0.001	<0.001	2.356	<0.05 *	
		Roost type open vs closed	-0.338	0.239	-1.405	0.172	F _{4,25} =
		Migratory nomadic yes vs no	-0.492	0.248	1.977	<0.05	4.577,
		Distribution area	<0.001	<0.001	1.644	0.112	0.330 (<0.01)

Figure 40: Timeline of the number of positive samples detected inside different families of bats over time: (A) Timeline for filoviruses in two bat families (Molossidae and Pteropodidae) and (B) Timeline for coronaviruses in three bat families (Hipposideridae, Pteropodidae and Rhinolophidae). Red arrows represent important events.

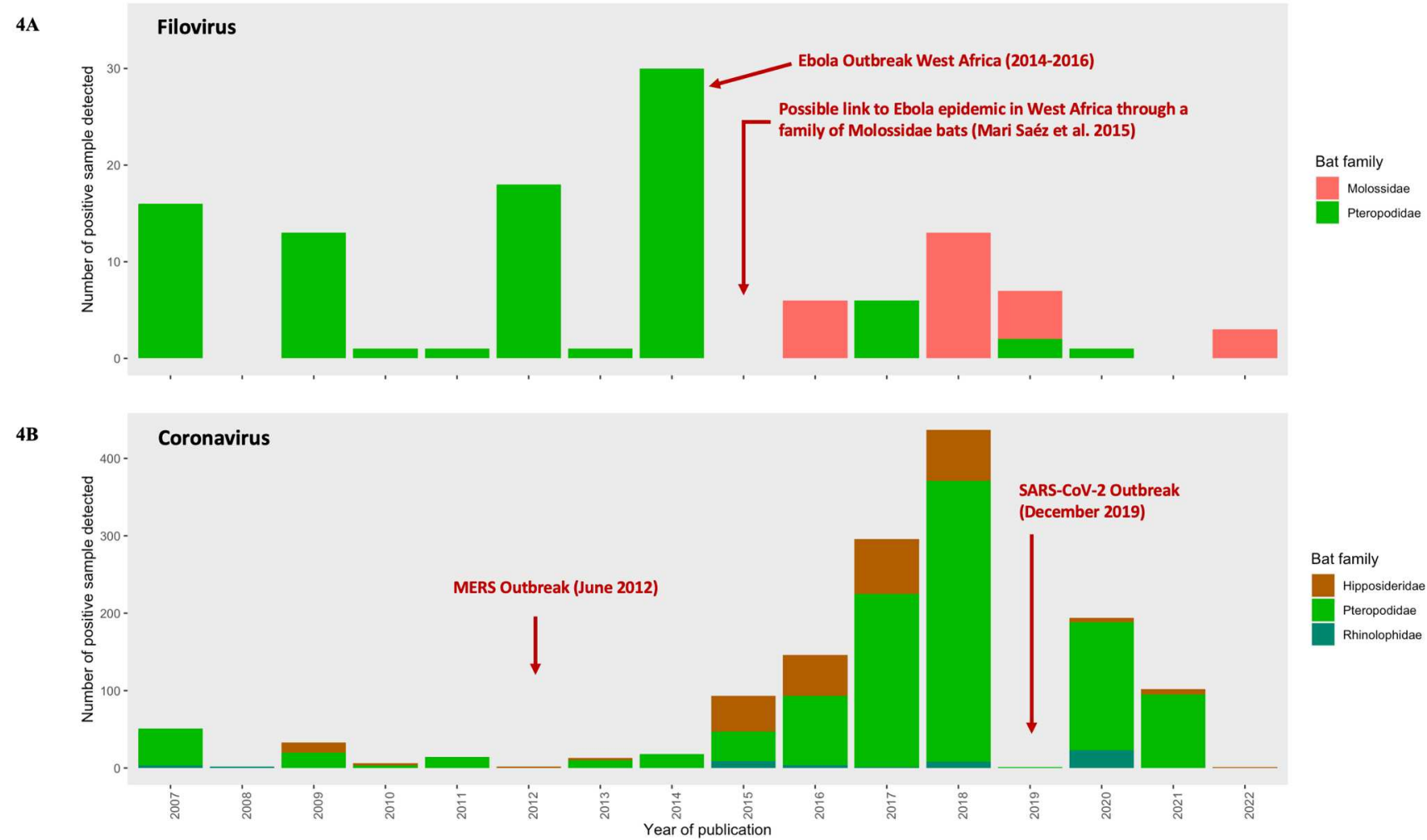
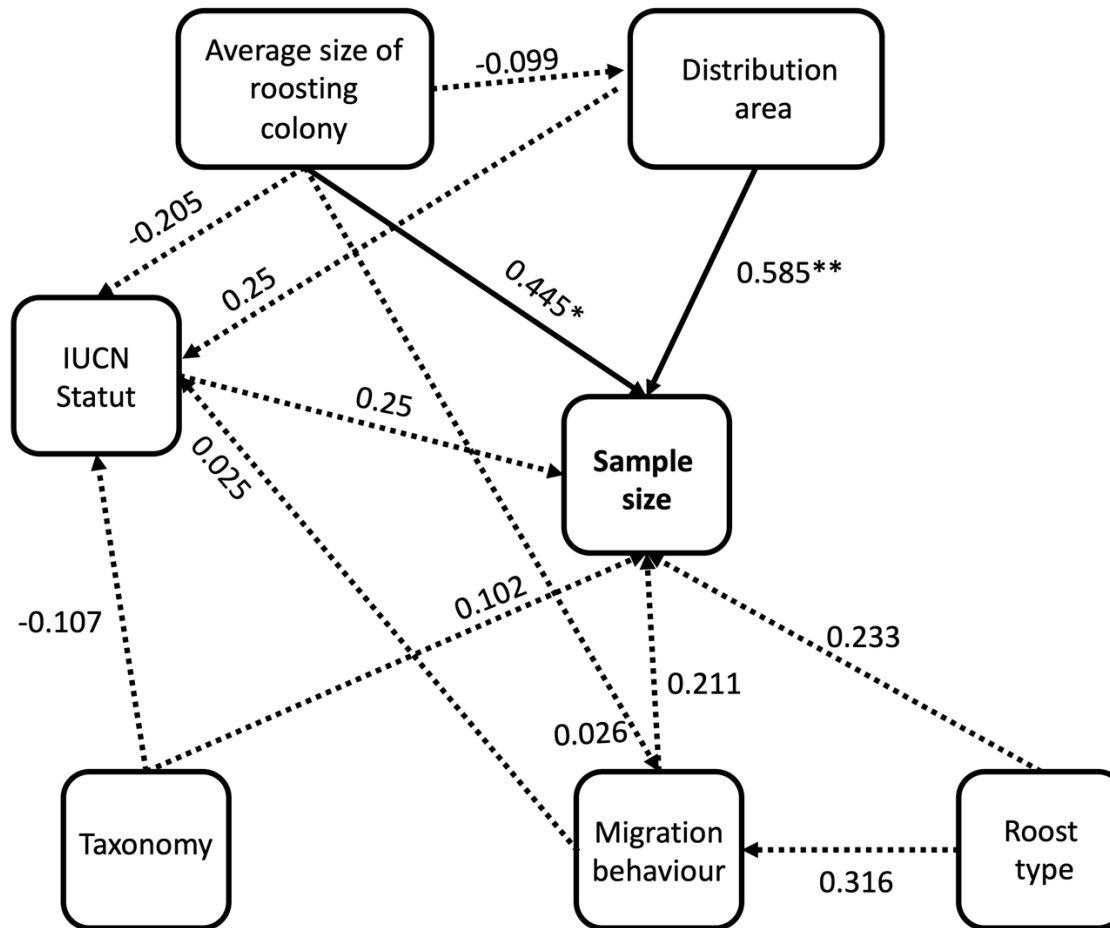


Figure 41: Results of the structural equation modelling (SEM) investigating the relationships between sample size and roost type, taxonomy (using the eigenvalues of the phylogenetic matrix distance), IUCN status, distribution area, migration behaviour and average size of roosting colony of 62 African bats (datasets of 62 species). Significant partial correlations are presented in continuous lines with values of standardized estimates (** = P value <0.01 and * = P value <0.05) and non-significant partial correlations are presented in dashed lines).



7.4.6 Factors influencing bat sample size

Distribution area and size of colony showed positive significant effects on bat sample size. A bat species living in a large colony with a large distribution range had more chance to be sampled extensively (**Fig. 41**).

7.4.7 Correlation of the trait with bat phylogeny

Variations in phylogenetic autocorrelation can be observed depending on ecological traits and species clades. The details and analyses are presented in the appendices (**Appendices 40, 41, 42 and 43**, as phylogenetic correlation is already largely covered by the PGLS).

7.5 Discussion

Due to their recognized or suspected roles in EIDs, bats have been extensively sampled in the last decades. Sampling wildlife is tedious, especially nocturnal animals such as bats, and logistical and field constraints can bias the information collected. The aim of this study was to access the determinants of viral richness in African bat species based on existing data. However, our study was unable to answer this question due to the biases present in the data. Our study identified three types of sampling bias: (1) a geographical bias, (2) a bias in the selection of bat families and species based on scientific interest, and (3) a bias due to the bats' life-traits. The shared viral data is also biased due to non-reporting of negative results, sample size and host ecological information. Scientists base their knowledge and conceptual models on snapshots taken at a given time at a given place, overseeing potential bias in the sampling design or representativeness. The problem of bias in bat viral sampling has been mentioned in numerous studies working on specific viral families in Africa (coronavirus research (Cohen et al., 2023); ebolavirus research (Han et al., 2016)) or on the viral diversity of bats throughout the world (Young & Olival, 2016). Cohen et al (2023) even demonstrated that the prevalence of coronaviruses is biased by the type and methodology used for sampling. The study of Weber et al (2023) confirms our results on sampling bias by showing that sampling of African bats is restricted to certain countries. Furthermore, in many virological studies, sampling is reduced to one specific species to the detriment of others (e.g. *R aegyptiacus* and *E. helvum* present in 46 of the 52 studies in their review of virological studies in Africa) (Weber et al., 2023).

In addition, our result showed an effect of the migratory or nomadic behaviour of bats on the viral richness of bat species (**Table 22**, $P = 0.05$). Several studies (Luis et al., 2015) have demonstrated the importance of bat migration behaviour, which favours the maintenance and transmission of viruses. Some bat species can travel long distances during migration (up to 1000 km for *Eidolon helvum* (Richter & Cumming, 2008)), which allows the geographical expansion of viruses and promote intra- and inter-species contacts (Letko et al., 2020). Interspecies transmission between bats and birds was suggested by Drexler et al. (2012) as bats host the paramyxovirus ancestors of all those of non-flying mammals and birds. In

contrast, other scientific studies emphasize the importance of migration behaviour as a mechanism for reducing or avoiding infections (Binning et al., 2022).

Our model for the 62 species does not indicate any significant effect of roost type or distribution area on viral family richness. In contrary, the study by Furey and Racey (2016) suggests that closed roosts, such as caves, will favour viral richness because they define specific ecological niches. Close roosts provide protection from climatic variations, allow breeding and serve as maternity colonies for many bat species and define good conditions for virus maintenance and evolution. Bats with a wide distribution area will be more prone to intra- and interspecies interactions, thus favoring viral richness (Gay et al., 2014). Finally, our study suggests that the viral richness of African bats is driven by host ecology as well as their phylogenetic relationships validating the conclusions of other studies on viral diversity (Guy et al., 2020).

Virus-positive samples on the African continent come mostly from countries which are home to the greatest diversity of bat species (**Fig. 38** and **Fig.39**). Research teams concentrated their efforts in areas of high biodiversity and/or close to human-wildlife interfaces. This bias is also related to the locations of epidemics of EIDs that attracted the main research effort to explore the origin and maintenance mechanism of virus ecology.

In other parts of the world, such as South America and Asia, the same trend can be observed (Tian et al., 2022).

Results from the PGLS and SEM analyses demonstrate the bias in sampling effort according to bat species (**Table 22, Fig. 41 and Appendix 39**). The SEM analysis also highlights two bat life traits that appear to have an impact on sampling effort, namely average size of roosting colony and bat distribution area (**Fig. 41**). For example, a frugivorous species such as Egyptian fruit bat (*Rousettus aegyptiacus*) is heavily sampled because of its gregarious behaviour in caves or tree, often in or near urban areas, living in large colonies over a large home range. In Central and West African ecosystems, this species is almost ubiquitous and the easiest to catch due to the life history traits presented above, in large groups in relatively accessible roosts close to human settlements. In addition, this species is intensively sampled because of its known link to virus families of interest, such as filoviruses (Towner et al., 2009). In contrast, insectivorous bat species such as Glen's long-fingered bat (*Miniopterus gleni*) or Griveaud's long-fingered bat (*Miniopterus griveaudi*), that are endemic to a specific country and carry leptospirosis or other

viruses of interest to human health (Hoarau et al., 2021; Lagadec et al., 2012), are much less studied, especially if their habitat is not easily accessible. Due to the difficulty of capturing some bat species (living in very small groups or hunting in high altitude) or rare species (i.e. Data deficient), viral sample collection is deficient for a 32.5% of bats. This sampling bias towards bat or virus? Families or species of interest to the scientific community is even apparent when focusing on the number of positive detections over time on filoviruses and coronaviruses as a function of the year of publication (**Fig. 40**). For example, in 2015, when Mari Saéz et al. (2015) discovered the potential link between the Molossidae family and the emergence of Ebola in West Africa (2014 epidemic outbreak), the following year saw a sharp increase in publications with positive detections of individuals from this family (**Fig. 40**).

Our efforts to identify the ecological determinants of bat viral family richness have been hampered by the lack of information on many bat species. According to IUCN, around 18 % of the 1,333 bat species recorded are considered data deficient in contrast to 0.38% for birds (Welch & Beaulieu, 2018). Knowledge on bat biology, behaviour and social ecology is deficient, especially for roost type, migration, average size of roosting colonies, home range, longevity, physiology and potential co-occurrence with other species. Besides, integrating potential changes in bat behaviour due to climate change or habitat loss is difficult (Chen et al., 2011) . Lastly, many African countries do not have a comprehensive inventory of their bat species. Filling these gaps in knowledge could be important to better understand viral richness in less known bat species.

Our study deliberately selected only positive samples in order to calculate viral family richness. Establishing a prevalence for each species was often impossible due to the fact that many studies do not publish the number of samples analyzed, negatives and/or positives samples. We also had to contend with small sample sizes for some viruses and species (< 3 samples). The aim of our study was to understand the effect of sample size on the viral richness of bat species. On the other hand, viral prevalence varies between viruses (e. g : prevalence Influenza viruses; Chauhan & Gordon, 2020) , so it may be acceptable to ignore sample size.

In addition, some families of viruses, such as coronaviruses, are very common and easy to detect in semi-invasive samples such as swabs and feces, unlike other families of viruses, such as filoviruses. Thus, the viral richness of certain common viral families (e.g., coronaviruses) is

difficult to compare with the viral richness of rarer families (e.g. filovirus). However, in this study, the coronavirus viral richness is much higher than filovirus.

We could analyze the determinants of viral family richness in only 62 African bat species, less than a third of African bat species (316 species). Our initial database contained 102 bat species with 40 species with less than five virus detection. If we focus on a larger number of species (PGLS subfiles 102 and 86 species, **Appendix 39**), factors such as the distribution range, average resting colony size and migratory behaviour also have an effect on viral family richness. However, to perform this type of analysis, a more homogeneous collection of sample sizes for each bat species, set at an analytical threshold, is required. Currently, resources are more allocated towards viral identification in samples and not enough towards bat species identification for each sample. Bat identification, often only possible through genetic analysis performed on samples (as morphology cannot identify between many species), should therefore be generalized.

The current viral screening strategy which focuses on the most accessible and abundant bats creates a bias in virus discovery. Our study distinguishes itself from other studies on this topic by clearly identifying a sampling bias, which is interconnected with the phylogeny and life history of African bat species. Variation in bat sampling can be explained by (1) the accessibility of the species and its distribution area, (2) knowledge about the species, (3) the roosting behaviour, (4) the abundance of the species, (5) the interest of researchers for this species (often based on their known or suspected role in virus ecology) and (6) the taxonomic group. However, the transmission and spillover of zoonotic diseases are the result of complex multi-factorial mechanisms. It is essential to work on a fine scale to identify more precisely the interlinkages of determinants that may play a role on viral dynamics.

The multiple biases identified in our study question the research strategy targeted at defining viral richness in bat species in Africa, and beyond. Is the current strategy focusing on bat species known to be exposed to important viruses, relatively easier to catch and in specific ecosystems and regions, the best to prevent viral epidemics and pandemic threat originating in bats? The available data is biased towards a small fraction of African bat species, in a few regions (e.g., mainly West and Central Africa) and often in places where the human/bat interfaces are thought to favour risk of pathogen spillovers. It is beyond the scope of this article

to provide guidelines to change or not this strategy as studies differ in their objectives and the diversity of studies should probably address the different sampling biases identified. However, thus biases should be taken into consideration when designing viral studies in African bats. Different approaches will answer different research needs: i) a better understanding of viral richness in the least known bat species that may harbour an unknown viral diversity, in already well-explored African regions but also in other regions hosting different bat communities (e.g., some parts of southern Africa); ii) continuing sampling the most accessible bat species for which hypotheses relative to maintenance of specific viral families exist, in order to better understand the ecology of pathogens in host; iii) focusing on the processes of viral spillover at bat/human and bat/non-human interfaces by using sampling methodologies that aim to characterize viral threat at the forefront of the species jump. These different strategies are not exclusive but the research effort should try to encompass all of them. Finally, as repeatedly requested, relevant ecological data and reports of negative results are of paramount importance to increase our knowledge of epidemic and pandemic threats of bat-borne diseases.

7.6 Authors' contributions

ML and SM conceived the ideas and designed methodology; ML collected the data; ML and SM analysed the data; ML, SM, AC, HDN, wrote the first draft manuscript. ML, SM, AC, HDN and MB contributed critically to the final version.

7.7 Acknowledgements

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7.8 Conflict of interest statement

The authors declare that they have no known competing interests or personal relationships that could have appeared to influence the work reported in this paper.

7.9 Appendix – Chapter 7

Appendix 34: Biological, ecological and environmental factors that may influence viral diversity in bats (+ = positive effect on viral diversity; □ = positive or negative effect on viral diversity depending on study).

Factor	Predicted effect on viral diversity and richness	Hypothesis advanced	References
Average size of roosting colony	□	Highly dense populations of bats increase contact between individuals (social behaviour), increasing viral persistence and transmission	(Luis et al., 2015; Webber et al., 2017; Willoughby et al., 2017)
Age structure	□	Juveniles play a key role in the viral dynamics (especially during the period when they are no longer protected by the maternal immune system). Adults, because of their longevity, can have a very efficient immune system but host many viruses.	(Anthony et al., 2017; Bergner et al., 2020; Turmelle & Olival, 2009)
Sex	□	Males are more likely to be infected due to their movement behaviour during the breeding season. Testosterone level may promote infection. Difference in gregarious behaviour according to sex. Females may congregate in maternity colonies, which may promote transmission and infection.	(Sjodin et al., 2020; Tidière et al., 2020)
IUCN Status and population genetic structure	□ higher viral richness in bat species with endangered IUCN status	Threatened bats are increasingly rare in their habitat. The likelihood of interaction between humans and bats is lower, unless the species lives in an anthropogenic environment or its interactions with humans are significant. Species that are endangered have their individuals subjected to anthropogenic disturbances that promote stress in individuals and favour viral infection.	(Johnson et al., 2020; Turmelle & Olival, 2009)
Season	□ Viral population dynamics vary seasonally	Bat populations vary over time in a given habitat (social behaviour, availability of resources). Gregarious behaviour varies according to season and gender.	(Bergner et al., 2020; Turmelle & Olival, 2009)

Factor	Predicted effect on viral diversity and richness	Hypothesis advanced	References
Sample size	<input type="checkbox"/> Some species are sampled more than others	Several factors such a scientific interest, accessibility, or habitat can play a part in the sampling effort	(Geldenhuys et al., 2021; Turmelle & Olival, 2009)
Roost type	<input type="checkbox"/> depending of the type of roost (caves, tree, ...)	Contact between individuals, secondary hosts and humans depends on the type of roost. “Closed” roosts such as caves may favour inter- and intra-species viral transmission. Bat maternity colonies can act as viral amplifiers.	(Amman et al., 2012; Dietrich et al., 2015; Luis et al., 2015; Mortlock et al., 2019; Plowright et al., 2008)
Distribution area	<input type="checkbox"/> Large distribution area favours high viral richness.	Individuals living over a large area of distribution will have a greater chance of multiplying their interactions with other species.	(Gay et al., 2014; Maganga et al., 2014)
Migration- nomadic	<input type="checkbox"/> Migration, nomadic or sedentary behaviour	The migratory and nomadic behaviour of bats, particularly those which are gregarious, can increase intra- and interspecies contacts and connect distant viruses with new populations (geographical expansion of viruses). However, the migration of individuals to climates hostile to the pathogen may contribute to its decline.	(Hayes & Piaggio, 2018; Luis et al., 2015; Markotter et al., 2020; Sherwin et al., 2013)
Diet type	<input type="checkbox"/> Viral richness is more important in frugivorous bats than other bat species with a different diet.	Some species of fruit bats may move closer to agricultural land or near urban areas in search of food. Bat diet is strongly correlated with bat phylogeny. But it may also point to another factor that is strongly correlated with diet, such as the immunological functions of these species	(Kessler et al., 2018; Luis et al., 2013, 2015; Schloesing et al., 2020; Willoughby et al., 2017)
Longevity	<input type="checkbox"/>	Long-living animals are more likely to accumulate parasites and to host high viral diversity. Multiple transmission events across communities are possible with a prolonged survival.	(Guy et al., 2020; Olival et al., 2017)

References for Appendix 34

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Appendix 35: 102 species of African bats and variables tested as potential determinants. Each specie has the following variables: sample size (log of number of hosts sampled for each species); roost type (1: closed or 2 : open); average size of roosting colonies (1: small, 2: medium or 3: large); IUCN status (1: Least concern, 2: Near threatened, 3: Vulnerable, 4: Data deficient); distribution area (geographical distribution range size in km²); migratory or nomadic (1: no and 2: yes); aggregation cluster while roosting (1: no and 2: yes) and bats diet type (1: insectivore or 2: frugivore).

Scientific Name	Sample size	Viral family richness	Viral family diversity	Colony size	Roost type	Aggregation clusters while roosting	Migratory nomadic	IUCN Status	Distribution area	Diet type
<i>Cardioderma cor</i>	4	3	1.61	2	1	1	2	1	2244479	1
<i>Chaerephon aloysiisabaudiae</i>	1	1	0.64	1	1			1	1116930	1
<i>Chaerephon leucogaster</i>	9	3	1.75	2	1	1		1	181997	1
<i>Chaerephon pumilus</i>	97	5	1.34	2	1	1	1	1	6859917	1
<i>Chaerephon pusillus</i>	6	1	0.69	1	1	1	2	4		1
<i>Cloeotis percivali</i>	1	1	0.41	2	1	1		1	528449	1
<i>Coleura afra</i>	18	5	1.64	2	1	2	2	1	2792269	1
<i>Coleura kibomalandy</i>	2	1	0.50		1			4	1480	1
<i>Doryrhina cyclops</i>	15	2	0.81	1	1	1	1	1	2322616	1
<i>Eidolon dupreanum</i>	13	4	1.27	1	1	1	2	3	458199	2
<i>Eidolon helvum</i>	3062	17	2.25	3	2	2	2	2	11708770	2
<i>Epomophorus gambianus</i>	162	4	0.95	2	2	1	2	1	3788360	2
<i>Epomophorus labiatus</i>	5	2	0.85	2	2	1	2	1	1765273	2
<i>Epomophorus pusillus</i>	60	5	1.78	1	2	1	2	1	5374840	2
<i>Epomophorus wahlbergi</i>	21	1	1.76	1	2	1	2	1	4994448	2
<i>Epomops buettikoferi</i>	2	1	0.35	1	2	1		1	1076554	2
<i>Epomops franqueti</i>	42	4	1.71	1	2	1	2	1	4502941	2
<i>Eptesicus hottentotus</i>	2	1	0.33	1	1	2		1	2853169	1
<i>Eptesicus isabellinus</i>	1	1	0.20	2	1		1	1	672741	1
<i>Glauconycteris poensis</i>	3	1	0.47	1	1	2		1	1524870	1
<i>Glauconycteris variegata</i>	7	2	0.85	1	1	2		1	8690762	1
<i>Hipposideros abae</i>	14	3	0.92	2	1	1		1	2323198	1

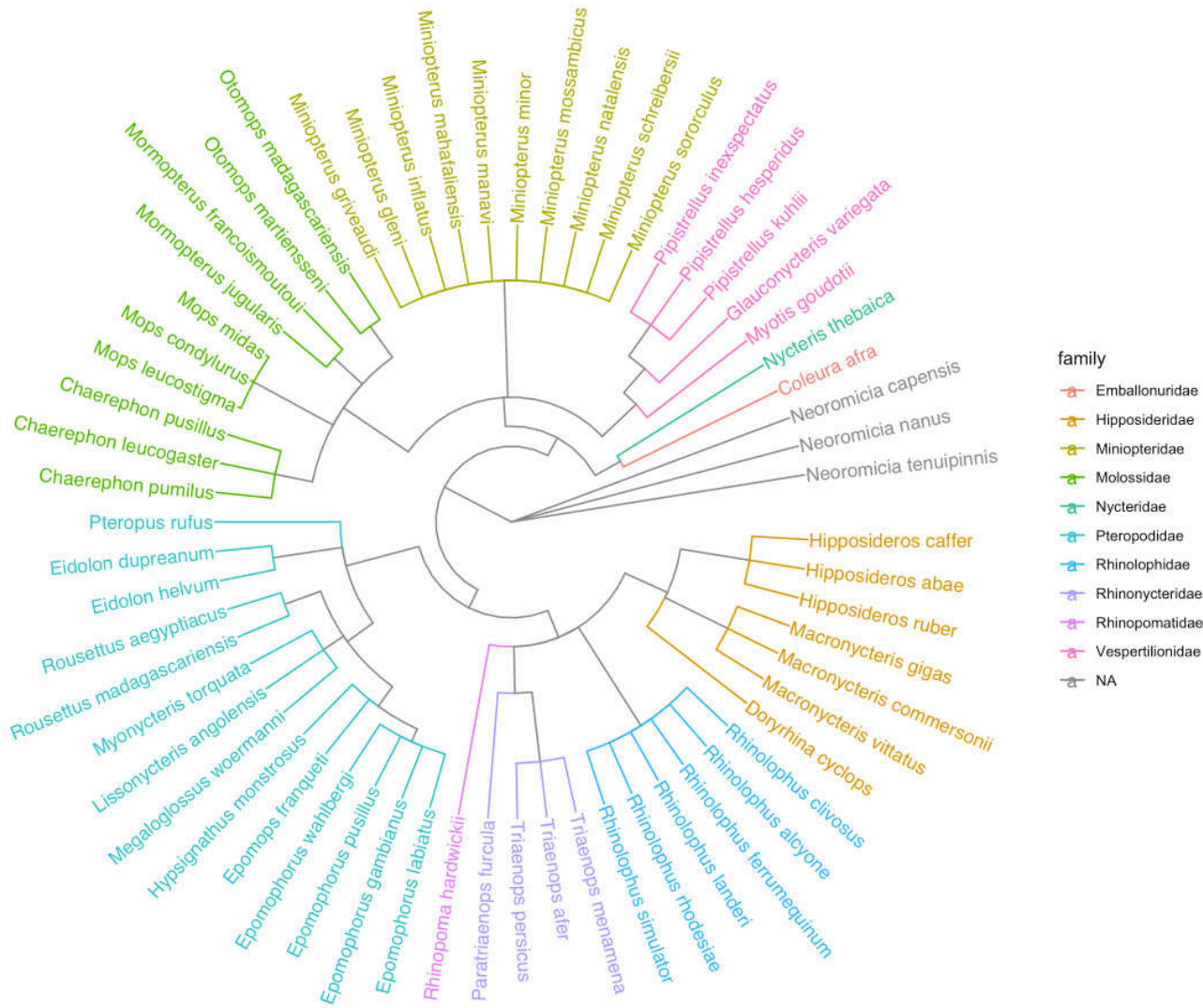
Scientific Name	Sample size	Viral family richness	Viral family diversity	Colony size	Roost type	Aggregation clusters while roosting	Migratory nomadic	IUCN Status	Distribution area	Diet type
<i>Hipposideros caffer</i>	87	7	2.51	3	1	1	2	1	1264379	1
<i>Hipposideros fuliginosus</i>	4	2	0.49	2	2	1		1	1332632	1
<i>Hipposideros jonesi</i>	1	1	0.16	2	1	1		2	1271016	1
<i>Hipposideros ruber</i>	256	5	2.76	3	1	1	1	1	9054875	1
<i>Hypsignathus monstrosus</i>	10	3	0.91	2	2	1	2	1	2854109	2
<i>Hypsugo musciculus</i>	1	1	0.15	1	1			4	606537	1
<i>Kerivoula argentata</i>	2	1	0.24	1	1	1		1	2952033	1
<i>Lissonycteris angolensis</i>	17	4	1.45	2	1	1		1	9075175	2
<i>Macronycteris commersonii</i>	11	4	1.06	3	1	1	2	2	364187	1
<i>Macronycteris gigas</i>	82	8	2.27	2	1	1	2	1	3572324	1
<i>Macronycteris vittatus</i>	9	2	0.70	2	1	2	2	2	4001648	1
<i>Megaloglossus woermanni</i>	39	4	1.55	1	2	1	2	1	3114671	2
<i>Miniopterus africanus</i>	3	3	0.36	1	1			4	2769838	1
<i>Miniopterus ambohitrensis</i>	1	1	0.12		1	2	2	1	1807	1
<i>Miniopterus gleni</i>	13	2	0.84	2	1	2		4	416347	1
<i>Miniopterus griveaudi</i>	35	2	1.52	2	1	2		1	929	1
<i>Miniopterus inflatus</i>	27	3	1.14	2	1	2	2	1	2348320	1
<i>Miniopterus mahafaliensis</i>	8	2	0.56	3	1			4	106932	1
<i>Miniopterus manavi</i>	14	2	0.74	3	1			1	509807	1
<i>Miniopterus minor</i>	19	3	0.84	3	1	2		1	139376	1
<i>Miniopterus mossambicus</i>	5	2	0.39	3	1	2	2	4		1
<i>Miniopterus natalensis</i>	121	6	1.90	3	1	2		1	3573828	1
<i>Miniopterus nimbae</i>	2	1	0.18	2	1			1		1
<i>Miniopterus schreibersii</i>	5	2	0.48	3	1	2	2	3	273162	1
<i>Miniopterus sororculus</i>	6	2	0.49	3	1	2	1	1	91203	1
<i>Mops condylurus</i>	311	8	1.77	2	1	2	1	1	9101058	1

Scientific Name	Sample size	Viral family richness	Viral family diversity	Colony size	Roost type	Aggregation clusters while roosting	Migratory nomadic	IUCN Status	Distribution area	Diet type
<i>Mops leucostigma</i>	13	2	0.78	2	1	1		1	212541	1
<i>Mops midas</i>	15	2	0.79	2	1	1		1	408475	1
<i>Mormopterus francoismoutoui</i>	53	2	1.54	3	1	2		1	2529	1
<i>Mormopterus jugularis</i>	15	2	0.82	2	1	1	1	1	234977	1
<i>Myonycteris torquata</i>	5	2	0.35	1	2	1	2	1	3647451	2
<i>Myotis goudotii</i>	8	2	0.55	1	1	1		1	502944	1
<i>Myotis punicus</i>	2	1	0.15	3	1	2	1	4	615972	1
<i>Myotis welwitschii</i>	3	1	0.23	1	2	1		1	4309448	1
<i>Nanonycteris veldkampii</i>	1	1	0.09	1	1	1	2	1	1707193	2
<i>Neoromicia capensis</i>	117	4	1.95	1	1			1	13734445	1
<i>Neoromicia helios</i>	3	2	0.25	1	1	1		4	585462	1
<i>Neoromicia nanus</i>	10	5	0.70	1	2	1	1	1	10431312	1
<i>Neoromicia somalica</i>	1	1	0.08	1	2	1		1	6068712	1
<i>Neoromicia tenuipinnis</i>	5	1	0.27	1	1			1	4238416	1
<i>Nycteris grandis</i>	1	1	0.08	1	1	1	1	1	4396522	1
<i>Nycteris hispida</i>	2	2	0.16	1	1	1		1	11497271	1
<i>Nycteris macrotis</i>	1	1	0.08	2	1	1		1	12079013	1
<i>Nycteris nana</i>	1	1	0.08	1	1	1		1	2934049	1
<i>Nycteris thebaica</i>	23	4	1.07	2	1	1		1	14165541	1
<i>Otomops harrisoni</i>	3	1	0.18	2	1	2	2	3	965793	1
<i>Otomops madagascariensis</i>	7	1	0.38	2	1	1		1	3539	1
<i>Otomops martiensseni</i>	53	5	1.53	2	1	2		2	5185684	1
<i>Paratriaenops furcula</i>	14	2	0.65		1	1		1	56529	1
<i>Pipistrellus hesperidus</i>	7	1	0.47	1	1	2		1	1600082	1
<i>Pipistrellus inexpectatus</i>	14	1	0.44	2	1			4	696309	1
<i>Pipistrellus kuhlii</i>	16	2	0.55	2	1		1	1	1335718	1

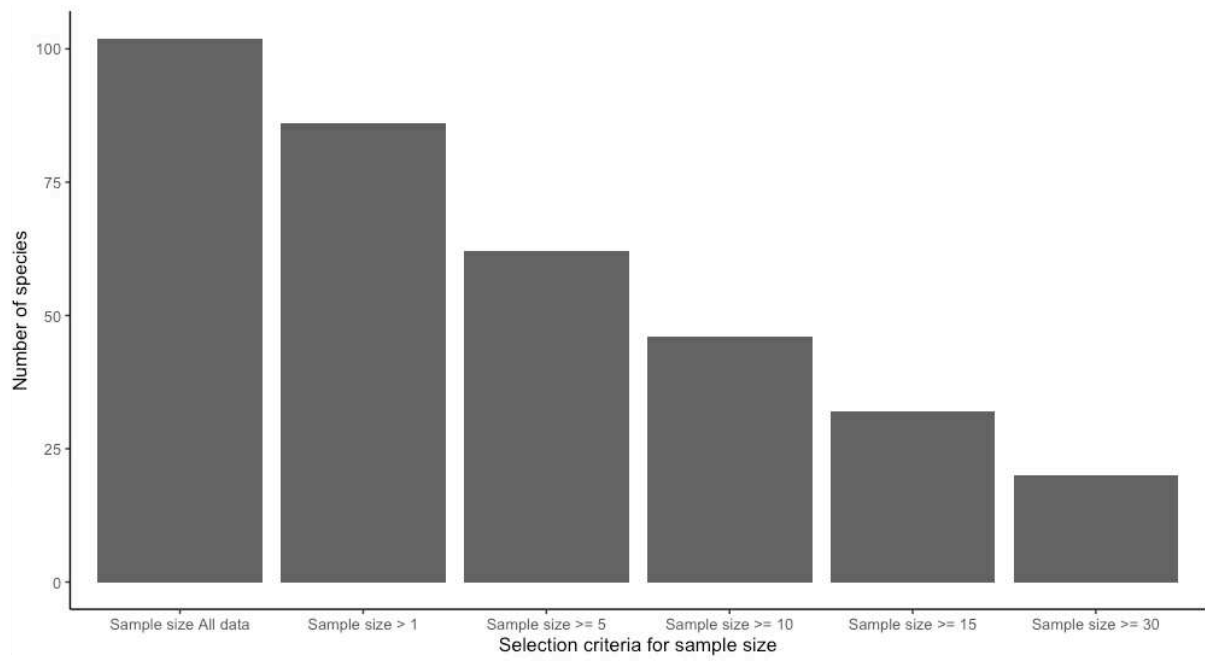
Scientific Name	Sample size	Viral family richness	Viral family diversity	Colony size	Roost type	Aggregation clusters while roosting	Migratory nomadic	IUCN Status	Distribution area	Diet type
<i>Pipistrellus nanulus</i>	1	1	0.07	2	1			1	1561171	1
<i>Pteropus rufus</i>	25	2	1.07	3	2	1	1	3	185389	2
<i>Rhinolophus alcyone</i>	12	3	0.58	1	1	1		1	2855031	1
<i>Rhinolophus blasii</i>	2	1	0.12	1	1	2	1	1	1108292	1
<i>Rhinolophus capensis</i>	1	1	0.07	2	1	2	1	1	241740	1
<i>Rhinolophus clivosus</i>	14	1	0.67	2	1	1		1	2856206	1
<i>Rhinolophus darlingi</i>	2	1	0.11	1	1	1		1	1359977	1
<i>Rhinolophus denti</i>	4	1	0.19	1	1	2		1	1703320	1
<i>Rhinolophus eloquens</i>	1	1	0.06					1	711334	1
<i>Rhinolophus euryale</i>	2	2	0.13	2	1	2	2	2	516639	1
<i>Rhinolophus ferrumequinum</i>	14	1	0.41	2	1	2	1	1	175208	1
<i>Rhinolophus fumigatus</i>	3	1	0.17	2	1	2		1	8361213	1
<i>Rhinolophus hildebrandtii</i>	3	2	0.18	2	1	2		1	3184054	1
<i>Rhinolophus landeri</i>	7	5	0.37	2	1	2	1	1	12111498	1
<i>Rhinolophus lobatus</i>	4	1	0.18	1	1	2		4		1
<i>Rhinolophus rhodesiae</i>	10	2	0.36	1	1	2		1		1
<i>Rhinolophus simulator</i>	19	4	0.73	2	1	2		1	1910941	1
<i>Rhinolophus smithersi</i>	2	2	0.12	1	1			2	81468	1
<i>Rhinopoma hardwickii</i>	158	1	0.68	2	1	1	1	1	11686644	1
<i>Rousettus aegyptiacus</i>	800	13	2.64	3	1	2	2	1	3482628	2
<i>Rousettus madagascariensis</i>	9	2	0.42	2	1	2	1	3	290933	2
<i>Scotophilus dinganii</i>	3	2	0.17	1	1	1		1	10946238	1
<i>Scotophilus nux</i>	1	1	0.06	1	1	1		1	949644	1
<i>Taphozous mauritanus</i>	4	2	0.21	1	2	1		1	10145238	1
<i>Taphozous perforatus</i>	1	1	0.06	2	1	1		1	1456037	1
<i>Trienops afer</i>	49	4	1.22	3	1	1		1	1639547	1
<i>Trienops menamena</i>	34	2	0.87	3	1	1		4		1

Scientific Name	Sample size	Viral family richness	Viral family diversity	Colony size	Roost type	Aggregation clusters while roosting	Migratory nomadic	IUCN Status	Distribution area	Diet type
<i>Triaenops persicus</i>	31	2	0.86	3	1	2		1	1036460	1

Appendix 36: Phylogeny of the 62 African bat species investigated in this study

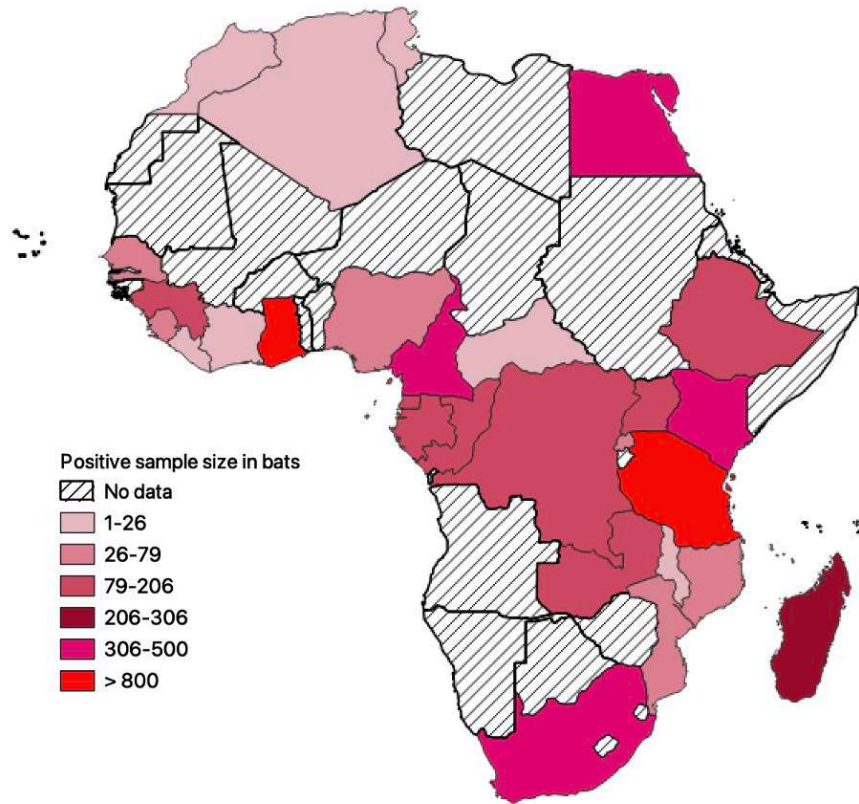


Appendix 37: Number of species according to the sample size

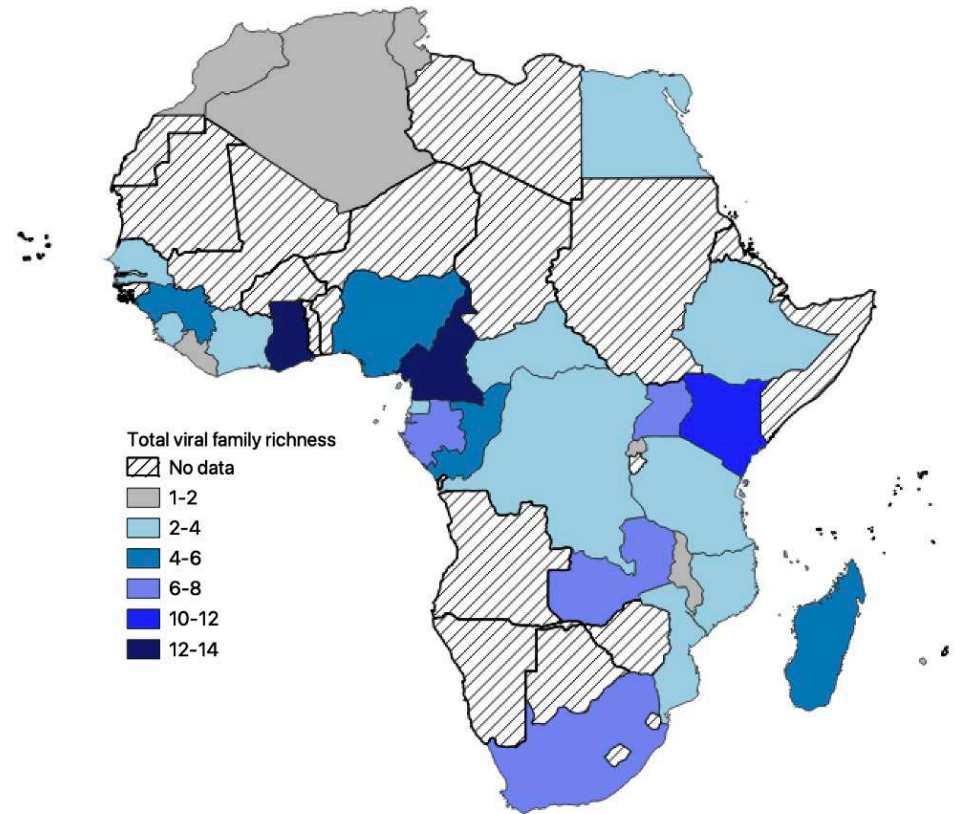


Appendix 38: Positive sample size and total viral family richness in datasets of 62 species by African countries: (A) Number of positive sample sizes in bats and (B) Total viral family richness

A



B



Appendix 39: Factors explaining viral family richness for the best PGLS models of each sub-groups of 102, 86 and 62 species of African bats (* significant differences).

Model	Dependant variable	Independent factor	Estimate	Std error	t value	Pr (> t)	F, R ² (P)
Model A: sub-group of 102 bat species							
	Viral family richness	Average size of roosting colony medium vs small	0.231	0.224	1.032	0.309	
		Average size of roosting colony large vs small	0.728	0.267	2.726	<0.01**	F _{4,35} = 7.77,
		Migratory nomadic yes vs no	0.622	0.190	3.26	<0.01**	0.409
		Distribution area	<0.001	<0.001	4.274	<0.001***	(<0.001)
Model B: sub-group of 86 bat species							
	Viral family richness	Sample size	<0.001	<0.001	2.583	<0.05 *	
		Roost type open vs closed	-0.400	0.269	-1.488	0.147	F _{5,29} = 4.885,
		Aggregation clusters while roosting yes vs no	-0.353	0.248	-1.424	0.165	0.364
		Migratory nomadic yes vs no	0.483	0.230	2.01	<0.05 *	(<0.01)
		Distribution area	<0.001	<0.001	2.010	0.053	
Model C: sub-group of 62 bat species							
	Viral family richness	Sample size	<0.001	<0.001	2.356	<0.05 *	
		Roost type open vs closed	-0.338	0.239	-1.405	0.172	F _{4,25} = 4.577,
		Migratory nomadic yes vs no	-0.492	0.248	1.977	<0.05 *	0.330
		Distribution area	<0.001	<0.001	1.644	0.112	(<0.01)

Appendix 40: Details of the correlation of the trait with bat phenology

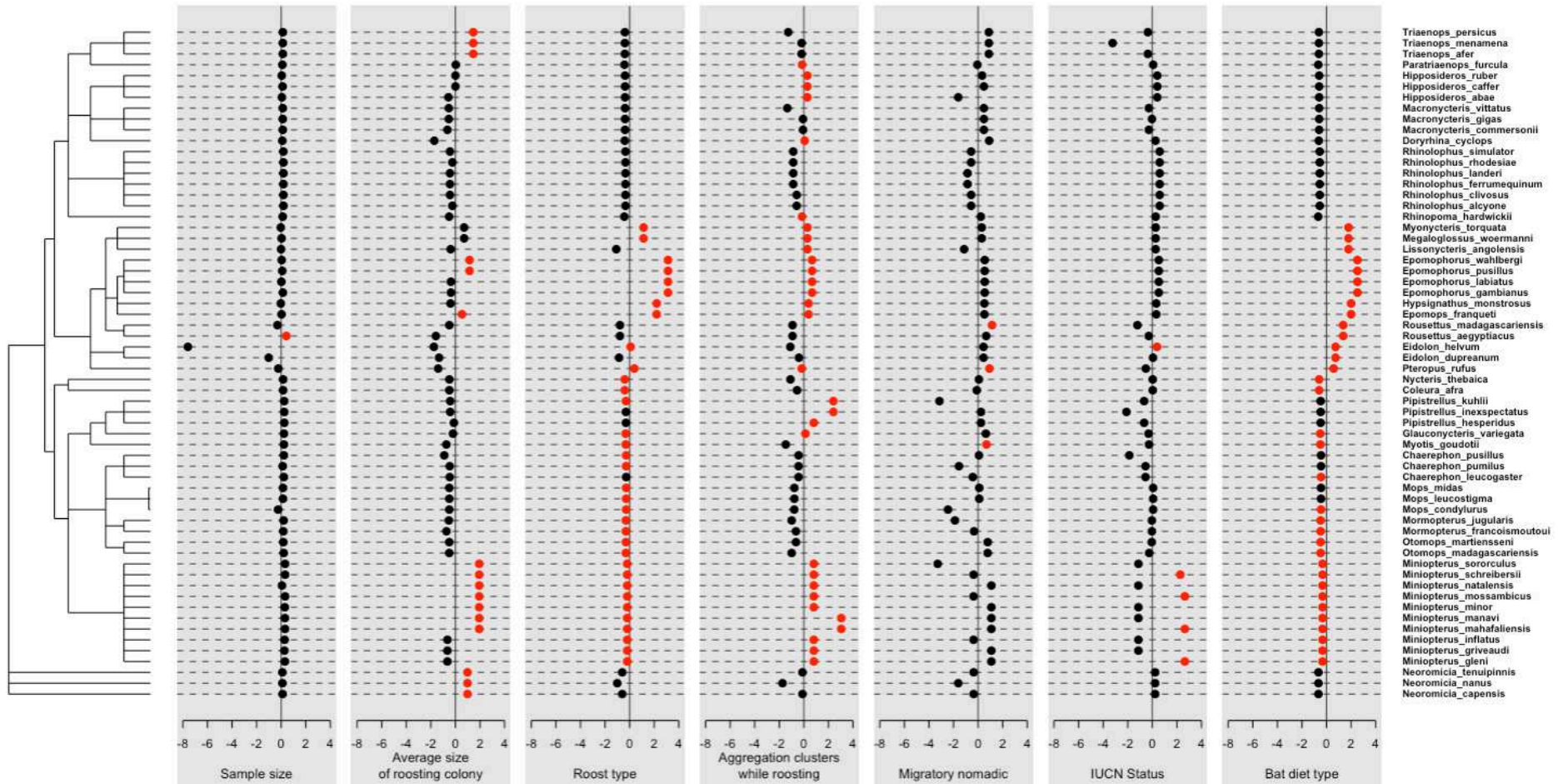
Correlation of the trait with bat phylogeny

Variations in phylogenetic autocorrelation can be observed depending on ecological traits and species clades (**Appendix 42 and 43**, several clades were not fully resolved in the phylogeny). For example, the trait "Bat diet type" showed strong phylogenetic autocorrelation with species of the same clade presenting the same diet (**Appendix 41, Appendix 42 and 43**).

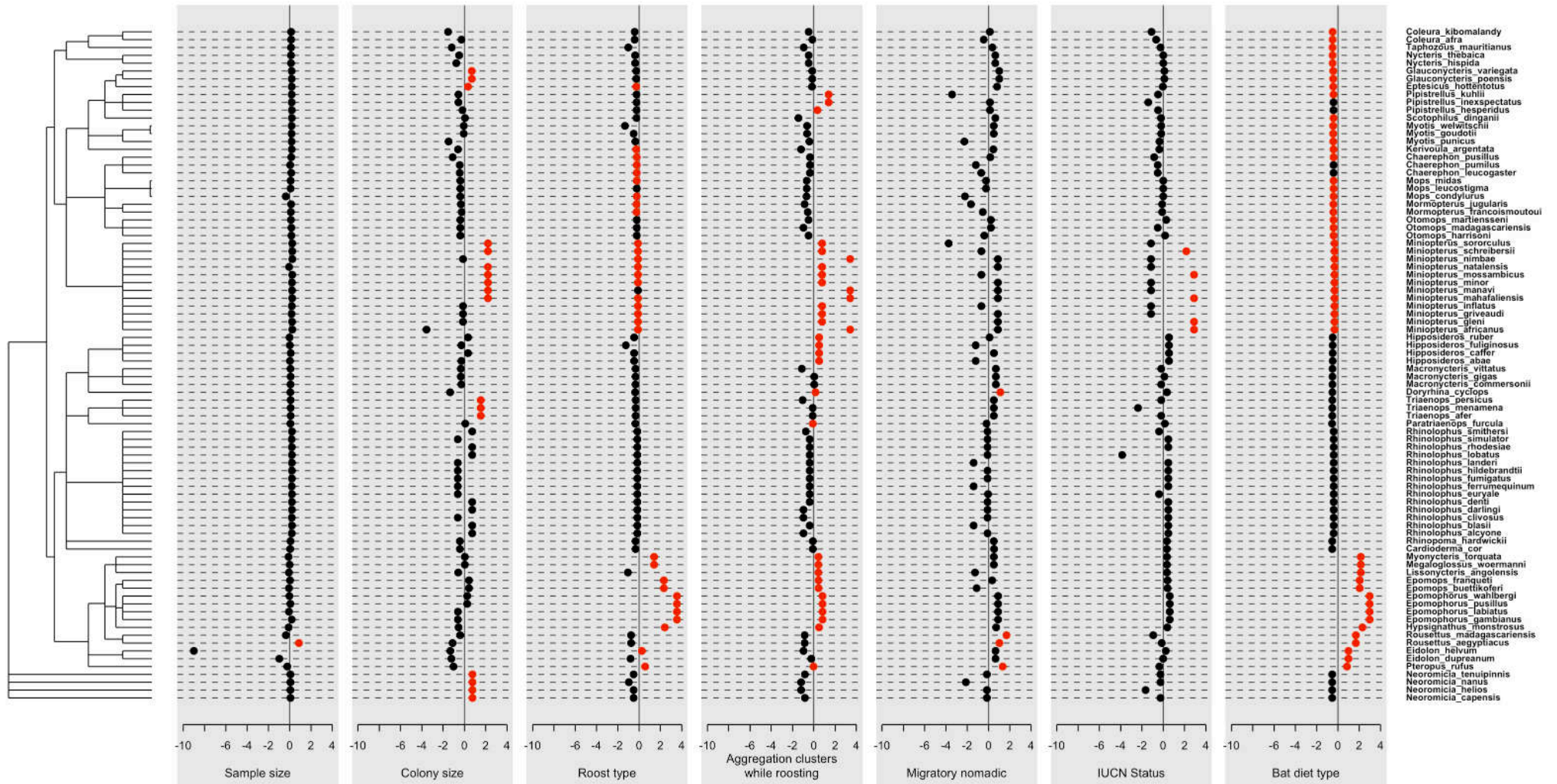
Regardless of the number of species, the trait "sample size" showed a strong phylogenetic autocorrelation for one species (*Roussettus aegyptiacus*) (**Appendix 41, Appendix 42 and 43**).

A phylogenetic effect on the migration or nomadic behaviour for two species (*Pteropus rufus* and *Roussettus madagascariensis*) (**Appendix 41**). There is also a phylogenetic effect that varies by clade for traits such as average size of roosting colonies, roost type and cluster aggregation while roosting for each dataset (102, 86 and 62 species) (**Appendix 41, Appendix 42 and 43**).

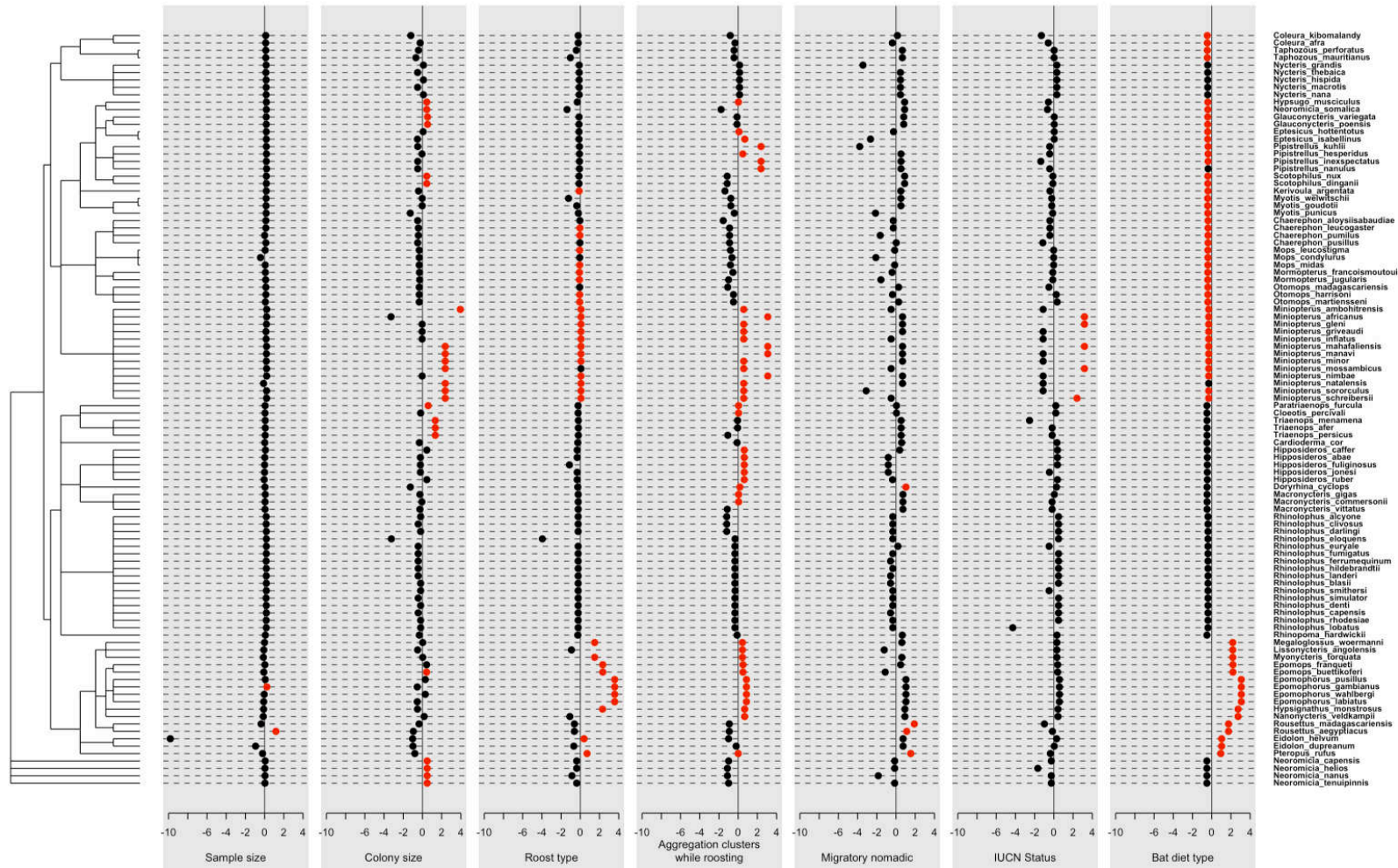
Appendix 41: Barplot of trait values (sample size, average size of roosting colonies, roost type, aggregation clusters while roosting, migratory or nomadic, IUCN Status, diet type) along the phylogeny of 62 African bats. Red points represent significant phylogenetic autocorrelation (< 0.05).



Appendix 42: Barplot of trait values (Sample size, Average size of roosting colonies, Roost type, Aggregation clusters while roosting, migratory or nomadic, IUCN Status, Bat diet type) along the phylogeny of 86 African bats. Red points represent significant phylogenetic autocorrelation (< 0.05).



Appendix 43: Barplot of trait values (Sample size, Average size of roosting colonies, Roost type, Aggregation clusters while roosting, migratory or nomadic, IUCN Status, Bat diet type) along the phylogeny of 102 African bats. Red points represent significant phylogenetic autocorrelation (< 0.05).



7.10 References Chapter 7

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Discussion Générale

8.1 Principaux résultats

L'objectif principal de cette étude était de contribuer à la connaissance de l'écologie des communautés et de la dynamique des populations de chauves-souris cavernicoles (**Chapitre 2** (en prép.), **Chapitre 3** (soumis) et **Chapitre 4** (en prép.)) et de l'écologie des interfaces entre les communautés de chauves-souris, la faune et l'homme à l'échelle temporelle (**Chapitre 5** et **6**, tous deux acceptés) dans un contexte d'émergence de pathogènes zoonotiques. Ces objectifs pourront être atteints par la mise en place d'un protocole innovant de suivi des colonies utilisant l'acoustique et les pièges photographiques afin de minimiser les perturbations des communautés de chauves-souris étudiées.

La première partie de cette thèse visait à décrire les communautés de chauves-souris cavernicoles présentes dans deux grottes (Mont Belo et Boundou) en République du Congo. Le **Chapitre 2** s'est concentré sur l'analyse des résultats de onze sessions de capture dans ces deux grottes, identifiant la présence régulière de six espèces de chauves-souris (*Rhinolophus cf. denti*, *Rhinolophus cf. alcyone-landeri*, *Miniopterus cf. minor*, *Miniopterus cf. inflatus*, *Hipposideros cf. caffer* et *Triaenops cf. afer* uniquement dans Boundou). Ces captures ont permis de collecter des données de référence sur la morphométrie, la génétique et l'acoustique, et d'identifier le statut reproducteur de ces individus.

La seconde partie de ce projet s'est appuyé sur un suivi acoustique passif (PAM), nous permettant de mettre en place une méthode innovante pour étudier l'activité annuelle et la phénologie des communautés de chauves-souris. Le **Chapitre 3** s'est concentré sur la description de la méthodologie utilisée et son application pour analyser les millions de fichiers acoustiques collectés pendant dix-neuf mois. A notre connaissance, c'est la première étude à utiliser un classificateur automatique de sonotypes (*Tadarida*) dans un pays ne disposant d'aucune information acoustique existante (ni de classificateur) pour identifier les genres ou groupes acoustiques présents. Ce classificateur de sonotypes a été couplé à une méthode de classification basée sur des critères a posteriori pour pallier les problèmes de chevauchement

des sonotypes de certaines espèces. Nous avons ainsi identifié la présence de cinq groupes acoustiques, dont trois sont communs aux deux grottes (groupe *Miniopterus*, *Hipposideros cf. caffer*, *Rhinolophus cf. denti*) et deux autres spécifiques à chaque grotte (groupe *Rhinolophus Macro tria* pour Boundou et *Rhinolophus landeri- alcyone* pour Mont Belo). Le **Chapitre 4** a utilisé une nouvelle méthode (comptage de nombre de « bat pass » à des périodes spécifiques) pour décrire les patrons d'activités et la phénologie annuelle des groupes acoustiques précédemment identifiés. Les résultats obtenus décèle une période de forte activité des groupes acoustiques sont entre octobre à mars, en raison de l'élevage et de l'envol des jeunes dans les grottes incluses dans cette étude.

La troisième partie de cette thèse, par l'intermédiaire du **Chapitres 5 et 6**, a fourni certains des premiers aperçus quantifiés des chaînes trophiques et des interfaces associées aux grottes au sein des communautés, y compris les humains. À l'extérieur de ces grottes, la richesse et la diversité en espèces étaient plus importantes qu'à l'intérieur de ces habitats. Nos résultats ont aussi montré des variations saisonnières dans l'utilisation de l'habitat par les taxons identifiés. Ce travail a également décrit plusieurs comportements d'intérêt, comme la prédation des genettes sur les chauves-souris, les rongeurs et les insectes présents dans la grotte de Boundou.

La quatrième section, constituée d'un seul chapitre (**Chapitre 7**, soumis), visait à identifier les facteurs écologiques pouvant expliquer la richesse virale des chauves-souris. Notre étude a montré la forte relation entre la richesse virale et l'effort d'échantillonnage. Cette étude met aussi en avant le biais dans l'échantillonnage des virus chez les chauves-souris africaines. Ainsi, les recherches futures devraient considérer ce biais et envisager avec soin la meilleure stratégie d'échantillonnage en fonction de question de recherche.

Cette thèse contribue à l'acquisition de connaissances dans trois domaines de recherche (i) l'écologie des chauves-souris africaines, (ii) l'écologie des interfaces et (iii) l'écologie de la santé. Une attention particulière sera également portée aux implications de ces résultats pour le projet 'EBOSURSY'. Enfin, les perspectives de recherche futures pour ce travail sont discutées.

8.2 Discussion sur les principaux résultats

8.2.1 Stratégie de recherche utilisée pour comprendre les mécanismes de transmission des pathogènes zoonotiques

Depuis plus de vingt ans, un effort de recherche important a été porté sur l'écologie et l'épidémiologie des chauves-souris en raison de plusieurs émergences zoonotiques liées à ce taxon (e.g., Ebola, Coronavirus). À ce jour, la recherche sur la transmission de pathogènes par les chauves-souris africaines souffre d'un fort biais d'échantillonnage (**Chapitre 7**, soumis). L'échantillonnage des chauves-souris est influencé par six facteurs : (i) l'accessibilité et la distribution des espèces de chauve-souris, (ii) la connaissance de l'espèce, (iii) le comportement de gîte, (iv) l'abondance, (v) l'implication potentielle de l'espèce dans le maintien ou la transmission de virus « d'intérêt » pour l'homme, et (vi) la phylogénie. En outre, l'effort d'échantillonnage entre pays africains varient grandement, avec une absence de recherche dans certains pays (ex : l'absence d'échantillonnage en Angola). Ce biais d'échantillonnage en Afrique a également été mis en évidence par une autre étude récente (Weber et al., 2023). Cette publication a montré que la République du Congo comptait un nombre assez important d'études différentes sur la virologie des chauves-souris (13 études différentes, le maximum étant de 20 études au Kenya) (Weber et al., 2023). Une des raisons de cet effort d'échantillonnage en République du Congo est sans doute lié à l'émergence de plusieurs épidémies d'Ebola dans le nord du pays entre 2001 et 2005 (Leroy et al., 2004 ; Leroy, 2015).

Cependant, malgré les efforts des chercheurs et une stratégie d'échantillonnage intensive, la question épineuse du mécanisme de circulation du virus Ebola chez les chauves-souris reste en suspens. En Asie, en revanche, ces dernière années, les mécanismes de transmission du virus Nipah par les chauves-souris ont été clarifié. La principale raison de cette émergence zoonotique est liée à la destruction de l'habitat des espèces du genre *Pteropus* et à leur nouvelle proximité avec les animaux d'élevage et les humains (Chua et al., 1999 ; Hasebe et al., 2012 ; Marsh & Wang, 2012 ; Pulliam et al., 2011). Les chercheurs qui travaillent sur ces questions doivent choisir la "meilleure stratégie méthodologique" pour comprendre les mécanismes de maintien et de transmission des agents pathogènes zoonotiques provenant de la faune sauvage.

La recherche actuelle est donc basée sur trois stratégies différentes pour comprendre les mécanismes de transmission : (1) en se concentrant sur la détection de pathogènes dans des populations de chauves-souris mal connues et sous-échantillonnées, (2) en échantillonnant les espèces les plus accessibles pour décrire quels pathogènes sont maintenus et transmis à une échelle plus fine, et/ou (3) en augmentant les efforts pour comparer et comprendre les mécanismes de transmission chez les espèces vivant à l'interface entre les chauves-souris, d'autres animaux (domestiques ou sauvages) et l'homme dans des habitats plus ou moins perturbés.

Idéalement, si les ressources financières, humaines et temporelles n'étaient pas limitées, nous pourrions appliquer ces trois stratégies. Cependant, compte tenu des contraintes actuelles qui pèsent sur les projets de recherche, il est plus approprié d'axer la stratégie d'échantillonnage sur les besoins du pays étudié. Par exemple, les efforts de surveillance et de recherche dans les pays à haut risque (en raison d'épidémies passées de maladies zoonotiques) sont souvent plus importants que dans les pays avec peu ou pas d'épidémies (Allen et al., 2017, Weber et al., 2023).

En République du Congo, les études se sont concentrées presque exclusivement sur la détection des virus, mais peu d'études ont adopté une approche multidisciplinaire ou pris en compte d'autres facteurs (par exemple Kumakamba et al., 2021, qui a pris en compte la saisonnalité). De plus, les données écologiques sur les espèces de chauves-souris en République du Congo sont très incomplètes (pas de clé d'identification, pas d'estimation des populations de chauves-souris par famille/espèce, dynamique des populations par saison, etc.) Le Gabon et la République Démocratique du Congo sont beaucoup plus avancés dans ce domaine.

Dans notre étude, nous avons choisi une stratégie de recherche à l'interface entre les chauves-souris, la faune sauvage et l'homme en étudiant l'écologie des chauves-souris dans un habitat abritant une forte concentration d'individus (grand nombre d'individus et facilité d'accès). Le choix d'une telle stratégie dans notre étude est lié à (i) l'importance écologique des grottes pour les chauves-souris, (ii) l'utilisation de ces grottes par les populations locales et (iii) une richesse et une diversité d'espèces dans un habitat "semi-fermé".

8.2.2 Des chauves-souris énigmatiques et insaisissables

Le **Chapitre 1** de cette thèse a fait un état des lieux des connaissances bibliographiques sur les chauves-souris, en se concentrant principalement sur les espèces africaines. Ce travail a mis en évidence les nombreuses questions qui restent en suspens sur ces espèces malgré leur grande diversité et leur importance écologique (dispersion des graines, pollinisation de nombreuses espèces à valeur économique et régulation des ravageurs des cultures). Leur importance sur le continent africain est d'autant plus cruciale que les enjeux de conservation sont nombreux et que les changements environnementaux sont importants (explosion démographique, exploitation des ressources, etc.) (Frick et al., 2020 ; Tanalgo et al., 2023). De manière surprenante, le voile de mystère qui entoure les chauves-souris pourrait être percé grâce aux nouvelles technologies (e.g. acoustique, GPS miniature, caméras) et aux multiples possibilités d'études qu'elles offrent sur le long terme. L'acoustique est l'une de ces nouvelles méthodes devenues très populaires sur d'autres continents, permettant de suivre passivement les populations de chauves-souris dans différents habitats sans causer de dérangement ou de stress, tout en collectant une quantité considérable de données même dans les endroits les plus reculés (Sugai et al., 2019, 2020 ; Zwerts et al., 2021). Cependant, l'acoustique, comme toute méthode, a aussi ses faiblesses (Sugai et al., 2019), et certaines données ne peuvent être collectées qu'en capturant des chauves-souris (Wilson et al., 1996).

8.2.3 La capture de chauves-souris, une méthode invasive pour collecter des données écologiques clés.

Onze sessions de capture ont été organisées à l'aide de pièges harpes à l'entrée principale des deux grottes pour collecter des individus et recueillir des données morphométriques (entre 11 et 12 mesures morphométriques différentes ont été collectées), des échantillons génétiques et acoustiques. Nous avons également pu déterminer le sexe et le statut reproducteur des individus capturés. Cependant, l'absence d'une clé d'identification des chauves-souris de la République du Congo et le manque de données morphologiques d'autres chauves-souris d'Afrique centrale ont été des obstacles à l'identification fiable des chauves-souris jusqu'au niveau de l'espèce. Dans cette étude, nous avons été confrontés à des espèces telles que *Hipposideros* et *Miniopterus*, qui sont difficiles à identifier jusqu'à l'espèce. Il est souvent difficile de se fier uniquement à la « forme de la tête », au nez et à la taille d'un individu pour différencier les espèces de chauves-souris (en particulier pour les espèces cryptiques). Par

conséquent, des études récentes ont utilisé la taxonomie intégrative (morphologie, génétique, acoustique, etc.) pour différencier ces espèces cryptiques (Chornelia et al., 2022 ; Filippi-Codaccioni et al., 2018 ; Ramasindrazana et al., 2011 ; Taylor et al., 2018). Dans notre étude, nous avons utilisé deux critères d'identifications issus de la taxonomie intégrative : la morphologie et l'acoustique.

Au total, notre étude a permis d'identifier la présence régulière de six espèces de chauves-souris (*Rhinolophus cf. denti*, *Rhinolophus cf. landeri-alcione*, *Miniopterus cf. minor*, *Miniopterus cf. inflatus*, *Hipposideros cf. caffer*, *Triaenops cf. afer* uniquement dans Boundou) et la détection épisodique de trois autres espèces dans la grotte de Boundou (*Macronycteris gigas*, *Coleura afra*, *Rousettus aegyptiacus*). Les espèces citées dans ce manuscrit sont le résultat de données morphométriques et acoustiques et d'une taxonomie préliminaire basée sur un échantillonnage génétique. L'analyse des données acoustiques à l'aide de l'analyse en composantes principales (ACP) a également confirmé la présence potentielle de deux groupes d'espèces dans la famille des *Hipposideros* (un minima de quatre individus avec des caractéristiques différentes des autres, voir **Chapitre 2**). Ce résultat confirmerait l'hypothèse de la présence de deux espèces dans le complexe *Hipposideros* et la nécessité d'utiliser l'acoustique comme l'un des éléments d'une taxonomie intégrative.

De plus, l'ACP avec les données acoustiques a également confirmé et validé les résultats d'autres études sur le dimorphisme sexuel dans les paramètres acoustiques chez *Triaenops* (Ramasindrazana et al., 2013 ; Rossoni et al., 2021). Ce dimorphisme sexuel acoustique permettrait et faciliterait une reconnaissance rapide et précise entre les chauves-souris lors de leurs différentes interactions sociales via les cris acoustiques. Les chauves-souris sont des animaux très sociaux, vivant en colonies avec de fortes interactions entre congénères. Les cris acoustiques des chauves-souris sont comme des codes permettant à un individu de transmettre de multiples informations, telle une "carte d'identité" (espèce, sexe, âge) (Grilliot et al., 2009 ; Jones et al., 1992 ; Jones & Ransome, 1993 ; Kazial et al., 2008 ; Masters et al., 1995 ; Russo et al., 2007 ; Schuchmann & Siemers, 2010 ; Voigt-Heucke et al., 2010 ; Yovel et al., 2011), les statuts reproductifs (Clarke, 2023 ; Knörnschild et al., 2012) et l'appartenance à un groupe (Grilliot et al., 2009 ; Jameson & Hare, 2009 ; Jones et al., 1992 ; Masters et al., 1995). Cependant, plusieurs questions restent en suspens, notamment si ces informations sont

volontairement partagées entre les congénères, l'effet des conditions environnementales sur le transfert d'informations, et la mesure dans laquelle ces informations influencent le comportement des congénères et même d'autres espèces de chauves-souris (Balcombe & Fenton, 1988 ; Barclay, 1982 ; M. B. Fenton, 2003 ; Jones & Siemers, 2010 ; Middleton et al., 2022).

Dans les deux grottes de l'étude, la richesse en espèces était presque identique, mais variait en fonction des mois échantillonnés. Cette variation de la richesse en espèces peut s'expliquer par plusieurs facteurs : (i) les conditions environnementales de la grotte (température, humidité, etc.), (ii) l'utilisation de la grotte par les espèces en fonction de leur cycle de reproduction (colonie de maternité), (iii) le type d'habitat présent autour de ces grottes, qui peut affecter la disponibilité des ressources, et (iv) les perturbations humaines dans la grotte et ses environs (Barros et al., 2020 ; Ferreira et al., 2020 ; Hughes et al., 2023 ; Nitzu et al., 2018 ; Tuttle & Stevenson, 2011). Cette étude s'est concentrée sur les chauves-souris insectivores, mais il est également intéressant de noter que ces deux grottes n'étaient pas des sites privilégiés par les chauves-souris frugivores (obs. pers.). De jeunes individus (adultes immatures) de *Roussettus aegyptiacus* ont été observés uniquement pendant la grande saison des pluies, lorsque les fruits sont abondants (Fleming, 2019 ; Thomas, 1983). Nous suspectons que la forte augmentation de la population de *Roussettus aegyptiacus* dans la région, du fait de la disponibilité des ressources, avait pour conséquence d'engendrer une compétition pour les roosts, obligeant certains individus à se réfugier dans des roosts annexes (ex : nos grottes) (Thomas, 1983).

Enfin, les captures de chauves-souris ont également permis de collecter 206 enregistrements acoustiques de référence des différentes espèces (dont 146 mâles et femelles liés à des données morphométriques et génétiques). Ces enregistrements ont été essentiels pour la deuxième partie de cette thèse, qui utilise les enregistrements acoustiques pour caractériser les patrons d'activité des chauves-souris à différentes saisons.

8.2.4 Le suivi acoustique passif, un "naturaliste électronique" pour étudier les colonies de chauves-souris

i. Application d'une méthode semi-automatique - l'apprentissage automatique

L'essor des nouvelles technologies et la démocratisation des méthodes d'échantillonnage non invasives sont l'occasion d'acquérir de nouvelles connaissances sur l'écologie des chauves-souris, leur comportement et leurs interactions. L'objectif premier des suivis acoustiques passifs était d'inventorier les espèces présentes dans un habitat et d'identifier les habitats de recherche de nourriture de ces espèces (Sugai et al., 2020). Le déclin mondial des populations de chauves-souris, causé par les multiples menaces qui pèsent sur elles (destruction des habitats, syndrome du nez blanc), a poussé les chercheurs à innover dans l'estimation des populations de chauves-souris (Almeida et al., 2021). L'acoustique a ainsi été proposée comme méthode pour estimer l'abondance et/ou l'activité dans différents habitats, dont les roosts (Brooks, 2011 ; Ford et al., 2011 ; Fraser et al., 2020 ; Muthersbaugh et al., 2019). A notre connaissance, notre étude est la première à suivre des grottes contenant des colonies de chauves-souris sur une période aussi longue (dix-neuf mois) (**Chapitres 3 et Chapitre 4**) (i) protocole dans un pays où il n'existe pas de bibliothèques de référence en acoustique, et (ii) trouver une solution pour analyser la grande quantité de données collectées, ce qui aurait nécessité des mois d'analyse manuelle (Gibb et al., 2019).

Actuellement, les classificateurs acoustiques automatiques les plus performants sont largement concentrés dans l'hémisphère nord (Europe, Amérique du Nord, Japon) (Clement et al., 2014 ; Kobayashi et al., 2021 ; Parsons & Jones, 2000), en raison de l'échantillonnage acoustique intensif dans ces pays, où le nombre d'espèces est limité. L'une des méthodes les plus efficaces pour automatiser la classification des espèces est l'utilisation de réseaux neuronaux, mais ils ont l'inconvénient de nécessiter une grande puissance de calcul et une très grande bibliothèque de référence (par exemple 54 525 appels pour entraîner un classificateur à reconnaître 30 espèces au Japon) (Kobayashi et al., 2021 ; Parsons & Jones, 2000). À l'inverse, dans les régions riches en espèces de chauves-souris, les données acoustiques sont très incomplètes (par exemple en Afrique et en Asie), ce qui limite la création de bibliothèques de référence et l'utilisation d'études acoustiques constantes basées sur des classificateurs

automatiques (Kershenbaum et al., 2016 ; Walters et al., 2013). Par conséquent, le fossé des connaissances entre les différents hémisphères continue de se creuser alors que de nombreuses espèces de chauves-souris tropicales sont confrontées à de nouvelles menaces (changements environnementaux, perturbations) (Meyer et al., 2016). Ces dernières années, de nouvelles méthodes ont émergé pour tenter de résoudre ce problème en créant des classificateurs semi-automatiques avec des processus de validation manuelle des fichiers ou en créant des classificateurs de sonotypes universels (López-Baucells et al., 2019 ; Roemer et al., 2021 ; Yoh et al., 2022).

Nous avons donc décrit une méthode " clé en main " de traitement des données acoustiques, permettant d'analyser les fichiers audio de manière semi-automatique et applicable à d'autres sites d'étude sans avoir besoin de milliers d'échantillons de référence. Comme mentionné cette étude est la première en Afrique à utiliser un classificateur automatique de sonotypes (Tadarida) dans un pays avec aucune donnée acoustique. Tadarida est entraîné à l'aide d'une méthode « random forest » de décision basée sur une base de données de référence (plus d'un million de fichiers déjà intégrés avec des fichiers provenant du monde entier) que nous avons enrichie en ajoutant 145 fichiers acoustiques (environ 695 cris étiquetés manuellement jusqu'au genre) provenant de nos enregistrements de référence sur le terrain. Ces données supplémentaires ont permis de limiter les erreurs du classificateur, en particulier pour les genres sous-représentés, comme *Hipposideros*. Cependant, pour surmonter les problèmes de chevauchement des sonotypes de certaines espèces (par exemple *Triaenops cf. afer* et *Macronycteris gigas*), nous avons également dû utiliser une classification a posteriori en utilisant des paramètres (type de sonotype, fréquence acoustique, présence d'harmoniques, etc.). Ainsi, nous avons pu isoler avec certitude (entre 82% et 98% de précision) cinq groupes acoustiques, dont trois communs aux deux grottes (groupe *Miniopterus*, *Hipposideros cf. caffer*, *Rhinolophus cf. denti*) et deux autres spécifiques à chaque grotte (groupe *Rhinolophus Macro Tria* pour Boundou et *Rhinolophus landeri-alcyone* pour Mont Belo).

8.2.5 Distribution de l'activité et phénologie annuelle des communautés de chauves-souris

i. Techniques de comptage des chauves-souris et analyse acoustique

L'estimation des populations de chauves-souris à l'aide de l'acoustique est encore une méthode récente, avec plusieurs méthodologies utilisées pour quantifier ou estimer l'abondance des populations. Dans l'hémisphère Nord, un certain nombre d'études ont utilisé diverses techniques pour estimer et décrire l'activité des populations présente dans les hibernacula (perchoirs utilisés par les chauves-souris pendant la période d'hibernation), en tenant compte des facteurs biotiques et abiotiques (Clark et al., 2002 ; Fraser et al., 2020 ; Gorman et al., 2021 ; Koch et al., 2020 ; Waag et al., 2022 ; Whiting et al., 2021). D'autres études, comme la nôtre, ont utilisé le nombre de "passages de chauves-souris" (occurrence du nombre de cris dans un fichier acoustique, dont le nombre de secondes peut varier) pour estimer les activités des chauves-souris (proxy de l'abondance) (Mariton et al., 2023 ; Revilla-Martín et al., 2020 ; R. Thomas & Davison, 2020). En revanche, Kloepper et al. (2016) ont estimé les colonies de chauves-souris cavernicoles en calculant un indice RMS (roost mean square) à l'aide d'une caméra et d'un enregistreur acoustique aux sorties des grottes. L'inconvénient de leur méthode était qu'elle nécessitait un minimum de dix chauves-souris sortant en même temps et qu'elle ne prenait en compte que les chauves-souris sortant du perchoir en ligne droite. Récemment, une étude de Hoggatt et al (2024) a utilisé un modèle de rencontre aléatoire généralisé pour estimer la densité des chauves-souris en se basant sur le nombre d'appels, les informations sur les signaux et les angles de détection, ainsi que sur les données météorologiques.

Dans cette thèse, nos données suggèrent que la méthode la plus efficace pour compter les populations de chauves-souris est lorsque les individus entrent dans la grotte (avant le lever du soleil) plutôt que lorsqu'ils en sortent (au coucher du soleil) (pic du nombre de « bat pass » qui semble plus important en entrée que lors de la sortie). Or, d'autres études utilisent pour comptabiliser les populations les données de sortie. Ces différences entre les deux pics pourraient s'expliquer par quatre différentes théories avec (i) une mauvaise couverture par l'enregistreur acoustique des sorties annexes, (ii) le comportement de sortie des chauves-souris pendant l'émergence qui est plus variable (certains individus ne sortant pas par l'entrée

principale), (iii) une surestimation du nombre d'individus au niveau de l'entrée en raison d'activité sociale entre différents individus pendant l'émergence et (iv) les individus sortent généralement par vagues en fonction de leur activité de recherche de nourriture. Or, ce comportement peut fausser la méthode de comptage utilisée (comptage du nombre de passages de chauves-souris 30 min avant le lever/coucher du soleil) en raison de la saturation des fichiers audio et donc lors de la détection avec le classificateur semi-automatique. Malgré les biais potentiels de cette méthode, l'utilisation du pic d'entrée semble être une méthode viable pour estimer la taille d'une population. Par exemple, dans la grotte de Boundou, nous avons pu enregistrer 850 passages de chauves-souris pour le groupe *Miniopterus* en février et seulement une dizaine d'individus pour *Rhinolophus denti*. L'avantage de cette méthode est qu'elle peut être appliquée dans n'importe quel gîte fermé ou semi-fermé (même les plus difficiles d'accès, comme les gîtes placés en hauteur dans des endroits difficiles d'accès). De plus, cette méthode ne nécessite pas une équipe importante, un niveau de formation élevé ou l'achat d'un équipement très coûteux (comme une caméra thermique). Cette méthode peut aider à la conservation des populations de chauves-souris en comblant les lacunes dans l'estimation des populations.

Au cours de notre étude, nous n'avons pas observé de différences majeures dans le temps d'émergence et d'entrée des chauves-souris. Ce résultat pourrait s'expliquer par notre choix de nous concentrer sur une analyse mensuelle de l'activité. Nous pourrions utiliser l'activité journalière pour identifier des variations plus fines de leur activité. En effet, l'activité des chauves-souris à l'émergence et à la rentrée des grottes peut être déterminée par un certain nombre de facteurs, tels que les conditions environnementales (e.g. climat, luminosité lunaire, etc.), la disponibilité des ressources, les traits d'histoire de vie individuels (e.g. âge, sexe, statut reproductif) et le comportement (Appel et al., 2019 ; Jones & Rydell, 1994 ; LaVal & Lawton, 2021 ; A. J. Thomas & Jacobs, 2013). Ces variations d'activité dans le temps des communautés de chauves-souris, et plus largement des populations, peuvent conduire à des différences d'interactions avec l'habitat.

ii. *Distribution de l'activité nocturne et phénologie mensuelle*

Notre étude a démontré la présence de deux distributions distinctes de l'activité durant la nuit, qui est soit bi-modale (un pic d'émergence et un pic de retour au repos dans le gîte) ou tri-modale/quadri-modale, selon le mois et le site. L'activité était principalement tri-modale dans les grottes en décembre et janvier, pendant la grande saison des pluies et le début de la saison sèche. Nous avons émis l'hypothèse que cette activité étalée tout au long de la nuit correspondait au retour des femelles dans la grotte pour allaiter leurs petits et se nourrir. Nos résultats sont cohérents avec les changements dans les patrons d'activité des femelles observés dans d'autres études (Erkert, 1982 ; M. B. Fenton et al., 1977 ; Gorman et al., 2021 ; Nurul-Ain et al., 2017).

Ces espèces ont également synchronisé la période d'élevage des jeunes avec la période de plus grande disponibilité des ressources alimentaires afin de minimiser le coût énergétique élevé de la lactation.

Pendant la grande saison des pluies (octobre, novembre et décembre), nous avons observé une forte augmentation de l'activité des chauves-souris. Cette augmentation de l'activité pendant la grande saison des pluies est liée à la période de gestation, de naissance et de lactation qui est synchronisée avec la saison de plus grande disponibilité des ressources (M. Fenton & Thomas, 1980 ; Jenber & Wili, 2021 ; Wolda, 1988). Cette synchronisation est essentielle pour limiter le coût énergétique de la lactation pour les femelles (Cumming & Bernard, 1997 ; Racey & Entwistle, 2000). De plus, les femelles se regroupent en colonies de maternité, ce qui les encourage à travailler ensemble pour élever leurs jeunes (ex : présence d'une " nounou " pour superviser un groupe de jeunes composé de plusieurs femelles) (Kunz & Hood, 2000 ; Rensel, 2021 ; Stirnemann, 2009). Pendant la petite saison sèche (janvier, février ; juste après la grande saison des pluies) et la petite saison des pluies (mars, avril, mai), nos résultats ont montré un fort pic d'activité des groupes acoustiques. Cette forte activité peut refléter (i) le début du vol juvénile (par exemple, les jeunes *Hipposideros* peuvent commencer à voler après un mois ; Wright, 2009)), (ii) le début de l'indépendance pour les juvéniles nés au début de la saison des pluies et (iii) une période d'accouplement pour certaines espèces (Wright, 2009).

Nos résultats ont montré une diminution de l'activité des différents groupes acoustiques dans les grottes pendant la longue saison sèche. Cette tendance peut s'expliquer en partie par la

réduction des ressources alimentaires (Barclay & Kurta, 2007 ; Patriquin & Ratcliffe, 2016 ; Patterson et al., 2007). Normalement, cette saison devrait également être l'une des périodes d'accouplement pour les espèces à cycle classique, donnant naissance pendant la saison des pluies. Malheureusement, l'une des faiblesses de notre étude est le manque d'informations sur l'état de la reproduction en mai, juillet et août pour toutes les espèces. .

Enfin, nous pouvons également souligner l'observation de périodes d'accouplement et d'élevage différentes selon les grottes et les groupes acoustiques. Une des hypothèses que nous émettons est la présence de plusieurs espèces dans un même groupe acoustique, comme le groupe *Rhinolophus Macro Tria* (voir chapitre 3) ou le groupe *Miniopterus*. Une autre hypothèse est la présence d'un complexe d'espèces dans une même colonie, qui n'aurait pas nécessairement les mêmes périodes d'accouplement et d'élevage. Par exemple, au Gabon, Brosset (1968) mentionne deux périodes de reproduction pour *H. caffer*, certaines femelles mettant bas en mars et d'autres en octobre. La dernière hypothèse pourrait être la présence de deux cycles de reproduction chez certaines espèces vivant près de l'équateur (Brosset, 1963, 1968 ; Menzies, 1973 ; Wright, 2009).

iii. Portée et implications de la méthode acoustique

Pour tester l'efficacité et la performance de cette méthode, il faudrait l'appliquer dans d'autres grottes avec différentes occurrences de populations de chauves-souris insectivores. A notre avis, cette méthode n'est pertinente que pour les roosts assez fermés (avec peu de sorties et d'entrées). Comme les chauves-souris frugivores utilisent très peu l'acoustique, il est peu probable que cette méthode soit précise et viable pour le suivi de ces populations.

Le temps nécessaire à la mise en œuvre de la méthode (enregistrement acoustique de référence, paramétrage du classificateur, analyse acoustique) peut être considérable pour une première étude dans une région. En revanche, l'investissement initial est largement compensé pour les études acoustiques futures, et notamment lorsque certains complexes d'espèces ont une distribution géographique étendue. Ainsi, cette méthode pourrait constituer une base de référence pour toutes les études acoustiques futures en République du Congo sur les chauves-souris insectivores, et plus largement pour le Bassin du Congo.

L'utilisation de cette méthode présente plusieurs avantages, car elle permet (i) d'explorer la dynamique des populations à l'échelle temporelle, (ii) de combler les lacunes dans la

connaissance de l'écologie des chauves-souris insectivores africaines, (iii) de quantifier les changements de population causés par les perturbations humaines ou les changements climatiques actuels, (iv) d'améliorer la conservation, (v) de permettre la collecte dans des zones éloignées, et (vi) de réduire les coûts par rapport à d'autres méthodes.

8.2.6 Grottes : Description de la niche écologique, de l'interface et de l'interaction entre les colonies de chauves-souris cavernicoles, la faune et l'homme au fil des saisons

La troisième partie de cette thèse, dans les **Chapitres 5 et 6**, s'est concentrée sur la description de la présence et du comportement de l'homme ou de la faune à l'intérieur et à l'extérieur de ces grottes, à l'aide d'un protocole de pièges photographiques sur une période de 19 mois. L'objectif était de caractériser l'interface chauves-souris-humains et non-humains peu explorée à l'intérieur et à l'extérieur des grottes dans un contexte de chaîne trophique et d'écologie des maladies.

i. Description de la faune à l'intérieur et à l'extérieur des grottes

Pour les deux grottes, la richesse et la diversité des espèces non chauves-souris étaient plus importantes à l'extérieur des grottes qu'à l'intérieur (**Chapitre 5**). En dehors de la détection des chauves-souris (présentes toute l'année et détectées par les caméras), à l'intérieur de la grotte du Mont Belo, les espèces les plus fréquemment détectées sont les petits rongeurs, suivis par les insectes (volants et non volants, et arachnides), les rats géants, les humains et les serpents. Dans la grotte du Boundou, les genettes sont les premières espèces détectées (à l'exclusion des chauves-souris), suivies par les insectes et les petits rongeurs. A l'extérieur des deux grottes, les espèces qui utilisaient l'intérieur de la grotte ont été redétectées, avec l'existence de nouveaux taxons comme les oiseaux (dont certains rapaces) et de nouvelles espèces de reptiles et de divers mammifères (dont des primates et le pangolin géant).

Les résultats de la courbe d'accumulation des espèces montrent que le nombre d'espèces atteint un plateau pour les espèces détectées à l'intérieur des grottes, mais pas à l'extérieur. Cette différence pourrait s'expliquer par le fait que les animaux utilisant l'intérieur des grottes sont moins nombreux que les animaux utilisant d'autres niches écologiques à l'extérieur des

grottes. Cependant, le nombre d'espèces présentes à l'intérieur et à l'extérieur des grottes a certainement été sous-estimé. En fonction d'un certain nombre de facteurs (par exemple, la disposition, les réglages, le type de modèle, etc.), les pièges photographiques peuvent ne pas être en mesure de détecter correctement certaines espèces de petits mammifères ou de reptiles.

La différence entre les espèces détectées à l'intérieur et à l'extérieur de la grotte pourrait s'expliquer par les types d'habitat à l'intérieur et à l'extérieur des grottes. A Mont Belo, la détection élevée de rongeurs pourrait potentiellement être corrélée à la présence de cultures qui offrent de multiples possibilités d'alimentation pour ces populations. En outre, la configuration de la grotte de Mont Belo (avec de nombreux trous dans la roche) pourrait offrir aux rongeurs des abris et des refuges suffisants contre les variations climatiques et les prédateurs.

La diversité et la richesse des espèces des deux grottes étaient plus importantes pendant la principale saison des pluies que pendant la principale saison sèche. Pendant la saison des pluies, l'abondance et la diversité des populations de chauves-souris étaient les plus élevées, en raison de la plus grande disponibilité des ressources et de l'utilisation des grottes pour l'élevage des jeunes. Cependant, leur présence peut favoriser la présence de nombreux autres animaux (prédateurs et animaux se nourrissant de la litière de guano). La longue saison sèche, qui est une période d'accouplement pour plusieurs espèces, a également été une période de forte abondance pour les populations de chauves-souris. Durant cette période, la végétation est moins dense, le climat chaud et sec, et les ressources alimentaires sont moins abondantes. Les grottes peuvent donc devenir un refuge contre les rigueurs du climat, apportant de la fraîcheur tout en offrant des possibilités de nourriture, notamment pour les prédateurs tels que les genettes et les hiboux.

ii. Description de comportements intéressants entre la faune et les chauves-souris

Le **Chapitre 6** décrit la consommation de chauves-souris par les genettes. Sur une période de 19 mois, plusieurs individus sont entrés dans cette grotte pour se nourrir d'insectes sur le sol de la litière de guano, de chauves-souris et éventuellement de rongeurs. Les vidéos montrent

un comportement de chasse évident à l'intérieur et à l'extérieur (à l'entrée) de la grotte. Des chauves-souris vivantes ayant des difficultés à se déplacer (tombant au sol dans la grotte) ou des individus décédés de cause inconnue ont également été consommés par les genettes. La configuration de la grotte du Boundou en une seule chambre favorise la promiscuité et une forte concentration d'individus au mètre carré. Cette forte concentration d'individus pourrait également favoriser la transmission de pathogènes entre chauves-souris et avec d'autres hôtes (Alcantara et al., 2023 ; Hoyt et al., 2018 ; Joffrin et al., 2018). À l'inverse, en mangeant les individus faibles, malades et les carcasses de chauves-souris, les genettes participent au maintien d'une population saine et peuvent limiter la propagation des maladies. Cette fonction de nettoyage est par exemple assurée par les vautours, qui limitent la propagation des maladies en nettoyant rapidement les carcasses à l'interface entre l'homme, le bétail et la faune sauvage (van den Heever et al., 2021 ; Ogada et al., 2012).

A Mont Belo, notre protocole de suivi a aussi permis de détecter la présence de nombreux petits rongeurs consommant des insectes sur la litière de guano. Nos résultats ont également révélé la présence du grand rat géant de Gambi dans la grotte, stockant potentiellement sa nourriture, et l'observation de serpents chassant vraisemblablement des chauves-souris (au sol ou sur les parois) (obs. pers.).

iii. Description des activités humaines

Dans la grotte du Boundou, en dehors des activités de notre équipe de recherche, peu d'activités humaines ont été observées. À l'inverse, dans la grotte de Mont Belo, plusieurs activités ont été enregistrées par les caméras. La perturbation anthropique la plus importante dans la grotte était les activités de prière, suivies par les activités de recherche, la collecte de guano, la chasse aux chauves-souris et les comportements non définis. Ces perturbations peuvent avoir un impact sur le comportement et les populations de chauves-souris, en déclenchant un stress et/ou un comportement de fuite de l'habitat. Cependant, la conception méthodologique (absence d'enregistrement acoustique diurne, mesure sonore des perturbations anthropogéniques) utilisée dans cette étude ne nous a pas permis de soutenir ou d'infirmer cette hypothèse. Un autre facteur pouvant avoir un impact sur le déclin des populations de chauves-souris est la consommation de viande de brousse. Cependant, dans

les pays du Sud, malgré l'importance de la conservation, peu de recherches ont été menées sur cette question à une échelle détaillée (Frick et al., 2019).

iv. Chevauchement des schémas d'activité entre la faune sauvage - les chauves-souris - les humains.

Plusieurs espèces de mammifères (chauves-souris, rongeurs, genettes), d'insectes et d'arachnides (volants ou non) semblaient suivre un rythme d'activités nocturnes. Une certaine synchronisation des activités entre espèces pourrait être expliquée par l'interconnexion entre les activités comportementales des proies et des prédateurs dans une chaîne trophique d'habitat (Monterroso et al., 2013 ; Vallejo-Vargas et al., 2022 ; Vilella et al., 2020). Cependant, nous avons observé au moins trois activités diurnes dans la grotte du Mont Belo. Tout d'abord, la collecte de guano et les activités de prière ont eu lieu pendant la journée. Ainsi, les chauves-souris des grottes étaient probablement plus visitées pendant la période de repos des chauves-souris. Deuxièmement, pendant les périodes de repos avec de fortes densités de chauves-souris dans les grottes, les rats géants et les genettes ont montré une activité diurne, soit pour explorer et déposer des ressources pour les premiers, soit pour chasser et/ou consommer des chauves-souris pour les secondes.

8. 3 Perspectives de recherche pour cette étude et contribution au projet EBOSURSY

8.3.1 Améliorer l'efficacité du classificateur jusqu'au niveau de l'espèce

Dans le cadre de cette étude, nous devrions améliorer la qualité du classificateur de sonotypes pour permettre l'identification au niveau de l'espèce. Cependant, pour atteindre cet objectif, il serait nécessaire d'ajouter des sons acoustiques supplémentaires après l'identification manuelle et l'ajout à la bibliothèque de sons de référence de Tadarida.

Une autre piste de recherche consiste à utiliser une méthode, telle que celle de Yoh et al (2022), qui a été testée sur des chauves-souris vivant à Bornéo. Nous pourrions alors comparer les deux méthodes et les résultats obtenus.

8.3.2 Collecte non invasive de guano

Au tout début de cette thèse, il était envisagé d'ajouter un chapitre supplémentaire pour décrire la richesse et la diversité virale en fonction des saisons, à partir de la collecte de guano. Malheureusement, en raison de contraintes d'analyse avec les partenaires responsables de cette section, ce chapitre n'a pas pu être finalisé. Ce n'est pas la première fois qu'une divergence entre les résultats écologiques et épidémiologiques se produit et elle semble inhérente à la dynamique et au fonctionnement des deux domaines qui utilisent des techniques différentes et subissent des pressions et des priorités différentes. Cependant, la pertinence de nos protocoles, de nos méthodes et des résultats obtenus fournit un cadre pour explorer la dynamique virale en relation avec l'écologie des communautés de chauves-souris. L'étude de la transmission des pathogènes dans les grottes abritant des chauves-souris doit être complétée et guidée par l'écologie des chauves-souris avec des indicateurs écophysologiques supplémentaires tels que les marqueurs immunologiques et les hormones de stress. Par exemple, au Mont Belo, une comparaison des mesures de stress dans les populations de chauves-souris avant et après une perturbation anthropogénique aiderait à comprendre à une échelle plus fine les impacts anthropologiques sur la physiologie des chauves-souris et potentiellement sur la virémie.

8.3.3 Enregistrement continu avec des enregistreurs acoustiques

Les enregistreurs acoustiques ont été réglés pour collecter des données seulement 30 minutes avant le coucher du soleil et jusqu'à 30 minutes après le coucher du soleil. Cependant, une perturbation survenant au milieu de la journée peut déclencher plus tard le comportement de vol. L'enregistrement continu avec des enregistreurs acoustiques permettra donc d'enregistrer ces effets potentiels et de calculer un indice de bruit de perturbation (indice d'anthropophonie) à l'aide des enregistreurs (Ross et al., 2021). Des études acoustiques ont déjà démontré l'impact des perturbations humaines sur la diversité acoustique dans les habitats perturbés (Hao et al., 2022 ; Pirota et al., 2015 ; Sangermano, 2022 ; Tuneu-Corral et al., 2020).

8.3.4 Comparaison de l'abondance des chauves-souris par l'acoustique et la vidéo

La mise en œuvre d'un protocole couplé acoustique et piège-caméra permet de comparer le nombre de chauves-souris pouvant émerger sur une vidéo de 30 secondes avec les données acoustiques pour la même période. L'utilisation d'un logiciel de comptage automatique des chauves-souris pourrait être envisagée afin d'éviter les erreurs humaines et de limiter le temps d'analyse (Bentley et al., 2023 ; Corcoran et al., 2021).

8.3.5 Collecte d'échantillons génétiques et acoustiques

Cette thèse a mis en évidence l'importance de poursuivre les efforts de collecte de données morphométriques, génétiques et acoustiques des espèces de chauves-souris. Ceci est particulièrement important sur le continent africain et en Afrique centrale, où peu d'études sur les chauves-souris ont été réalisées. La collecte de données morphométriques et génétiques permettra (i) de confirmer la découverte de nouvelles espèces, (ii) de contribuer à la taxonomie des chauves-souris africaines, (iii) de distinguer les espèces présentes dans le complexe d'espèces *Hipposideros* de cette étude. De plus, les enregistrements acoustiques de référence collectés sur le terrain et liés aux échantillons seront des données cruciales pour augmenter la fiabilité des classificateurs acoustiques automatiques sur le long terme.

8.3.6 Vers une étude encore plus multidisciplinaire

Afin d'étudier et de comprendre l'interface chauve-souris-faune-homme, nous pourrions ajouter d'autres protocoles pour couvrir un spectre plus large de disciplines scientifiques. Par exemple, nous pourrions coupler ce projet de thèse avec un travail sur les dimensions sociales des interactions homme-chauve-souris afin de déterminer l'exposition humaine aux chauves-souris et à leurs pathogènes. Tout d'abord, une étude anthropologique pourrait fournir des informations supplémentaires sur l'utilisation des grottes par les communautés (ex. femmes, hommes, enfants, adultes, etc.), leurs perceptions de ces environnements particuliers et de leurs habitants, y compris les chauves-souris. Deuxièmement, une étude comparative des viromes partagés entre les humains, les chauves-souris et d'autres animaux sauvages permettra de mieux évaluer l'existence d'une propagation du virus tout en améliorant la prévention au niveau de l'interface.

8.4 Contribution de ce travail au projet EBOSURSY.

L'objectif principal du projet EBOSURSY était de renforcer les systèmes de surveillance et les capacités des pays à risque d'épidémies zoonotiques en Afrique. La République du Congo est l'un de ces pays cibles en raison de plusieurs épidémies zoonotiques telles qu'Ebola, la fièvre hémorragique de Crimée et le mpox. Le pays fait partie du bassin du Congo, qui contient la deuxième plus grande forêt tropicale après l'Amazonie et abrite une riche biodiversité.

Dans ce contexte, ce travail s'est concentré sur l'écologie des chauves-souris cavernicoles en relation avec l'émergence de pathogènes zoonotiques. Ce travail participe à l'acquisition de connaissances sur l'identification, la phénologie et la dynamique des communautés de chauves-souris insectivores présentes dans les grottes. Ces nouvelles connaissances sur l'écologie des chauves-souris pourront être intégrées dans de futurs travaux de modélisation épidémiologique (nécessitant des données locales ou régionales) et/ou des analyses visant à comprendre les déterminants écologiques responsables de l'émergence et de la propagation des pathogènes (un exemple de ce type d'analyse est fourni au **Chapitre 7**).

Enfin, par son approche écologique, ce travail a permis de définir des hypothèses épidémiologiques sur la circulation et le maintien des pathogènes en ciblant l'échantillonnage nécessaire à l'interface chauve-souris-faune-homme. Les échantillons collectés au cours de ce travail en collaboration avec une équipe de l'IRD permettront également le criblage de virus. Ainsi, les résultats virologiques pourront être interprétés à la lumière des résultats écologiques de cette thèse.

8.5 Conclusion

La dernière pandémie de Covid-19, qui a paralysé la quasi-totalité de la planète, a été un choc pour nos sociétés, nous rappelant les risques épidémiologiques auxquels nos sociétés peuvent être confrontées à l'Anthropocène. La crise Covid aurait pu être le tsunami qui détruit "l'organisation globale" de nos sociétés centrées sur l'économie, où l'homme et la nature sont imbriqués l'un dans l'autre. Toujours aussi mystérieuses et craintes, malgré les grands services écosystémiques qu'elles rendent, les chauves-souris, qui sont nos voisines silencieuses et "les fantômes de nos nuits" depuis des milliers d'années, sont la parfaite représentation du problème "faune - pandémie". En conséquence, les humains ont de plus en plus peur de la faune et des êtres vivants, et trouvent plus facile d'éviter le danger en les détruisant. Par exemple, en tuant des centaines de chauves-souris pour éviter une "prochaine pandémie mondiale" qui, ironiquement, pourrait en déclencher une (Amman et al., 2014). En outre, une grande partie des efforts de recherche se concentre sur la manière de se préparer et de réagir à la prochaine pandémie. Cependant, la solution n'est pas de se préparer au pire, mais plutôt de s'attaquer à la "racine du mal" et d'éliminer les risques à la source. Pour ce faire, nous devons porter un regard pluridisciplinaire sur les dysfonctionnements que nous, en tant que société humaine, causons au monde naturel. Nous devons poursuivre nos recherches sur les mécanismes de propagation des zoonoses et la science des interfaces, tout en continuant à explorer la biologie et l'écologie des organismes vivants. Cet enjeu est confirmé par le dernier rapport Eklipse (2023) sur les relations entre biodiversité et pandémies, qui préconise une recherche globale et participative sur l'interface faune sauvage-animaux domestiques-humains, intégrant les interactions à l'aide des sciences sociales et naturelles.

Ainsi, cette thèse contribue à l'écologie des chauves-souris cavernicoles africaines en s'appuyant principalement sur des protocoles non invasifs à l'interface écologique entre humains et animaux non-humains tout en prenant en compte le facteur temporel. L'approche développée ici permet d'identifier précisément les facteurs épidémiologiques contribuant à la circulation et au maintien des pathogènes. De plus, les grottes pourraient constituer un modèle d'étude plus "simple et fermé" adapté à la compréhension des risques sanitaires et des mécanismes de transmission à l'interface chauve-souris-faune-homme. Enfin, ce travail souligne l'importance écologique des grottes en tant qu'habitats uniques, souvent négligés et menacés.

General discussion (ENGLISH VERSION)

8.1 Principal results

The main aim of this study was to contribute to the knowledge of the community ecology and population dynamics of cave bats (**Chapter 2 (in prep.)**, **Chapter 3 (submit)** and **Chapter 4 (in prep.)**) and the ecology of the interfaces between bat communities, wildlife and humans on a temporal scale (**Chapter 5 and 6, both accepted**) in a context of zoonotic pathogen emergence. These objectives could be achieved by implementing an innovative colony monitoring protocol using acoustics and camera traps to minimize disturbance to the bat communities studied.

The first part of this thesis aimed to describing the cave bat communities present in two caves (Mont Belo and Boundou) in the Republic of Congo. **Chapter 2** focused on analysing the results of eleven capture sessions in these two caves, identifying the regular presence of six bat species (*Rhinolophus cf. denti*, *Rhinolophus cf. alcyone-landeri*, *Miniopterus cf. minor*, *Miniopterus cf. inflatus*, *Hipposideros cf. caffer* and *Triaenops cf. afer* only in Boundou). These captures enabled us to collect reference data on morphometry, genetics and acoustics, and to identify the reproductive status of these individuals.

The second part of this thesis, based on a passive acoustic monitoring (PAM), allowed us to establish an innovative method for studying the annual activity and phenology of bat communities. **Chapter 3** focused on the description of the methodology used and its application to analyse the millions of acoustic files collected over nineteen months. This study is the first to use an automatic sonotype classifier (Tadarida) in a country where no classifier exists to identify the acoustic genera or groups present in a cave. This sonotype classifier was coupled with a classification method based on a posteriori criteria to overcome the problems of overlapping sonotypes of certain species. We thus identified the presence of five acoustic groups, three of which are common to both caves (*Miniopterus group*, *Hipposideros cf. caffer*, *Rhinolophus cf. denti*) and two others specific to each cave (*Rhinolophus Macro tria* group for Boundou and *Rhinolophus landeri- alcyone* for Mont Belo). **Chapter 4** used a new method to

describe the activity patterns and annual phenology of the acoustic groups previously identified. Thus, the acoustic groups are very active from October to March, as a result of the rearing and fledging of young in the caves included in this study.

The third part of this thesis, in **Chapters 5 and 6**, provided some of the first quantified insights on trophic chains and interfaces within communities, including humans, associated with caves. This study contributes to our understanding of the interfaces and interactions between animal species, including humans. Within these caves, species richness and diversity were greater outside than inside the caves. Our results showed seasonal variations in habitat use by the identified taxa. This work also described several behaviours of interest, such as predation of genets on bats, rodents and insects presents in the Boundou cave.

The fourth section, consisting of a single chapter (**Chapter 7, submit**), aimed at identifying the ecological factors that could explain the viral richness of bats. Our study showed the strong relationship between virus richness and sampling effort. However, this study also showed the bias in virus sampling in African bats. Future research must therefore take this bias into account and the best sampling strategy will be discussed.

This thesis contributes to the acquisition of knowledge in three research areas (i) the ecology of African bats, (ii) the ecology of interfaces and (iii) the ecology of health. Particular attention will also be paid to the implications of these results for the 'EBOSURSY' project. Finally, future research prospects for this work are discussed.

8.2 Discussion on main results

8.2.1 Research strategy used to understand the transmission mechanisms of zoonotic pathogens

For more than twenty years, a major research effort has been placed on bat ecology and epidemiology due to several zoonotic emergences linked to this taxon (e.g., ebola, coronavirus). To date, research on the transmission of pathogens from African bats suffers from a strong sampling bias (**Chapter 7, submit**). Bat sampling is affected by six factors: (i)

accessibility and distribution of the bat species, (ii) knowledge of the species, (iii) roosting behavior, (iv) abundance, (v) potential involvement of the species in the maintenance or transmission of a virus of interest, and (vi) phylogeny. In addition, some African countries were less sampled than others, for example the lack of sampling in Angola. This sampling bias in Africa has also been highlighted by another recent study (Weber et al., 2023). This study also showed that the Republic of Congo had a fairly large number of different studies on bat virology (13 different studies, the maximum being 20 studies in Kenya) (Weber et al., 2023). One of the reasons for this sampling effort in the Republic of Congo was undoubtedly the emergence of several Ebola epidemics in the North of the country between 2001 and 2005 (Leroy et al., 2004; Leroy, 2015).

Despite the best efforts of researchers, sampling strategy has failed to identify the reservoir of the Ebola virus in bats, while the maintenance of this virus in Africa remains unclear. In Asia, on the other hand, the mechanisms by which the Nipah virus is transmitted from bats have been clarified in recent years. The main reason for this zoonotic emergence is linked to the destruction of the habitat of species of the genus *Pteropus* and their new proximity to farm animals and humans (Chua et al., 1999; Hasebe et al., 2012; Marsh & Wang, 2012; Pulliam et al., 2011). Researchers working on these issues have to select the 'best methodological strategy' to understand the mechanisms of maintenance and transmission of zoonotic pathogens from wildlife.

Current research is therefore based on three different strategies to understand the transmission mechanisms by: (1) focusing on detecting pathogens in poorly known and under-sampled bat populations, (2) sampling the most accessible species to describe which pathogens are maintained and transmitted at a finer scale, and/or (3) increasing efforts to compare and understand transmission mechanisms in species living at the interface between bats, other animals (domestic or wild) and humans in more or less disturbed habitats.

Ideally, if there were no limits to financial, human and time resources, we would be able to apply all three strategies. However, given the current constraints on research projects, it is more appropriate to focus the sampling strategy on the needs of the country being studied. For example, surveillance and research efforts in high-risk countries (due to past outbreaks of

zoonotic diseases) are often greater than in countries with few or no outbreaks (Allen et al., 2017, Weber et al., 2023).

In the Republic of Congo, studies have focused almost exclusively on virus detection, but few studies have taken a multidisciplinary approach or considered other factors (e.g. Kumakamba et al., 2021, which considered seasonality). Furthermore, ecological data on bat species in the Republic of Congo are very incomplete (e.g. no identification key, no estimation of bat populations by family/species, population dynamics by season, etc.). Gabon and the Democratic Republic of Congo are much more advanced in this field.

In our study, we chose a research strategy at the interface between bats, wildlife and humans by studying the ecology of bats in a habitat with a high concentration of bats (large number of individuals and easy access). The choice of such a strategy in our study relates to (i) the ecological importance of the caves for bats, (ii) the use of these caves by local populations and (iii) a richness and diversity of species in a "semi-enclosed" habitat.

8.2.2 Enigmatic and elusive bats

Chapter 1 of this thesis reviewed the literature on bats, mainly African species, and highlighted the many questions that remain unanswered about these species despite their great diversity and ecological importance (seed dispersal, pollination of many species of economic value and regulation of crop pests). Their importance on the African continent is all the more crucial because of the many conservation issues at stake and the major changes in the environment (demographic explosion, exploitation of resources, etc.) (Frick et al., 2020; Tanalgo et al., 2023). Surprisingly, the veil of mystery that surrounds bats could be pierced with the help of new technologies (e.g., acoustics, miniature GPS, cameras) and the multiple study possibilities they offer over the long term. Acoustics is one of these new methods that has become very popular on other continents, making it possible to passively monitor bat populations in different habitats without causing any disturbance or stress, while collecting a considerable amount of data even in the most remote locations (Sugai et al., 2019, 2020; Zwerts et al., 2021). However, acoustics, like any method, also has its weaknesses (Sugai et al., 2019), and some data can only be collected by capturing bats (Wilson et al., 1996).

8.2.3 Capturing bats, an invasive method for collecting key ecological data

Eleven capture sessions were organised using harp traps at the main entrance of the two caves to collect individuals and gather morphometric data (between 11 and 12 different morphometric measurements were collected), genetic and acoustic samples. We were also able to determine the sex and reproductive status of the captured individuals. However, the absence of an identification key for bats in the Republic of Congo and the lack of morphological data from other Central African bats were obstacles to the reliable identification of bats down to species level. In this study, we were confronted with species such as *Hipposideros* and *Miniopterus*, which are difficult to identify to the species level. It is often difficult to rely solely on the shape of the head, nose and size of an individual to differentiate between bat species (particularly for cryptic species). As a result, recent studies used integrative taxonomy (morphology, genetics, acoustics, etc.) to differentiate these cryptic species (Chornelia et al., 2022; Filippi-Codaccioni et al., 2018; Ramasindrazana et al., 2011; Taylor et al., 2018). In our study, we used two identification criteria from integrative taxonomy: morphology and acoustics.

In total, our study identified the regular presence of six bat species (*Rhinolophus cf. denti*, *Rhinolophus cf. landeri-alcyone*, *Miniopterus cf. minor*, *Miniopterus cf. inflatus*, *Hipposideros cf. caffer*, *Triaenops cf. afer* only in Boundou) and the episodic detection of three other species in Boundou (*Macronycteris gigas*, *Coleura afra*, *Rousettus aegyptiacus*). The species quoted in this manuscript were the result of morphometric and acoustic data and preliminary taxonomy based on genetic sampling. Analysis of the acoustic data using principal component analysis (PCA) also confirmed the potential presence of two groups of species in the *Hipposideros* family (a minima of four individuals with different characteristics to the others, see **Chapter 2**). This result would confirm the hypothesis of the presence of two species in *Hipposideros complex*, and the need to use acoustics as one of the elements for an integrative taxonomy.

In addition, PCA with acoustic data also confirmed and validated the results of other studies on sexual dimorphism in acoustic parameters in *Triaenops* (Ramasindrazana et al., 2013; Rossoni et al., 2021). This acoustic sexual dimorphism would allow and facilitate rapid and precise recognition between bats during their various social interactions via acoustic calls. Bats

are highly social animals, living in colonies with strong interactions between conspecifics. Bat acoustic calls are like codes enabling an individual to transmit multiple pieces of information, such as an "identity card" (species, sex, age) (Grilliot et al., 2009; Jones et al., 1992; Jones & Ransome, 1993; Kazial et al., 2008; Masters et al., 1995; Russo et al., 2007; Schuchmann & Siemers, 2010; Voigt-Heucke et al., 2010; Yovel et al., 2011), reproductive status (Clarke, 2023; Knörnschild et al., 2012) and group membership (Grilliot et al., 2009; Jameson & Hare, 2009; Jones et al., 1992; Masters et al., 1995). However, several questions remain such as if this information is voluntarily shared between conspecifics, the effect of environmental conditions on information transfer, and the extent to which this information influences the behaviour of conspecifics and even other bat species (Balcombe & Fenton, 1988; Barclay, 1982; Fenton, 2003; Jones & Siemers, 2010; Middleton et al., 2022).

Species richness was almost identical between the two caves of the study, but varied according to the months sampled. This variation in species richness can be explained by several factors: (i) the environmental conditions of the cave (temperature, humidity, etc.), (ii) the use of the cave by species according to their reproductive cycle (maternity colony), (iii) the type of habitat present around these caves, which can affect the availability of resources, and (iv) human disturbance in the cave and its surroundings area (Barros et al., 2020; Ferreira et al., 2020; Hughes et al., 2023; Nitzu et al., 2018; Tuttle & Stevenson, 2011). This study focused on insectivorous bats, but it is also interesting to note that these two caves were not preferred sites for fruit bats (pers. obs.). Young (immature adult) individuals of *Rousettus aegyptiacus* were only observed during the long rainy season, when fruit is abundant (Fleming, 2019; Thomas, 1983). We suspect that the sharp increase in the population of *Rousettus aegyptiacus* in the region, as a result of resource availability, is leading to competition for roosts and forcing some individuals to seek refuge in other roosts such as the caves in our study (Thomas, 1983).

Finally, the bat captures also enabled the collection of 206 reference acoustic recordings of individuals of the various species captured (with 146 males and females linked to morphometric and genetic data). These recordings were essential for the second part of this thesis, which uses acoustic recordings to characterize bat activity patterns at different seasons.

8.2.4 Passive acoustic monitoring, an “electronic naturalist” to study bat colonies

i. Application of a semi-automatic method - machine learning

The booming of new technologies and the democratization of non-invasive sampling methods is an opportunity to acquire new knowledge on the ecology of bats, their behavior and their interactions. The primary purpose of passive acoustic monitoring was to inventory species in a habitat and identify foraging habitats (Sugai et al., 2020). The worldwide decline in bat populations, caused by the multiple threats they face (habitat destruction, white nose syndrome), has prompted researchers to innovate in estimating bat populations (Almeida et al., 2021). Acoustics has thus been proposed as a method to estimate abundance and/or activity in different habitats, including roosts (Brooks, 2011; Ford et al., 2011; Fraser et al., 2020; Muthersbaugh et al., 2019). To our knowledge, our study is the first to monitor caves containing bat colonies over such a long period (nineteen months) (**Chapters 3 and Chapter 4**). The difficulty of this type of study is twofold: setting up this (i) protocol in a country where there are no acoustics reference libraries, and (ii) finding a solution to analyse the large amount of data collected, which would have taken months of manual analysis (Gibb et al., 2019).

Currently, the best performing automatic acoustic classifiers are largely concentrated in the northern hemisphere (Europe, North America, Japan) (Clement et al., 2014; Kobayashi et al., 2021; Parsons & Jones, 2000), due to intensive acoustic sampling in these countries, where the number of species is limited. One of the most effective methods for automating species classification is the use of neural networks, but they have the disadvantage of requiring a lot of computational power and a very large reference library (e.g. 54,525 calls to train a classifier to recognise 30 species in Japan) (Kobayashi et al., 2021; Parsons & Jones, 2000). Conversely, in regions rich in bat species, acoustic data are very incomplete (e.g. Africa and Asia), which limits the creation of reference libraries and the use of constant acoustic studies based on automatic classifiers (Kershenbaum et al., 2016; Walters et al., 2013). As a result, the knowledge gap between different hemispheres continues to widen at a time when many tropical bat species are facing new threats (environmental change, disturbance) (Meyer et al., 2016). In recent years, new methods have emerged to try and solve this problem by creating

semi-automatic classifiers with manual file validation processes or by creating universal sonotype classifiers (López-Baucells et al., 2019; Roemer et al., 2021; Yoh et al., 2022).

We have therefore described a 'turnkey' method for processing acoustic data, enabling audio files to be analysed semi-automatically and applicable to other study sites without the need for thousands of reference samples. As mentioned, this study is the first in Africa to use an automatic sonotype classifier (Tadarida) in a country that does not have a classifier to identify the genera or acoustic groups present in a cave. Tadarida is trained using a decision tree forest method based on a reference database (over a million files already integrated with acoustic files from around the world) that we have enhanced by adding 145 acoustic files (around 695 calls manually labelled down to gender) from our reference field recordings. These additional data have limited the errors of the classifier, particularly for genera that were under-represented, such as *Hipposideros*. However, to overcome the problems of overlapping sonotypes of certain species (e.g., *Triaenops cf. afer* and *Macronycteris gigas*), we also had to use *a posteriori* classification using acoustic criteria (type of sonotype, acoustic frequency, presence of harmonics, etc.). Thus, we were able to isolate with certainty (between 82% and 98% accuracy) five acoustic groups, three of which were common to both caves (*Miniopterus* group, *Hipposideros cf. caffer*, *Rhinolophus cf. denti*) and two others specific to each cave (*Rhinolophus* Macro Tria group for Boundou and *Rhinolophus landeri-alcyone* for Mont Belo).

8.2.5 Distribution of activity and annual phenology of bat communities

i. Bat counting techniques and acoustics analysis

Estimating bat populations using acoustics is still a recent method, with several methodologies being used to quantify or estimate population abundance. In the Northern hemisphere, a number of studies have operated a variety of techniques to estimate and describe the activity of bat hibernacula populations (roosts used by bats during the hibernation period), taking into account biotic and abiotic factors (Clark et al., 2002; Fraser et al., 2020; Gorman et al., 2021; Koch et al., 2020; Waag et al., 2022; Whiting et al., 2021). Some other studies, such as ours have used the number of "bat passes" (occurrence of the number of calls in an acoustic file, the number of seconds of which can vary) to estimate bats' activities (proxy of abundance) (Mariton et al., 2023; Revilla-Martín et al., 2020; R. Thomas & Davison, 2020). In contrast, Kloepper et al. (2016) estimated colonies of cave bats by calculating a roost mean square

(RMS) index using a camera and an acoustic recorder at cave exits. The disadvantage of their method was that it required a minimum of ten bats exiting at the same time, and only took into account bats exiting the roost in a straight line. Recently, a study by Hoggatt et al (2024) used a generalized random encounter model to estimate bat density based on the number of calls, signal information and detection angles, and meteorological data.

In this thesis, our data suggest that the most effective method of counting bat populations is when individuals enter the cave (before sunrise) rather than when they leave (at sunset) (peak in the number of "bat passes", which appears to be greater on entry than on exit). However, other studies use exit data to count populations. These differences between the two peaks could be explained by four different theories: (i) poor coverage by the acoustic recorder of ancillary exits, (ii) the exit behaviour of bats during emergence, which is more variable (some individuals do not exit via the main entrance), (iii) overestimation of the number of individuals at the entrance due to social activity between different individuals during emergence and (iv) individuals generally exit in waves depending on their foraging activity. However, this behaviour can distort the counting method used (counting the number of bat passages 30 min before sunrise/sunset) due to saturation of the audio files and therefore during detection with the semi-automatic classifier.

Despite the potential biases of this method, the use of peak entry appears to be a viable method for estimating the size of a population. For example, in the Boundou cave, we were able to record 850 bat passes for *Miniopterus* group in February and only around ten individuals for *Rhinolophus denti*. The advantage of this method is that it can be applied in any closed or semi-closed roost (even the most difficult to access, such as roosts positioned high up in places difficult to access). In addition, this method does not require a large team, a high level of training or the purchase of very expensive equipment (such as a thermal camera). This method can help in the conservation of bat populations by filling gaps in population estimates.

During our study, we did not observe any major differences in the time of emergence and entry of the bats. This result could be explained by our decision to concentrate on a monthly analysis of activity. We could use daily activity to identify finer variations. Indeed, bat activity at emergence and re-entry from caves can be determined by a number of factors, such as environmental conditions (e.g. climate, lunar luminosity, etc.), resource availability, individual

life history traits (e.g. age, sex, reproductive status) and behavior (Appel et al., 2019; Jones & Rydell, 1994; LaVal & Lawton, 2021; A. J. Thomas & Jacobs, 2013). These variations in the activity over time of bat communities, and more broadly of populations, can lead to differences in interactions with the habitat.

ii. Distribution of activity during the night and monthly phenology

Our study demonstrated the presence of two distinct distributions of activity during the night, which is either bi-modal (an emergence peak and a return peak to rest in the roost) or tri-modal/quadri-modal, depending on the month and site. Activity was mainly tri-modal in the caves in December and January, during the main rainy season and the start of the dry season. We hypothesized that this activity spread throughout the night corresponds to the females returning to the cave to nurse their young and feed. Our results are consistent with changes in female activity patterns observed in other studies (Erkert, 1982; M. B. Fenton et al., 1977; Gorman et al., 2021; Nurul-Ain et al., 2017).

These species have also synchronised the period of rearing young with the period of greatest availability of food resources in order to minimize the energy cost of rearing young (high energy cost of lactation).

During the main rainy season (October, November and December), we observed a sharp increase in bat activity. This increase in activity during the long rainy season is linked to the gestation, birth and lactation period, which is synchronized with the season of greatest resource availability (Fenton & Thomas, 1980; Jenber & Wili, 2021; Wolda, 1988). This synchronization is essential to limit the energy cost of lactation for females (Cumming & Bernard, 1997; Racey & Entwistle, 2000). In addition, the females gather in maternity colonies, encouraging them to work together to raise their young. (e.g. the presence of a 'nanny' to supervise a group of young made up of several females) (Kunz & Hood, 2000; Rensel, 2021; Stirnemann, 2009). During the short dry season (January, February; just after the main rainy season) and the short rainy season (March, April, May), our results showed a strong peak in acoustic group activity. This high activity may reflect (i) the start of juvenile flight (e.g. young *Hipposideros* may start flying after one month; Wright, 2009)), (ii) the beginning of

independence for juveniles born at the start of the rainy season and (iii) a mating period for some species (Wright, 2009).

Our results showed a decrease in the activity of the different acoustic groups in the caves during the long dry season. This trend can be partly explained by the reduction in food resources (Barclay & Kurta, 2007; Patriquin & Ratcliffe, 2016; Patterson et al., 2007). Normally, this season should also be one of the mating period for species with a classical cycle, giving birth during the rainy season. Unfortunately, one of the weaknesses of our study is the lack of information on the reproductive status in May, July and August for all the species. .

Finally, we can also highlight the observation of different mating and rearing periods depending on the caves and acoustic groups. One of the hypotheses we put forward is the presence of several species in the same acoustic group, such as the *Rhinolophus Macro Tria* group (see Chapter 3) or the *Miniopterus* group. Another hypothesis is the presence of a complex of species in the same colony, which would not necessarily have the same mating and rearing periods. For example, in Gabon, Brosset (1968) mentioned two reproductive periods for *H. caffer*, with some females giving birth in March and others in October. The final hypothesis could be the presence of two reproductive cycles in certain species living near the equator (Brosset, 1963, 1968; Menzies, 1973; Wright, 2009).

iii. Scope and implications of the acoustic method

To test the effectiveness and performance of this method, we should apply it in other caves with different occurrences of insectivorous bat populations. In our opinion, this method is only relevant in fairly closed roosts (with few exits and entrances). As fruit bats use acoustics very little, this method is unlikely to be accurate and viable for monitoring these populations.

The time required to implement the method (reference acoustic recording, setting the classifier parameters, acoustic analysis) can be considerable for a first study in a region. On the other hand, the initial investment is largely compensated for future acoustic studies, and particularly when certain species complexes have extended geographical distribution. Thus, this method could be a reference base for all future acoustic studies in the Republic of Congo on insectivorous bats, and more largely for the Congo Basin.

The use of this method has several advantages, as it (i) helps to explore population dynamics on a temporal scale, (ii) fills gaps in the knowledge of the ecology of African insectivorous bats, (iii) quantifies population changes caused by human disturbance or current climate change, (iv) improves conservation, (v) allows collection in remote areas, and (vi) reduces the costs compared to other methods.

8.2.6 Caves: Description of the ecological niche, interface and interaction between cave bat colonies, wildlife and humans over the seasons

The third part of this thesis, in **Chapter 5** and **Chapter 6**, focused on the description of human or wildlife presence and behavior inside and outside these caves, using a camera trap protocol over a 19-month period. The objective was to characterize the little explored bat-human and non-humans interface in and outside caves in a context of trophic chain and disease ecology.

i. Description of the fauna inside and outside the caves

For both caves, non-bat species richness and diversity were greater outside the caves than inside (**Chapter 5**). Excluding the detection of bats (present all year round and detected by the cameras), inside the Mont Belo cave, the most commonly detected species were small rodents, followed by insects (flying and non-flying, and arachnids), giant rats, humans and snakes. Inside the Boundou cave, genets were the first species detected (excluding bats), followed by insects and small rodents. Outside the two caves, the species that used the interior of the cave were re-detected, with the existence of new taxa such as birds (including some birds of prey) and new species of reptiles and various mammals (including primates and the giant pangolin). The results of the species accumulation curve showed that the number of species reached a plateau for species detected inside the caves, but not outside. This difference could be explained by the fact that animals using the inside of the caves are less numerous than animals using other ecological niches outside the caves. However, the number of species present inside and outside the caves were certainly underestimated. Depending on a number of factors (e.g.,

layout, settings, model type, etc.), camera traps may not be able to detect certain species of small mammals or reptiles correctly.

The difference between species detected inside and outside the cave could be explained by the types of habitat inside and outside the caves. At Mont Belo, the high detection of rodents could potentially be correlated with the presence of crops that offer multiple feeding opportunities for these populations. In addition, the configuration of Mont Belo cave (with many holes in the rock) could provide sufficient shelter and refuge for rodents from climate variability and predators.

The diversity and species richness of both caves were greater during the main rainy season than during the main dry season. During the rainy season, the abundance and diversity of bat populations were highest, due to the greater availability of resources and the use of the caves for rearing youngsters. However, their presence could favor the presence of many other animals (predators and animals feeding on guano litter). The long dry season, which is a mating season for several species, was also a period of high abundance for bat populations. During this period, the vegetation is less dense, the climate hot and dry, and food resources are less abundant. The caves thus may become a refuge from the harsh climate, providing coolness while offering food opportunities, especially for predators such as genets and owls.

ii. Description of interesting behavior between wildlife and bats

Chapter 6 describes the consumption of bats by genets. Over a 19-month period, several individuals entered this cave to feed on insects on the guano litter floor, bats and potentially rodents. The videos showed clear hunting behavior both inside and outside (at the entrance) the cave. Live bats having difficulty moving (falling to the ground in the cave) or deceased individuals of unknown cause were also consumed by genets. The Boundou cave's single-chamber configuration favors promiscuity and a high concentration of individuals per square meter. This high concentration of individuals could also favour pathogen transmission between bats and with other hosts (Alcantara et al., 2023; Hoyt et al., 2018; Joffrin et al., 2018). Conversely, by eating weak, sick individuals and bat carcasses, genets help maintain a healthy population and may limit the spread of disease. This cleaning function, for example, is

performed by vultures, who limit the spread of disease by rapidly cleaning carcasses at the interface between man, livestock and wildlife (van den Heever et al., 2021; Ogada et al., 2012). Other interesting behaviors were observed at Mont Belo, with our monitoring protocol that detected the presence of numerous small rodents consuming insects on the guano litter. Our results also revealed the presence of the large giant rat in the cave, potentially storing its food, and the observation of snakes presumably hunting bats (on the ground or on the walls) (pers. obs.).

iii. Description of human activities

In Boundou cave, apart from the activities of our research team, little human activity was observed. Conversely, in Mont Belo cave, several activities were recorded by the cameras. The greatest anthropogenic disturbance in the cave was prayer activities, followed by research activities, guano collection, bat hunting and undefined behaviors. These disturbances can impact bat behavior and populations, triggering stress and/or habitat flight behavior. However, the methodological design (absence of day-time acoustic recording, sound measurement of anthropogenic disturbance) used in this study did not allow us to support or refute this hypothesis. Another factor that can have an impact on the decline of bat populations is bushmeat consumption. However, in southern countries, despite the importance of conservation, little research has been carried out on this issue on a detailed scale (Frick et al., 2019).

iv. Overlapping patterns of activity between wildlife - bats - humans

Several mammal species (bats, rodents, genets), insects and arachnids (both flying and non-flying) seemed to follow a rhythm of nocturnal activities. Some synchronization of activities between species could be explained by the interconnection between prey and predator behavioral activities in a habitat trophic chain (Monterroso et al., 2013; Vallejo-Vargas et al., 2022; Vilella et al., 2020). However, we observed at least three diurnal activities in Mont Belo cave. Firstly, guano collection and prayer activities occurred during the day. Thus, cave bats were probably more visited during the bat resting period. Secondly, during resting periods with high bat densities in caves, giant rats and genets showed mid-day activity, either to explore and deposit resources for the former or hunt and/or consume bats for the latter.

8.3 Research prospects for this study and contribution to EBOSURSY project

8.3.1 Improving the efficiency of the classifier down to the species level

As part of this study, we should improve the quality of the sonotype classifier to enable identification to species level. However, to achieve this objective it would be necessary to add additional acoustic sounds after manual identification and addition to the *Tadarida* reference sound library.

Another avenue of research is to use a method, such as Yoh et al (2022), which has been tested on bats living in Borneo. We could then compare both methods and the results obtained.

8.3.2 Non-invasive guano collection

At the very beginning of this thesis, it was envisaged to add an additional chapter to describe viral richness and diversity according to season, based on guano collection. Unfortunately, due to analysis constraints with the partners responsible for this section, this chapter could not be finalized. This is not the first time that a discrepancy between ecological and epidemiological results occurs and it seems inherent to the dynamics and functioning of both fields using different techniques and under different pressures and priorities. However, the relevance of our protocols, methods and the results obtained provide a framework to explore viral dynamics in relation to bat community ecology. Exploring pathogen transmission in bat caves needs to be complemented and driven by the ecology of bats with additional eco-physiological indicators such as immunological markers and stress hormones. For example, at Mont Belo, a comparison of stress measurements in bat populations before and after an anthropogenic disturbance would help to understand at a finer scale the anthropological impacts on bat physiology and potentially on viremia.

8.3.3 Continuous recording with acoustic recorders

The acoustic recorders were set up to collect data only 30 minutes before sunset and up to 30 minutes after sunset. However, a disturbance occurring in the middle of the day may later

trigger the flight behavior. Continuous recording with acoustic recorders will therefore provide an opportunity to record these potential effects and compute a disturbance noise index (anthropophony index) using the recorders (Ross et al., 2021). Acoustic studies have already demonstrated the impact of human disturbance on acoustic diversity in disturbed habitats (Hao et al., 2022; Pirodda et al., 2015; Sangermano, 2022; Tuneu-Corral et al., 2020).

8.3.4 Comparison of bat abundance using acoustics vs video

The implementation of a coupled acoustic and camera trap protocol provides an opportunity to compare the number of bats that can emerge on a 30-second video with the acoustic data for the same period. The use of automatic bat-counting software could be envisaged in order to avoid human error and limit analysis time (Bentley et al., 2023; Corcoran et al., 2021).

8.3.5 Genetic and acoustic sample collection

This thesis has highlighted the importance of continuing efforts to collect morphometric, genetic and acoustic data of bat species. This is particularly important on the African continent and in Central Africa, where few studies on bats have been carried out. The collection of morphometric and genetic data will (i) confirm the discovery of new species, (ii) contribute to the taxonomy of African bats, (iii) distinguish the species present in the *Hipposideros* species complex of this study. In addition, reference acoustic recordings collected in the field and linked to samples will be crucial data for increasing the reliability of automatic acoustic classifiers over the long term.

8.3.6 Towards an even more multidisciplinary study

In order to study and understand the bat-wildlife-human interface, we could add further protocols to cover a wider spectrum of scientific disciplines. For example, we could couple this thesis project with work on the social dimensions of human-bat interactions in order to determine human exposure to bats and their pathogens. Firstly, an anthropological study could provide additional information on the use of caves by communities (e.g., women, men, children, adults, etc), their perceptions of these particular environment and inhabitants

including bats. Secondly, a comparative study of the sharing viromes between humans, bats and other wildlife will better assess the existence of virus spillover while addressing better prevention at the interface.

8.4 Contribution of this work to the EBOSURSY project.

The main objective of the EBOSURSY project was to strengthen the surveillance systems and capacities of countries at risk of zoonotic epidemics in Africa. The Republic of Congo is one of these target countries due to several zoonotic outbreaks such as Ebola, Crimean hemorrhagic fever and mpox. The country is part of the Congo Basin, which contains the second largest rainforest after the Amazon and is home of a rich biodiversity.

In this context, this work focused on the ecology of cave bats in relation to the emergence of zoonotic pathogens. This work participates in the acquisition of knowledge on the identification, phenology and dynamics of communities of insectivorous bats present in caves. This new knowledge of bat ecology could be incorporated into future epidemiological modelling work (requiring local or regional data) and/or analyses aimed at understanding the ecological determinants responsible for pathogen emergence and spread (an example of this type of analysis is provided in **Chapter 7**).

Finally, through its ecological approach, this work has made it possible to define epidemiological hypotheses for the circulation and maintenance of pathogens by targeting the sampling required at the bat-wildlife-human interface. The samples collected during this work in collaboration with a team from the IRD will also allow the screening of viruses. In this way, virological results can be interpreted in the light of the ecological findings of this thesis.

8.5 Conclusion

The latest Covid-19 pandemic, which brought almost the entire world to a standstill, was a shock to our societies, reminding us the epidemiological risks our societies may face in the Anthropocene. The Covid crisis could have been the tsunami destroying the "global organization" of our economy-centric societies, where humans and nature are intertwined. As mysterious and feared as ever, despite the great ecosystem services they provide, bats, which have been our silent neighbors and "the ghosts of our nights" for thousands of years, are the perfect representation of the "wildlife - pandemic" problem. As a result, humans are becoming increasingly fearful of wildlife and living things, finding it easier to avoid danger by destroying them. For example, by killing hundreds of bats to avoid a "next global pandemic" which, ironically, could trigger one (Amman et al., 2014). In addition, much of the research effort is focused on how to prepare and response for the next pandemic. However, the solution is not to prepare for the worst, but rather to attack "the root of the evil" and eliminate risks at the source. To achieve this, we need to take a multidisciplinary look at the malfunctions that we, as a human society, are causing to the natural world. We need to pursue our research into the mechanisms by which zoonoses spread and the science of interfaces, while continuing to explore the biology and ecology of living organisms. This issue is confirmed by the latest Eklipse report (2023) on the relationship between biodiversity and pandemics, which recommends global, participatory research into the wildlife-domestic animal-human interface, integrating interactions using social and natural sciences.

Thus, this thesis contributes to the ecology of African cave bats, relying mainly on non-invasive protocols at the ecological interface between humans and non-human animals while taking the temporal factor into account. The approach developed here makes it possible to accurately identify epidemiological factors contributing to the circulation and maintenance of pathogens.

In addition, caves could be a more 'simple and closed' suitable study model to understanding the sanitary risks and transmission mechanisms at the bat-fauna-human interface. Finally, this work highlights the ecological importance of caves as unique and often neglected and threatened habitats.

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