Health monitoring and medicinal plant use by bonobos at LuiKotale in the Democratic Republic of the Congo

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Thesis submitted in partial fulfilment of the requirements of Liverpool John Moores University for the degree of PhD of Philosophy

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September 2022

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ABSTRACT

Infectious diseases are a major threat to the survival and fitness of all great ape species. Because of their close genetic relatedness, great apes share human susceptibility to a variety of viral, bacterial and parasitic infections. In the context of a growing human population and increasing habitat encroachment, pathogen transmission is of great concern for both humans and great apes. Therefore, monitoring great ape health status is key to understanding pathogen-host dynamics.

Here, I studied two communities of habituated bonobos (*Pan paniscus*) at the LuiKotale field site in the Democratic Republic of the Congo (DRC) between December 2018 and July 2020. I first looked at health impairments and how they influence feeding behaviour and food choice in bonobos. I then investigated the consumption of *Manniophyton fulvum* leaves and stem bark by bonobos, and its association with parasite presence in faeces. Lastly, I measured individual urinary neopterin levels as a marker for the activation of cellular immunity, and studied how urinary neopterin varied with season, individual age, sex, reproductive status, and the presence of visual sickness symptoms.

I found the presence of health impairments predicted neither a change in feeding behaviour nor an increased consumption of medicinal plant candidates. I hypothesized that bonobos used medicinal plant candidates as prophylaxis rather than cures. The consumption of *M. fulvum* leaves and stem bark was not associated with the presence of strongyle worms and cestode proglottids in faeces. My results suggest alternative triggers of the consumption of *M. fulvum*, including intestinal pain and discomfort. Finally, I found urinary neopterin levels increased with the presence of respiratory

symptoms and fluctuated over a one-year period. I discussed the possible link to malaria and other infectious diseases in bonobo's neopterin variation.

This study highlights the extent of medicinal plant use by bonobos at LuiKotale and sheds light on ecoimmunological aspects of the species. Further research is needed to understand the nature of diseases and infections carried by and affecting bonobos in order to better understand how individuals use medicinal plant candidates to maintain and restore health in the wild.

RESUME

Les maladies infectieuses représentent une menace majeure pour la survie et la condition physique des grands singes. Du fait de leur forte proximité génétique, les grands singes et l'Homme partagent une susceptibilité à un grand nombre de virus, bactéries et infections parasitaires. Dans le contexte d'une croissance démographique humaine et d'une diminution de la disponibilité de l'habitat des grands singes, la transmission de pathogènes est préoccupante à la fois pour l'Homme et les grands singes. Par conséquent, le suivi de l'état sanitaire des populations de grands singes est essentiel pour mieux comprendre les dynamiques hôte-pathogènes.

J'ai étudié deux communautés de bonobos (*Pan paniscus*) habitués à la station de recherche de LuiKotale en République Démocratique du Congo (RDC) entre Décembre 2018 et Juillet 2020. Je me concentre en premier lieu sur les problèmes de santé et leurs effets sur les comportements et choix alimentaires du bonobo. Ensuite, j'étudie la consommation des feuilles et écorces de la plante *Manniophyton fulvum* par les bonobos, et son association avec la présence de parasites dans les fèces. Enfin, je mesure les niveaux de néoptérine urinaire, un marqueur de l'activation de l'immunité cellulaire, et j'étudie comment la néoptérine urinaire varie au fil des saisons, en fonction de l'âge, du sexe, et du statut reproductif des individus, ainsi que de la présence de symptômes.

Je montre que la présence des problèmes de santé ne prédit ni un changement du comportement alimentaire ni une augmentation de la consommation de plantes médicinales. J'émets ainsi l'hypothèse que les bonobos utilisent les plantes médicinales à des fins prophylactiques plutôt que curatives. Je démontre que la consommation des feuilles et écorces de *M. fulvum* est dissociée de la présence de

strongles adultes et de proglottis de cestodes dans les fèces. Mes résultats suggèrent des déclencheurs alternatifs à la consommation de *M. fulvum*, comme les douleurs et troubles intestinaux. En dernier lieu, je trouve que la concentration de néoptérine urinaire augmente avec la présence de symptômes respiratoires et varie sur une période d'un an. Je discute le lien possible entre ces variations et la prévalence de maladies infectieuses, y compris le paludisme.

Cette étude souligne l'étendue de l'utilisation des plantes médicinales par les bonobos à LuiKotale et met en lumière les aspects éco-immunologiques de l'espèce. Des recherches complémentaires sont nécessaires pour identifier les maladies qui affectent les bonobos dans le but de mieux comprendre comment les individus utilisent les plantes médicinales pour maintenir et restaurer leur santé dans leur habitat naturel.

DECLARATION

I declare that no portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

ACKNOWLEDGMENTS

I want to start by thanking Barbara Fruth, who believed in me from the start, when I joined LK in 2016. Thanks for many fascinating discussions, for always pushing me forward, for being continuously present even during COVID-times, and for the valuable teaching moments. I will always be grateful to you and to Gottfried for giving me the chance to spend time in LuiKotale, and of course with the bonobos.

Thanks to Shelly Masi and Torsten Wronsky for dedicating your time reading and revising this work, for your pertinent suggestions for improvements and for your kindness.

To Verena and Caro thank you for sharing your experience with me and for teaching me about scientific writing and reasoning. Thanks to Gillian as well for her support navigating through the tumultuous administration of LJMU. Thanks also to Katie, Denise, and Aly for their kind assistance with administrative matters. Thanks to Susanne for welcoming me in Osnabruck and teaching me about parasitology. To Michael Huffman, Victor Narat, Hideo Hasegawa, a warm thank you for sharing some of your valuable knowledge on parasites and medicinal plants with me, and thanks to all who have participated directly or indirectly to the completion of this project. Thanks for long-term support go to Gottfried Hohmann and Zjef Pereboom.

I thank the following institutions, namely the Liverpool John Moores University, the Centre for Research and Conservation/KMDA and the Max Planck Institute for Animal Behavior for their respective financial and logistical support. Meg Crofoot, Keith George, Michael Heistermann, Richard McElreath, Andy Tattersall, Peter Wheeler and Martin Wikelski are thanked for their support in named institutions.

Of course, to Sonya and Giulia, the two best assistants I could have asked for, a massive thank you! Thanks again for your dedication, curiosity, for being my friends, and for all the efforts, sweat and blood you gave to our project, while running through swamps, crawling in lokosa patches, and freezing under rainstorms. I know, aside the awesomeness of it all, it wasn't easy, and you rocked it.

I also want to acknowledge and thank the LK team, Nico, Tommy, Francesca, Maisie, Louise, Alexis, Nathan and all the other I don't name here, with whom I fought for the last piece of fried makemba, and shared single-person snickers. Being 'stuck' in the middle of Congo in the beginning of a global pandemic with you wasn't so bad! To all the Congolese, Lambert, Tsi-Kosse, Innocent, Bowo, Lovis, Baduze and the others in camp and in the village, for your support, your hard work and for sharing your knowledge with me, natondo mingi!

A massive thanks and my eternal admiration to Kat because you're such an inspiration. Your energy is impressive and communicative, you don't know how much your help meant to me in the field and in the office! Thanks also to all my friends in Liverpool, particularly Yannis, Adeline and Lucho, the best housemates; and to those in Konstanz, especially Nadia and Ed for your support and your kindness., and all those in France. Thanks for the laughs, the encouragements, and the support. I carry you in my heart wherever I go.

And last but not least, merci à mes parents et à ma famille pour votre soutien sans limite, pour m'avoir toujours permis de poursuivre mes passions, que vous les compreniez ou non, et merci d'être venu me voir quand vous pouviez, même à l'autre bout du monde. Maman, j'aurais adoré t'emmener au Congo voir les bonobos, et je le ferais si les conditions étaient différentes. Marie, Nico, Hervé et Amandine, merci pour

tout, je n'en serais pas là aujourd'hui, sur le point de devenir docteur en « bébés animaux » si ce n'était pour la force que vous me donnez chaque jour.

AUTHOR CONTRIBUTIONS

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M. Kreyer	Writing – original draft, review & editing
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CHAPTER 2

M. Kreyer	Conceptualisation, Methodology, Data collection, Formal analyses, Writing – original draft, review & editing
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CHAPTER 5

M. Kreyer	Writing – original draft, review & editing
B. Fruth	Writing – review and editing

Work published prior to the submission of the present thesis has been reformatted and language adjusted to British English to fit the requirements of Liverpool John Moores University.

LIST OF ABBREVIATIONS AND NOTATIONS

χ^2	Chi square
BpE	Bompusa East
BpW	Bompusa West
CRC	Centre for Research and Conservation
DRC	Democratic Republic of the Congo
GAMM	Generalized Additive Mixed Model
GLM	Generalized Linear Model
GLMM	Generalized Linear Mixed Model
HIV	Human Immunodeficiency Virus
ICCN	Institut Congolais de Conservation de la Nature
IUCN	International Union for Conservation of Nature
LJMU	Liverpool John Moores University
LKBP	LuiKotale Bonobo Project
LMM	Linear Mixed Model
MHC	Major Histocompatibility Complex
PI	Perhumidity Index
rs	Spearman's rank correlation coefficient
SD	Standard Deviation
SG	Specific Gravity
SIV	Simian Immunodeficiency Virus
uNeo	Urinary Neopterin
VIF	Variance Inflation Factor

CHAPTER 1
GENERAL INTRODUCTION

1.1. Great apes

Great apes, also called hominids, are a taxonomic group of primates comprising eight species from four genera (Figure 1.1). While orangutans (*Pongo* spp) are restricted to the islands of Borneo and Sumatra in Indonesia and Malaysia, gorillas and chimpanzees live in several countries across equatorial Africa. Bonobos (*Pan paniscus*) are endemic to the Democratic Republic of the Congo (DRC), south of the Congo River and their distribution range does not overlap with other African great apes. In the subfamily Homininae (i.e. African great apes and hominids), gorillas only diverged from the *Pan-Homo-Gorilla* ancestor around 10-15 million years ago (Moorjani et al., 2016; Reis et al., 2018) and the *Homo-Pan* lineage diverged as recently as 5 to 7 million years ago (Munch et al., 2012). Chimpanzees and bonobos split from their common ancestor between 2.5 and 0.9 million years ago (Gagneux et al., 1999; Won & Hey, 2005) and are often referred to as human's closest living relatives.

The study of wild great apes took a major step forward thanks to the effort and dedication of three women in the 1960s and 1970s: Jane Goodall, Dian Fossey and Biruté Galdikas, supported and mentored by then world-leading primatologist and anthropologist Louis Leakey. Goodall, Fossey and Galdikas spent years in tropical forests in following and studying the behaviour of chimpanzees, gorillas, and orangutans, respectively. For example, Jane Goodall (1986) was the first to report

behaviours never observed before in animal in general, and great apes in particular, such as tool making and tool use, empathy, and specific aggressive behaviours (e.g. inter-group aggressions and warfare), resembling what anthropologists thought was unique to humans. The work of those three women not only shed light on our understanding of great ape and human behaviours but also revolutionized the scientific methods of primatology. Their studies also raised awareness of increasing threats on wild great ape populations, notably infectious diseases, poaching and habitat loss.

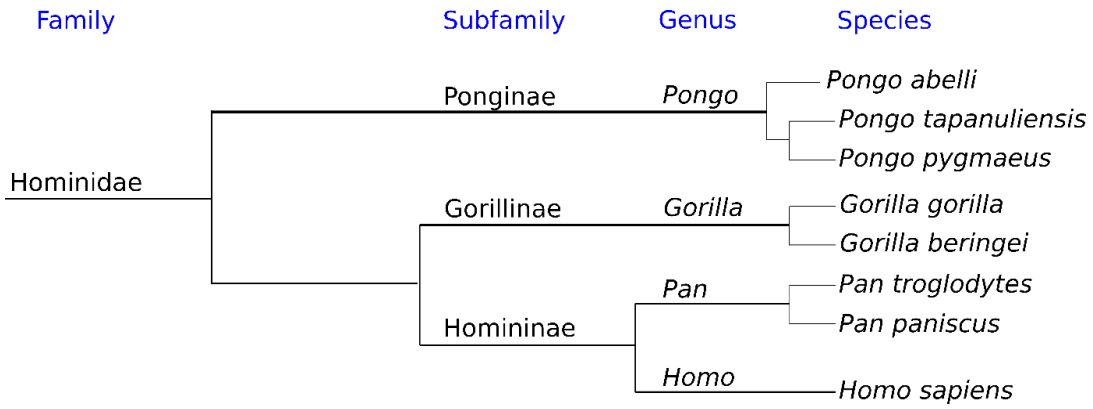

Figure 1.1 | Dendrogram of the Hominidae family or great apes. Only non-extinct species are represented. Branch lengths are independent from evolutive time (adapted from de Sousa & Wood, 2007).

1.2. Why do great apes matter?

Great ape species are of a tremendous importance for their landscapes and ecosystems. In addition to being umbrella species, the protection of which should benefit other species within their habitat (Wrangham et al., 2008), great apes deliver important ecological services (Beaune et al., 2013a; Haurez et al., 2015; Petre et al., 2013). For example, apes play a major role in maintaining the structure and ecological functions

of forest habitats in their range by dispersing seeds of the fruit species they feed on (Chancellor et al., 2017; Tarszisz et al., 2018; Wrangham et al., 1994). Gut passage also optimizes germination success and germination time for many tree species (Tarszisz et al., 2018). In some cases, great apes even benefit the local population indirectly by aiding in the dispersal and early stages of seed recruitment of economically important fruit resources (Aguado et al., 2022). Bonobos are thought to disperse the seeds of 40% of the tree species in the LuiKotale area in central DRC, representing 65% of the local biomass (Beaune et al., 2013a). As such, all great ape species play a major role in maintaining ecosystemic structure and services.

From an anthropological, genetic, behavioural, and physiological point of view, *Pan* species and humans are closely related. Chimpanzees and bonobos have often been used as models for early human ecology and behaviour (Domınguez-Rodrigo, 2002; Sayers & Lovejoy, 2008; Wrangham & Pilbeam, 2001). At the nucleotide level, *Pan* and human genomes are estimated to be 98 to 99% identical (Mikkelsen et al., 2005; Munch et al., 2012). Studies of great ape culture (Whiten, 2010), tool use (Haslam, 2014; Yamakoshi, 1998), hunting strategies (Pruetz & Bertolani, 2007; Stanford, 1995), medicinal plant use (Huffman, 2001), and comparative studies between chimpanzee (*Pan troglodytes*) and bonobo social systems (Ghiglieri, 1987; Parish et al., 2000) exemplify how studying our closest living relatives have helped us understanding where we, humans, come from.

1.3. Past and present threats on great apes' survival

All great ape species are endangered (*Pan* spp.) or critically endangered (*Gorilla* and *Pongo* spp.) according to the IUCN Red List (IUCN, 2021). Major threats include

environmental and land-use change, human population growth, and infectious diseases (Hockings et al., 2015; Junker et al., 2012; Leendertz et al., 2006a). Researchers have long tried to draw more attention to the rapidly declining populations and urge more effective, evidence-based conservation strategies of wild populations (Walsh et al., 2003). However, local politics, lack of people power and funding, and a paucity of knowledge remain major obstacles to stopping and reversing the decline of great ape populations (Benz-Schwarzburg & Benz, 2012; Breuer & Mavinga, 2010). The impact of human threats differs between ape species and populations but overall, all great apes are negatively affected by hunting (Freund et al., 2017; Yamagiwa, 2003) and habitat loss resulting from commercial logging and land-use conversion for agriculture and mining (Miles et al., 2005). Great apes, particularly orangutans, have long inter-birth intervals, from 4.4 y in gorillas to 8.5 y in orangutans on average (Nakahashi et al., 2018; van Noordwijk et al., 2018), putting them at greater risk of short- and long-term extinction from these threats (Meijaard et al., 2012; Rizkalla et al., 2007).

1.4. Infectious diseases in great apes

The risk of infectious diseases to the survival of wild great ape populations became tangible from observations of poliomyelitis-like cases in Gombe chimpanzees (Goodall, 1986), and later from Ebola outbreaks in gorilla (*G. gorilla gorilla*) and chimpanzee (*P. troglodytes troglodytes*) populations in Gabon and the Republic of Congo from 1994-1996 and 2001-2003 (Huijbregts et al., 2003; Leroy et al., 2004). These outbreaks were estimated to have been responsible for a decline of about 80% of the local populations (Leendertz et al., 2006a).

Investigating the origin of those epidemics and optimising strategies to mitigate them in the future are keys to reversing the current decline of great apes' populations. One way to attain this objective is by gaining a better understanding of the health challenges great apes face in the wild, and how they cope with them (Azevedo et al., 2021; Dunay et al., 2018). Preventing epidemics can also be achieved by rigorously implementing preventive measures and health monitoring that reduce pathogen transmission (Azevedo et al., 2021; Leendertz et al., 2017). Those ways happen to fit within the frame of the 'One Health' initiative aiming at understanding the connections between the health of humans, wild animals, and the environment (Azevedo et al., 2021; Craddock & Hinchliffe, 2015; Evans & Leighton, 2014).

Terminology of infectious disease transmission from human to non-human animals and vice versa is heterogeneous in the literature. The WHO/FAO Expert Committee on Zoonoses (World Health Organization, 1959) defines zoonoses as diseases and infections transmitted between vertebrate animals and humans without specifying the direction of transmission. I use zoonosis to describe disease and infection transferred from non-human animals to humans and reverse-zoonosis for disease and infection transferred from humans to non-human animals (e.g. as per Britton et al., 2019; Jia et al., 2021). Several reports described reverse-zoonoses cases that affected great ape populations (Bermejo et al., 2006; Leendertz et al., 2017; Morbeck et al., 1991; Scully et al., 2018). Because of their common evolutionary history, modern humans and hominins exhibit similar immune systems and sensitivity to similar infectious diseases (Leendertz et al., 2006a; Woodford et al., 2002). For example, humans and *Pan* species share functionally similar allele versions of their respective major histocompatibility complex, or MHC class I (De Groot et al., 2002; Maibach et al., 2017), which is responsible for antigen recognition.

African great ape populations have been found suffering heavy consequences from viral epidemics, such as Ebola (Bermejo et al., 2006; Formenty et al., 1999; Leroy et al., 2004), poliomyelitis (Morbeck et al., 1991), respiratory outbreaks (Hanamura et al., 2008; Patrono et al., 2018; Scully et al., 2018), and bacterial epidemics such as anthrax (Leendertz et al., 2004, 2006b). The risk of zoonoses and reverse-zoonoses has long been a cause for concern when habituating great apes for ecotourism and research (Ryan & Walsh, 2011; Woodford et al., 2002). This risk became an even bigger concern following the emergence of COVID-19 in 2019, a coronavirus affecting not only humans, but also many other mammals such as non-human primates, bats, felines, and some mustelids (He et al., 2021; Parolin et al., 2021). Gorillas, chimpanzees and some cercopithecoids primates are as susceptible as humans to COVID-19 infection because they possess the same protein receptors found in humans that the severe acute respiratory syndrome coronavirus 2 (SARS-COV2), the virus responsible for the COVID-19, attaches to (Melin et al., 2020). There has been evidence of infection by and transmission of a strain of the SARS-COV2 in at least four captive gorillas in Czechia (*G. gorilla gorilla*), likely transmitted by humans, which caused fatigue, coughing, and appetite loss (Nagy et al., 2022). At the San Diego Zoo Safari Park, eight gorillas were infected with COVID-19, from which a 48 year-old male with pre-existing cardiac condition developed severe signs, and younger individuals developed mild symptoms (Kalema-Zikusoka et al., 2021). Based on these reports and recent literature (Melin et al., 2020; Morrison et al., 2021; Murphy & Ly, 2021), we can speculate on the impacts of a COVID-19 outbreak on the fitness and survival of wild great apes. Intensified conservation measures need to be implemented to prevent such scenario in the first place (Casal & Singer, 2021).

1.5. Health monitoring of wild great apes

In exceptional cases, human intervention, treatment, and vaccines are used as practical conservation strategies; however, they remain controversial (Ryan & Walsh, 2011). Beyond the direct beneficial effect for the individual, in practice, human interventions can have negative consequences on the study of great apes, particularly when looking at the individual health and diseases in a natural socio-ecological context. This further highlights the high value of remote research sites, where, so far, great apes are preserved from human encroachment and live in almost undisturbed habitats. Except for a few great ape populations followed by veterinarians with human intervention in place when necessary (Fedigan, 2010; Gruen et al., 2013), health monitoring technologies in wild great apes, rely mostly on behavioural observations and analyses of non-invasively collected samples, such as urine, faeces, hair from nests, and saliva (Macfie et al., 2015). Various health related information can be extracted from these samples, including measures of stress, nutritional status, and pathogen load (Jensen et al., 2009; Köndgen et al., 2010; Leendertz et al., 2004).

Commonly used health monitoring methods include the use of urinary dipsticks to measure urine pH, leukocytes, protein, ketones and other urinalysis parameters (Krief et al., 2005; Leendertz et al., 2010; but see also Kaur & Huffman, 2004); endocrinological and immune-indicator measurements in urine, such as neopterin, oxidative stress, cortisol and c-peptide (Behringer & Deschner, 2017; González et al., 2020; Löhrich et al., 2018); gastrointestinal parasite analyses from faecal samples (Labes et al., 2010; McLennan et al., 2017; Vlčková et al., 2018); or the study of individual behaviour (Alados & Huffman, 2000; Ghai et al., 2015; MacIntosh et al.,

2011). In the following section, I review three methods used in the frame of the present work: the behavioural analyses of health, the analysis of gastrointestinal parasites in faeces, and the use of urinary neopterin as a marker of cellular-immune activation.

1.5.1 Behavioural analyses of health

Although commonly used in zoo and sanctuary-housed animals to assess wellbeing (Birkett & Newton-Fisher, 2011; Cabana et al., 2018; Rietkerk & Pereboom, 2018), behavioural studies are typically the least common method used to assess health in wild animals. In natural environments, it can be extremely hard or impossible to identify and measure all factors influencing a wild animal's behaviour. Behaviour is often affected by an individual's health status (Alados et al., 1996; Hart, 1988). For instance, Krief and colleagues (2005) reported in one study on chimpanzees in the Kibale National Park, Uganda, that individuals show symptoms typical of a respiratory infection (coughing, dyspnoea, and lethargy) associated with 1) abnormal feeding pattern (decreased feeding time compared to the rest of the group) , 2) abnormal food choice (feeding on more unripe figs, *Ficus capensis*, than other party members), 3) presence of gastrointestinal parasites in stools, and/or 4) abnormal urinalysis results. In the same study, the authors reported an individual chimpanzee with abnormal urinalysis and high parasitic load feeding on unidentified seeds extracted from fresh elephant dung, soil, and fibrous material from a hollowed dead trunk, as well as another individual with parasitic infection and intestinal disorder feeding on bark soft tissues of *Albizia grandibracteata*. All reported behaviours were unusual in this community and the conditions of those individuals improved significantly following

ingestion of mentioned items. These examples illustrate the possible relation between behaviour and health restoration.

Another method using behavioural analysis was described by Alados and Huffman (2000) and aims to evaluate the general health status of an individual by measuring the complexity of its behavioural sequence, applicable to wild habituated animals. Authors found female chimpanzees had more complex social behavioural sequences, compared to males, and this complexity decreased in sick individuals. These studies (Alados & Huffman, 2000; Krief et al., 2005) show that using behaviour to assess an individual's health status is possible but typically requires controlling for other correlates (e.g. parasite load, urinalysis). The study of health through a behavioural scope is however, often based on anecdotal observations or require very fine continuous observations.

1.5.2 Analyses of gastrointestinal parasites from faeces

Many methods of determining gastrointestinal parasitic load using faecal sample analysis exist (Ballweber et al., 2014; Dib et al., 2019; McNabb et al., 1985; Pouillevet et al., 2017). Selecting a method depends on the nature of the study (for example, whether presence/absence provides enough information or whether identification and quantification is required); the parasite species of interest; field or laboratory conditions; and operator skill. Methods can be categorised under macro- and microscopical investigations. Microscopical analyses include direct smear and concentration methods (i.e. flotation and sedimentation), with and without centrifugation. Parasite species richness and abundance vary widely between great ape species, populations, and seasons, meaning it is necessary to carry out investigations

at a site specific level (McLennan et al., 2017; Narat et al., 2015). For example, studies in bonobos reported inter-site parasite diversity between the Wamba, Lomako and Manzano populations (Dupain et al., 2009; Hasegawa et al., 1983; Narat et al., 2015). Temporal abundance variability was also reported in Manzano, with higher egg counts per gram of faeces in the wet season compared to the dry season for three out of six parasite species (*Strongyloides* sp, Oxyuridae gen. sp. and Dicrocoeliidae gen. sp.), while egg counts from two parasite species were higher in the dry season (Strongylida fam. gen. sp. and *Capillaria* sp.) (Narat et al., 2015).

1.5.3 Use of urinary neopterin as a marker of the cellular immune response

Neopterin is a biomarker of the cellular-immune activation produced by activated macrophages in response to infection by intracellular pathogens, such as viruses and intracellular bacteria and parasites (Berdowska & Zwirska-Korczala, 2001; Hamerlinck, 1999; Widner et al., 2002). Since neopterin is excreted untransformed by the kidneys it can be reliably measured in urine (Müller et al., 1991), and it has been shown to be stable under field conditions (Heistermann & Higham, 2015). Methods for the measurement of urinary neopterin levels have been validated in macaques, chimpanzees, and bonobos (Behringer et al., 2017; Danish et al., 2015). Studies found that neopterin levels typically vary with age, sex, and reproductive status, and follow climatic and/or disease seasonality. In humans and non-human primate species, urinary neopterin levels were higher 1) in early and late life stages (Behringer et al., 2021; Müller et al., 2017; Murr et al., 2003), 2) in fertile and pregnant females compared to other reproductive stages (Boyunağa et al., 2005; Negrey et al., 2021), 3)

with lower ambient temperatures (Löhrich et al., 2018; Mohyuddin et al., 2017), and 4), with increased malaria transmission rates (Picot et al., 1993; also see Altizer et al., 2006). Few studies to date have measured urinary neopterin levels in bonobos (Behringer et al., 2018, 2021; Behringer & Deschner, 2017), and none that I know of in wild adult individuals.

1.6. Zoopharmacognosy and medicinal plant use

With the aim of understanding animal-plant interactions, Janzen (1978) was the first to describe the concept of zoopharmacognosy. Janzen defined zoopharmacognosy as the use of plant items with pharmacological properties by animals. The term zoopharmacognosy was thereafter used by Rodriguez and Wrangham (1993). More research in the field shed light on new behaviours, expanding on the original definition with observations of the use of non-plant material and non-pharmacological means of parasite control using plants. This behaviour has been defined as self-medication in the literature, and generally refers to the avoidance of disease transmission (i.e. prophylaxis) or disease treatment that directly or indirectly enhances an individual's health status and reproductive fitness (Huffman & Vitazkova, 2007). To date, self-medication has been described in diverse taxa such as insects (Abbott, 2014), birds (Clark & Mason, 1985, 1988), and mammals (reviewed in Huffman, 2003), including great apes, from which most data have been collected (Huffman, 1997, 2016; Masi et al., 2012b). Medicinal plant use can be prophylactic or curative, internal or external, and can use chemical and/or mechanical (e.g. presence of trichome on the surface of swallowed leaves) properties of plants. The most researched forms of self-medicative

behaviours have been associated with parasite control; specifically, gastrointestinal parasite control (Huffman, 2003; Huffman & Vitazkova, 2007).

In his study of medicinal plant use by chimpanzees, Huffman (2016) defined four basic criteria to demonstrate curative self-medication. They consist of 1) identifying the disease or symptom(s) being treated, 2) distinguishing the use of the plant item of interest from that of everyday food items, 3) demonstrating a positive change in health condition, and 4) providing evidence for the plant item's activity (Huffman, 1997, 2016). Huffman and others extensively studied two forms of self-medicative behaviour in chimpanzees: bitter pith chewing and leaf-swallowing (Huffman, 2003; Huffman et al., 1996; Koshimizu et al., 1994; Krief et al., 2005). While the former relies on the activity of the biochemical content of the plant part ingested (detailed in Huffman, 2001), the medicative role in parasite expulsion of leaf-swallowing would rely on the mechanical properties of the leaves being swallowed whole and passing through the digestive tract undigested (Huffman & Caton, 2001).

Medicinal plant use has also been suggested to occur in bonobos as an antiparasitic treatment (Dupain et al., 2002; Fruth et al., 2014). So far, reports focused on anecdotal leaf-swallowing of rough surfaced leaves of *Cola* sp., as well as *Manniophyton fulvum* (Figure 1.2). Fruth and colleagues also described the ingestion of stem bark of *M. fulvum*, likely serving the same purpose of parasitic worm expulsion, given the presence of similar trichomes on the bark surface (Fruth, in press; Fruth et al., 2014; Figure 1.3). Hominoids (great apes and humans) are hosts to strongyle species, internal parasites including *Oesophagostomum* spp., and infections can have, in severe cases, consequences on the fitness and survival of the host (Krief et al., 2008; Terio et al., 2011). Because of their life cycle, strongyles should be more infectious during the rainy season (Krepel, 1994; but see Masi et al., 2012a). Previous studies showed that

leaf-swallowing was seasonal, with more observations being recorded during the rainy season (Dupain et al., 2002) or with low ambient temperatures (Fruth et al., 2014). Dupain and colleagues (2002) also found the prevalence of *Oesophagostomum* infections increased after the onset of the rainy season, although it was not explicit if eggs or worms were found in the faeces.

In other studies, Fruth and others (Fruth et al., 2010, 2011; Muganza et al., 2012) showed the extent of overlap between bonobo food repertoire at LuiKotale and the pharmacopeia of the Nkundo indigenous people, inhabiting the area in the proximity of the LuiKotale field site (Fruth et al., 2011; Muganza et al., 2012; Fruth in press; see also Appendix 1). The Nkundo are reported to use at least 226 plant species, of which 180 from 64 families were used for prevention and treatment of different health conditions and disease (Fruth et al., 2010, 2011; Muganza et al., 2012). From those 180 plant species, 32 from 20 families were also consumed by bonobos in LuiKotale, in the central DRC. Such overlap calls for further investigations into whether or not bonobos too, consume those plant items for their medicinal purpose.

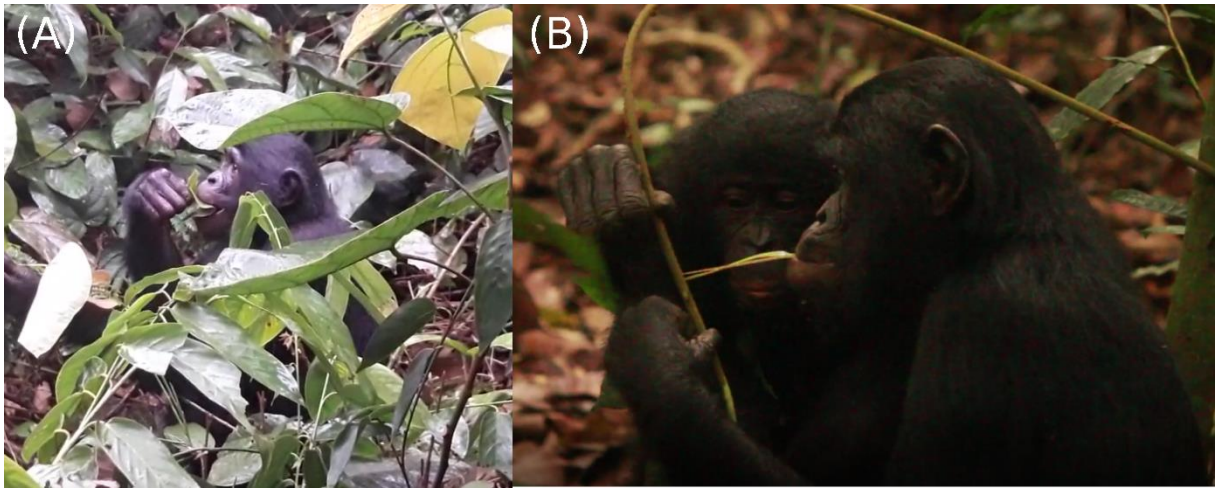

Figure 1.2 | **A)** Adolescent male carefully folding a leaf of *Manniophyton fulvum* before swallowing it whole. **B**) Adult female stem-stripping *M. fulvum* before forming an intricate ball of it in her mouth and swallowing it without chewing. Juvenile daughter was peering at her during the process. *M. fulvum* is used by the Nkundo people for numerous medicinal applications (Muganza et al., 2012) and is thought to play a role in gastrointestinal parasite expulsion in *Pan* species (Boesch, 1995; Fruth et al., 2014; Huffman, 1997). Pictures by M. Kreyer © LKBP.

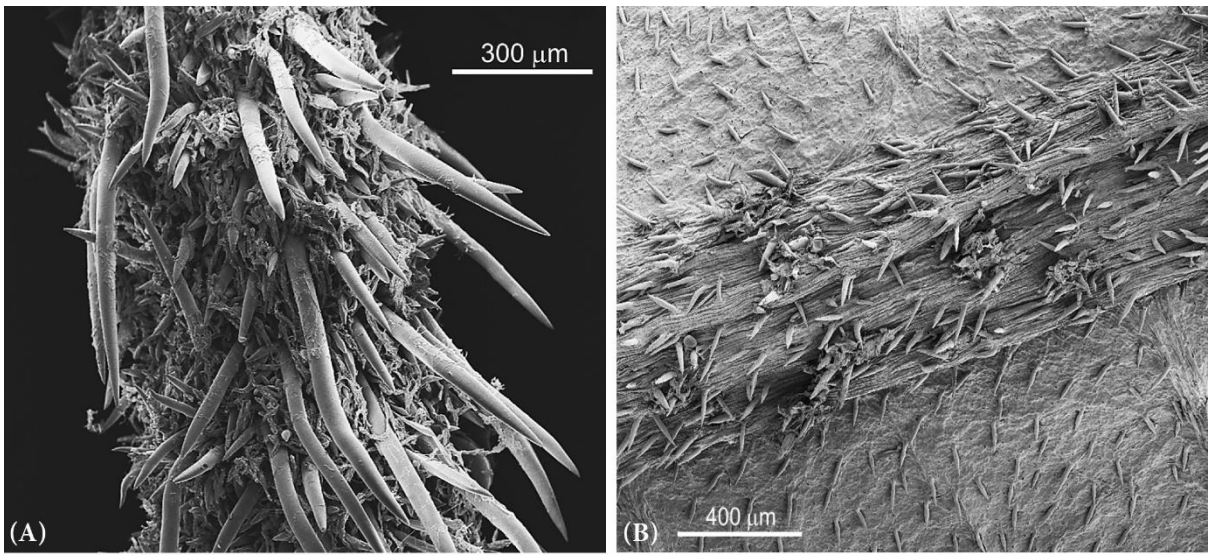

Figure 1.3 | Scanning electron micrographs of *Manniophyton fulvum* (A) stem and (B) upper-leaf surface showing the presence of trichomes. Both plant parts are carefully folded in the mouth and swallowed unchewed. In most cases undigested leaf-packet are found in faeces and, occasionally stem-strip remains (photo from Fruth, in press).

1.7. Bonobo research

The bonobo is the least studied of all great apes, primarily because it was first described and distinguished from its sister species, the chimpanzee, as recently as 1929 (Coolidge, 1933; Schwarz, 1929). Its range, still not fully surveyed to this day, is restricted to one country only, the Democratic Republic of the Congo, where until recently conducting research was extremely challenging. After the DRC achieved independency from Belgium in the mid-sixties, the country slowly fell into corruption and devastation, for almost 30 years, under the dictatorship of Mobutu Sese Seko, only to continue declining further, under the regime of Laurent-Désiré Kabila, third president of the DRC between 1997 and 2001, during which Congo underwent a destructive civil war (Marijnen, 2018; Reinartz & Inogwabini, 2000). Laurent-Désiré Kabila was assassinated in 2001 after what his son, Joseph Kabila, succeeded him at the head of the country, allowing a semblance of peace to return.

In the seventies, the first field research facilities were established in Wamba and Lomako (Badrian & Badrian, 1977; Furuichi et al., 2012; Kano, 1980), both located around a hundred kilometres south of the Congo River in the Tshuapa district (Figure 1.4). However, political instability and threats from armed groups interrupted research, considerably reducing data collected on wild bonobos until the early 2000s and the cessation of armed conflict. More research sites were established again after this, including LuiKotale, founded in 2002 by Gottfried Hohmann and Barbara Fruth outside the western border of the Salonga National Park Block South (Hohmann & Fruth, 2003b; Figure 1.4). Two bonobo communities have since been habituated to researcher presence and are continuously followed by local and international assistants. The habituation of a third community is ongoing since 2017.

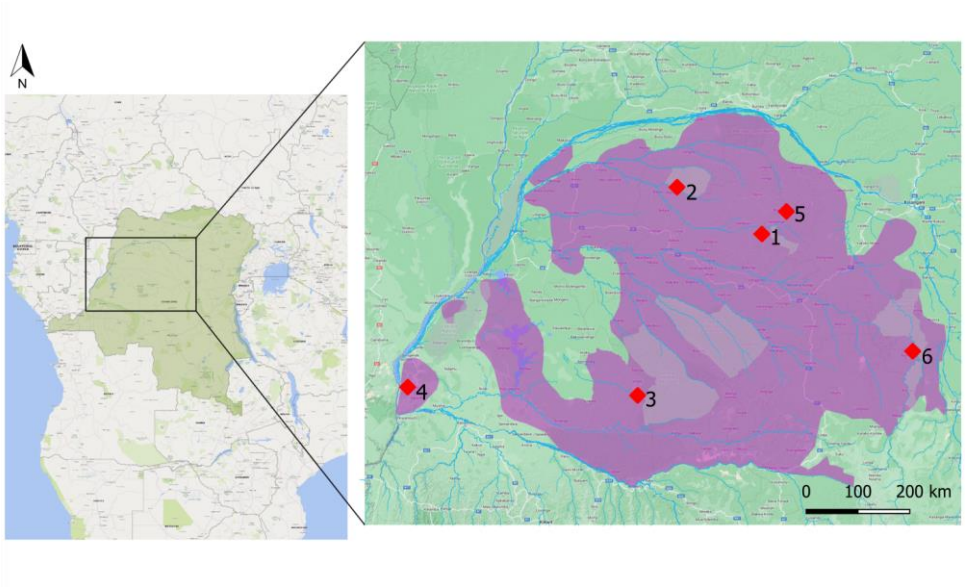

Figure 1.4 | Map of active bonobo study sites (1: Wamba, 2: Lomako, 3: LuiKotale, 4: Malebo, 5: Kokolopori/Lonoa and 6: TL2). Purple area represents the bonobo distribution (IUCN, 2021) and faded grey shapes the protected areas in the Democratic Republic of the Congo (UNEP-WCMC & IUCN, 2022).

Bonobos, like chimpanzees, have a multi-female and multi-male social system with male philopatry, meaning that males remain in their native community and females, around adolescence (i.e. 6-13 y), emigrate to other communities (Furuichi et al., 2012). Social structure is characterized by a fusion-fission dynamic, with individuals splitting to form temporary smaller groups called parties, fluctuating in size and composition. Estimations of home range size are highly variable, ranging from 2 to over 80 km² (summarized in Pennec et al., 2020). Bonobos range exclusively south of the Congo River, preferring dry, *terra firma* forest habitats but also using swamp and heterogenous forests with Marantaceae understorey (Hashimoto et al., 1998; Terada et al., 2015).

Bonobos are mostly frugivorous, with fruits comprising 55-83% of their diet (Beaune et al., 2013a; Kano & Mulavwa, 1984), but also feed from a diversity of food sources,

including leaves, flowers, terrestrial herbaceous vegetation (THV), and meat. At Lomako and Wamba, bonobos have been reported to feed from 110 plant species (Badrian & Malenky, 1984; Kano, 1992; Kano & Mulavwa, 1984 *in* Rafert & Ono Vineberg, 1997). In Manzano, where the habitat is more fragmented compared to other bonobo research sites, bonobos were reported to feed on 78 fruit species (from seeds retrieved in faeces; Serckx et al., 2014). In LuiKotale, Beaune et al. (2013a) found bonobos to feed from at least 149 plant items, from 134 plant species. The observed inter-site variability can be explained by differences in methodology (direct observation versus faecal analysis), variability in phenology of food species, and possibly by a difference in biodiversity indexes, which remain to be tested. The count of food items is still growing, with the more time is spent on observing wild bonobos feeding.

1.8. The LuiKotale field site

LuiKotale (2°45'36"S; 20°22'43"E) is located at the fringe of the Salonga National Park in central DRC, south of the Lokoro river (Hohmann & Fruth, 2003b). The research site originally oversighted over 50 km² of primary evergreen lowland rainforest, in the forest belonging to the village of Lompole and has since expanding to neighbouring forests to cover about 500 km² to date (Bekombo and Mbungusani to the west and Mbongo to the east). The habitat in the LuiKotale area is mostly composed of *terra firma* forest, but also includes periodically and permanently inundated forests as well as swamps. Aside from bonobos, this forest is home of a diverse fauna, including forest elephants (*Loxodonta cyclotis*), African golden cats (*Caracal aurata*), leopards (*Panthera pardus*), giant ground pangolins (*Smutsia*

gigantea) and many others (see Bessone et al., 2020). The nearest village is located 25 km away, and agreements have been made with villagers so that the part of the forest where bonobos are studied is strictly dedicated to research with no hunting and snaring allowed.

Two communities have been habituated to human observers, the Bompusa West (BpW) community since 2002 and the Bompusa East (BpE) community since 2012 and are followed daily from dawn to dusk by local and international assistants. Nest sites are geolocated each day so that observers can find the party the next morning. With permanent presence of a changing team of researchers and assistants on ground, data on bonobo's ecology, behaviour, health and movement along with phenological data on bonobo food tree species are recorded since almost 20 years. Urine and faecal samples are also collected for monthly pregnancy tests on cycling females, as well as for endocrinological and genetic analyses. As of July 2020, the two fully habituated communities BpE and BpW comprised 17 and 25 sexually mature individuals, respectively. Details of community composition are provided in Table 1.1.

Table 1.1 | Size and composition for the Bompusa East (BpE) and Bompusa West (BpW) bonobo communities in July 2020. Females were defined as post-reproductive when they were over 35 y and had not given birth for at least twice the average bonobo interbirth interval.

Community	Sex	Immature (0-10 y)	Mature (>10 y)	Post-reproductive	Total
BpE	Female	6	8	2	**16**
	Male	6	7		**13**
BpW	Female	19	16	1	**36**
	Male	8	8		**16**

1.9. Project aims and significance

From a theoretical standpoint, Inogwabini and Leader-Williams (2012) studied how the occurrence of diseases, namely anthrax, Ebola, monkeypox and trypanosomiasis may have shaped bonobo distribution in DRC. Their results suggested 1) bonobos tolerated the presence of monkeypox and 2) trypanosomiasis was the most important disease in shaping bonobo's distribution, since they did not overlap geographically. Studies have also shown that bonobos possess allotypes of their MHC class I that are functionally similar to those conferring protection against the simian immunodeficiency and human immunodeficiency viruses (SIV/HIV) and, independently, malaria in humans (De Groot et al., 2017, 2018). These studies support a long evolutionary history between bonobos and their pathogens, which may have resulted in species specific resistance to certain diseases, likely excluding trypanosomiasis, and immunological variation. Apart from this and anecdotal reports of respiratory outbreaks and parasitological analyses (Grützmacher et al., 2018; Narat et al., 2015; Ryu et al., 2020), we know little about the health status of bonobos in the wild. As previously mentioned, infectious diseases are one of the major threats to bonobo populations (IUCN, 2021). Gaining a better understanding on individual health is, therefore, of crucial importance for their conservation. This involves monitoring the health status of individuals using innovative tools that can shed light on bonobo strategies to maintain and restore health in their natural habitat.

The concept behind this project comes from the field itself. Since virtually the dawn of time, humans have observed animals and noticed unusual plants consumed for medicine and narcotics (Huffman & Vitazkova, 2007). The idea of zoopharmacognosy emerged in the 1970s and in the late 1980s, and 1990s when observations of medicinal plant use in wild great apes, mostly chimpanzees, accumulated (Huffman et al., 1993;

Huffman & Seifu, 1989; Wrangham, 1995). In the frame of her post-doctoral research, Barbara Fruth (2013) started to study the overlap in the use of plants by riverine human populations and apes. Her work was interrupted by the second Congo war in 1998. When she and her husband, Gottfried Hohmann, finally returned to DRC in 2002, opening a new site (LuiKotale), she focused on the plants used by the Nkundo (Fruth et al., 2010, 2011; Muganza et al., 2012), the ethnic group living traditionally in the central part of DRC, in the Maï-Ndombe region. This work allowed to appreciate the extent of the overlap between the medicinal plant repertoire of the local population with plants consumed by bonobos. Fruth and colleagues' work (2014) on the ingestion of *M. fulvum* leaves and stem bark in LuiKotale bonobos, which she previously observed in Lomako, was a steppingstone into the study of medicinal plant use by bonobos at this site paving the way for further investigations, thus, this project came as a logical follow up.

1.10. Thesis structure

This thesis is based on a continuous 20-month field season of data and sample collection by Sonya Pashchevskaya and Giulia Rossi, my assistants, and myself, at the LuiKotale field site in DRC from December 2018 to July 2020. When necessary, I included data and samples from the long-term database of the LuiKotale Bonobo Project (LKBP).

The second chapter presents 1) a review of the overlap between the Nkundo pharmacopeia and the bonobo food repertoire; 2) observations of sickness behaviours, sickness signs and injuries in LuiKotale bonobos between December 2018 and July

2020 and 3) analyses of how their presence influenced individual's feeding pattern and food choice with the aim of shedding light on medicinal plant use by bonobos.

The third chapter is a follow up from the study by Fruth et al. (2014) of the ingestion of *M. fulvum* leaves and stem bark by bonobos. We revisited the original study design by performing individual focal follows from nest to nest to increase chances of observing the behaviour and collect adequate samples throughout the day. We also performed macroscopic and microscopic analyses on faecal samples to determine the relation between the presence of macro- and microparasites with the ingestion of leaves and stem bark of *M. fulvum*.

In the fourth chapter, we measured urinary neopterin as a marker of inflammation and assessed its variability with health, sex, age, season, and reproductive status.

In the last chapter, I summarise main findings in light of my objectives to better understand bonobo health maintenance and restoration in the wild and the use of non-invasive methods to assess individual's health status.

In the following pages, I present each research chapter (**2** to **4**) as a journal manuscript adapted to LJMU PhD-thesis requirements, either already published (**Chapter 3**), submitted (**Chapter 4**) or currently in preparation for submission (**Chapter 2**).

CHAPTER 2

BETTER SAFE THAN SORRY:

WHAT CAN WE LEARN ABOUT BONOBO'S (*Pan paniscus)* SELF-MEDICATION FROM LOOKING INTO HEALTH STATUS, FEEDING BEHAVIOUR AND FOOD CHOICE?

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Keywords: *Pan paniscus* – bonobo – traditional medicine – feeding behaviour – sickness behaviours – non-invasive health monitoring

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2.1. Abstract

Zoopharmacognosy is the non-human animal use of medicinal plants for the prevention or treatment of disease or symptom. Zoopharmacognosy can be prophylactic or curative to treat symptoms, infectious diseases, or pathogen infections for instance. This behaviour is found in various taxa and explains the occasional consumption of plant parts with high concentration in secondary compounds, which could otherwise negatively impact individual fitness. Health impaired individuals typically display sickness behaviour. Sickness behaviour includes an array of adaptive trade-off behaviours, which reallocate an individual resources into the immune response. The aim of this study was to investigate feeding patterns and food choice in individuals showing sickness behaviours, sickness signs and injuries, to shed light on potential medicinal plant use. We collected data on health and feeding behaviour in habituated bonobos (*Pan paniscus*) at the LuiKotale field site in the Democratic Republic of the Congo between December 2018 and July 2020. We predicted 1) that the prevalence of sickness behaviour would not relate to sex and community, and, that health impaired individuals would consume 2) more vegetative plant parts, and 3) plant items used by local people as traditional medicines. We observed health impaired individuals on 58 days (19% of observation days). We found an overlap with traditional pharmacopeias for 20 out of 313 identified food items bonobos fed on. Our results showed injured individuals, but not those displaying sickness behaviour and signs, consumed more vegetative plant parts than fruits compared to visually healthy group members. Consumption of medicinal plant candidates was independent of the presence of health impairments, suggesting that either those health conditions did not require the use of medicinal plants or medicinal plant candidates were ingested as prophylaxis in the context of health maintenance.

2.2. Introduction

All great ape species, namely chimpanzees (*Pan troglodytes*), gorillas (*Gorilla* spp.), orangutans (*Pongo* spp.) and bonobos (*Pan paniscus*), are endangered or critically endangered according to the IUCN Red List (IUCN, 2021). Threats include environmental changes, human population growth and infectious diseases. Studying and monitoring great apes' health status across populations is critically important to lower the risk of zoonoses (Dunay et al., 2018; Leendertz et al., 2006a; Ryan & Walsh, 2011). While most studies focused on lethal diseases with direct short-term impacts on wild populations (Caillaud et al., 2006; Formenty et al., 1999; Negrey et al., 2019), the understanding of how mild diseases impact the fitness of individuals, and by that the long term survival of these endangered species, is still limited (Behringer et al., 2020; McLennan et al., 2017; Wu, 2019). Monitoring mild diseases requires complex logistics in wild great ape species and usually relies on information extracted from non-invasive samples such as urine and faeces (Krief et al., 2005; Leendertz et al., 2006a). Complementary research has focused on the study of specific behaviours in relation to an individual's health and disease, also called sickness behaviour (Alados & Huffman, 2000; Ghai et al., 2015; MacIntosh et al., 2011).

Sickness behaviour refers to an array of behaviours shown by humans and non-human animals in the course of an immune challenge (Hart, 1988; Lopes, 2014; Tizard, 2008). Are included, behaviours such as inactivity, anorexia, sleepiness, and in social species, withdrawal from social interactions until complete isolation from the group (Marais et al., 2013; McFarland et al., 2021; Stockmaier et al., 2018). Sickness behaviour has been suggested as an adaptive behaviour because it enhances the chances of recovery by re-allocating a sick individual‘s resources to immune functions (Hart 1988). Sickness behaviour may be induced by physiological mediators such as

proinflammatory cytokines interleukin-1β, interleukin-6, and tumour necrosis factor-α (Dantzer, 2004). Experiments have contributed the most to our current knowledge of sickness behaviour (Dantzer, 2006; Johnson & von Borell, 1994; McLinden et al., 2012). However, we know very little about sickness behaviour in wild non-human animals, which generally experience different immunological challenges and socio-ecological constraints. In particular, it is unclear how sickness behaviour influences the individual life history strategies (Stockmaier et al., 2021).

Although great ape food repertoires and associated nutritional requirements have been extensively studied in the past decades, little attention has been given to rare food items they ingest (Hohmann et al., 2012; Nishida & Uehara, 1983; Yamagiwa et al., 2005). Some of these rarely consumed plants are not widely available or, are only consumed occasionally and/or by only a few individuals in a group, despite their widespread availability (Huffman, 2001; Masi et al., 2012b). A plant is primarily ingested for its macro- (carbohydrates, fats and proteins) and micronutrients (vitamins and minerals) (Provenza et al., 2003; Westoby, 1974). In addition, plants contain secondary compounds (e.g. phenolics, terpenoids, alkaloids) that mostly serve to defend the plant against herbivory (Glander, 1982). Secondary compounds may impact animal feeding behaviour (Chapman & Chapman, 2002; Freeland & Janzen, 1974; Glander, 1982), with negative effects ranging from deterrence of feeding activity due to the impact on herbivores fitness to increased mortality (Ginane et al., 2005; Jacobsen, 2021; Oates et al., 1977). Usually, herbivorous species avoid plant parts with significant quantities of secondary compounds and typically seasonally avoid parts they normally consume when these contain increased proportions of antifeedants, such as tannins (Chapman et al., 2003; Glander, 1982; Schoonhoven et al., 2005).

Some plants depend on animal species to disperse. Dispersion via ingestion and defecation of entire seeds is called endozoochory. Endozoochoric plants typically produce fruit containing substances such as sugar, lipids, proteins, or vitamins, that are attractive to disperser species. As such, ripe fruit usually contains low concentrations of secondary compounds (Cipollini & Levey, 1997). Nonetheless, some fruits contain variable concentrations of secondary compounds to optimize dispersion (Whitehead & Bowers, 2013). These fruits are adapted to specific consumers tolerating their secondary compounds and even using it to their advantage (Huffman, 2003; Rhoades & Cates, 1976; Villalba et al., 2014). For example, when ingested, appropriate dosage of secondary compounds may help prevent or reduce bacterial, parasitological, and fungal infestations in a consumer's body, which otherwise cause health impairment (Glander, 1982; Janzen, 1978; Provenza et al., 2007).

Animal self-medication is defined as the search and use of substances by animals to enhance their own health (Costa-Neto, 2012; de Roode et al., 2013; Janzen, 1978). To date, self-medication has been documented in various animal taxa, from insects to great apes (Costa-Neto, 2012; Raman & Kandula, 2008; Rodriguez & Wrangham, 1993). It is classified as 1) preventative, such as when birds use pathogen repellent material for nest-building (Dubiec et al., 2013) or 2) curative. Furthermore, two forms of curative self-medication can be distinguished: the external and internal use of plants with medicinal properties. As an example of external medicinal plant use, Morrogh-Bernard (2008) described processing and subsequent fur-rubbing by orangutans of *Commelina* sp. leaves, commonly used by local indigenous people externally on muscular pain and swellings. Examples of internal use of medicinal plants include the chewing of bitter pith by chimpanzees (Huffman et al., 1997), and the swallowing of

whole leaves by chimpanzees, gorillas and bonobos (Dupain et al., 2002; Fruth et al., 2014; Huffman et al., 1996; Huffman, 2003). The latter is suggested to be an adaptive anti-parasitic behaviour that relies on the mechanical properties of the leaves rather than on their chemical content (Huffman & Caton, 2001).

So far, studies in bonobos focused on the ingestion of leaves and stems of the Euphorbiaceae *Manniophyton fulvum* (Dupain et al., 2002; Fruth et al., 2014; Kreyer et al., 2021), and on the overlap between the bonobo food repertoire and the pharmacopeia of local human populations (Fruth, in press; Ngbolua et al., 2015). The Nkundo people in the central Democratic Republic of the Congo (DRC) use at least 226 plants species, of which 180 from 64 families were for prevention and cure of different health conditions and diseases (Fruth et al., 2010, 2011; Muganza et al., 2012). Some of these were shown to contain bioactive compounds known for their medicinal properties (Fruth et al., 2011; Muganza et al., 2012; Ngbolua et al., 2014). From the medicinal plants used by the Nkundo, 32 species from 20 families are also ingested by LuiKotale bonobos (Fruth, in press), although different parts were used by either humans or bonobos.

Here, we focused on LuiKotale bonobo' health impairments in relation to plant consumption, with specific focus on plants and plant parts listed in the Nkundo pharmacopeia. We assessed the frequency and nature of health impairments (i.e. sickness behaviour, signs and injuries); and whether or not individuals showing health impairment adapted their feeding behaviour and food choice. Specifically, we first predicted that 1) sickness behaviours and sickness signs were not associated with sex or community. Second, we predicted that an individual's health impairment would be

associated with 2) higher rates of vegetative plant part consumption than that of fruits, and 3) increased consumption of food items with medicinal properties.

2.3. Material and methods

2.3.1 Study site and subjects

This study was conducted between December 2018 and July 2020 at the LuiKotale field site (2°45'36"S; 20°22'43"E), located in the buffer zone of Salonga National Park, Block South, DRC (Hohmann & Fruth, 2003b). We collected behavioural data on individuals from two habituated bonobo communities (Fruth & Hohmann, 2018), Bompusa East (BpE) and Bompusa West (BpW). Community sizes and composition are summarized in Table 2.1.

Table 2.1: Size and composition of the Bompusa East (BpE) and Bompusa West (BpW) bonobo communities in July 2020.

Community	Sex	Immature (0-10 y)	Mature (>10 y)	Total
BpE	Female	6	10	**16**
	Male	6	7	**13**
BpW	Female	19	17	**36**
	Male	8	8	**16**

2.3.2 Scan and focal data collection

We followed 18 focal individuals (12 adult females, 6 adult males) of both communities across 359 days, typically on a nest-to-nest basis (n= 3,323 focal hours). Using scan sampling at 5-min intervals (Altmann, 1974), we recorded the activity performed by the focal individual, the distance between the focal individual and its neighbours and the activity performed by each independent individual in sight (except dependent offspring). We retained all focal follows longer than four hours (n= 304; median= 9.5 h, range [4-12.3]). We aimed at following each focal individual once every month, randomizing sex, and community. When we observed an individual exhibiting sickness behaviour (see below) and/or signs, we terminated the ongoing focal follow and started a new one on the sick individual for the rest of the day.

2.3.3 Health impairments

An individual's health was defined as the absence of visual sickness behaviour (Hart, 1988) and/or sickness sign and/or injury. Sickness behaviour was defined as one of the following in combination with sickness signs and/or injury or the combination of at least two of the following: 1) fatigue (increased resting time compared to other group members and/or the individual exited the night nest later in the morning or nested earlier in the evening, increased day nest construction), 2) loss of appetite (the individual fed less quantity and spent less time feeding compared to the rest of the group), and 3) social disinterest (the individual spent less time involved in social interactions compared to the rest of the group). Sickness signs included diarrhoea and intestinal discomfort (upset stomach, gazes, intestinal rumbling) visibly impairing an individual's daily routine in or without combination of sickness behaviour. Injury was

recorded when the integrity of the skin was compromised, such as a cut or bite mark with open flesh or bleeding or when the individual was limping or not using a foot or hand for basic activity (feeding or traveling). Health impairment hereafter is used to refer to sickness behaviours, sickness signs and/or injuries.

For the scan data collection, we used an ethogram including the following activities: 'feed', 'forage', 'rest', 'socialize', 'travel', and 'other'. Definitions are provided in Table 2.2.

Table 2.2 | Ethogram for focal sampling, definition of behaviours.

Behaviour	**Definition**
Feed	Processing and ingestion of a food item
Forage	Active search of food within a feeding patch
Rest	Inactivity with (i.e. sleep) or without closed eyes
Socialize	Affiliative and agonistic interactions with at least one other individual (i.e. grooming, playing, aggressive or sexual interactions)
Travel	Long distance movement (between two food patches for instance)
Other	Any other activity that does not fit the other behaviour described here (e.g. nest construction, drinking, urinating…)

2.3.4 Feeding data collection

In addition to the scan sampling data, we collected all-occurrences of feeding for the focal individual and other individual in sight, recording plant species and part ingested. If necessary, we collected plant specimens and identified them in camp or had them identified by experienced botanists of the Biology Department, Faculty of Science, at the Kinshasa University. In the absence of availability data for most food items, we simplified the concepts of preferred, staple fallback and filler fallback food (defined in Yamagiwa & Basabose, 2009), and distinguished staple from rare food items. For this, we used the frequency of consumption of all food items recorded in feeding scans during the study period and calculated the median. All items with a scan count above the median were considered as staples and those below as rare, being fed on occasionally by few individuals or for a very short period (Figure 2.1).

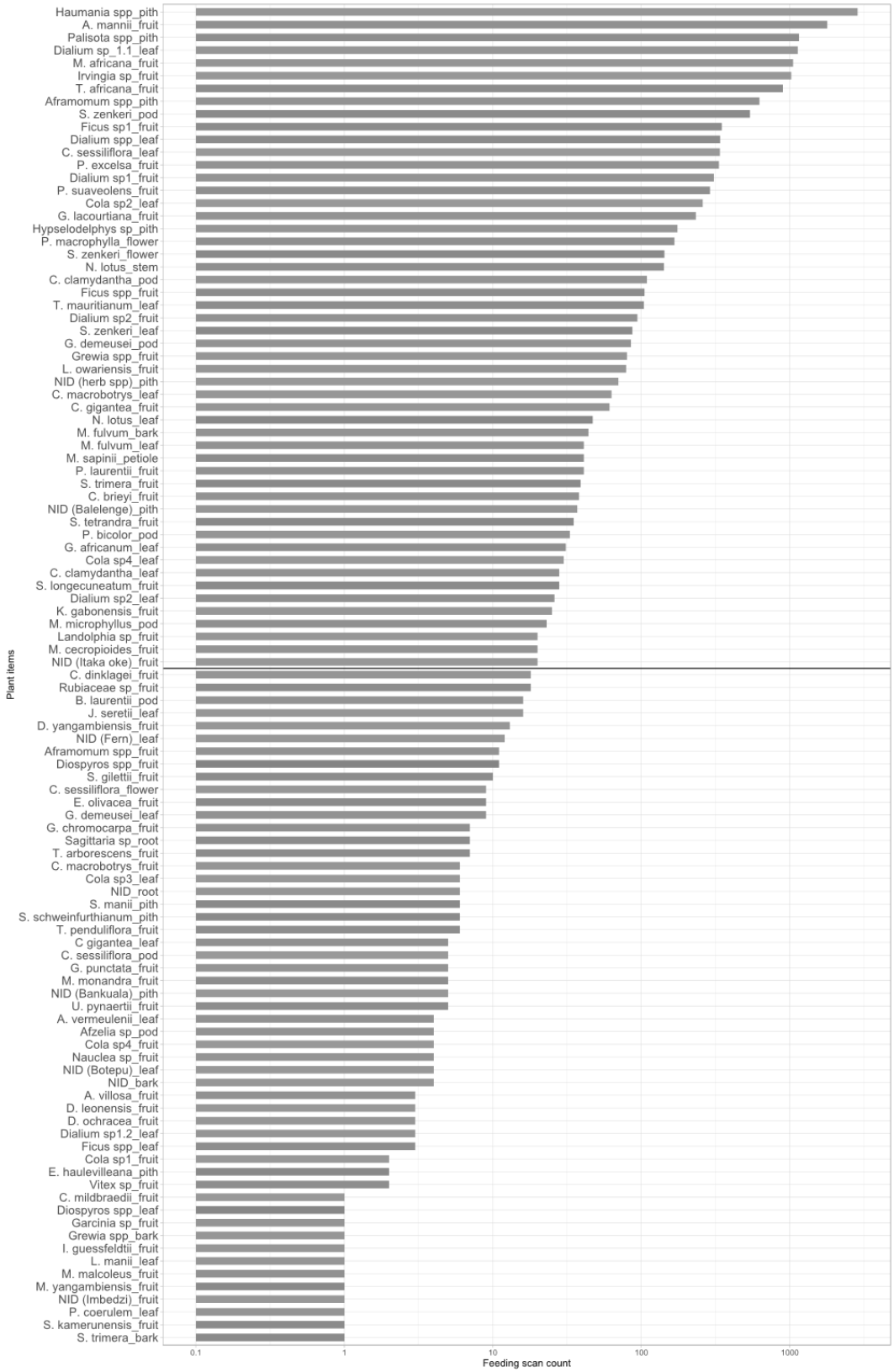

Figure 2.1: Feeding scan count per food item consumed by LuiKotale bonobos between December 2018 and July 2020. Scan counts (n= 22,194) are extracted from scan sampling data on all mature individuals (n= 42). The black line represents the

median value of scan counts (median= 24). All food items above the median line are defined as staples, those below as rare food items.

2.3.5 Medicinal plant candidates

We investigated overlap between the bonobo plant food repertoire (Appendix 1) and plants used in traditional medicine by reviewing literature using the Google Scholar search engine. We focused on traditional medicinal plants used by the Nkundo (Fruth et al., 2011; Muganza et al., 2012, 2016), in DRC (Ali et al., 2011; Eto, 2013; Ngbolua et al., 2014; Nia et al., 2005) and more generally across Central Africa (Betti, 2004; Cousins & Huffman, 2002; Hulstaert, 1966; Iwu, 2014; Ndukwu & Ben-Nwadibia, 2005; Oliver-Bever, 1986; Tcheghebe et al., 2016). We excluded plant items that required specific processing (where specified) such as decoction, ointment, and boiling. We also focused on plant items noticeable by bonobos' processing behaviour, or, as referred to thereafter, unusually processed food items such as bark- and stem-stripping, leaf-swallowing, and consumption of roots and petioles.

2.3.6 Statistical analyses

Using the scan data, we calculated activity budget as the relative frequency of activity categories, using the formula: $y = (n_y / N) \times 100$, where n_y = number of records of category y, and N = total number of records collected during a focal follow (Pinheiro et al. 2013). Using Fisher exact tests with a significance level of $\alpha = 0.05$, we compared activity budgets and defined sickness behaviour as the combination of 1) lower feeding and higher resting rates, 2) higher resting and lower socializing rates, or 3) lower feeding and socializing and higher resting rates, in the focal individual compared to

the nearest neighbour. We used chi square tests to evaluate if the presence of sickness behaviour and sickness signs was independent from 1) sex and 2) community.

2.3.6.1 Party size model

We fitted a linear mixed-effects model (LMM), using the *lmer* function from the 'lme4' package in R (Bates et al., 2015) to test the likelihood that health impaired individuals were more often associated with large or small parties [M0]. In this model, we used the daily mean party size as the response variable and the presence and nature of health impairment as fixed effect. We used sex of the focal individual as a control variable because we suspected males to have increased costs of staying in a large party while suffering health impairment (increased sexual competition, increased aggression rate), and we expected females to seek the safety of a large party (Hohmann & Fruth, 2003a; McFarland et al., 2021; Stockmaier et al., 2021). We also fitted month as a random effect which attests to the natural seasonal fluctuation of party sizes.

2.3.6.2 Feeding behaviour model

We fitted two generalized linear mixed models (GLMM), using the *glmer* function from the 'lme4' package, to investigate whether or not health impaired individuals feed on more vegetative plant parts than fruits compared to the nearest neighbour [M1]. For this model we used the scan data collected from the focal individual and the visually healthy nearest neighbour. The nearest neighbour's behaviour served as reference reflecting behaviour of healthy party members (based on the absence of visual health impairment). Activities of the focal individual and the nearest neighbour were categorized for each scan as follows: 1= focal individual resting; nearest

neighbour feeding; 2= focal individual feeding on vegetative plant parts; nearest neighbour feeding on fruit OR performing other activities; and 3= any other combination of activities not indicating potential sickness behaviour in the focal individual. We categorised "fruits" as fleshy fruits and pods of which seeds were ingested, and "vegetative plant parts" as terrestrial herbaceous vegetation (THV), leaves, bark, and roots. We fitted focal individual and the study month as random intercepts. We included only scans with at least one other individual neighbour being present in addition to the focal individual.

2.3.6.3 Individual feeding behaviour model

We fitted a LMM using the *lmer* function to test if the focal individual consumed more vegetative plant parts in relation to fruits in the presence and absence of health impairment [M2]. For this model, we used the same scan data as in [M1] (see above) and also included scans when the focal individual was without neighbour (individuals were not included in scans when they were out of sight of the observer). As the response variable, we calculated the feeding ratio as the sum of fruit feeding scans minus the sum of vegetative plant parts feeding scans in each focal follow for the focal individual. We used the presence and type of health impairment as fixed effects and individual and study month as random intercepts.

2.3.6.4 Food choice models

We built three binomial GLMMs, using the *glmer* function from the 'lme4' package, to test if the presence and nature of health impairment predicted the consumption of 1) staple [M3] and 2) rare plant items used as traditional medicine [M4], and 3)

unusually processed food items [M5]. To fit those models, we used the feeding data on focal individuals. Bark-stripping episodes were excluded when they were on tree species that bonobos usually and obviously consume for their nutritive cambium (including *Xylopia aethiopica* and *Macaranga* spp.; Fruth pers. comm.) or when they involved all party members, as it is at the opposite of a self-medicative behaviour according to Huffman's definition (2016). We fitted focal individual and study month as random intercepts in all three models.

We conducted all statistical analyses and models in R (R Core Team, 2021). Where appropriate, we checked for the absence of multicollinearity between independent model's factors using the *check_collinearity* function in the 'performance' package (Lüdecke et al., 2021). In all models, we performed a comparison of a full model, containing the fixed and random effects, to a null model containing only control and/or random effects. The significance of the full model to the null model comparison was derived using a likelihood ratio test with the *lrtest* function from the 'lmtest' package (Dobson & Barnett, 2018). Assumptions of normally and homogenously distributed residuals were met in all LMMs, and we inspected diagnostic plots to verify, amongst others, the normal distribution and homoskedasticity of residuals.

2.4. Results

2.4.1 Health impairments

Overall, we observed 147 injuries on 37 different individuals on 103 follow days (15.4%) between December 2018 and July 2020 in both communities (n_{BpE}= 93; n_{BpW}= 54). Most of those injuries were the result of aggressive intra- or inter-community

interactions. We never observed disease outbreaks at the party or community level during the study period. Based on activity budgets and opportunistic observations, 15 of 18 focal individuals showed health impairment on 58 focal follows (n= 304; Table 2.3). Of these, the most observed sickness behaviour was fatigue (8.0% of focal days), and the most observed symptom was diarrhoea (6.4% of focal days), either as an isolated symptom or associated with sickness behaviour. Affected individuals were from both sexes (males: n= 8 observations from 5 individuals; females: n= 50 observations from 10 individuals) and communities (BpE: n= 35 observations from 8 individuals; BpW: n= 23 observations from 7 individuals). The frequency of sickness behaviours and signs did not differ between communities (χ^2= 0, df= 1, p= 1) but females were observed with sickness behaviours and signs more often compared to males (χ^2= 5.21, df= 1, p= 0.02). We observed injuries, mostly bite marks and cuts on limbs and genitals 27 times across 299 focal days (8.7%: BpE: n_{male}= 2, n_{female}= 18; BpW: n_{male}= 1, n_{female}= 6), generally as results of aggressions. The model of party size [M0] did not differ significantly from the null model ([M0] lrtest: χ^2= 2.75; df= 3; p= 0.431), indicating health impaired individuals of either sex were not more likely found in larger or smaller parties.

We compared the activity budget of the focal individual to that of the nearest neighbour for each focal follow (n= 304, within 299 focal days with five days split into two focal follows) using Fisher tests. Individuals fed less and rested more on 6 days, rested more and socialized less on 11 days, and fed less, rested more and socialized less on 1 day, compared to the nearest neighbour.

Table 2.3 | Sickness behaviour, sickness signs and injuries observed on focal individuals between December 2018 and July 2020 (n_{BpE}= 164 and n_{BpW}= 140 focal days). Focal individuals comprise six adult males (n_{BpE}= 3; n_{BpW}= 3) and twelve adult females (n_{BpE}= 6; n_{BpW}= 6). Numbers in brackets represent the percentage of days with health impairment compared to the total focal days per community.

Community	**Sex**	**S. Be**				**S. Si**	**Inj**	**Total**
		Fa	La	Sd	**All S. Be**			
BpE	F	11 (6.7)	6 (3.7)	5 (3.0)	**11 (6.7)**	6 (3.7)	17 (10.4)	**33 (20.1)**
	M	3 (1.8)	2 (1.2)	-	**3 (1.8)**	3 (1.8)	2 (1.2)	**7 (4.3)**
BpW	F	10 (7.1)	1 (0.7)	8 (5.7)	**10 (7.1)**	10 (7.1)	6 (4.3)	**24 (17.4)**
	M	1 (0.7)	-	1 (0.7)	**1(0.7)**	-	1 (0.7)	**2 (1.4)**

Community: **BpE**: Bompusa East, **BpW**: Bompusa West; Sex: **F**: Female, **M**: Male, Health impairment: **S. Be**: Sickness Behaviour; **Fa**: Fatigue; **La**: Lack of appetite; **Sd**: Social disinterest; **S. Si**: Sickness sign; **Inj**: Injury

2.4.2 Food repertoire & medicinal plant candidates

Between December 2018 and July 2020, we observed LuiKotale bonobos ingest 313 plant items from 165 plant species and 43 families (Appendix 1). Of these 165 species, 144 were identified to the genus (n= 99) and 114 to the species level. We recorded 115 plant food items fed on in scans (n= 22,194) and calculated a median of scan counts for all food items of 24 (range [1-2,883]), therefore defining 58 items as "staples" and 57 items as "rare items" (Figure 2.1). Some plant items were not recorded during scans, therefore we could not measure their consumption frequency. We categorized those items as rare. A total of 20 plant parts from 19 species consumed by bonobos were used by the Nkundo or people in surrounding regions for their medicinal properties (Table 2.4). Of those 20 items, eleven were fruits (including one fruit of which latex is used as ethnomedicine), four were leaves, one was stem bark and four were piths. In addition, seven items were defined as rare. For processed food items,

we included feeding scans of the inner bark / cambium of *Santiria trimera*, *Grewia* spp. and one other unknown tree species, the leaf-swallowing and stem-stripping of *Manniophyton fulvum*, the consumption of root / rhizome of *Sagittaria* sp. and an unidentified species, and of petioles from *Millettia sapinii*.

2.4.3 Feeding behaviour

The [M1] full model did not differ significantly from the null model ([M1] lrtest: χ^2= 4.99, df= 6, p= 0.546), indicating that health impaired individuals were not more likely to feed on more vegetative plant parts than fruits compared to their nearest neighbour on a given focal day. In line with results from [M1], the full model for [M2] was not significantly different from the null model ([M2] lrtest: χ^2= 6.41, df= 3, p= 0.093), indicating that health impaired individuals were not more likely to feed on more vegetative plants or fruits compared to visually health ones across the study period. However, we noticed that the estimate from the injured individuals' category was 10-fold that of visually healthy ones and ran an additional model [M2b] including only visually healthy and injured individuals (n= 267). The likelihood ratio tests comparing the full versus null model in [M2b] showed the full model was significantly different from the null model ([M2b] lrtest: χ^2= 5.98, df= 1, p= 0.014), suggesting injured individuals were more likely to feed on vegetative plant parts than fruits in comparison to visually healthy ones.

Table 2.4 | Medicinal plant candidates used as traditional medicine and ingested by bonobos between December 2018 and July 2020 at LuiKotale. Food items: 'staple' = median of ingestion scans ≥ 24; 'rare'= median of ingestion scans < 24.

Local name	Family	Genus	Species	Staple/ rare food item	Part eaten by bonobos	Use(s) in traditional medicine
Baole	Apocynaceae	*Landolphia*	*forestiana*	s	mc; lx	Latex used to treat abscesses * [A]
Bodzinda	Annonaceae	*Polyalthia*	*suaveolens*	s	mc; se	Fruit, root and leaf used as anti-parasitic, anti-inflammatory, and for rheumatism and toothache [B, C]
Bodzungu	Annonaceae	*Isolona*	*hexaloba*	r	mc; se	Masticated seed used to treat abscesses * [A]
Boeko	Ulmaceae	*Celtis*	*brieyi*	s	mc; se	Fruit used to treat tuberculosis [B]
Boele	Burseraceae	*Canarium*	*schweinfurthii*	NA	mc; se	Fruit, stem and bark used as laxative, and to treat cough, venereal diseases and skin eruptions [B, D]
Bokolombe	Myristicaceae	*Staudtia*	*kamerunensis*	r	mc; se	Seed, bark and leaf used to treat gonorrhoea, rheumatism, snakebite, cough, wounds (…) [E]. Exudate used to treat wounds and mycosis * [A, F]

Boleleko	Piperaceae	*Piper*	*guineense*	NA	mc; se	Leaf and/or fruit and fruit juice used to treat cough, stomach aches and as antiemetic, anti-parasitic [G, H, I]
Boseki ya moindo	Irvingiaceae	*Klainedoxa*	*gabonensis*	s	mc; se	Fruit used to treat abscesses * [A]
Botendo	Clusiaceae	*Garcinia*	*chromocarpa*	r	mc; se	Seed used as analgesic [J]
Botumbe (leaves)	Cecropiaceae	*Musanga*	*cecropioides*	NA	mc; se; lf	Leaf used to treat abscesses * [A]
Fumbwa	Gnetaceae	*Gnetum*	*africanum*	s	lf	Aerial parts used to treat diabetes, piles, high blood pressure. Leaf used as antiemetic and antidote for certain types of poisons [K]
Koka	Euphorbiaceae	*Maesobotrya*	*floribunda*	NA	lf	Leaf used as is on wound (circumcision)* [A]
Lokosa	Euphorbiaceae	*Manniophyton*	*fulvum*	s	sb	Stem bark used to treat cough [L]
Lokosa	Euphorbiaceae	*Manniophyton*	*fulvum*	s	lf	Leaf used to treat wounds, dysentery and diarrhoea and used as anti-inflammatory [L]
Ntetele	Commelinaceae	*Palisota*	*ambigua*	s	pi; rt	Stem used pounded to treat snake bites * [M]
Ntetele	Commelinaceae	*Palisota*	*brachythyrsa*	s	pi; rt	Inner stem used to treat tropical ulcers * [N]
Ntetele	Commelinaceae	*Palisota*	*hirsuta*	s	pi; rt	Leaf and stem used to treat sore throat, cough, and toothache and sap used as anthelmintic [B]

Ntetele	Commelinaceae	*Palisota*	*schweinfurthii*	s	pi; rt	Stem used pounded to treat wounds * [A]
Saake	Flacourtiaceae	*Caloncoba*	*welwitschii*	r	mc; se	Fruit used to treat mental illness, asthma, gall bladder infections and worms [B]
Tondolo	Zingiberaceae	*Aframomum*	sp.	s	mc; se	*A. giganteum* seed used as anthelmintic and the fruit pulp as a light laxative O; A. melegueta fruit and seed used as anthelmintic and to treat several infectious diseases H; A.sanguineum seed used as anthelmintic and to treat stomach-aches B

* Topical application, mc: mesocarp, lx: latex, se: seed, lf: leaf, sb: stem bark, pi: pith, rt: root; References: [A] Fruth et al., 2011, [B] Cousins & Huffman, 2002, [C] Muganza et al., 2016, [D] Tcheghebe et al., 2016, [E] Iwu, 2014, [F] Eto, 2013, [G] Muganza et al., 2012, [H] Ndukwu & Ben-Nwadibia, 2005, [I] Oliver-Bever, 1986, [J] Lambert Booto pers. comm., [K] Ali et al., 2011, [L] Nia et al., 2005, [M] Betti, 2004, [N] Hulstaert, 1966, [O] Raponda-Walker & Silans 1961 *in* Cousins & Huffman 2002

2.4.4 Food choice

From the rare items we retained as medicinal plant candidates (n= 8), three did not appear in the feeding data (fruits of *Piper guineense*, and leaves of *Musanga cecropioides and Maesobotrya floribunda*), meaning they were either consumed by non-focal individuals or on non-focal days. The other five (fruits of *Canarium schweinfurthii*, *Isolona hexaloba*, *Staudtia kamerunensis*, *Garcinia chromocarpa* and *Caloncoba welwitschii*) were rarely consumed by bonobos (median= 2 focal follows, range [1-4]). The likelihood ratio tests comparing the full versus null models showed the full models for the binomial GLMMs [M3] to [M5] did not differ significantly from null models (lrtest: [M3]: χ^2= 0.46, df= 3, p= 0.928; [M4]: χ^2= 2.04, df= 2, p= 0.564; [M5]: χ^2= 3;03, df= 3, p=0.386), indicating that the consumption of medicinal plant candidates and processed plants was not significantly associated with the presence of health impairments.

2.5. Discussion

Here, we investigated food choice and feeding behaviour in relation to the presence of health impairments in habituated bonobos at LuiKotale, providing the first observations for this species. Focusing on the food repertoire, bonobos consumed 20 of 313 plant items ingested across 20 months of observation, known to be traditionally used by the Nkundo people and other Central African local communities. We observed sickness behaviours, sickness signs and injuries in focal individuals from both communities 58 times during the study period (19.4% of follow days). Results showed a sex bias, with females being observed more often with sickness behaviour and signs than males. We found injured individuals – but not those displaying sickness behaviours and signs – were more likely to consume more vegetative plant parts than

fruits compared to visually healthy ones. Lastly, our results showed that the presence of health impairment did not predict a higher ingestion of ethnomedicinal plants or unusually processed food items.

2.5.1 Health impairments

The most observed sickness behaviour was fatigue or lethargy, a decreased activity level or increased resting rate when compared to other members in the party. Reducing activity is a common sickness behaviour and is said to re-allocate individual resources to the immune system (Hart, 1988). Some individuals showing sickness behaviours and signs such as fatigue and diarrhoea had potentially pathogenic parasites in their faeces (Kreyer et al., 2021), while others did not. However, the absence of parasite egg/cysts/larvae in the faeces is not necessarily evidence for the absence of parasitic infection (Muehlenbein, 2006).

Parasite infection may therefore be a cause for sickness behaviour even in the absence of parasites in faeces. These observations corroborate observations from wild red colobus monkeys, in which individuals suffering from whipworm infections showing increased resting and a decrease of energetically costly behaviours compared to individuals with no whipworms found in their faeces (Ghai et al., 2015). Previous studies showed whipworms can modulate the host immune response by reducing secretion of interleukin-1β and tumour necrosis factor α, two inducers of sickness behaviours (Darlan et al., 2021). In this case, the origin of the sickness behaviour is likely physiologically different, but nevertheless similar in nature, with infected individuals showing reduced grooming and increased resting compared to non-infected ones (Friant et al., 2016; Ghai et al., 2015; Wren et al., 2021).

The nature of sickness behaviour also varied with the infecting parasite species in vervet monkeys (Blersch et al 2021). In individuals infected with *Protospirura* sp., higher egg counts in faeces were associated with lethargy and anorexia. However, when individuals were infected with *Trichostrongylus* sp., higher egg counts were associated with decreased resting and more time spent foraging, which the authors explained as a possible mechanism to increase energy intake to fight off the infection. Although the lack of physiological correlates in our study did not allow us to determine the origin for the sickness behaviours and signs observed with certainty, further study could shed light on the distinction between different induction mechanisms that cause various sickness behaviours.

During the study period, we also measured urinary neopterin levels (**Chapter 4**) which is a marker of the cell mediated immune response. Elevated neopterin levels suggested individuals suffered infectious diseases at times, such as respiratory infections, caused by unidentified intracellular pathogens (Kreyer et al. submitted/Chapter 4). During the study period, we measured elevated urinary neopterin levels for six individuals displaying sickness behaviour and/or signs (median= 4,048.7 nmol/L corrected for specific gravity (corr. SG), range [1,284-8,100.9], median $_{healthy}$= 820.5 nmol/L corr. SG, range $_{healthy}$ [83.9-6,316.2]).

Both gastrointestinal parasite and intra-cellular pathogen infections were shown to induce sickness behaviour in chimpanzees (Krief et al., 2008; Wu et al., 2018), vervet monkeys (Blersch et al., 2021) and red colobus monkeys (Ghai et al., 2015). Further analyses are required to identify the infectious diseases and pathogens bonobos carry and/or suffer from at LuiKotale, and to assess their influence on individual sickness behaviour.

Our results showed a bias in sickness behaviours and signs prevalence in females compared to males that may be explained by variation in party composition, given bonobo parties are typically comprised mostly of females (Furuichi, 2009, 2011; Hohmann & Fruth, 2002). Mating opportunities trigger male party attendance and male-male aggression, with aggressors having higher mating rates compared to targets (Hohmann & Fruth, 2003a). Health impaired males should therefore avoid large parties, as they risk becoming target of directed aggression and costs of associating with other males may exceed the benefits (Hohmann & Fruth, 2003a; McFarland et al., 2021; Stockmaier et al., 2021).

In vervet monkeys, febrile individuals displaying sickness behaviours were targeted with twice as much aggression by other individuals and were more likely to receive injuries compared to afebrile monkeys (McFarland et al., 2021). In contrast, female bonobos are highly cohesive leading to a high party attendance (Furuichi, 2011). While males may become more solitary when sick, females may seek the safety of the group when immune challenged or carrying injuries even when engaging into less social interactions. Thus, the observed bias may be system inherent and not an actual sexual difference in sickness. Looking at fitness consequences (e.g. paternity and number of surviving offspring) of displaying sickness behaviours in bonobos should provide additional information as to why it is seen so rarely in males compared to females.

Nevertheless, other studies showed that an animal's sex can influence the expression of sickness behaviour. For instance, an injection of interleukin-1 β, responsible for inducing sickness behaviour, inhibited sexual behaviour in normally cycling female, but not male rats (Yirmiya et al., 1995). Sickness behaviour likely involves trade-offs. Individuals must re-allocate energy to the immune system at the cost of other

behaviours, such as sexual behaviours that are important for an individual's fitness. In males, it may be advantageous to suppress infection cues when presented with a chance to mate, therefore gaining immediate fitness advantages (Lopes, 2014; Owen-Ashley & Wingfield, 2006; but see Weil et al., 2006).

Our results suggested health impaired individuals did not attend smaller or larger parties compared to visually healthy individuals. In other species, social context modulates the intensity of sickness behaviours. For example, after an immune challenge, zebra finches exhibited reduced activity levels when isolated from the group but not when they were kept in colonies (Lopes et al., 2012). As such, different parameters likely influence the occurrence and nature of sickness behaviours. Although sickness behaviours are likely not disease-specific, observations can still provide useful insights into self-medicative behaviours (Ghai et al., 2015). Our results provide a useful foundation for improving our understanding of social triggers of sickness behaviours in wild bonobos.

2.5.2 Food repertoire & medicinal plant candidates

During the study, bonobos fed on 165 plant species, representing a relatively small subset of their food repertoire. We found an overlap of 48.7% with the food repertoire detailed by Beaune et al. (2013a), who observed bonobos feeding on 133 plant species over 22 months (September 2009 and June 2011). This highlights the high degree of variation in both bonobo food choice, and likely plant part phenology in the study area (Hohmann et al., 2010; Fruth & Hohmann unpublished data). Including phenology records of bonobo plant foods, particularly those of items rarely eaten and non-fruit

items, would allow a more detailed investigation of the relationship between availability and consumption in future studies.

During the study period, 20 plant items from 19 species that we observed bonobos feed on overlapped with ethnomedicinal plants used by the Nkundo or traditional healers in surrounding regions. We included these items in our model despite some of them (n= 10) being used externally in traditional remedies. Of those ten items, six were defined as staples in our study (two fruit and four THV pith items). Even though they are consumed regularly, these items likely contain bioactive compounds and could also have beneficial health effects when ingested by bonobos. However, depending on the nature and combination of the active compounds, they may be poorly absorbed or metabolized taken orally, resulting in a low bioavailability (Zhang et al., 2012).

2.5.3 Feeding behaviour

Vegetative plant parts, namely roots, stem and root bark, and leaves are often used in traditional medicines instead of fruits because they likely contain higher concentrations of secondary compounds (Ngbolua et al., 2014). Bonobos that were health impaired were not more likely than visually healthy individuals to increase the proportion of vegetative plant parts in their diet relative to their fruit intake, in contrast to our prediction. Because we wanted to compare the behaviour from the focal individual to that of visually healthy individuals, we removed all scans when no neighbour behaviour was recorded, possibly including those in which the focal individual had left a party to search for medicinal plants. Increasing the density of

scans or using continuous animal sampling should improve the chance to capture rare and short-lasting events in future studies.

Nevertheless, when we included all focal scans the health impaired individuals did not exhibit significantly different feeding behaviour compared to visually healthy individuals, except for injured ones, that consumed more vegetative plant parts than fruit. Because we did not observe any change in the feeding behaviour of non-injured individuals, it is possible this difference was a consequence of debilitating injuries, which could result in individuals having difficulty travelling arboreally and preferring to feed on more easily accessible terrestrial vegetation, including THV and shrub leaves.

2.5.4 Food choice

Amongst the rare medicinal plant candidates retained for the food choice model, only five of eight species were consumed by a focal individual on eleven focal days. This 'rare event bias' could have masked an effect of the presence of health impairments on the consumption of ethnomedicinal plants (King & Zeng, 2001). However, although those plant items may influence bonobo health, their properties will likely be specific to a condition or symptom which would make the opportunistic observation even rarer, and sample sizes insufficient for statistical power. We also did not account for quantity ingested, or bioavailability, and possible food / substances combinations on those days, given the rarity of the feeding bouts.

Given our data, we were not able to conclude that the presence of any of the health impairment predicted a tendency to a higher consumption of either plants with pharmacological properties, whether rare or not, or "processed" food items, in

contradiction to our third prediction. Although, we noted a link when opportunistically observing individuals with sickness behaviours ingesting ethnomedicinal plant parts (e.g. fruits of *Greenwayodendron suaveolens*, *Isolona hexaloba* and *Aframomum* sp.). These observations – that is, consumption of ripe fruit while associating with other apparently healthy individuals – did not indicate a curative self-medicative behaviour (Huffman, 2016). Instead, the absence of association between health impairment and consumption of medicinal plant candidates suggests that consumption may have a prophylactic benefit. Although difficult to test, future studies could focus on looking at whether or not individuals ingesting medicinal plant candidates show less frequent sickness behaviour and signs compared to those that do not consume them.

Given our records of health impairments, and the fact they did not appear highly debilitating, we may speculate that most cases did not call for medicinal plant use. In addition, bonobos exhibit pathogen avoidance behaviours, such as removing ectoparasites by grooming, avoiding of contaminated food, constructing new sleeping nests daily and nomadism (Hart & Hart, 2018; Sarabian et al., 2021). The use of medicinal plant items in bonobos may be a 'last recourse' when facing an immune challenge or very specific health conditions and symptoms. A similar behavioural paradox exists with respect to tool use in bonobos. Captive bonobos have been shown to use a vast array of and apply tools efficiently (Bardo et al., 2016; Jordan, 1982), while any use of tools is rare in wild populations (Furuichi, 2015; Koops et al., 2015; Samuni et al., 2021).

2.6. Conclusion

Our study is the first to investigate sickness behaviours, sickness signs and injuries in wild bonobos and estimate the influence of their presence on unusual food choice and feeding behaviour. During the study period we did not observe group epidemics, and most sickness behaviours and signs lasted only briefly. We observed a sex bias in the prevalence of sickness behaviour and signs, with females displaying higher rates than males. Whether females are more likely than males to be sick or to display sickness behaviour at the same infection level requires further investigation, using physiological markers. We highlighted the complexity of social and environmental factors that may influence the prevalence and frequency of sickness behaviours. During the study, both communities appeared relatively healthy, but we lack reports from other sites to allow comparison. We did not find substantial evidence that feeding behaviour is an adaptive response to sickness, although injured individuals consumed more vegetative plant parts than fruits compared to visually healthy individuals. Vegetative plant parts may contain higher concentrations of secondary compounds and investigation on their specific content is required in future studies. We found that the presence of health impairment did not predict the consumption of ethnomedicinal plant items. The consumption of medicinal plant candidates in bonobos may play a preventative role in health maintenance. We surmised that LuiKotale bonobos did not develop severe infectious diseases that necessitated the use of medicinal plants. Instead, sickness behaviours were likely sufficient to re-allocate resources in immune defences, allowing individuals to recover quickly. In conclusion, LuiKotale bonobos ingest plant items with medicinal potential, but ingestion does not appear to be related to visible health impairment.

2.7. Ethics statement

All research activities complied with protocols approved by the Institut Congolais pour la Conservation de la Nature (ICCN) and adhered to the legal requirements of the Democratic Republic of the Congo (DRC). The research project was approved by the ethics committee of Liverpool John Moores University (LJMU). This study also complied with the Guidelines for health monitoring of great ape populations (Macfie et al., 2015).

2.8. Acknowledgments

We thank the Institut Congolais pour la Conservation de la Nature (ICCN) for granting permission to work at the LuiKotale field site. We also thank all villages in the study area for facilitating research in their forest. Funding for research in the field came from the Centre for Research and Conservation of the Royal Zoological Society of Antwerp (CRC/ KMDA), the Max-Planck Institute of Animal Behavior, Department for the Ecology of Animal Societies (MPIAB), and Liverpool John Moores University. Special thanks for long-term support go to Gottfried Hohmann and Zjef Pereboom. Giulia Rossi, Lambert Booto, and the LuiKotale Bonobo Project team are thanked for their assistance in the field, and Kathrine Stewart and Ed McLester for their advice on statistical analyses. Andy Tattersall, Keith George, Peter Wheeler, Richard McElreath, and Meg Crofoot are thanked for their support in named institutions. Last but not least, we thank Verena Behringer for her help on the statistical approach and suggestions that allowed significant improvements on this manuscript.

CHAPTER 3

WHAT FAECAL ANALYSES REVEAL ABOUT *MANNIOPHYTON FULVUM* CONSUMPTION IN LUIKOTALE BONOBOS (*PAN PANISCUS*): A MEDICINAL PLANT REVISITED

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Kreyer, M., Stewart, K., Pashchevskaya, S., & Fruth, B. (2021). What fecal analyses reveal about *Manniophyton fulvum* consumption in LuiKotale bonobos (*Pan paniscus*): A medicinal plant revisited. *American Journal of Primatology*, e23318. https://doi.org/10.1002/ajp.23318

This chapter was published in August 2021 and reformatted for consistency and LJMU thesis requirements. All supplementary materials are included in the chapter.

3.1. Abstract

Observations of animals in the wild can result in the discovery of plants for human medicinal purposes. In this context, our closest relatives, the great apes, are of particular interest. The Euphorbiaceae *Manniophyton fulvum* possesses both phytochemical and biomechanical properties. Its use in the genus *Pan* (*P. troglodytes*; *P. paniscus*) is thought to be based on its mechanical properties promoting the egestion of intestinal parasites, but additional observations from different habitats where the behaviour is performed may shed more light on its true purpose. To improve our understanding of what triggers this behaviour, we investigated *M. fulvum* consumption in wild bonobos at LuiKotale, Democratic Republic of the Congo between December 2018 and July 2020. Specifically, we tested the hypothesis that *M. fulvum* ingestion is related to gastrointestinal parasite expulsion. Of 649 focal follows of 37 individuals from two habituated communities, consumption of *M. fulvum* was observed on 111 days (n= 507), independent of seasons, environmental factors, and the plant's availability. A total of 588 faecal samples were assessed for the presence/absence of gastrointestinal parasites. We found strongyle eggs in 2.89% of samples and their presence was not associated with the ingestion of *M. fulvum* or environmental conditions. We discuss the importance of seasonality in the life cycle of strongyle species that may influence the pattern of *M. fulvum* consumption observed at LuiKotale. Our data open additional perspectives concerning behavioural parameters such as the existence of a cultural component when comparing ingestion behaviour between communities.

Keywords: bonobo, leaf swallowing, *Manniophyton fulvum*, *Pan paniscus*, self-medication, zoopharmacognosy

3.2. Introduction

Humans (*Homo sapiens*) use medicinal plants (Bailung & Puzari, 2016; Efferth & Greten, 2014; Hardy et al., 2013). Medicinal plant use, however, is not specific to humans. Other animal species also choose organic or inorganic items from their environment that contain pharmacological substances to treat diseases or symptoms (Costa-Neto, 2012; Huffman, 2003; Lozano, 1998). Rodriguez and Wrangham (1993) proposed the term "zoopharmacognosy" to describe the process by which wild animals select and use specific plants with medicinal properties for the treatment and prevention of disease. Zoopharmacognosy in animals describes the use of plants and other items ingested or applied topically in a manner that differs from a species' typical use of these items. The term is often associated with, but not limited to, antiparasitic behaviours (Costa-Neto, 2012). Great apes (orangutans: *Pongo* spp.; gorillas: *Gorilla* spp.; bonobos: *Pan paniscus*; and chimpanzees: *P. troglodytes*) are particularly useful as models for investigating the evolution of medicinal plant use in humans due to their phylogenetic relatedness. Indeed, the *Homo-Pan* lineage only diverged 5 to 7 million years ago (Prüfer et al., 2012), thus making *Pan* species our closest relatives. Since chimpanzees and bonobos use habitats that likely resemble those of early hominins (Sommer & Ross, 2011), inferences about early hominin medicinal plant use can be drawn from studying the overlap in that of indigenous people and great ape species (Cousins & Huffman, 2002; Fruth et al., 2011; Obbo et al., 2013).

One example of ape medicinal plant use that has received considerable attention is the swallowing and subsequent excretion of intact leaves by chimpanzees (e.g., Huffman et al., 1996; Mclennan & Huffman, 2012; Wrangham, 1995). Since these leaves are excreted intact, the purpose of performing this behaviour must extend beyond the uptake of nutrients or secondary compounds (Messner & Wrangham, 1996). In 1996,

Huffman and colleagues ended speculations concerning the mode of action of those leaves, reducing them to their only common denominator: the presence of a rough surface bristled with trichomes (1996). Huffman and Caton (2001) explained the antiparasitic role of this property with the leaves' bristly surface increasing intestinal tract motility when swallowed without chewing, particularly when ingested on an empty stomach. By that, gut passage time was said to be reduced to 6.5 h, mechanically inducing larvae expulsion by increased peristalsis and "flushing" of the bowel.

African great apes are commonly infected with several species of gastrointestinal parasites. The leaf swallowing behaviour has been suggested to control existing infections by the nematode *Oesophagostomum stephanostomum* and the cestode *Bertiella studeri* (Huffman et al., 2009; Mclennan & Huffman, 2012; Wrangham, 1995). *Oesophagostomum* spp. (Chabertiidae) infection occurs by ingesting food on the ground that is contaminated with L3 larvae which is more typical at the onset of the rainy season when climatic conditions favour larval infectivity (Polderman & Blotkamp, 1995). In the wild, great apes can become infected with *Oesophagostomum* sp. larvae and subsequently develop nodules in their gastrointestinal tract without necessarily presenting severe clinical signs (Krief et al., 2008). However, infection may cause oesophagostomiasis, which can cause increased weight loss, intermittent diarrhoea and/or hepatic complications, leading to fatal outcomes in some cases (e.g., in captive conspecifics: Krief et al., 2008; Rousselot & Pellissier, 1952). In the case of *B. studeri*, infection occurs when larvae-carrying orbatid mites (order Oribatida) are ingested and the larvae subsequently migrate to the intestines and develop into adult (Huffman et al., 2009; Metzger, 2015). Pathogenicity in nonhuman primates is comparatively low, with possible symptoms including abdominal pain, diarrhoea, loss of appetite, and fatigue (Metzger, 2015). In chimpanzees, the seasonality of *B. studeri*

proglottid egestion varies between sites (Huffman et al., 2009). Thus far, there is no published record of *B. studeri* infections in wild bonobos.

Manniophyton fulvum (Euphorbiaceae) is a straggling shrub that is widely distributed across Africa's tropical evergreen forests. The surface of its large leaves and young stems is bristled with trichomes, resulting in a rough, sandpaper-like texture that irritates the skin upon contact (Fruth, in press). Across tropical Africa, people use its leaves, sap, roots, bark, and stems *inter alia* as medicine (Bellomaria & Kacou, 1995; Burkill, 1994; Fruth et al., 2014). Plant parts contain antioxidant, antidiarrheal, antibiotic, antiviral, and/or anti-inflammatory properties (e.g., Mbeunkeu et al., 2018; Muganza et al., 2012; Nia et al., 2005) depending on their associated secondary compounds (e.g., alkaloids, saponins, and terpenes: Agbaire et al., 2013). The Nkundo indigenous people in the Democratic Republic of the Congo (DRC) use *M. fulvum* for treating wounds, gastrointestinal spasms, diarrhoea, malaria-induced fever, and eye infections (Muganza et al., 2012).

M. fulvum is ingested by chimpanzees (Boesch, 1995; Huffman & Wrangham, 1994) and bonobos (Dupain et al., 2002; Fruth et al., 2014) without it being part of their regular diet. Both species ingest young leaves, and bonobos in LuiKotale and Kokolopori also ingest the stembark of the plant (Fruth et al., 2014; Surbeck, pers. comm.). *M. fulvum* ingestion has been observed at all bonobo research sites active to date: Lomako (Dupain et al., 2002; Fruth, 2013; Henry, 1998), Wamba (Kano & Mulavwa, 1984), LuiKotale (Fruth et al., 2014), Kokolopori (Surbeck, pers. comm.), and Manzano (Narat, 2014). Bonobos typically pick an entire leaf with their hand or front teeth. Depending on its size, they bite off pieces or take one or two additional leaves, folding them slowly and meticulously into an intricate leaf package that they swallow without chewing. Leaf packages are excreted undigested when defecating.

The number of leaves ingested per bout ranges from one to a dozen, which significantly distinguishes *M. fulvum* ingestion from habitual leaf ingestion (Fruth et al., 2014). For bonobos at LuiKotale, Fruth and colleagues (2014) described the ingestion of 15-30 cm long strips peeled from young *M. fulvum* rough and hairy shoots. Here, bonobos twist stem-strips within their mouth to form balls that they swallow without chewing.

Analysing faecal samples can provide insight into the frequency and seasonality of *M. fulvum* ingestion and is the only non-invasive approach to diagnose an infection by gastrointestinal parasites. In 1.5% (n= 131), 3.4% (n = 1094) and 6.4% (n = 110) of the faecal samples analysed in Lomako (Dupain et al., 2002), LuiKotale (Fruth et al., 2014) and Manzano (Narat, 2014), respectively, researchers found undigested *M. fulvum* leaf-packages. At LuiKotale, stem-strip remains had not been found before the onset of this study. Although site-specific variation is expected, these differences may stem from methodological differences. For example, the first two studies mainly analysed faecal samples collected beneath night nests, which likely led to an underestimation of the consumption rate. Indeed, hispid leaves swallowed in the early morning are expected to be shed within 6.5 h due to accelerated gut passage and are therefore less likely to occur in samples collected beneath night nests (Huffman & Caton, 2001). Methodology may have also unduly influenced inferences about the seasonality of *M. fulvum* consumption. At Lomako, none of the 33 faecal samples collected during the dry season contained *M. fulvum* remains and only two of 98 samples collected during the rainy season did (Dupain et al., 2002). Nevertheless, since 50% of a separate set of faecal samples (n = 86) collected at the onset of the rainy season contained *Oesophagostomum* sp. eggs, this study inferred *M. fulvum* ingestion was associated with the rainy season. However, Fruth et al. (2014) found

that *M. fulvum* ingestion by bonobos in LuiKotale was more common when ambient temperatures were low, which is a characteristic of the dry season. In Manzano, *M. fulvum* remains were found in faeces collected both in the rainy and dry seasons and when the parasitic load was high (Narat, 2014). Based on previous findings (Fruth et al., 2014) testing Huffman's criteria (1997, 2016) to assess self-medication of LuiKotale bonobos' *M. fulvum* ingestion, we continued investigations to better understand its trigger(s). We focused on the health condition of an individual, before and after ingestion of *M. fulvum*. We also investigated if the consumption of *M. fulvum* affected gut transit time and the consistency of faeces.

With regard to *M. fulvum*, we predicted that the frequency of consumption was (1.1) higher during the rainy season; (1.2) higher following periods of heavy rainfall and/or low temperatures and/or when individuals spent more time feeding terrestrially; and (1.3) independent of the plant's availability. Regarding the prevalence of gastrointestinal parasites in LuiKotale bonobos, we predicted that the frequency with which we found strongyle eggs and/or worms in faecal samples would be greater (2.1) after the onset of the rainy season, and (2.2) when bonobos fed terrestrially; also, (2.3) the egestion of *B. studeri* proglottids and eggs would not follow a clear seasonal pattern. In line with the above predictions, we expected (3.1) *M. fulvum* ingestion to be positively associated with the egestion of strongyle worms and/or *B. studeri* proglottids while (3.2) the absence of *M. fulvum* ingestion to be positively associated with the presence of strongyle eggs in faeces.

3.3. Materials and methods

3.3.1 Ethics statement

All research activities complied with protocols approved by the Institut Congolais pour la Conservation de la Nature (ICCN) and adhered to the legal requirements of the Democratic Republic of the Congo (DRC). The research project was approved by the ethics committee of Liverpool John Moores University (LJMU). This study also complied with the American Society of Primatologists Principles for the Treatment of Non-Human Primates and Code for Best Practices in Field Research.

3.3.2 Study site and species

Data were collected at the LuiKotale field site (2°45'36"S; 20°22'43"E), located west of Salonga National Park block South, DRC (Hohmann & Fruth, 2003b). The site consists of pristine evergreen lowland rainforest and is extremely remote. Two communities of wild, habituated bonobos (n_{BpW}= 22 adults; n_{BpE}= 15 adults; July 2020) were followed between December 2018 and July 2020. All individuals were identified (Fruth & Hohmann, 2018).

3.3.3 Climatological data

We used LuiKotale long-term data covering eleven years from January 2010 to December 2020. Daily cumulative rainfall was measured using a rain gauge (mm/m2) open to the sky, and minimum and maximum temperatures using a Bresser 5 in 1 Weather station deployed in the forest. Following Newton-Fisher (1999), we delineated seasons during the study period using a Walter–Lieth climate diagram

(Walter & Lieth, 1967). This diagram characterizes monthly moisture conditions based on the relationship between cumulative monthly rainfall and mean monthly temperature. Months are classified as "wet" when monthly rainfall is >100mm; "transient" when monthly rainfall is ≤ 100mm; and "dry" when the rainfall figures below the mean temperature line. Whenever possible (n= 472 days), forest temperature was directly used. When forest temperature was not available (n= 147 days), we used a general linear model (GLM) to predict forest temperature from available camp temperature. We characterized the extent of seasonality during the study period using the Perhumidity Index (PI), which measures the annual "continuity of wetness" (Walsh, 1996). PI scores were applied as follows: very wet month (≥ 200 mm)= +2; wet month (100-199mm)= +1; dry month (50-199mm)= -1; drought month (< 50mm)= -2; first dry month after a wet month= -0.5; and first drought month following a wet month= -1.5.

3.3.4 Behavioural observations

We recorded data ad libitum using focal animal sampling (Altmann, 1974), complemented by health data visually assessed for each individual in sight within the party for a minimum of 15 min, recording symptoms related to typical gastrointestinal parasitic infections (e.g., diarrhoea; fatigue; slow locomotion; Krief et al., 2008; Metzger, 2015). Individuals were followed from nest to nest (n= 383 follows > 8 h) and during shifts (n= 266 shifts 4–8 h; n_{BpW}= 311; n_{BpE}= 338; n= 9145 observation hours including several observers). Independent of the *ad-libitum* focal sampling, we recorded all occurrences of *M. fulvum* consumption, specifying individual, time of day, part and age of plant, procedure, and duration of ingestion. Following Fruth et al.

(2014) we defined an ingestion bout as one or more ingestion events uninterrupted by other activities (e.g., resting, grooming, ingestion of other food). Using behavioural data collected during 5-min activity scans of mature (>10 years) individuals (n= 617 observation days), we calculated the time each community fed terrestrially as the percentage of fruit-feeding scans. All behavioural data were collected using CyberTracker 3 (Steventon et al., 2011) on a Samsung Xcover 5 smartphone.

3.3.5 *Manniophyton fulvum* availability

We assessed the availability of *M. fulvum* up to three times a day (n= 841): at the nest site, at the first stop of the day (e.g., feeding patch) and at a randomly generated stop point during the day. For each stop, we scored the visibility of *M. fulvum* to party members by scanning the surrounding area for two minutes. When plants were visible, we recorded their location (terrestrial, arboreal), visible plant parts (stem, leaf), and stage of maturity (young, old).

3.3.6 Faecal sampling

Faecal samples were collected systematically from focal individuals and opportunistically from other party members. For each individual, we collected 1-5 faecal samples per day to control for intermittent egestion of parasites (Van Gool et al., 2003). Faeces were collected immediately after defecation using latex gloves and stored in a sealed bag until they could be processed at camp. Time between collection and processing averaged 6.51 h (sd± 3.06 h). We did not collect faeces contaminated with urine or shed near water (Garcia et al., 2018). For all faecal samples, we described consistency and content, and searched for macroscopically visible worms or

proglottids. Packages of folded undigested leaves were unfolded and taxonomically identified. For parasitological investigation, we retrieved 2-5 g of the faecal matrix from the centre of the stool to avoid contamination from soil micro-organisms (Krauth et al., 2012). Using a 15 ml tube, the matrix was mixed with a 10% formaldehyde solution, allowing preservation for later investigation.

3.3.7 Parasitological analyses

We investigated formol samples (n= 588) for the presence of gastrointestinal parasites. Of these, 298 samples were analysed in camp, while 290 were analysed in the laboratory. Time between collection and analysis was 163 days on average (range [0-517], sd± 155 days). All samples were analysed following the same protocol with minor adaptations depending on available resources (e.g. manual/ electric centrifuge, analytical scale). We performed flotations with a modified Sheather' sugar solution following protocol described in Modrý et al. (2015). Eggs were identified using morphological features such as shape and size (Garcia et al., 2018; Hasegawa et al., 1983, 2009; Modrý et al., 2015). To allow for control and later identification, specimen photos were taken using a graticule scale. With the exception of *O. stephanostomum*, morphological features are not considered being useful for identification of *Oesophagostomum* spp. (Chabertiidae) and *Necator* spp. (Ancylostomatidae) (Blotkamp et al., 1993; Narat, pers. comm.). Thus, we hereafter refer to them as strongyles (Pit et al., 1999; Polderman & Blotkamp, 1995; Strait et al., 2012).

3.3.8 Statistical analyses

We used χ^2 tests to compare the occurrence of *M. fulvum* consumption events between (1) sexes, (2) communities, (3) plant parts, and (4) availability. We also used χ^2 tests to test for differences in (5) faecal consistency; and (6) health status before and after ingestion of *M. fulvum*. We ran a Spearman rank correlation to test for the independence of *M. fulvum* availability in both communities and between months.

To determine if *M. fulvum* consumption was seasonal, we used a logistic regression to assess whether the occurrence (Y/N) of *M. fulvum* consumption per observation day (n= 507) depended on the season (wet, transient or dry as defined by a Walter–Lieth climate diagram sensu Newton-Fisher, 1999) [**M1**]. We then used a Bernoulli generalized additive mixed model (GAMM) to investigate whether the probability of observing *M. fulvum* consumption during a shift (n= 526) depended on (1) cumulative rain; (2) mean minimum temperature, and (3) the percentage of terrestrial feeding time [**M2**]. All values for fixed co-variates were calculated over a period of 14 days before the date of observation to account for the time between potential contamination with L3 infective *Oesophagostomum* larvae and the final moult from L4 stage to adult (Anderson, 2000). We predicted the hatching of the L4 larvae from the nodules embedded in the mucosa causing bleeding and intestinal discomfort (Stewart & Gasbarre, 1989). For this model, we fit community as a random intercept to account for the hierarchical sampling design, and terrestrial feeding tie as a random slope to account for community-level differences in the influence of time spent feeding terrestrially on the parasitic infection risk.

To determine whether the presence of strongyle eggs in faecal samples depended on climatic conditions, we used a second Bernoulli GAMM whereby faecal egg presence

(Y/N) was fit as the response; cumulative rain and mean minimum temperature as fixed covariates; and individual ID inside community as a nested random effect [**M3**]. For this model, we averaged values of fixed covariates over the month before sample collection to account for the time between infection by strongyle larvae and production of eggs by adult females (Anderson, 2000). Finally, we used a Bernoulli generalized linear mixed model (GLMM) to assess whether there was an association between the presence of parasite eggs in faecal samples and *M. fulvum* consumption [**M4**]. Since *M. fulvum* consumption was expected to significantly accelerate gut passage time (Huffman & Caton, 2001), we limited the observation window for *M. fulvum* consumption to the same day the faecal sample was collected and included focal individuals (n= 375) only. All models were fit using the packages 'brms' (Bürkner, 2017) and 'rstan' (Stan Developers Team, 2016) in R version 4.0.5 (R Core Team, 2021). Before modelling, all continuous variables were Z-transformed. After running each model, we checked for autocorrelation in the residuals, and used trace plots to check for chain convergence. We evaluated model performance using posterior predictive checks and checked prior sensitivity by re-running the model with alternate priors. Models are summarized in Table 3.1.

Table 3.1 | List of logistic regression models used to test hypotheses related to self-medication in response to parasite infection or re-infection in bonobos (*Pan paniscus*) at LuiKotale, DRC.

Model	Hypotheses	Response	Predictors	Random Effects
M1	*M. fulvum* consumption is more frequent in the wet season	Occurrence of *M. fulvum* consumption (Y/N) during an observation day	• season (dry, transition, wet)	NA
M2	*M. fulvum* consumption varies with climate conditions (cumulative rainfall and mean minimum temperatures in the past 14 days) and bonobo behaviour (terrestrial feeding rate in the past 14 days)	Occurrence of *M. fulvum* consumption (Y/N) during a shift	• cumulative rain (bi-weekly) • mean minimum temperature (bi-weekly) • percentage of time spent feeding terrestrially (bi-weekly)	• community (BpE, BpW)
M3	Strongyle eggs are more likely to be present in faeces as cumulative rain increases due to increased re-infection and/or as mean minimum temperature decreases	Presence of strongyle eggs in faeces (Y/N)	• cumulative rain (monthly) • mean minimum temperature (monthly)	• community (BpE, BpW) • individual bonobo ID (n= 32)
M4	Strongyle eggs are more likely to be present in faeces in the absence of *M. fulvum* consumption	Presence of strongyle eggs in faeces (Y/N) (focal samples only)	• *M. fulvum* consumption in the past 24 h (Y/N)	• community (BpE, BpW) • individual bonobo ID (n= 18)

3.4. Results

3.4.1 Behavioural observations

Overall, *M. fulvum* consumption was observed in 60 individuals of different age and sex (Table 3.2). Considering mature individuals only, *M. fulvum* consumption occurred on 111 out of 507 days (Figure 3.1B), with one or more individuals ingesting part of the plant's leaf and/or stembark. Consumption often occurred early in the morning (median time= 06:26 h, range [05:31-15:32], n= 166 observations) on an empty stomach. In most cases (92.31%, n= 104), individuals that ingested *M. fulvum* did not show symptoms of infection on the day of consumption, nor did they isolate themselves from the group. In 89.7% of cases (n= 68) focal individuals did not show any symptoms before or after ingestion. In the remaining 10.3% of observations, we observed individuals with asthenia (n= 3) combined with an obvious lack of appetite in one case, diarrhoea (n= 2), or a combination of both symptoms (n= 2). For these seven individuals, we noted an improvement of visually detectable symptoms within a few days (0-6 days) after ingestion of *M. fulvum* (normal faeces consistency, normal feeding rate, or energy level).

Individuals from both communities consumed leaves and/or stem-strips throughout the study period (median= 2 bouts/individual, range [1-12]). Adult females consumed *M. fulvum* more often than adult males (median= 3 bouts/female, range [1-12]; median= 2 bouts/male, range [1-7]). *M. fulvum* consumption occurred independent from the plant's availability (χ^2_{BpW}= 1.74, p> 0.05; χ^2_{BpE}= 0.92, p> 0.05).

Table 3.2 | *Manniophyton fulvum* ingestion at LuiKotale by sex and age class; December 2018 - July 2020.

	Infant	**Juvenile**	**Adolescent**	**Adult**	**Total**
	(0-5 y) (n= 14)	(>5-10 y) (n= 14)	(>10-15 y) (n= 13)	(>15 y) (n= 37)	
Female (n= 51)	11	23	7	118	**159**
Male (n= 27)	2	5	6	34	**47**
Total	**13**	**28**	**13**	**152**	**206**

3.4.2 Seasonality

LuiKotale climate (2010-2020) was equatorial with abundant annual rainfall (1,884 mm/m2, sd± 225 mm, n= 11). Temperatures ranged from 16°C to 39°C, with night temperatures being colder in the small dry season. The PI ranged between 0.5 in 2012 (seasonality) and 13.5 in 2010 (homogenous wetness) averaging 6.40± sd 3.77 (n= 10 years) between 2010 and 2020. During the study period 70% of the months were rainy, 15% transient, and 15% dry (Figure 3.1A). With the exception of two months (December 2018 and March 2020), *M. fulvum* consumption was observed every month when combining consumption of both communities (Figure 3.1B). We observed two peak periods of *M. fulvum* consumption, one in March-April 2019 (average= 31.58% of monthly follow days with *M. fulvum* bouts), and one in September-December 2019 (average= 29.77%). This was true for both the count of individuals involved and the frequency of consumption bouts. From **M1**, the posterior distributions for the wet and transition seasons both overlapped zero (Figure 3.2), meaning there was no significant difference in the likelihood of observing *M. fulvum* consumption between seasons. However, since the probability of the wet season's beta coefficient being positive and nonzero was 0.14, there was an 86% chance that the behaviour was less common in the wet season. The occurrence of *M. fulvum* consumption was not predicted by

cumulative rain, minimum temperature or time spent feeding terrestrially (Figure 3.3, Table 3.3).

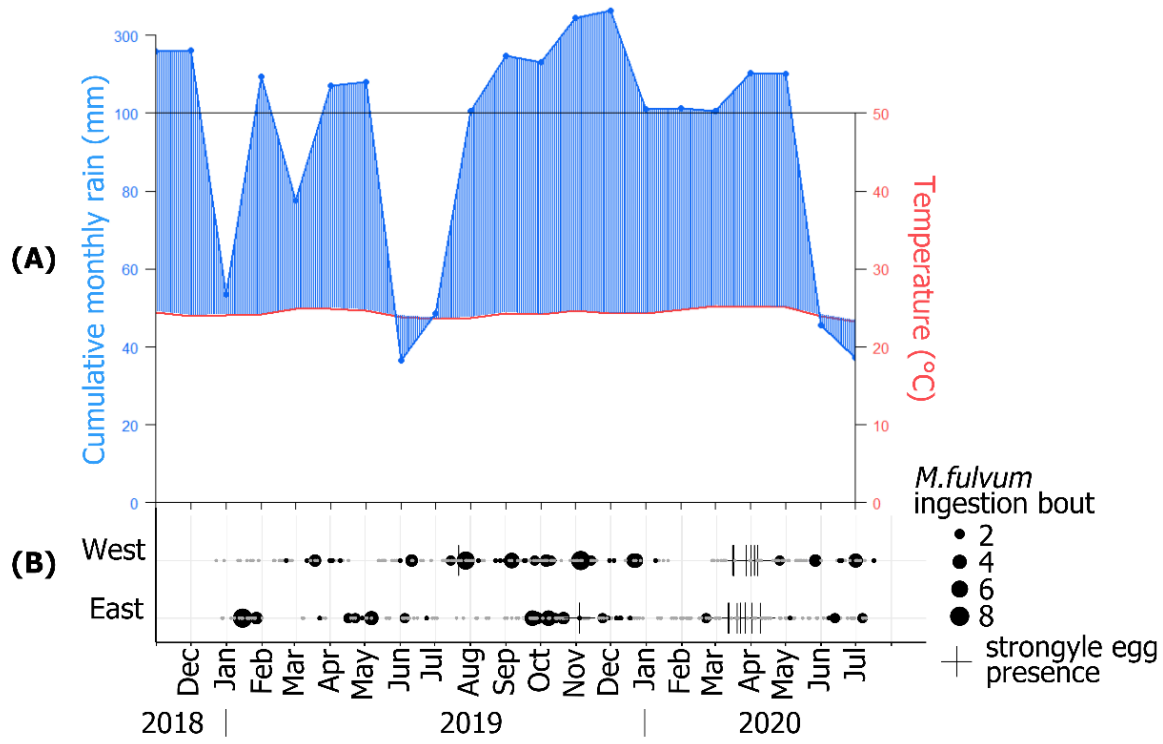

Figure 3.1 | Seasonality, *Manniophyton fulvum* ingestion and strongyle egg presence in bonobo (*Pan paniscus*) faeces collected between December 2018 and July 2020 at LuiKotale, DRC: (A) Walter–Lieth climate diagram with cumulative monthly rainfall in mm/m² (blue) and smoothed monthly mean temperatures in °C (red). (B) Occurrence of *M. fulvum* ingestion per day (black dots with size representing number of bouts) and presence of strongyle eggs in faeces (cross) observed in the BpW (top) and BpE (bottom) communities.

Table 3.3 | Posterior distributions of the population-level (fixed effects) for all models (**M1** to **M4** see Table 3.1 for model description). The Estimate is the mean of the posterior distribution (i.e. the beta-coefficient) and the Error is the estimated standard deviation from the mean, The Lower and Upper C.I.s represent lower and upper bounds of the 95% Credible Intervals. 'Prob> 0' and 'Prob< 0' are the Probabilities of the true value of the beta-coefficient being non-zero and positive or negative respectively. For **M1**, the dry season was set as the reference category against which estimates for the transition and wet seasons were compared. For **M4**, the absence of *M. fulvum* consumption (i.e., behaviour not observed) was set as the reference category.

Model	Fixed Effect	Estimate	Error	Lower C. I.	Upper C. I.	Prob < 0	Prob > 0	Reference Category
M1	Season: transition	0,005	0,350	-0,580	0,580	0,510	0,490	dry season
	Season: wet	-0,310	0,280	-0,770	0,160	0,860	0,140	dry season
M2	Cumulative Rainfall	-0,010	2,065	-3,303	3,613	0,525	0,475	na
	Mean Min. Temp.	-0,193	2,005	-3,513	3,113	0,541	0,459	na
	% Terrestrial Feeding	1,510	2,637	-3,127	5,490	0,268	0,732	na
M3	Cumulative Rainfall	-1,089	3,039	-5,924	4,100	0,650	0,350	na
	Mean Min. Temp.	2,123	3,015	-2,842	7,067	0,247	0,753	na
M4	*M. fulvum* consumption: yes	-0,817	0,325	-1,360	-0,291	0,996	0,004	*M. fulvum* consumption: no

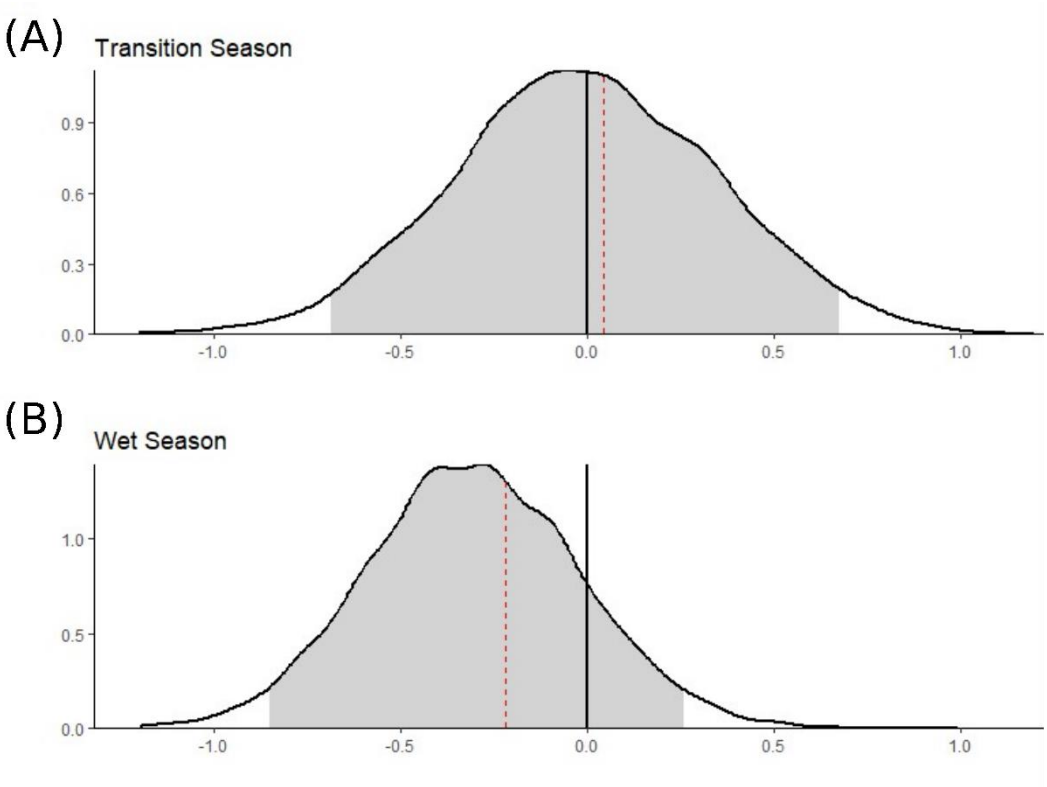

Figure 3.2 | Posterior distributions of the population-level effects for **M1**, which compared *M. fulvum* consumption by LuiKotale bonobos, DRC, in the dry, transition and wet seasons. Dry season was set as reference category against which transition (A) and wet (B) seasons were compared. The shaded area represents the 95% credible interval and the dashed red line is the median value for the posterior distribution.

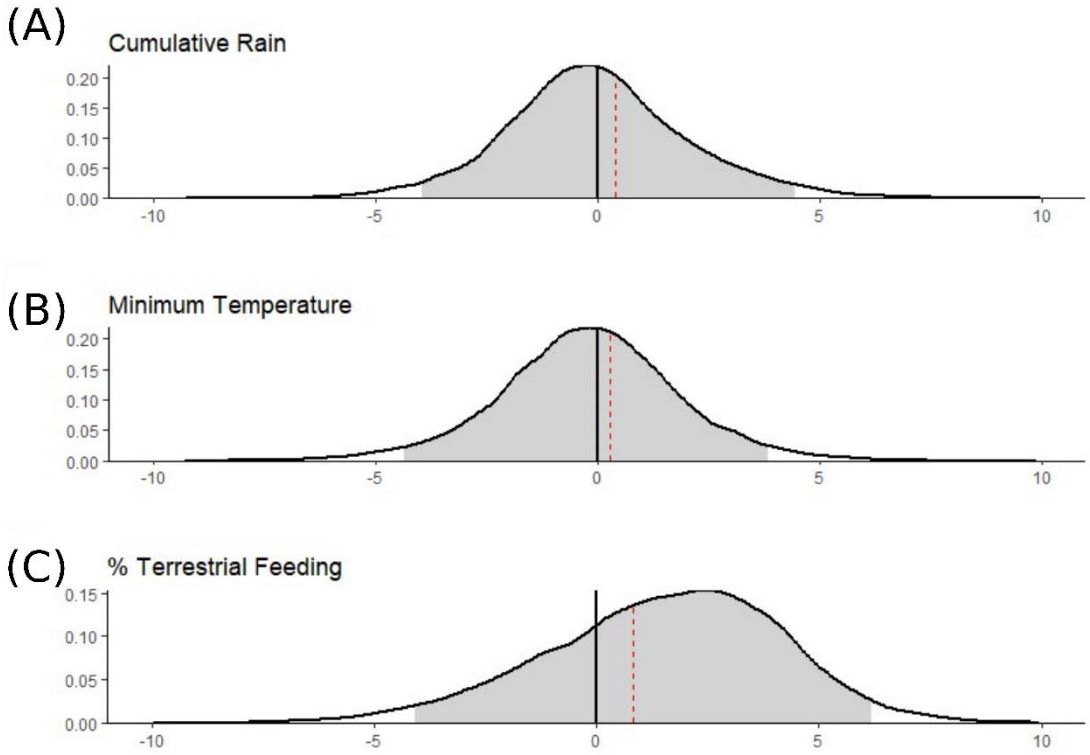

Figure 3.3 | Posterior distributions of the population-level effects for **M2**, which investigated whether cumulative rainfall (**A**), mean minimum temperature (**B**) and time spent feeding terrestrially (**C**) predicted *M. fulvum* consumption. The shaded area represents the 95% credible interval and the dashed red line is the median value for the posterior distribution.

3.4.3 Cultural behaviour

M. fulvum consumption periods partially overlapped between the two communities, with three and four periods for the BpW and BpE community, respectively (Figure 3.1B). The *M. fulvum* intake frequency in mature individuals was slightly higher, but not significantly, in the BpE compared to the BpW community (0.013 vs. 0.008 bouts/follow day/individual, Wilcoxon sign ranked test: W= 331, p> 0.05). However, the frequency of plant part consumption (leaf or stembark) differed significantly between communities, with BpW choosing stem-strips over leaves and BpE choosing

leaves over stem-strips (χ^2= 16.4, p< 0.001). This difference was independent of the plant's availability (leaves: rs= 87.27, p> 0.05; stem-strips: rs= 89.25, p> 0.05).

3.4.4 Faecal examination

We collected and described 969 faecal samples from 32 individuals (median= 21.5 samples/individual, range [1-49]). We found *M. fulvum* remains in 5.3% of samples. No leaves from species other than *M. fulvum* were found intact and folded, even though other species presenting similar characteristics were available. We also found remains of stem-strips (n= 7) still identifiable by their typical rough surface and dimensions easily distinguishable from fibres of terrestrial herbaceous vegetation. *M. fulvum* egestion occurred on average 8.04 h after ingestion (n= 18, sd± 1.39 h; leaves: 8.14 h (sd± 1.27 h); stem-strips: 7.29 h (sd± 1.52 h); both: 7.53 h (sd± 2.46 h); Figure 3.4). When focusing on faecal samples (n= 355) collected from beneath night nests only (19-05 to 25-07-019), 2.25% contained *M. fulvum*. Twenty-five ingestions were directly observed during that time (n= 483 observation hours). When focusing on 28 direct observations of *M. fulvum* ingestion during focal follows, inspecting n= 33 faecal samples collected thereafter (5.30-11.00 h after ingestion), *M. fulvum* remains were present in 42.4% of these samples. We did not observe the egestion of adult worms or cestode proglottids in any of the faecal samples (n= 969) during the study period. Presence or absence of *M. fulvum* remains in faecal samples were unrelated to consistency (χ^2= 3.67, p> 0.05), as was consumption of a specific *M. fulvum* part to the change of faecal consistency when compared with the absence of consumption (χ^2= 3.84, p> 0.05). Based on the comparison between samples collected within 48 h before *M. fulvum* ingestion and after 6 to 24 h after ingestion (n= 10), we observed

faeces to be harder in consistency after ingestion than before (Figure 3.5), though the difference was not significant (χ^2= 3.02, p> 0.05).

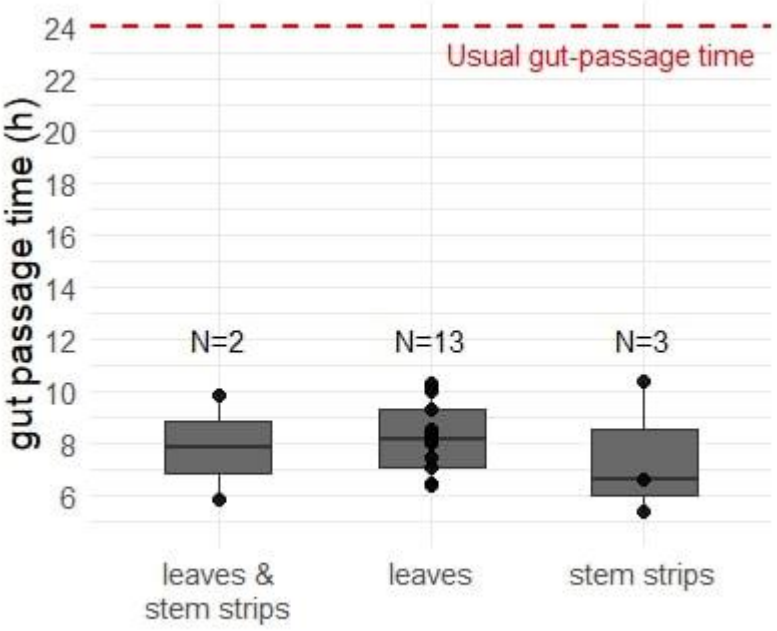

Figure 3.4 | LuiKotale bonobo gut-passage time from ingestion to shedding (in h) by *Manniophyton fulvum* plant part. Boxplots show median (horizontal line), interquartile range (box) and range (vertical line). Dots indicate observed cases. Dashed line represents the 24 h habitual gut passage time (Beaune et al., 2013b).

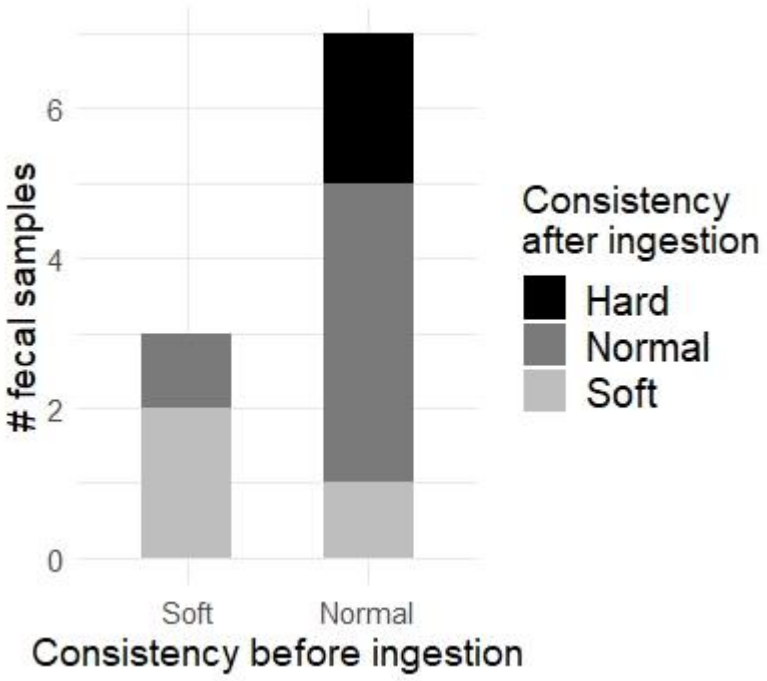

Figure 3.5 | LuiKotale bonobo faecal sample consistency (soft; normal; hard) within 48 h before and between 6 and 48 h after ingestion of *Manniophyton fulvum.*

3.4.5 Parasitological analyses

The flotation experiments revealed the presence of several gastrointestinal parasite species, but here we focused solely on strongyle species (i.e. *Oesophagostomum* spp. and hookworms). Based on shape and dimensions (Hasegawa et al., 1983; Metzger, 2015) we identified strongyle eggs in 2.89% (n= 588) of samples from 12 individuals. Specific morphological features and dimensions of the eggs found in four of these samples resembled those of *O. stephanostomum* (75-80 μm length; 43-48 μm width) (Hasegawa et al., 1983). We only found them in faeces from female individuals (n_{BpE}= 1; n_{BpW}= 3) collected between March and April 2020 (Figure 3.1B). Based on egg dimensions and morphology, other samples contained unidentified strongyles (n= 10) and hookworms (n= 2). The presence of strongyle eggs in faeces was not predicted by rainfall, minimum temperature, or the percentage of terrestrial feeding time (Figure

3.6, Table 3.3). Finally, faeces collected the day of *M. fulvum* ingestion were less likely to contain strongyle eggs (probability of sample containing no egg when collected after consumption of *M. fulvum*= 0.96; Figure 3.7, Table 3.3). Between the first (17-03-2020) and the last (09-04-2020) positive sample of *O. stephanostomum* eggs presence, one sample contained hookworms and seven samples contained unidentified strongyles. The remainder (n= 29) did not contain additional strongyles. In April 2020, we observed a significant increase in the records of diarrhoea in many party members from both communities (χ^2= 123.62, p< 0.001).

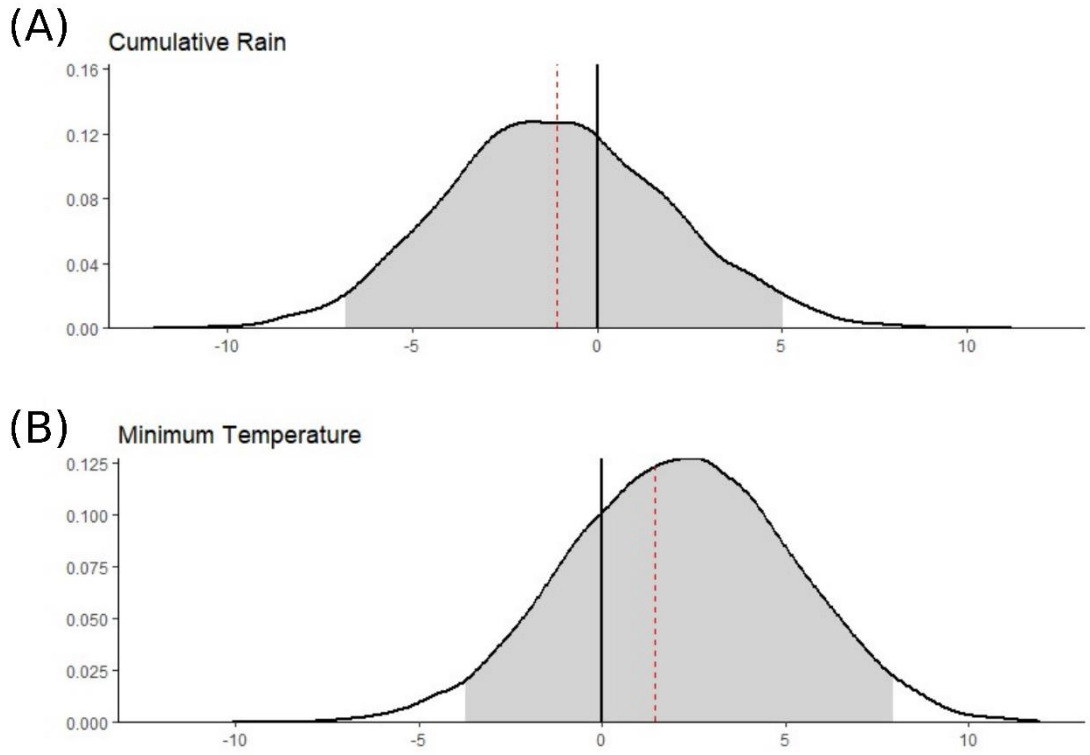

Figure 3.6 | Posterior distributions of the population-level effects for **M3**, which investigated whether cumulative rainfall (A) and mean minimum temperature (B) predicted the presence of strongyle eggs in bonobo fecal samples. The shaded area represents the 95% credible interval and the dashed red line is the median value for the posterior distribution.

Figure 3.7 | Posterior distribution of the population-level effect for **M4**, which investigated whether *M. fulvum* consumption (measured as a binary 'yes' or 'no' variable) predicted the presence of strongyle eggs in bonobo faecal samples. For this model, 'no' was set as the reference category against which incidences of *M. fulvum* consumption were compared. The shaded area represents the 95% credible interval and the dashed red line is the median value for the posterior distribution.

3.5. Discussion

Here, we set out to understand potential triggers for *M. fulvum* consumption by LuiKotale bonobos, testing the hypothesis that the plant's ingestion is related to (re)-infection by and egestion of strongyle eggs or *B. studeri* proglottids. Between December 2018 and July 2020, we investigated 969 faecal samples at the macroscopic level, 588 of which were also analysed at the microscopic level.

3.5.1 Behavioural observations

We observed more *M. fulvum* ingestion occurrences by LuiKotale bonobos than in previous studies (Lomako: Dupain et al., 2002; LuiKotale: Fruth et al., 2014). Considering individuals across both communities, we witnessed leaf swallowing and/or stem-stripping of *M. fulvum* almost every month. Independent of the timing of faecal sample collection (i.e., during day or from night nests), we observed *M. fulvum* ingestion more often than we found remains in faecal samples. It is likely we missed faecal samples containing whole leaves and stem-strip remains, even following *M. fulvum* consumption. Some leaves may remain in the digestive tract for some time before being egested and were therefore not present in the inspected faeces within the expected time window. Alternatively, leaves and stem-strips may have been digested when not well folded or too small. Observations were biased to focal versus non-focal individuals with all 18 focal individuals ingesting *M. fulvum* at least once and more regularly than non-focal individuals, emphasizing again how cryptic this behaviour is. In addition, these 18 individuals were adults, representing 23.7% of the studied population (48.6% of all adults), meaning we cannot make age-specific inferences as neither infants nor juveniles were focal individuals.

3.5.2 Seasonality

In contrast to our prediction and expectations based on previous studies in chimpanzees and bonobos (Dupain et al., 2002; Wrangham & Nishida, 1983), the frequency of *M. fulvum* consumption was neither seasonal nor predicted by rainfall, temperature or terrestriality. However, the likelihood of observing *M. fulvum* ingestion during the wet season relative to the dry season was seemingly lower, which aligns

with previous results from the LuiKotale research site (Fruth et al., 2014). In the past, leaf swallowing of entire leaves by African great apes has been associated with parasite expulsion (Fowler et al., 2007; Huffman et al., 1996; Huffman & Caton, 2001; Wrangham, 1995). Because re-infection with strongyles likely occurs 1-2 months after the onset of the rainy season, we expected to observe more leaf swallowing and stem-stripping at this time. At LuiKotale however, bonobos consumed *M. fulvum* throughout the year, independent of wet, transient, and dry months (Figure 3.1B). When looking at the communities separately, we observed periodic consumption by single individuals interspersed with short periods during which *M. fulvum* was ingested by a larger proportion of the party. Similar to Budongo (Huffman et al., 2009; Newton-Fisher, 1999), the seasonality at LuiKotale showed extended humid and short dry periods. At Budongo, adults of *Oesophagostomum* spp. were not retrieved from chimpanzees' faecal samples, nor was egestion of eggs associated with rain, or leaf swallowing correlated with monthly rainfall (Huffman et al., 2009). In contrast, at Bulindi, which is located close to Budongo and hence has a similar climate, *Oesophagostomum* eggs were found at higher frequencies in wet compared to transient or dry months, with their presence being associated with low temperatures. However, leaf swallowing here had no clear seasonality.

Seasonality may play a critical role in the development and life cycle of parasite species because changing seasonal climate conditions alter the host-parasite dynamic. Strongyles, in particular, are known to be typically seasonal in some areas of their distribution. At LuiKotale, Budongo and Bulindi dry seasons are short, meaning the soil environment may never be particularly hostile for *Oesophagostomum* eggs to mature into the infective larval stage. Accordingly, bonobos at LuiKotale may not suffer a high burden of re-infection at any particular time during the year (e.g., after

the onset of the rainy season) because larvae remain infective over longer periods. Thus, bonobos cope with re-infection all year around by regularly swallowing *M. fulvum* thereby maintaining low levels of infection.

3.5.3 Cultural behaviour

As shown previously by Huffman and colleagues (2010) in captive chimpanzees, naïve zoo-housed chimpanzees started using two different techniques of leaf swallowing to insert and fold the rough leaves into their mouth. These techniques, which have also been observed in the wild, were propagated spontaneously within the respective social groups (see also Tennie et al., 2008). Swallowing without chewing is an acquired behaviour as shown by Huffman and Hirata (2004). Youngsters learn it from their mother or other members of the community. In the present study, we observed immature individuals ingesting *M. fulvum* alongside adults and other group members, and it was not uncommon to observe a juvenile peering at another individual processing and ingesting leaves or stem-strips of *M. fulvum*. Bonobos at LuiKotale and Kokolopori ingest leaves and stem bark of *M. fulvum* while bonobos at Lomako and Manzano were only reported to ingest the leaves. This suggests stem-stripping is a cultural behaviour. However, even between the two communities in LuiKotale, we documented differences in the plant part chosen, with BpE choosing leaves over stem-strips and BpW choosing stem-strips over leaves, independent from plant part availability. This may be due to individual preferences or need if different plant parts serve different purpose (with the sap ingested along *M. fulvum* stem-strips possibly containing more biochemical compounds), though our data did not allow us to define these. We observed individuals combining ingestion of leaves and stem-strips in the

same bout, which leads us to speculate whether the two parts act synergistically. In some cases, we observed individuals seeking leaves while ignoring abundant stems and vice-versa. To better understand the function of leaves and stem-strips and whether techniques for ingestion are cultural, future studies should target immigrant females.

3.5.4 Faecal examination

During the study period we did not find adult nematodes or cestode proglottids in faecal samples, independent of the presence of *M. fulvum*. Nevertheless, we cannot exclude bonobos being infected with these parasite species. Unlike at other sites (Huffman et al., 2009; Huffman, pers. comm.), leaf swallowing by LuiKotale bonobos does not appear to be associated with the expulsion of *B. studeri*. Therefore, we consider it very unlikely any of the individuals sampled during the study period were heavily infected with this species. We found leaf packages in 5.3% of collected faecal samples compared to 1.5% at Lomako (Dupain et al., 2002) and 3.5% at LuiKotale in a previous study (Fruth et al., 2014). The fact our sample collection was more systematic and focused on samples collected during all-day follows rather than nest sites may explain this difference. However, we found less *M. fulvum* remains in faecal samples than were found in Manzano (6.4%) (Narat, 2014), despite a larger sample size. Given the discrepancy between the proportion of *M. fulvum* found in faeces collected beneath night nests (2.3%) and those during focal follows (42.4%), it is evident that studies relying on faecal collection independent of direct observations considerably underestimate the frequency of this behaviour. Swallowing intact leaves without chewing is generally rare, although variation exists between chimpanzee

populations. For study sites in Kibale National Park, Uganda, Wrangham (1995) reported that 2.5% of faeces at Kanyawara (n= 1,696), and 0.4% at Ngogo (n= 1,198) contained intact leaves. In Gashaka, Nigeria, Fowler and colleagues (2007) found intact leaves in 3.7% of samples (n= 299); and Huffman and colleagues (1996) found intact leaves in 2.4% (n= 245) of samples collected in Mahale, Tanzania. In all the above-mentioned studies, the swallowing of rough-surfaced leaves was associated with parasite infection and/or expulsion. In Bulindi, Uganda, this behaviour was more frequent, with McLennan and colleagues (2017) finding intact leaf remains in 11.8% (n= 406) of faecal samples. Bulindi is a very disturbed, fragmented area where the risk of parasite transmission between chimpanzees and villagers is high (McLennan et al., 2017); thus, the frequency of *M. fulvum* ingestion may be related to the risk of (re)-infection.

3.5.5 Parasitological analyses

O. stephanostomum is the most predominant species in African great apes (Hasegawa et al., 1983; Huffman et al., 1996, 2009; Makouloutou et al., 2014). Following previous observations in wild chimpanzees with moderate *O. stephanostomum* infections (Krief et al., 2008), bonobos at LuiKotale did not show obvious symptoms when egesting strongyle eggs, suggesting they do not suffer heavy infection by *O. stephanostomum* or unidentified strongyles. We retrieved strongyle eggs from 17 of 588 samples analysed using a flotation technique common in veterinary diagnostics for retrieving helminth eggs (Broussard, 2003; Pouillevet et al., 2017; Vlčková et al., 2018). It is sensitive for detecting strongyle eggs, but has some drawbacks, including the potential to distort the eggs (making identification complicated) and not being

adaptable for other egg types (Ballweber et al., 2014; Salvador et al., 2014). It also does not allow identification of larvae making taxonomic identification speculative in our study. In addition, the limitations of field conditions or sample storage may have reduced the chances to retrieve parasite eggs using this method.

While some specialists claim that *Oesophagostomum* and hookworm eggs cannot be distinguished based on egg visualization only (Blotkamp et al., 1993; Narat, pers. comm.), others distinguish between *O. stephanostomum* and other strongyle species microscopically (Hasegawa, pers. comm.). Combining our method with alternative ones such as coproculture or isolation of larval stages would have been pertinent and is encouraged for future studies. However, accurate identification of parasite species can only happen when molecular analyses are used. Lacking genetic identification, we cannot confirm which strongyle genus and species occur at LuiKotale and what their abundance is compared to other sites. Moreover, as there is no published record of parasitological analyses of humans inhabiting the study region, we cannot exclude the presence of helminth species, and if present, whether or not they are shared between humans and bonobos.

At Lomako, researchers found 16.1% and 50.6% of faecal samples (n= 87) contained undetermined strongyles and *Oesophagostomum* sp. respectively (Dupain et al., 2009). At Wamba these rates were 21% and 17.9% (Hasegawa et al., 1983) respectively, while at Manzano 44% of samples had undetermined strongyles (Narat et al., 2015). Researchers used different methods of faecal analyses (i.e., sedimentation in Lomako and Wamba; direct smears in Manzano) which might partly explain these differences. In Ugandan chimpanzees, McLennan and colleagues (2017) found 58% and 66% of all samples (n= 38) containing *Oesophagostomum* sp. and hookworm respectively using coproculture. One of the main differences between sites where

chimpanzees and bonobos have been studied so far and the LuiKotale study site is the remoteness of the latter. In LuiKotale the nearest village is located approximately 20 km away, with a large river in between; thus, the only humans in regular contact with the bonobos are researchers that adhere to a strict hygienic protocol. In this context, cross-species parasite transmission is limited, which could partly explain the very low rate of parasite infections observed in bonobo faeces. Another limitation that we need to consider when analysing our results is the phenomenon of intermittent shedding (e.g., Khurana & Sethi, 2017; McLennan et al., 2018; Van Gool et al., 2003). Sometimes, parasites are not shed in faecal samples despite the host being infected. Indeed, when analysing our samples, we found that consecutive faecal samples collected on the same day from the same individual did not have consistent parasite diversity. To account for intermittent parasite and parasite egg egestion and, thereby, obtain a more accurate assessment of an individual's infection status, we recommend increasing the frequency of faecal sample collection and performing molecular analyses in conjunction with classical observational methods.

3.5.6 Potential role of *M. fulvum* leaf and stem-strip swallowing

So far, the swallowing of unchewed leaves observed in bonobos is known for two species only, *M. fulvum* and *Cola* spp. (Dupain et al., 2002). In the case of *M. fulvum* at least, it is unlikely the plant is consumed for nutritional purposes considering the mode of ingestion (i.e., leaf folding and swallowing without chewing) by bonobos (Messner & Wrangham, 1996) and the absence of taste or odour from any plant part to human taste. We confirmed that *M. fulvum* increases gut motility, with boluses being expulsed three times faster than usual without significant modification of faecal

consistency and regardless of the plant part consumed. We observed a slight antidiarrheal effect, contradicting what we expected from the increased gut motility; however, this antidiarrheal effect was not evident in all samples and not supported statistically, possibly due to the small sample size.

As found in previous studies, our observations confirmed *M. fulvum* consumption occurs primarily in the early morning and often as the first ingestion of the day. In most cases, bonobos ingesting *M. fulvum* did not present any symptoms of infection on the day of consumption. On only seven occasions, we recorded symptoms such as diarrhoea and/or asthenia that were absent after ingestion of *M. fulvum*. In these cases, bonobos showed moderate sociality but remained in the group. Although we cannot prove that the symptoms disappeared as a result of *M. fulvum* ingestion, we also never observed a deterioration in health following ingestion. Our data showed no association between the consumption or egestion of *M. fulvum* and the egestion of strongyle eggs or adults. As mentioned earlier, independent of leaf presence in faeces, we never observed adult worms in faeces, which suggests severe parasite infections are rare at LuiKotale.

Interestingly, the frequency of *M. fulvum* ingestion was lower during a period when we found strongyle eggs in faeces (between March and May 2020), suggesting a negative association between *M. fulvum* ingestion and the presence of parasite eggs in faeces. If *M. fulvum* ingestion helps dislodge larvae from the intestinal wall or expulse adults present in the lumen, we would expect to see a positive association (i.e., worms jointly detected with intact leaves). However, if parasitic load (i.e., estimated by egg count) was very low across individuals and time, this may explain why we did not jointly detect worms and intact leaves in faecal samples. As observed in a previous study on chimpanzees, infection intensity can be very high in one community and low

in another one when the prevalence of the given parasite species is comparable between the two communities (Huffman et al., 2009). It is likely that higher infection intensities trigger more leaf swallowing, supporting the need to incorporate egg counts in future studies.

After experiments with captive chimpanzees, Huffman and Hirata (2004) concluded that leaf swallowing is most likely not an innate response to parasite infection as symptom-free individuals performed the behaviour. This suggests alternative triggers for *M. fulvum* swallowing by LuiKotale bonobos, such as bloating, intestinal discomfort and spasms observed in chimpanzees (McLennan et al., 2017; Mclennan & Huffman, 2012). We speculate that the wound induced by the burst of L4 *Oesophagostomum* larvae from their nodules causes intestinal pain and discomfort that triggers *M. fulvum* swallowing. If true, this suggests the association between the egestion of *Oesophagostomum* worms and leaf swallowing being a by-product of the self-medicative behaviour. It would also support our observation that we detected strongyle eggs in faeces more frequently in the absence of *M. fulvum* consumption, as larvae had time to develop into adult reproductive stages.

3.6. Conclusion

Our results provide new insights into the leaf swallowing behaviour. We showed that across 20 months of bonobo observation at LuiKotale, leaf swallowing of *M. fulvum* was not seasonal or predicted by environmental variables or associated with the egestion of *Oesophagostomum* worms or proglottids of *B. studeri*. However, we cannot exclude gastrointestinal infections caused by other parasite species triggering *M. fulvum* ingestion. We showed that leaf swallowing and stem-stripping of *M. fulvum*

occurred more often than previously reported. When *M. fulvum* was not ingested, diarrhoea and the presence of strongyle eggs in faeces were more frequent. Thus, our results support the hypothesis that ingesting *M. fulvum* flushes out larvae before they develop into adults, thereby reducing the likelihood of pathologic nodules and related severe symptoms. We cannot exclude that *M. fulvum* acts differently from other bristly leaved species possessing specific characteristics and mechanical and chemical properties. It is possible that its ingestion is triggered by symptoms we cannot detect with the non-invasive methods used here. In future, a more comprehensive study on the health condition of single individuals, including a thorough molecular screening of their intestinal parasite and microbial diversity and abundance, may provide a better understanding of this behaviour. Independent of the search for triggers, the evidence that *M. fulvum* ingestion is a cultural trait is a fascinating aspect that warrants further exploration. Studies investigating different bonobo populations may shed light on new components of this potential cultural aspect.

3.7. Acknowledgments

We thank the Institut Congolais pour la Conservation de la Nature (ICCN) for granting permission to work at LuiKotale, and Lompole and Bekombo villages for facilitating research in their forest. Funding for research in both field and lab came from the Centre for Research and Conservation of the Royal Zoological Society of Antwerp (CRC/ KMDA), the Max-Planck Institute of Animal Behavior, Department for the Ecology of Animal Societies (MPIAB), and Liverpool John Moores University. Special thanks for long-term support go to Gottfried Hohmann and Zjef Pereboom. Giulia Rossi, Lambert Booto, and the LuiKotale Bonobo Project team are thanked for their

assistance in the field. Andy Tattersall, Keith George, Peter Wheeler, Richard McElreath, and Meg Crofoot are thanked for their support in named institutions. We thank Michael Huffman and one anonymous reviewer whose comments greatly improved this manuscript. Thanks to Julien Grizi and OREDUI for granting access to their facilities. Hideo Hasegawa, Victor Narat, and Susanne Klomburg are thanked for their advice in parasitological techniques and identification, and Mattia Bessone and Shauhin Alavi for their help with statistical analyses. Open Access funding enabled and organized by Projekt DEAL.

3.8. Conflict of interests

The authors declare that there is no conflict of interests.

3.9. Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request and will be made available in MPI data repository upon acceptance.

CHAPTER 4

URINARY NEOPTERIN LEVELS INDICATE SPECIES-SPECIFIC IMMUNITY IN WILD BONOBOS

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This chapter was submitted for publication in *EcoHealth* in January 2022. All supplementary materials are included in the chapter.

4.1. Abstract

As environmental changes emphasise the threat coming from infectious diseases in wild species, monitoring their health and gaining a better understanding of the species-specific immune functioning has become critically important. Chimpanzees (*Pan troglodytes*) and bonobos (*Pan paniscus*) are closely related species, however, they differ in their MHC class I repertoire. Bonobo's diminished repertoire likely make them more sensitive to novel pathogens but resistant to others (i.e. malaria, HIV/SIV). Neopterin is a biomarker of cell-mediated immune responses to intracellular infections. We studied the variation of the urinary neopterin (uNeo) levels in 309 samples collected from wild, habituated bonobos at the LuiKotale field site, DRC, with the aim of comparing it to that in chimpanzees. Based on current knowledge of zoo-housed conspecifics and closely related species, we predicted uNeo levels to increase 1) during infections, 2) with increasing age, 3) over the gestation period and in fertile females; and 4) vary seasonally. Our results showed uNeo levels varied over a one-year period and increased in individuals showing respiratory symptoms. Our study provides a baseline to a better understanding of bonobo's immunocompetence in the context of socio-ecological pressures and to optimizing the use of biomarkers to monitor the health of wild populations.

Key words: *Pan paniscus*, urinary neopterin, health monitoring, non-invasive, species-specific immune response, ecoimmunology, seasonal infectious diseases

4.2. Introduction

Environmental pathogen exposure shapes and challenges the immune system of animals (Nunn et al., 2006; Brinkworth & Pechenkina, 2013). Species coevolve with their pathogens and minimise the cost of immune defense strategies by developing and maintaining immunocompetence (Zuk & Stoehr, 2002; Gandon et al., 2008; Brock et al., 2014), likely altered by environmental changes shifting and modifying the incidence and prevalence of infectious diseases (Acevedo-Whitehouse & Duffus, 2009; Altizer et al., 2013). The immune functioning plasticity thus presents obvious advantages in our increasingly globalised world with changing environments and disease risks (Capri et al., 2014). A better understanding of wildlife health is of major importance in the context of ecosystem change with ecoimmunology aiming to comprehend the plasticity of a host's immunocompetence in the context of socio-ecological pressures (Adelman et al., 2014; Schoenle et al., 2015; Bowden et al., 2017).

Disease outbreaks can pose serious risks to the conservation and survival of endangered species (Ryan & Walsh, 2011). All great ape species are listed as endangered or critically endangered on the IUCN Red List (IUCN, 2021). Of concern is the transmission of infectious diseases, particularly from humans (Dunay et al., 2018), threatening wild populations, as documented for gorillas (*Gorilla* spp.) and chimpanzees (*Pan troglodytes*) (Leendertz et al., 2006a; Köndgen et al., 2008; Grützmacher et al., 2016). In bonobos (*Pan paniscus*), little is known about infectious diseases so far (Behringer et al., 2018; Maibach & Vigilant, 2019). Only a few, mainly anecdotal reports are available from the wild, describing cases of flu-like and respiratory diseases apparently without detrimental impact (Ryu et al., 2020; Sakamaki et al., 2009). Why are outbreaks in wild bonobos under-represented in the

literature compared to those affecting chimpanzee populations? Could it just be a sample size effect, or is there a difference in the immune functioning between the *Pan* species?

Chimpanzees, bonobos, and humans (*Homo sapiens*) share some major histocompatibility complex (MHC) class I allotypes (De Groot et al., 2002; Prüfer et al., 2012), suggesting that these allotypes were already present in the last common ancestor. Thereafter, two selective sweeps resulted in a considerably diminished, but specialized MHC class I repertoire in bonobos (De Groot et al., 2002, 2017; Maibach et al., 2017; Wroblewski et al., 2017; Maibach & Vigilant, 2019): the first took place after the *Homo-Pan* divergence (i.e. 7 to 6 million years ago; Takemoto et al., 2015) (Carrington & Bontrop, 2002; De Groot et al., 2017), the second one after the chimpanzee-bonobo split (De Groot et al., 2017). Compared to humans and chimpanzees, the MHC class I repertoire of bonobos may provide an advantage in coping with specific infectious diseases such as the human and simian immunodeficiency viruses (HIV/SIV), and malaria (De Groot et al., 2018). But these selective sweeps have also diminished the bonobos' MHC class I repertoire, possibly making them more susceptible to emerging and unfamiliar pathogens. It is therefore important to understand the consequences these differences in immune allotypes have on the immunocompetence of this species, as this can have important implications for conservation efforts.

Non-invasive biomarkers of immune functioning can be used to investigate immunocompetence in wild animals (Murr et al., 2002; Negrey et al., 2021). Neopterin is a biomarker of the cell-mediated immune response and its level typically increases during infections caused by viruses, and intracellular bacteria and parasites (Hamerlinck, 1999; Berdowska & Zwirska-Korczala, 2001; Widner et al., 2002).

Neopterin can be measured from non-invasively collected samples such as urine. Urinary neopterin (uNeo) has been used in several studies on non-human primates (Behringer et al., 2021; González et al., 2020; Negrey et al., 2021). Neopterin levels in primates and humans typically vary with age, sex and reproductive state, and follow climatic and/or disease seasonality. Urinary neopterin levels were higher in early and late life stages (Murr et al., 2003; Müller et al., 2017; Behringer et al., 2021), and in potentially fertile and pregnant females compared to lactating and cycling ones (Boyunağa et al., 2005; Negrey et al., 2021). Increased uNeo levels were also found in response to low ambient temperatures (Mohyuddin et al., 2017; Löhrich et al., 2018), and increased malaria transmission rates (Picot et al., 1993; also see Altizer et al., 2006). Sex differences have been reported to influence uNeo levels in some species and/or studies (Behringer et al., 2017; Negrey et al., 2021), but not in others (Behringer et al., 2019; González et al., 2020).

In the past decades, much attention has been given to elucidating the uNeo response to different individual and environmental factors in humans and chimpanzees, but so far, we know very little on the uNeo response in bonobos. However, we expected bonobos to show a species-specific response of their cell-mediated immunity compared to chimpanzees, given the possible immunological repercussions of bonobo's reduced MHC class I repertoire, and the differences on other levels, such as environmental and social ones. We used uNeo levels as proxy for cell-mediated immunity of wild bonobos investigating its variability, and the effect of potential pathogens, age, sex, season, and reproductive state. Based on the findings in primates, we predicted uNeo levels in wild bonobos to **1)** increase during infections; **2)** vary seasonally; **3)** increase with age; and **4)** increase over the gestation period and in potentially fertile females.

4.3. Materials and methods

4.3.1 Ethics statement

All methods applied were strictly non-invasive and noncontact as required by the IUCN guidelines. The Institut Congolaise pour la Conservation de la Nature (ICCN) granted permission to conduct research at LuiKotale, Salonga National Park, Democratic Republic of the Congo (DRC). The research project was approved by the ethics committee of Liverpool John Moores University (LJMU).

4.3.2 Study site and study subjects

Data were collected between 2010-2011 and 2016-2019 at the LuiKotale field site (2°45'36''S; 20°22'43''E; Hohmann & Fruth, 2003), located west of the southern block's border of Salonga National Park, DRC. Two bonobo communities were habituated to the presence of human observers, the Bompusa West (BpW) and the Bompusa East (BpE) community. In January 2020, BpW and BpE communities were composed of 23 (16 females, 7 males) and 16 (10 females, 6 males) sexually mature individuals, respectively.

During focal follows, individuals were visually assessed for the presence of sickness behaviours (i.e. asthenia, anorexia) and symptoms of infectious and/or other diseases for at least 15 minutes. We recorded activity level, appetite, locomotion, presence/absence of diarrhea, repetitive respiratory symptoms (e.g cough, sneeze), and injuries. Bonobo age estimate was based on the project's long-term records and reflects estimates based on an individual's life history (offspring number and age, female sexual swelling cycling…) and morphological features (facial and postural). Age estimates at the time of sample collection ranged from 10 to 42 years (Table 4.1).

Females' sexual swellings, reflecting the likelihood of their ovulation's timing (Douglas et al., 2016), were assessed during follows and scored into four categories from 1 (not swollen) to 4 (fully swollen) following Furuichi (1987).

Table 4.1 | Sex (F: Female, M: Male), sample count and mean age estimate and standard error for individuals (3 letters code) sampled in (**m1**).

Individual (3 letters code)	Sex	Sample count	Mean age estimate [years (sd)]
Lna	F	2	12.0 (0)
Rit	F	15	13.7 (0.6)
Gwe	F	2	17.0 (0)
Agb	F	1	19.0
Sus	F	2	19.0 (2.8)
Wma	F	12	19.7 (3.1)
Uma	F	22	23.5 (3.2)
Nin	F	2	26.0 (0)
Pau	F	4	27.0 (0)
Rio	F	2	27.0 (0)
Olg	F	21	28.2 (2.4)
Kim	F	11	28.4 (0.7)
Pem	F	20	29.5 (0.7)
Evi	F	22	33.5 (0.7)
Iri	F	17	34.0 (2.6)
Sor	F	12	35.8 (0.4)
Zoe	F	17	35.9 (2.6)
May	F	17	39.4 (0.7)
Mar	F	19	41.2 (2.5)
Roq	M	1	10.0
Pet	M	1	11.0
Ben	M	2	16.5 (0.7)
Cam	M	2	19 (0)
Emi	M	10	19.3 (3.6)
Ban	M	19	21.5 (0.8)
Lit	M	13	21.5 (0.7)
Dgo	M	11	24.3 (0.9)
Jac	M	14	30.6(2.8)

4.3.3 Climatological data

We retrieved climatic data for 2010-2011, and 2016-2019 from the LuiKotale long-term database. We measured daily cumulative rainfall using a rain gauge (mm/m^2) open to the sky. Temperatures were measured using a min-max thermometer and a Bresser 5 in 1 Weather station deployed in the forest. We modelled climatic conditions at LuiKotale using Walter-Lieth climatic diagrams (Walter & Lieth, 1967), characterizing monthly moisture conditions based on the relationship between cumulative monthly rainfall and mean monthly temperatures. Months are classified as "wet" when monthly rainfall is > 100 mm; "transient" when monthly rainfall is ≤ 100 mm; and "dry" when the rainfall figures below the mean temperature line.

4.3.4 Urine sample collection

Individual urine samples were collected directly into a leaf or indirectly from the surrounding vegetation or the ground (Surbeck et al., 2012). Urine was pipetted into 2 ml cryogenic tubes, marked, and protected from sunlight and body heat to avoid neopterin degradation (Behringer et al., 2017). In camp, cryotubes were transferred into liquid nitrogen, and shipped frozen to the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, where they were stored at -20°C. All cycling females were tested for their reproductive state monthly using urinary pregnancy tests (Artron Hcg-Test sticks). The pregnancy stage was assessed retrospectively based on the offsprings' birthdate.

4.3.5 Urinary neopterin measurement

Frozen urine samples were shipped from the Max Planck Institute for Evolutionary Anthropology on dry ice to the German Primate Center in Göttingen, Germany. All samples, assay controls, and standards were thawed to room temperature, vortexed and measured in duplicate with a commercial competitive neopterin ELISA kit (Neopterin ELISA, Ref. 59321, IBL International GmbH, Germany) validated for bonobo urine samples (Behringer et al., 2017). Inter-assay variation was 6.74% (n= 10) and intra-assay variation was 3.66% (n= 309). To account for variation in hydration status, the urine's density (urine specific gravity, SG) was measured using a digital handheld refractometer (TEC++ Dr. Volker Schmidt GmbH: TR35U). All samples with a SG< 1.003 (n= 2) and >1.05 (n= 3) were removed from the dataset to avoid over- or underestimating the uNeo level (Thompson et al., 2016). To assure that most samples were within the range of the neopterin assay's standard curve, SG was used to determine dilution factors between 1:10 to 1:400 for each sample. Samples that fell outside of the measurement range were remeasured at an appropriate dilution factor. Final urinary neopterin levels are expressed in nmol/L corrected for SG and were calculated following Miller et al. (2004), by correcting for dilution factor, and individual and population urine density (SG_{pop}= 1.015).

4.3.6 Statistical analyses

All models were fitted in R 4.0.3 (R Core Team, 2021). We ran a linear mixed effect model (LMM) using the *lme* function from the 'nlme' package in R (Laird & Ware, 1982) to assess the effects of age, sex, health status, and season, on the uNeo levels (**m1;** n= 293). To account for potential seasonal variation in uNeo levels, we added

the sine and cosine of the collection date converted into a continuous circular variable. The sine and cosine predictors represent a generic seasonal term and allow to model a wave like periodic pattern throughout a year (Stolwijk et al., 1999). Finally, we added community and sample collection time as controls for inter-community differences and circadian rhythm of the uNeo level (Auzeby et al., 1988), respectively. We included the day and year of the sample collection per individual as nested random effects to account for multiple samples collected for the same individual on the same day but unevenly between years in the study period. We checked for the absence of collinearity between predictors using the *check_collinearity* function from the 'performance' package (Lüdecke et al., 2021, VIF≤ 1.24 for all predictors). We found a slight kurtosis and heteroskedasticity from the qq-plot of the residuals of the expected values versus observed values that can be explained by the failure of our model to predict the few very low and very high values.

To prevent redundancy between sex and reproductive state predictors, we ran a separate LMM (**m2**) with female only samples (n= 220) including reproductive state with the following levels: lactating, pregnant, post-reproductive and two states of cycling with swelling stages 1) 1-2 and 2) 3-4, as a predictor and keeping all other factors from (**m1**) as controls. In female bonobos, ovulation mostly occurs when swellings are at stage 3 or 4 (Douglas et al., 2016). In (**m2**) we also found no correlation between predictors (VIF≤ 2.10 for all predictors).

The significance of the full model to the null model comparison was derived using a likelihood ratio test (anova with argument test set to "Chisq"; (Dobson & Barnett, 2018). Null models only contained control variables and random effects in the respective models. In both models, the response variable uNeo was log-transformed and all other quantitative variables were z-transformed to a mean of zero and a

standard deviation of one (Schielzeth, 2010). Assumptions of normally and homogenously distributed residuals were met in both models, and diagnostic plots to verify there was no concerning impediment were run.

4.4. Results

Overall, 309 urine samples (230 female and 79 male samples) from 28 individuals (mean= 11 samples/individual, sd± 8.1) collected in 2010-2011 (n= 37) and 2016-2019 (n= 272) were analysed. Of these, samples from injured individuals (n= 16) were excluded from analysis, as injuries can also cause an increase of the uNeo level (González et al., 2020). Amongst 309 samples, 27 were collected from pregnant females (n= 5). We collected eight samples from six individuals showing signs of respiratory infection and eight samples from four individuals with symptoms of diarrhoea and/or asthenia. Levels of uNeo varied between 83.9 and 8,100.9 nmol/L corr. SG (median= 832.4; n= 293 samples). In uNeo levels we found variability between individuals (anova: $F= 1.55$, $p< 0.05$, n= 293; Figure 4.1).

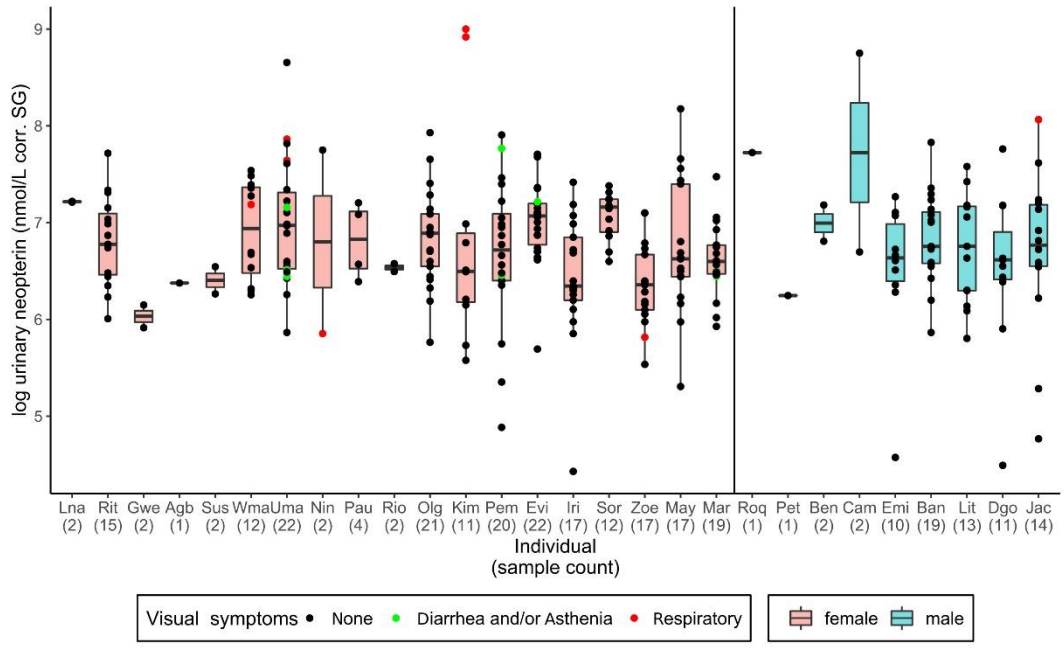


Figure 4.1 | Log-transformed urinary neopterin (uNeo) level of LuiKotale bonobos between 2010 and 2019. Samples (n= 293) of individuals showing respiratory symptoms (red dots), diarrhoea and/or asthenia (green dots) and asymptomatic individuals (black dots) are ordered by sex (red boxplot: female and blue boxplot: male), and within sex by mean individual estimated age on the sampling date. Numbers in brackets show sample count per individual. Boxplots show median (horizontal line), with 3rd and 1st quartiles (upper and lower limit of the box), range (vertical line) and outliers (black dots).

In **m1**, the full-null model comparison was significant (χ^2= 30.5, df= 5, p< 0.001). Only the presence of symptoms (Figure 4.2) and the sine function were significant predictors of uNeo levels (Table 4.2). Bonobos with respiratory symptoms, had higher uNeo levels compared to visually asymptomatic individuals (anova: F= 6.65, p< 0.01; posthoc test Tukey HSD between presence of respiratory symptoms versus no symptom: p< 0.001). However, some individuals showed elevated uNeo levels (n= 12) despite being visually asymptomatic (max $_{asymptomatic}$= 6,316.2 nmol/L corr. SG; n= 278).

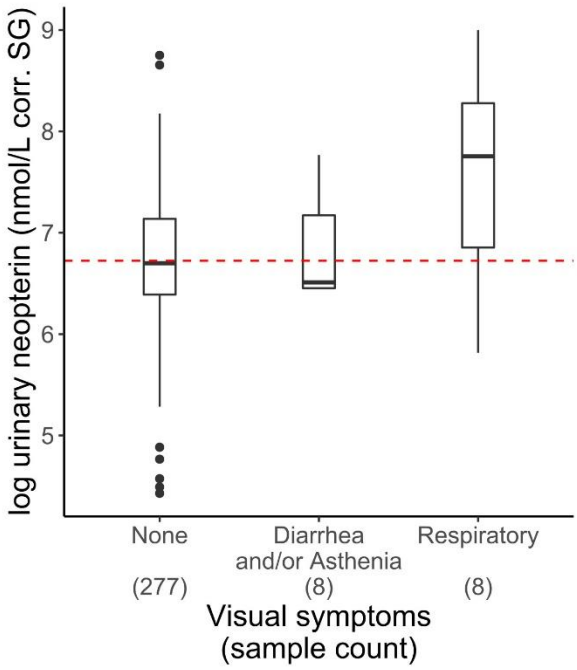
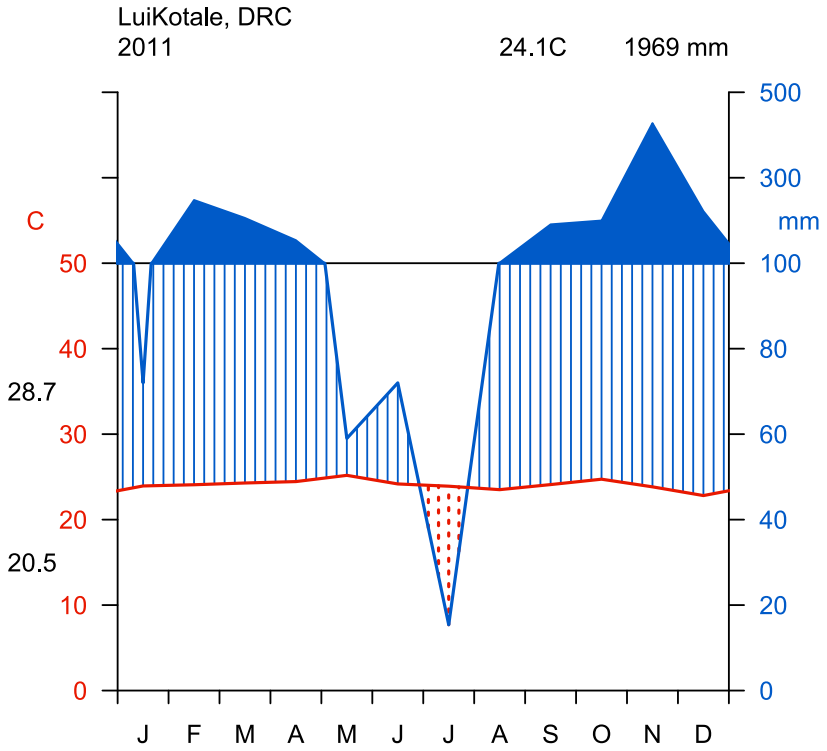


Figure 4.2 | Log-transformed urinary neopterin (uNeo) level of LuiKotale bonobos between 2010 and 2019 in the presence or absence of visual symptoms. Numbers in brackets are the sample count per category. Red dashed line: median of uNeo levels across all samples (n= 293). Boxplots show median (horizontal line), with 3rd and 1st quartiles (upper and lower limit of the box), range (vertical line) and outliers (black dots).

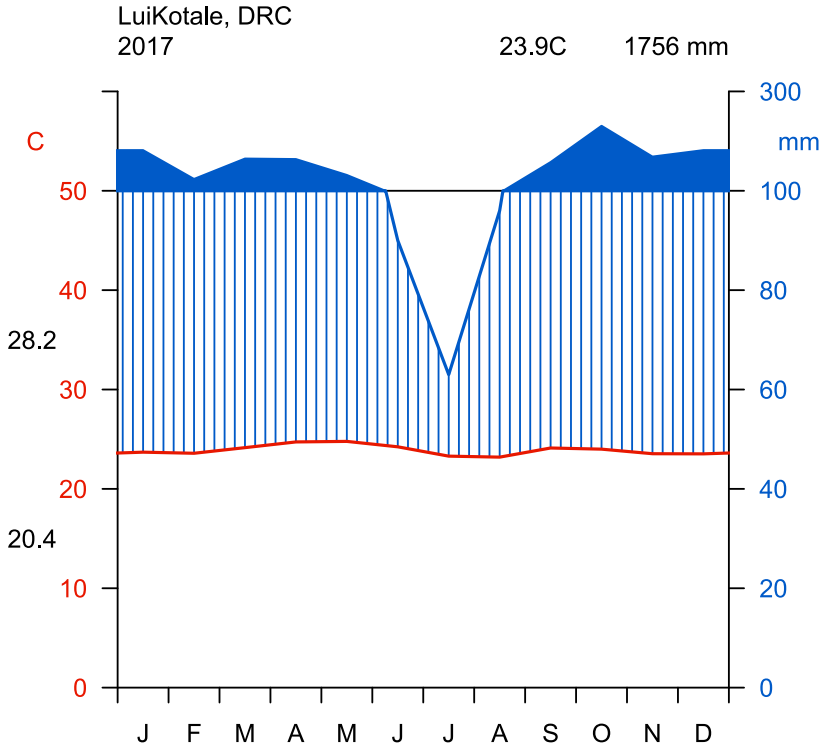
Table 4.2 | Model output for fixed effects in (**m1**) testing for the effect of age, sex, visual symptoms, and season on the urinary neopterin level.

	Estimate	Std. Error	df	t value	p value
(Intercept)	6.760	0.073	178	92.566	0.0000
Age (Z-transformed)	-0.055	0.049	49	-1.121	0.2677
Sex	-0.070	0.108	25	-0.643	0.5258
Visual symptoms	0.590	0.189	49	3.118	**0.0030**
Sine (date)	0.218	0.052	49	4.153	**0.0001**
Cosine (date)	-0.045	0.054	49	-0.835	0.4077

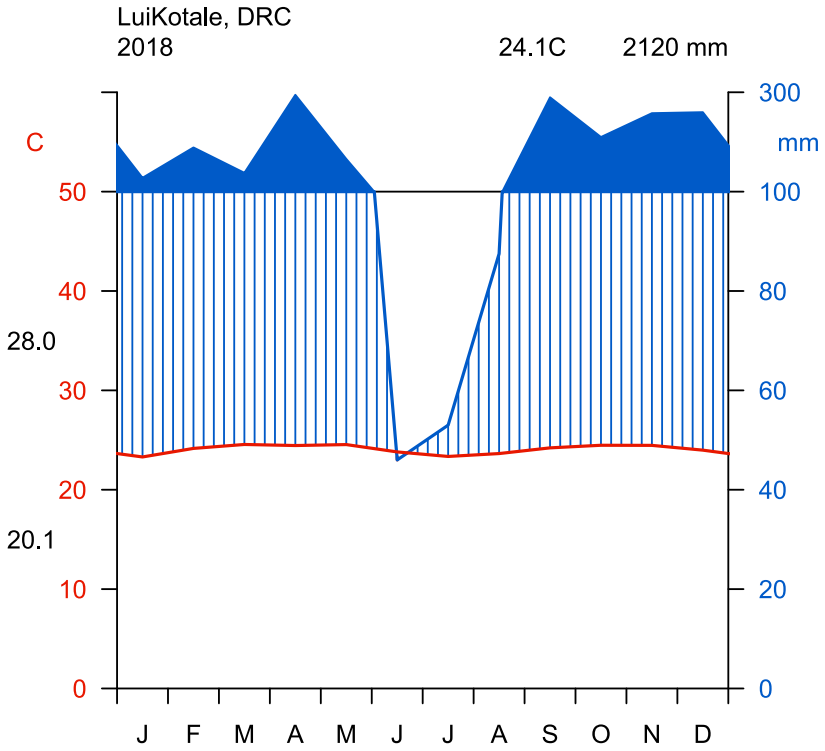
A


LuiKotale, DRC
2011
24.1C
1969 mm
C
mm
50
40
30
20
10
0
500
300
100
80
60
40
20
0
28.7
20.5
J F M A M J J A S O N D

B


LuiKotale, DRC
2017
23.9C
1756 mm
C
mm
50
40
30
20
10
0
300
100
80
60
40
20
0
28.2
20.4
J F M A M J J A S O N D

C


LuiKotale, DRC
2018
24.1C
2120 mm
C
mm
300
100
80
60
40
20
0
50
40
30
20
10
0
28.0
20.1
J F M A M J J A S O N D

D

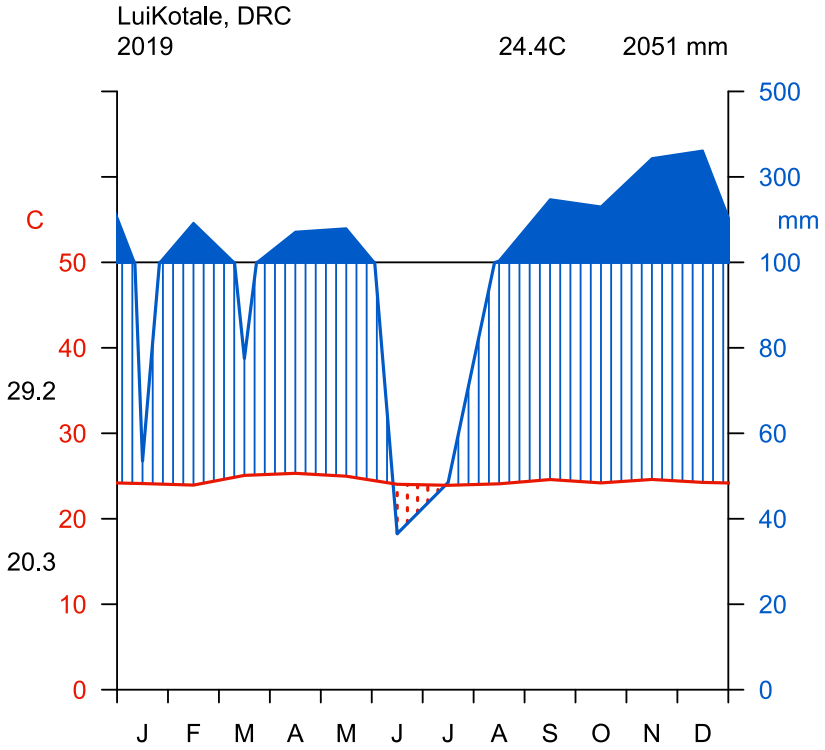

LuiKotale, DRC
2019
24.4C
2051 mm
C
mm
500
300
100
80
60
40
20
0
50
40
30
20
10
0
29.2
20.3
J F M A M J J A S O N D

Figure 4.3 | Walter-Lieth climate diagrams in A) 2011, B) 2017, C), 2018 and D) 2019 based on averaged monthly temperatures and rainfall data collected at LuiKotale, DRC. Red line: mean monthly temperature; blue line: monthly cumulative rainfall. Months are classified as "wet" when monthly rainfall is > 100 mm; "transient" when monthly rainfall is ≤ 100 mm; and "dry" when the rainfall figures below the mean temperature line (Walter & Lieth, 1967).

According to our model (**m1**) uNeo levels fluctuated seasonally following a one-year oscillation period, with higher uNeo levels in March-April and lower levels in September-October. This pattern seems to be unrelated to the climatic seasonality observed at the study site (Figure 4.4). The Walter-Lieth climatic diagrams showed seasonality in LuiKotale was not pronounced (Figure 4.3) with 6.45% of the study months (n= 31) being dry, 32.3% being transient and 61.3% being wet, suggesting the absence of prolonged dry seasons between the two annual rainy seasons.

For the second model (**m2**), the full-null model comparison was not significant (χ^2= 7.1, df= 4, p= 0.13), indicating that variation in female uNeo levels was not explained by reproductive state in our samples (Figure 4.5).

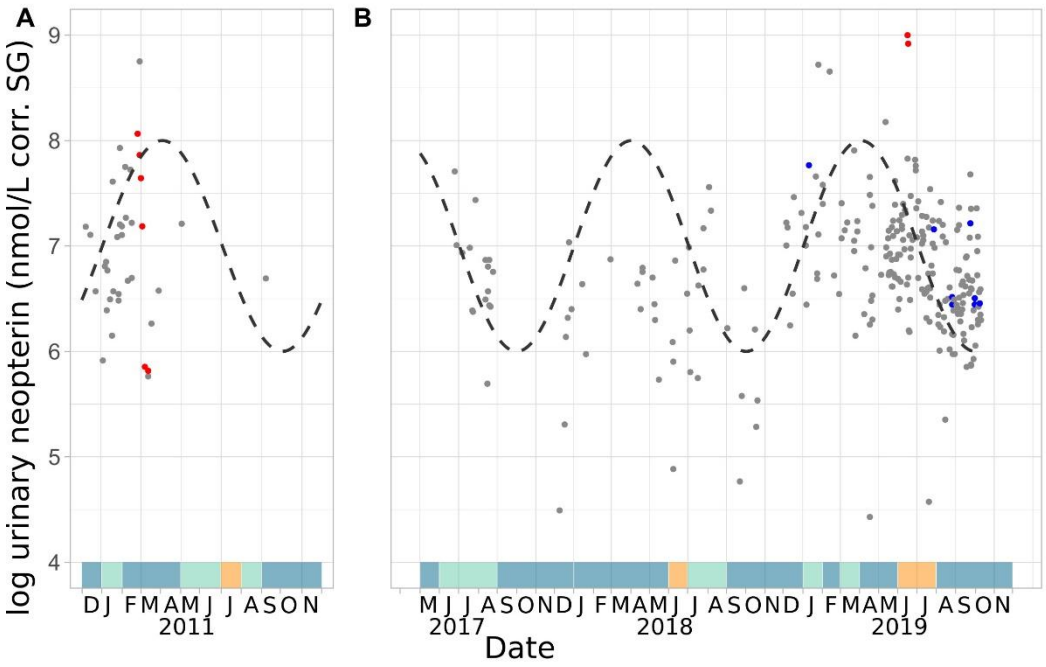


Figure 4.4 | Log-transformed urinary neopterin (uNeo) level in samples collected from (A) 2011 and (B) 2017 to 2019 (n= 292). Dots represent uNeo measurements from visually asymptomatic (grey), symptomatic with unknown infection (blue) and symptomatic with respiratory infection (red) individuals. Dashed line represents one year period sinusoidal oscillation. Areas above the x axis coloured by month represent dry (orange), transient (turquoise) and wet (blue) months as defined by the Walter-Lieth climate diagrams (Figure 4.3).

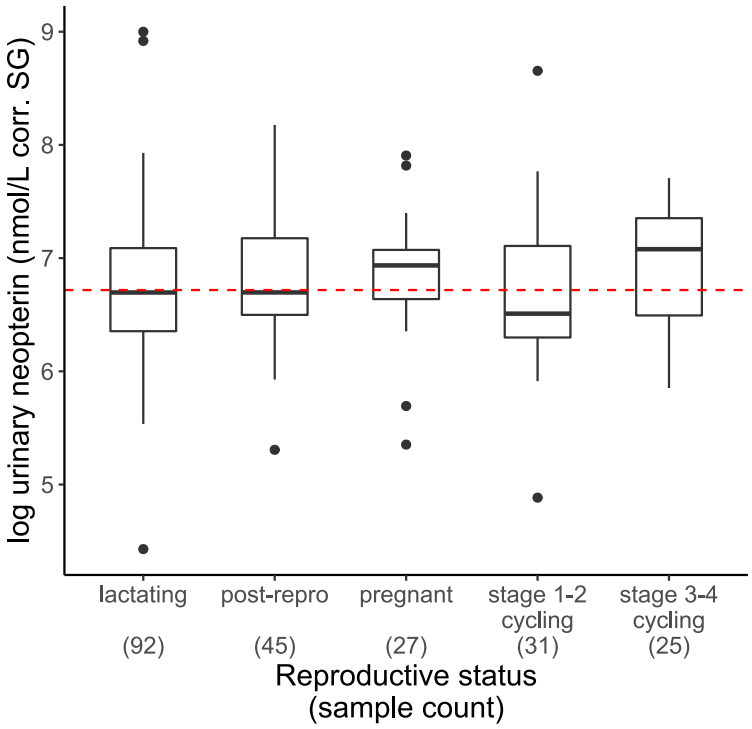


Figure 4.5 | Log-transformed urinary neopterin level (uNeo) of LuiKotale bonobos between 2011 and 2019 in different reproductive states. Boxplots show median (horizontal line), with third and first quartiles (upper and lower limit of the box), range (vertical line) and outliers (black dots). The dashed line represents the median of the log(uNeo) levels.

4.5. Discussion

In this study, we analysed uNeo levels as a proxy for the activity of cell-mediated immunity in relation to seasonality and individual's age, sex, reproductive status, and presence of visual symptoms, to assess whether the uNeo response was species specific in bonobos. We found that urinary neopterin levels were elevated in individuals with symptoms of respiratory infection, and showed a yearly seasonal pattern, supporting our first and second predictions. However, prediction (3) was not supported, in contrast to chimpanzees (Negrey et al., 2021) uNeo levels in bonobos were not related to age. However, our focal individuals were adults only, and estimated

ages may have caused a range of error potentially concealing a tendency. At the time of sampling, the oldest female was estimated to be around 42 y and the oldest male around 32 y. Although life expectancy in wild bonobos is not yet well documented, it is thought to be around 45-50 y (Furuichi, 2019). Similarly, our prediction (4) was not supported either, with female's reproductive state not being related to uNeo levels. This is in contrast to other studies (Bichler et al., 1983; Negrey et al., 2021). However, female bonobos were not sampled across all reproductive states, making intra- and inter-individual comparisons limited, and possibly explaining the absence of the usually robust increase of uNeo level during pregnancy.

In line with findings from humans and chimpanzees, uNeo levels were often, but not systematically, higher in bonobos with respiratory symptoms, compared to asymptomatic individuals (Behringer et al., 2018; Denz et al., 1990; Wu et al., 2018). Some samples collected in 2010-2011 from individuals showing respiratory symptoms showed lower uNeo levels than expected. It is possible those samples were not properly protected from the sunlight after collection, which would have resulted in the degradation of the uNeo (Behringer et al., 2017).

However, we also found elevated uNeo levels in samples (n= 12) from visually asymptomatic individuals, possibly caused by an underlying infection (Eisenhut, 2013). Infections can cause an increase in neopterin production in humans preceding the onset of symptoms by 24 to 48 h (Giovannoni et al., 1997; Murr et al., 2002). Individuals with non-respiratory symptoms such as asthenia and diarrhoea did not differ in their uNeo levels from visually asymptomatic individuals. The immune reaction that increases neopterin levels after infection is specific to intracellular

pathogens such as viruses and malaria parasites (Fuchs et al., 1988). Gastrointestinal symptoms can also be caused by extracellular pathogens that do not activate this pathway of cell-mediated immunity, and thus do not result in elevated neopterin levels (Plata-Nazar et al., 2019). *Oesophagostomum* spp. and *Trichuris* spp. have been identified in samples from LuiKotale bonobos (Kreyer et al., 2021). These extracellular parasites do not stimulate neopterin production. Therefore, this result supports the specificity of uNeo as a biomarker for intracellular infections.

Our results showed uNeo levels follow a seasonal pattern with a one-year periodicity. Urinary neopterin levels were highest at the beginning of the short rainy season around March-April, and lowest at the start of the long rainy season in September/ October. This pattern is different from the seasonal pattern observed in uNeo levels of chimpanzees in West Africa. In Taï chimpanzees, Côte d'Ivoire, Löhrich et al. (2018) found highest uNeo levels during the long dry season, when the minimum ambient temperatures were decreasing, and lowest uNeo levels at the end of the rainy season (Anderson et al., 2006). One explanation for uNeo seasonality could be underlying seasonality in the prevalence of viral respiratory diseases. In the tropics, respiratory infections such as the respiratory syncytial virus or influenza, are more prevalent during rainy seasons (Shek & Lee, 2003; Tamerius et al., 2013). However, the few cases of symptomatic respiratory disease we observed in wild bonobos occurred during a short rainy (February-March 2011) as well as during a dry season (June 2019; Figure 4.4), suggesting that at our field site, the seasonality of uNeo levels detected by our model is not linked to seasonally fluctuating climatic conditions. Moreover, in the 20 years of observation at LuiKotale, only one respiratory outbreak of non-anthropogenic origin was observed (Leendertz pers. comm.; Fruth unpublished data),

suggesting that respiratory infections are not very prevalent in these communities and are therefore unlikely to explain the observed variation in uNeo levels.

Seasonal fluctuations in tropical rainfall can increase the abundance of vectors with aquatic larval stages such as mosquitoes that transmit vector borne diseases such as malaria (Altizer et al., 2006). Wu et al. (2018b) showed that malaria infections occurred seasonally in one community of Taï chimpanzees, where malaria detection rates were highest three months after the start of the rainy season, and lowest at the end of the dry/ beginning of the rainy season. This pattern resembles the seasonality of uNeo levels observed in our study area, where humans get malaria (Muganza et al., 2012; Tajuddeen & Van Heerden, 2019). Rate and timing of malaria infections are not well documented in wild bonobos although malaria seems to be less prevalent than in wild chimpanzees (Liu et al., 2017; Loy et al., 2017). At one bonobo field site, the Tshuapa-Lomami-Lualaba area, but not at 10 other sites, a high prevalence of infections caused by *Laverania*, a subgenus of the genus *Plasmodium* was found in bonobos (Liu et al., 2017). Bonobos also possess MHC class I allotypes resembling allotypes that provide malaria resistance in humans (De Groot et al., 2018). Therefore, bonobos may respond to *Plasmodium* infections with a less pronounced response in neopterin production. If indeed MHC class I allotypes in bonobos provide protection against malaria, both the seasonal fluctuation in uNeo levels, and the absence of a drastic increase in uNeo levels can be explained. In future, it would be interesting to assess uNeo levels and malaria prevalence in bonobos in parallel to assess their specific immunity.

Other infectious diseases, such as monkeypox (Mandja et al., 2019) or measles (Martinez, 2018), are also potentially present in the study area. They are known to occur during the dry months, with an irregular or absent annual cyclicity (Mandja et al., 2019; Martinez, 2018). Further studies are necessary to identify pathogens that affect bonobos at LuiKotale and the uNeo response they elicit. Also, testing seasonality over longer periods of time than one year may uncover trends possibly matching the cyclicity of infectious diseases other than those mentioned above. The irregularity of our sample collection did not allow for this. The uNeo levels reported in our study could thus reflect specific conditions of our study population (prevalence, frequency, and type of pathogens), and/or a species-specific trait that has emerged as an adaptation to environmental conditions of the central Congo basin (e.g. more selective sweeps in the MHC class I repertoire).

LuiKotale bonobos are isolated from human settlements with researchers being the only humans in regular proximity to the study communities. Researchers wear surgical masks when following bonobos to limit transmission of pathogens. Therefore, it is very unlikely that LuiKotale bonobos encounter human borne novel pathogens to which their immune system is completely naive, while captive conspecifics are regularly exposed and show a high sensitivity to human transmitted pathogens (reviewed in Stevens, 2020). This is in line with studies on wild and zoo-housed chimpanzees (Glasser et al., 2021; Negrey et al., 2019). Examining pathogen susceptibility and prevalence in wild bonobos will require integrating neopterin measurements with local disease ecology. Nevertheless, human pathogen transmission remains a serious threat for Africa's wild great ape populations (Boesch, 2008; Fruth

et al., 2016; Leendertz et al., 2006a) and understanding baseline levels and factors triggering their deviation is crucial for future population management.

In conclusion, our study supports the use of uNeo levels as a marker of the cell-mediated immune response as an efficient way to monitor outbreaks of infectious diseases most likely caused by viruses and other intracellular pathogens. Disease monitoring in wild bonobos in particular, and great ape populations in general, is essential in the context of rapid environmental changes that change the pathogenic environment of wild populations. We recommend systematizing the use of uNeo levels as health monitoring tool of individual habituated great apes, considering the influence of the different individual and environmental factors discussed here. Our results showed that uNeo levels were more elevated in individuals with symptoms associated with respiratory infections, and cyclically at the beginning of the short rainy season, but not in older individuals or pregnant and fertile females. Our results revealed a yearly seasonality in the variation of the uNeo levels that may be related to seasonality in the prevalence of malaria, and/or other unknown infectious diseases differing from what is observed in other species, including chimpanzees, bonobo's closest relative.

4.6. Acknowledgments

We thank the Institut Congolais pour la Conservation de la Nature (ICCN) for granting permission to work at LuiKotale, Lompole and Bekombo villages for facilitating research in their forest. Funding for research in field and lab came from the Centre for Research and Conservation of the Royal Zoological Society of Antwerp (CRC/ KMDA), the Max-Planck Institute of Animal Behavior (MPIAB), Department for the

Ecology of Animal Societies, and Liverpool John Moores University (LJMU). We thank Michaela Hau and her team of the Evolutionary Physiology research group, Max-Planck Institute for Ornithology, Seewiesen, for their help with laboratory analyses on a preliminary data set. Likewise, we thank the German Primate Center for their assistance and support with laboratory analyses. Special thanks for long-term support go to Gottfried Hohmann and Zjef Pereboom. Meg Crofoot, Keith George, Michael Heistermann, Richard McElreath, Andy Tattersall, Peter Wheeler and Martin Wikelski are thanked for their support in named institutions. Giulia Rossi, Sonya Pashchevskaya, Lambert Booto, and the LuiKotale Bonobo Project team are thanked for their assistance in the field, and Mattia Bessone and Alexander Vining for their help with statistical analyses.

CHAPTER 5
GENERAL CONCLUSION

The main objectives of this thesis were to investigate 1) wild bonobo health status by using non-invasive methods and 2) the extent of medicinal plant use in wild bonobos. During a 20-month study period between December 2018 and July 2020, we followed individuals from two habituated bonobo communities, at the LuiKotale field site in the Democratic Republic of the Congo (DRC). We carried out 319 focal individual follows of at least four hours on 18 adult individuals, collecting behavioural (scan sampling and all-occurrences), socio-ecological and phenological data as well as urine and faecal samples (n_{urine}= 776, n_{faeces}= 1088). We investigated 1) bonobo food repertoire and its overlap with the pharmacopeia of traditional healers to identify medicinal plant candidates and explore their use by bonobos in the presence and absence of sickness behaviour and health impairments (**Chapter 2**), 2) the consumption of *Manniophyton fulvum* (Euphorbiaceae) by bonobos and its association with gastrointestinal parasite infection (**Chapter 3**), and 3) the association between urinary neopterin levels and season, individual age-sex class, presence of sickness signs, and reproductive status (**Chapter 4**).

5.1. Main findings

In **Chapter 2** we highlighted the overlap between the traditional pharmacopeia of people from tropical Africa, with a focus on the Nkundo, and the bonobo food repertoire. We also compared food intake by bonobos in the context of health impairments. During the 20-month study period, we identified 20 items (6.4%) from

19 plant species (11.5%) consumed by bonobos that are also used as ethnomedicine. 12 of these 20 plant items were defined as staples, meaning they were consumed regularly throughout the study period or by several party members. We observed sickness behaviour and sickness signs on 8% of focal days, with no group epidemics. Individuals usually recovered within 1-4 days. We found that sickness behaviour and signs did not predict feeding behaviour; that is, individuals did not consume more vegetative plant parts than fruits compared to visually healthy individuals, contrary to our prediction. The presence of health impairments also was not associated with the consumption of medicinal plant candidates or processed plant items. Nevertheless, we observed individuals consuming plant items used as medicine by humans regardless of the presence of health impairment. We hypothesized the consumption of those plant items can serve either a preventative or curative role in the treatment of diseases indicating the need for future investigations of the phytochemical content of medicinal plant candidates and their relevance for self-medication in bonobos. Alternatively, we suggested that the absence of association between the consumption of medicinal plant candidates and the presence of health impairment was because health impaired individuals did not need to use them. Behavioural adjustments such as less time spent travelling, feeding, and engaging in social interactions, may have been sufficient in those cases to re-allocate enough energy into the immune system to fight pathogens.

In **Chapter 3** we focused on the consumption of *Manniophyton fulvum* leaves and stem bark, previously investigated for its postulated antiparasitic properties at both LuiKotale and Lomako field sites (Dupain et al., 2002; Fruth et al., 2014). *M. fulvum* has antioxidant, anti-inflammatory, antibiotic, and antiviral phytochemical properties (Anthony et al., 2013; Nia et al., 2005), and is traditionally used by the Nkundo as an

anti-diarrheic, antipyretic, antispasmodic, and wound curative (Muganza et al., 2012). We focused on the leaf-swallowing and stem-stripping of *M. fulvum* using parasitological analyses. Bonobos consumed *M. fulvum* at higher rates in the early morning on an empty stomach and excreted between 7.5 to 8.25 hours later depending on the plant part(s) ingested, similar to observations of medicinal plant use in chimpanzees (Huffman et al., 1996). We observed *M. fulvum* consumption on 22% of observation days, a four fold increase compared to a previous study at this site (Fruth et al., 2014), emphasizing the importance of research design for the investigation of inconspicuous behaviours such as self-medication. We also showed that relying on indirect (faecal) rather than direct (focal) observations resulted in underestimating the frequency of the behaviour, particularly when collecting night nest faecal samples which were significantly less likely to contain *M. fulvum* remains than day samples. Interestingly, we also found a discrepancy in *M. fulvum* leaf and stem-bark preference between communities, which for the first time suggests a cultural aspect to using this plant to self-medicate in great apes.

We analysed 969 faecal samples macroscopically and 588 microscopically, the largest sample set analysed so far in bonobos, in search for 1) *M. fulvum* remains and macro and 2) microscopic parasites respectively. We found no parasitic worm or fragment, such as helminth larva and tapeworm proglottid at the macroscopic level in those samples. As such, the theory that leaf-swallowing aids the expulsion of adult larvae of *Oesophagostomum stephanostomum* nematodes (Huffman et al., 1996; Huffman & Caton, 2001) and proglottid fragments of *Bertiella studeri* tapeworms (Wrangham, 1995) was not supported. However, it is important to note that the absence of evidence of intestinal parasites in faeces is not evidence for the absence of the possible intestinal parasitic burden an individual may carry (Van Gool et al., 2003). Microscopic analyses

revealed the presence of potentially pathogenic parasite species and particularly strongyle eggs, of which what resembled *O. stephanostomum* eggs. We found strongyle eggs in 2.9% of faecal samples, representing a 13, 15 and 23-fold decrease compared to results from reports at Wamba, Manzano and Lomako, respectively (Dupain et al., 2009; Hasegawa et al., 1983; Narat et al., 2015). Our results showed the presence of strongyle eggs in faeces was not associated to the consumption of *M. fulvum* leaves and stem bark and, interestingly, the frequency of *M. fulvum* consumption was lower during the period in which we found strongyle eggs. Our results support an alternative hypothesis from that of Huffman and Caton (2001), suggesting that trichomes present on the surface of leaves being swallowed whole increase gastrointestinal motility and aid physical removal of larvae resulting in their expulsion. Huffman and Hirata (2004) showed leaf-swallowing of rough-surfaced leaves was not an innate response to infection in zoo-housed chimpanzees, since symptom-free individuals performed the behaviour as often as infected ones. If the consumption of *M. fulvum* in LuiKotale was triggered by the burst of L4 larvae from their nodules, in the case of *O. stephanostomum* infection, we would have expected to find macroscopic worms associated with *M. fulvum* remains in the faeces. Instead, *M. fulvum* consumption may be explained by intestinal pain and discomfort induced by different conditions. In sum, our work supported Huffman and Hirata's (Huffman & Hirata, 2004) findings and encourages to keep an open mind in future when looking at the rational of this behaviour in great apes.

In **Chapter 4** we measured urinary neopterin levels in bonobos, as a marker of the cell-mediated immune response, focusing on the impact of environmental and individual factors on the marker's variation. Except for our recent study looking at

juvenile cell-mediated immune ontogeny (Behringer et al., 2021), this study was the first to measure urinary neopterin level in adult wild bonobos and its relationship with age-sex class, season, presence of visual symptoms, and reproductive status. In line with previous studies on primate species, urinary neopterin levels did not relate to age-sex class or reproductive status of an individual (Behringer et al., 2021; Müller et al., 2017; Negrey et al., 2021). However, urinary neopterin levels were higher in individuals showing signs of respiratory infection and varied seasonally over a one-year period. Urinary neopterin levels were highest at the end of the short rainy season and lowest at the start of the long rainy season, contradicting results in chimpanzees (Löhrich et al., 2018). Climate can cause a seasonal pattern in infectious diseases (Altizer et al., 2006; Picot et al., 1993). We suggested seasonal variation in neopterin levels being a response to seasonally occurring infectious diseases, including malaria and mild infections. We explained the lack of conspicuous symptoms with a specificity inherent in bonobo physiology (of their MHC class I repertoire), likely rendering them resistant to malaria infection. Our study, in support of previous clues, hinted at bonobo cell-mediated immune specificity (De Groot et al., 2018; Maibach & Vigilant, 2019).

5.2. Future prospects

Although I was able to observe the consumption of medicinal plant candidates and evidence for health impairment in wild bonobos, some important aspects of bonobo ecophysiology remain to be tested in the future. Given the extremely diverse food repertoire of bonobos at LuiKotale, we need to investigate the use of items for their nutritional versus non-nutritional purpose. While our results did not suggest health impaired individuals consumed more plants requiring specific processing, we

observed bonobos consuming plant items without any obvious nutritional value, including the inner-bark or cambium of *Santiria trimera* and *Grewia* sp. and the ligneous petioles of *Millettia sapinii*. *S. trimera* bark is traditionally used by healers as a medicine for various conditions, including tuberculosis, and scabies (Martins et al., 2003; Ndah et al., 2013). However, when used as medicine, bark of this species is processed (typically through decoction) and examining its effect on bonobos is therefore problematic.

Further research of plant but also non-plant items with potential pharmacological properties are needed. Bonobos consume soil of different composition, mostly from *Cubitermes* and *Macrotermes* termite mounds (Kano & Mulavwa, 1984; Fruth unpublished data). Geophagy is widespread in non-human primate species (reviewed by Pebsworth et al., 2019). One hypothesis for the evolution of geophagy is its protective role on the gastrointestinal tract. Geophagy assists animals to absorb toxins and alleviate diarrhoea, and mediates infection by parasites (Pebsworth et al., 2019). Given geophagy is likely an important aspect of self-medication, this behaviour may complement and/or synergize the potential role of *M. fulvum* (see **Chapter 3**) and requires further investigations in bonobos.

Results from the parasitological analyses in **Chapter 3** confirmed the presence of pathogenic parasite species in bonobo faeces. Molecular screening of parasite species in faecal samples (originally part of the study design) is an important next step for quantifying and identifying gastrointestinal parasite species at the genus and species level in bonobos faeces (Chapman et al., 2005). Such molecular analyses would ultimately help us understand, by quantifying and qualifying parasite prevalence and diversity, if the prevalence of parasitic infections influences bonobo behaviour, including the consumption of *M. fulvum* and other medicinal plant candidates. For

example, innovative technologies are being developed that allow the sequencing and species-level identification of parasitic nematodes from faecal samples using a portable molecular biology lab (Knot et al., 2020; Knott et al., 2021; Piel et al., 2022). Given the remoteness of many ape populations and the logistic difficulties of sample storage and transportation, this technology could revolutionize parasitological analyses in field studies in the future.

In **Chapter 4** we suggested that seasonal variation of neopterin levels in LuiKotale bonobos was the result of the seasonal pattern of unidentified pathogen infection(s). A more comprehensive understanding of the ecoimmunology of bonobos requires linking local disease ecology with neopterin measurements. In this thesis we used methods allowing detection of individual sickness (**Chapter 2**) and identification of pathogens affecting bonobos such as faecal parasitological analysis (**Chapter 3**). Other recent methods, including molecular diagnostics of viral, bacterial, or parasitological pathogens that can provide rapid accurate diagnostics could be used in future studies to determine what type of infectious diseases and pathogens wild bonobos carry (Patrono et al., 2022; Vlčková, Kreisinger, et al., 2018; Zimmerman et al., 2022).

5.3. Relevance

Non-human primates serve as reservoir species to many zoonotic pathogens (Kebede et al., 2020; Kotait et al., 2019; Li et al., 2012). Non-human primate viruses represent a major threat to humans, such as those responsible for epidemics of HIV/SIV, monkeypox, rabies, and yellow fever (Devaux et al., 2019; Reynolds et al., 2019). Moreover, the acquired immunodeficiency syndrome (AIDS) pandemic is thought to have originated from cross-species transmission of the non-human primates' SIV and has considerably increased public health concerns about zoonoses from primate species (Corbet et al., 2000; Van Heuverswyn & Peeters, 2007). HIV is still considered one of the deadliest pathogens for humans (Makam & Matsa, 2021). Aside from viruses, other pathogenic bacteria and parasites have raised concerns for their zoonotic potential across non-human primates range countries, including malaria, another of the deadliest diseases in humans (Adrus et al., 2019; Carrillo-Bilbao et al., 2021; Ramasamy, 2014). As the result of population growth and globalisation and the many contexts in which humans interact with primates (e.g. research, tourism, and cultural / spiritual gatherings), the physical distance between wild primates and humans has never been so slim.

While guidelines for research and tourism involving wild great apes are continuously updated based on research advances and implemented to populations under research as best as possible, such measures are extremely difficult to enforce in touristic places and contexts, where people may spend substantial amounts of money to see non-human primates in their natural habitats. In such situations, it is common for tourists to get up close to primates, and rangers to allow such behaviour for the sake of improving visitor experiences. In Bwindi Impenetrable National Park in Uganda, a study showed the average minimum distance between tourists visiting mountain

gorillas under the supervision of park rangers was as short as 2.76 m, being critically smaller than the recommended 7 m, and increasing the risk of airborne disease transmissions (Sandbrook & Semple, 2006).

The ongoing (at the time of writing) SARS-COV 2 pandemic has been instrumental in highlighting that social distancing is effective in limiting and preventing transmission of airborne viruses. Respiratory infections are a major mortality risk for non-human primate populations. Keeping appropriate distances and using surgical masks, which are already the standard measures in use by researchers following great apes (Devaux et al., 2019; Macfie et al., 2015), should become the norm where non-human primates and humans are in close proximity.

Some bonobo research sites are experiencing increasing human presence at the interface of the populations' range. Such encroachment increases the risk of pathogen transmission, and overall, progressively changes the pathogenic environment of bonobos. This could result in long-term consequences on the fitness and survival of such populations, similar to local extinctions of chimpanzee and gorilla populations in the past (Bermejo et al., 2006; Hanamura et al., 2008; Walsh et al., 2003). In an effort to conserve and reduce the health risks facing bonobos, it will be critical to develop standardized methods to study and monitor bonobos health so as to be able to compare results between sites and ultimately provide ongoing surveillance of the health status of populations living near humans.

Researching what diseases affect bonobos represents a crucial step in the optimization of conservation strategies and in our understanding of bonobo's life-history trade-offs and interactions with their environment. While fatal diseases should be given priority, given the high risks of population extinction, mild diseases are also important to

account for, since they can impact populations in the long term by decreasing individual fitness and reproductive abilities (Löhrich, 2020).

In conclusion, history has taught us that disasters can result from anthropogenic impact on wild great ape populations. Now more than ever, it is critical to enforce guidelines for the study of wild great apes in particular, and primates in general, and develop standardized health monitoring methods for the long-term surveillance of populations with the possibility of range-wide comparative studies. Only by doing so we can minimize and prevent our impact on wild populations and continue studying fascinating behaviours, including cryptic ones like the use of medicinal plants, in our closest living relatives.

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APPENDIX 1

Plant food repertoire and plant parts ingested by bonobos at LuiKotale, Democratic Republic of the Congo (DRC). Local names refer to the dialect of the Nkundo people inhabiting central DRC. Local names are not systematically temporarily or geographically consistent, and may refer to different plant species or genera. One genus/species may have different local names. Plant parts ingested by bonobos at LuiKotale (only for species observed in the present study and in Beaune et al., 2013a): **ar**: arylum, **sb**: stem bark, **ca**: cambium/inner bark/sap, **fl**: flower, **le**: leaf, **me**: mesocarp (fruit), **se**: seed, **pe**: petiole, **pi**: pith/stem, **ro**: root. Ref:* all plant species (identified and unidentified) were recorded in the frame of the long term study between 2002 and 2020 (Fruth & Hohmann, unpublished data), 1: present study, 2: Beaune et al., 2013a

Family	Genus	Species	Local name	ar	sb	ca	fl	le	me	se	pe	pi	ro	Ref*
Achariaceae	*Lindackeria*	*dentata*	Saake						x	x				1
Agavaceae	*Dracaena*	*arboerea*	Bokaka					x				x		1
Amarylcidaceae	*Scadoxus*	sp.	Esandza Wale											
Anacardiaceae	*Antrocaryon*	*nannanii*	Bonkongwende											
Anacardiaceae	*Sorindeia*	*gilettii*	Ipambwa						x	x				1
Anacardiaceae	*Sorindeia*	*multifoliolata*	Ipambwa ya pembe						x	x				1
Anacardiaceae	*Sorindeia*	*zenkeri*							x	x				2
Anacardiaceae	*Trichoscypha*	*acuminata*							x	x				2
Anacardiaceae	*Trichoscypha*	*arborescens*	Bohungwu					x	x	x				1,2
Annonaceae	*Anonidium*	*mannii*	Bodzingo						x	x				1,2
Annonaceae	*Enantia*	*olivacea*	Ikodzi konga			x			x	x				1,2
Annonaceae	*Enantia*	*pilosa*							x	x				2
Annonaceae	*Friesodielsia*	*enghiana*	Bodzingo kodzi						x	x				1
Annonaceae	*Greenwayodendron*	*suaveolens*	Bodzinda						x	x				1

Family	Genus	Species	Local name	ar	sb	ca	fl	le	me	se	pe	pi	ro	Ref*
Annonaceae	*Isolona*	*hexaloba*	Bodzungu						x	x				1,2
Annonaceae	*Monanthotaxis*	*myristicifolia*	Bombende						x	x				1,2
Annonaceae	*Thonnera*	*congolana*	Bodzungu (Bumbanama)											
Annonaceae	*Uvaria*	*acabrida*	Bombende						x	x				1,2
Annonaceae	*Uvaria*	*engleriana*							x	x				2
Annonaceae	*Uvaria*	sp.	Bodzingo kodzi II						x	x				1
Annonaceae	*Uvariastrum*	*pynaertii*	Bomposo						x	x				1,2
Annonaceae	*Xylopia*	*aethiopica*	Bosange			x								1
Annonaceae	*Xylopia*	*chrysophylla*	Iyenzelo					x						1
Annonaceae			Unknown Annoncaceae					x						1
Apocynaceae	*Dictyophleba*	*ochracea*	Idiki ya ntolo					x	x	x				1
Apocynaceae	*Landolphia*	*congolensis*	Batope						x					1,2
Apocynaceae	*Landolphia*	*forestiana*	Baole						x					1,2
Apocynaceae	*Landolphia*	*owariensis*	Batope						x					1,2
Apocynaceae	*Rauwolfia*	*mannii*	Bosenge walenge											
Apocynaceae	*Tabernaemontana*	*crassa*	Bonkole ya pembe						x	x				1
Apocynaceae	*Tabernaemontana*	*iboga*	Somaentele											
Apocynaceae	*Tabernaemontana*	*penduliflora*	Bonkole						x	x				1
Apocynaceae			Bokanampambi											
Apocynaceae			Bototolo											
Apocynaceae			Ikandze											
Araceae	*Anchomanes*	*giganteum*	Ezamvula											

Family	Genus	Species	Local name	ar	sb	ca	fl	le	me	se	pe	pi	ro	Ref*
Araceae	*Culcasia*	*scandens*	Yolama (fern epiphyte)									x		1
Arecaceae	*Eremosptha*	*haulevilleana*	Sapa									x		1
Arecaceae	*Sclerosperma*	*manii*	Ndua						x	x		x		1
Asclepiadaceae	*Perichasme*	*nigrescens*	Lodziki											
Asteraceae	*Vernonia*	*conferta*	Bopopko ya pembe											
Burseraceae	*Canarium*	*schweinfurthii*	Boele						x	x				1,2
Burseraceae	*Dacryodes*	*buettneri*	Iyele nsaw											
Burseraceae	*Dacryodes*	*edulis*	Bosaw											
Burseraceae	*Dacryodes*	sp.	Safu						x	x				1,2
Burseraceae	*Dacryodes*	*yangambiensis*	Sawsaw					x	x	x				1,2
Burseraceae	*Santiria*	*trimera*	Botalala						x	x				1,2
Caesalpiniaceae	*Anthonotha*	*fragrans*	Atsangila							x				1
Caesalpiniaceae	*Antonotha*	*gilletii*	Bootodzi											
Caesalpiniaceae	*Brachystegia*	*laurentii*	Manga							x				1
Caesalpiniaceae	*Cynometra*	*alexandri*	Botuna						x	x				1,2
Caesalpiniaceae	*Cynometra*	*pallestris*	Eaka							x				1,2
Caesalpiniaceae	*Cynometra*	*sessiliflora*	Kfumo				x	x		x				1,2
Caesalpiniaceae	*Erithrophloeum*	*suaveoleus*	Epomi (Efomi)											
Caesalpiniaceae	*Gilbertiodendron*	*dewevrei*	Bolapa				x			x				1,2
Caesalpiniaceae	*Gilbertiodendron*	*ogouense*								x				2
Caesalpiniaceae	*Guibourtia*	*demeusei*	Bokongo					x		x				1
Caesalpiniaceae	*Hymenostegia*	*mundungu*	Bokote											
Caesalpiniaceae	*Jesmania*	*africana*	Waka											

Family	Genus	Species	Local name	ar	sb	ca	fl	le	me	se	pe	pi	ro	Ref*
Caesalpiniaceae	*Julbernardia*	*seretii*	Tutsa ya moindo					x						1,2
Caesalpiniaceae	*Monopetalanthus*	*microphyllus*	Bokese ya pembe				x	x		x				1,2
Caesalpiniaceae	*Monopetalanthus*	sp.	Bokese ya moindo							x				1
Caesalpiniaceae	*Paramacolobium*	*coerulem*	Boongo					x						1
Caesalpiniaceae	*Prioria*	*balsamifera*	Bonkuku (Ntola rouge)											
Caesalpiniaceae	*Prioria*	*oxyphylla*	Tolo blanc (Paki)											
Caesalpiniaceae	*Scorodophloeus*	*zenkeri*	Bopidji				x	x		x				1,2
Caesalpiniaceae	*Tessmannia*	*africana*	Iyala Atwa (Waka)											
Caesalpiniaceae			Bolodzu											
Cecropiaceae	*Musanga*	*cecropioides*	Botumbe					x	x	x	x			1,2
Chrysobalanaceae	*Parinari*	*congensis*	Bondzale											
Chrysobalanaceae	*Parinari*	*excelsa*	Bodzilo mpongo						x					1,2
Clusiaceae	*Garcinia*	*chromocarpa*	Botendo						x	x				1,2
Clusiaceae	*Garcinia*	*ovalifolia*							x	x				2
Clusiaceae	*Harungana*	*madagascariensis*	Bontone											
Clusiaceae	*Mammea*	*africana*	Bokodzi						x					1,2
Clusiaceae	*Psorospermum*	*staudtii*	Impino						x	x				1
Combretaceae			Bompfunde											
Commelinaceae	*Palisota*	*ambigua*	Ntetele									x		1
Commelinaceae	*Palisota*	*brachythyrsa*	Ntetele									x		1
Commelinaceae	*Palisota*	*hirsuta*	Ntetele									x		1
Commelinaceae	*Palisota*	*schweinfurthii*	Ntetele									x		1
Commelinaceae	*Palisota*	sp.	Ntetele									x	x	1

Family	Genus	Species	Local name	ar	sb	ca	fl	le	me	se	pe	pi	ro	Ref*
Connaraceae	*Algelaea*	*dewevrei*	Nkanga te ya moindo											
Connaraceae	*Crestis*	*crestisiomala*	Ikodzua yap											
Connaraceae	*Crestis*	*ferruginea*	Ikodzua yap											
Connaraceae	*Crestis*	*iomalla*	Ikodzwa											
Connaraceae	*Jaundea*	*pubescens*	Iyedza (Yamoinso)											
Connaraceae	*Roureopsis*	*obliquifoliolata*	Botengi											
Cucurbitaceae	*Cogniauxia*	*trilobata*	Saasaka											
Cucurbitaceae	*Momordica*	*foetida*					x							2
Cyperaceae	*Rhynchospora*	*corymbosa*	Ndelenge									x		1
Ebenaceae	*Diospyros*	*hoyleana*	Ikungu						x	x				1,2
Ebenaceae	*Diospyros*	sp.	Mandza					x	x	x				1,2
Ebenaceae	*Diospyros*	sp.	Mandza						x	x				1,2
Ebenaceae	*Diospyros*	sp.	Mandza						x	x				1
Ebenaceae	*Diospyros*	sp.	Bopepeleke											
Euphorbiaceae	*Alchornea*	*floribunda*	Ngombo											
Euphorbiaceae	*Chaetocampus*	*africanus*	Impampale											
Euphorbiaceae	*Cleistanthus*	*mildbraedii*	Boombe						x	x				1
Euphorbiaceae	*Cleistanthus*	sp.	Nzau											
Euphorbiaceae	*Drypetes*	*cinnabarina*	Kalanga ya moindo						x	x				1,2
Euphorbiaceae	*Drypetes*	*leonensis*	Kalanga ya pembe						x	x				1,2
Euphorbiaceae	*Drypetes*	sp.	Boseke					x		x				1,2
Euphorbiaceae	*Macaranga*	*monandra*	Enge nkoso			x			x	x				1
Euphorbiaceae	*Maesobotrya*	*bertramiana*							x					2

Family	Genus	Species	Local name	ar	sb	ca	fl	le	me	se	pe	pi	ro	Ref*
Euphorbiaceae	*Maesobotrya*	*floribunda*	Koka					x	x	x				1
Euphorbiaceae	*Manniophyton*	*fulvum*	Lokosa		x	x		x						1,2
Euphorbiaceae	*Phyllanthus*	*muellerianus*							x					2
Euphorbiaceae	*Phyllantus*	*pynaertii*	Bontepfu					x						1
Euphorbiaceae	*Plagiostyles*	*africana*	Bondenge ya dzamba					x						1,2
Euphorbiaceae	*Spondianthus*	*preussi*	Bodenga ya mai											
Euphorbiaceae	*Uapaca*	*heudelotii*	Bosenge ya mai						x					1
Fabaceae	*Afzelia*	*bella*	Afzelia	x						x				1
Fabaceae	*Angylocalyx*	*pynaertii*	Bwanda ya makita						x	x				1
Fabaceae	*Angylocalyx*	*vermeulenii*	Imanga					x	x	x				1
Fabaceae	*Baphia*	sp.	Lipa											
Fabaceae	*Dalhousiea*	*africana*	Lopusa											
Fabaceae	*Dialium*	*corbisieri*	Maku rouge 5 folioles			x		x	x	x				1,2
Fabaceae	*Dialium*	sp.	Maku pembe 11 folioles						x	x				1,2
Fabaceae	*Dialium*	sp.	Maku pembe 3 folioles					x	x	x				1,2
Fabaceae	*Dialium*	sp.	Maku pembe 3 folioles velvet					x	x	x				1,2
Fabaceae	*Dialium*	sp.	Maku pembe 7 folioles					x	x	x				1,2
Fabaceae	*Dialium*	sp.	Maku rouge 7 folioles			x		x	x	x				1,2
Fabaceae	*Leptoderris*	*gilletii*	Mpute					x						1

Family	Genus	Species	Local name	ar	sb	ca	fl	le	me	se	pe	pi	ro	Ref*
Fabaceae	*Lonchocarpus*	*griffonianus*	Wenge			x								1
Fabaceae	*Millettia*	*sapinii*	Bompuwa ya pembe				x				x			1
Fabaceae	*Millettia*	sp.	Mpute					x						1
Fabaceae	*Parkia*	*bicolor*	Walanga				x			x				1
Fabaceae	*Pterocarpus*	*soyauxii*	Bofulo							x				1
Fabaceae			Bompfete											
Flacourtiaceae	*Caloncoba*	*welwitschii*	Saake						x	x				1,2
Flacourtiaceae	*Camptostylus*	*mannii*	Bonkasa ya dzamba					x	x	x				1
Flacourtiaceae	*Homalium*	*gilletii*	Booyo						x	x				1
Flacourtiaceae			Koba											
Gnetaceae	*Gnetum*	*africanum*	Itakaoke					x						1
Guttiferae	*Garcinia*	*punctata*	Bosepe						x	x				1,2
Guttiferae	*Garcinia*	*smeathmannii*	Itatalongo						x	x				1,2
Guttiferae	*Garcinia*	sp.	Itatalongo ya moindo											
Guttiferae	*Symphonia*	*globulifera*	Bolongo											
Hippocrataceae	*Salacia*	*lehmbachii*	Boluanteke											
Hippocrataceae	*Salacia*	*longipes*	Boluanteke (Bonua wa enteke)											
Huaceae	*Afrostyrax*	*camerunensis*	Isiidza ya moindo											
Huaceae	*Afrostyrax*	sp.	Isiidza ya pembe											
Hymenocardiaceae	*Hymenocardia*	*ulnoides*	Bopanzo					x						1

Family	Genus	Species	Local name	ar	sb	ca	fl	le	me	se	pe	pi	ro	Ref*
Icacinaceae	*Icacina*	*guessfeldtii*	Bonkoto ya moindo						x	x				1,2
Icacinaceae			Bonkoto ya pembe											
Irvingiaceae	*Irvingia*	*gabonensis*	Boseki ya pembe						x					1,2
Irvingiaceae	*Irvingia*	*grandifolia*	Loote											
Irvingiaceae	*Klainedoxa*	*gabonensis*	Boseki ya moindo						x	x				1,2
Lecythidaceae	*Napoleona*	*vogellii*	Elenkete											
Linaceae	*Hugonia*	*gilletii*	Bomposo ya pembe											
Marantaceae	*Haumania*	*leonardiana*	Nkombe									x		1,2
Marantaceae	*Haumania*	*liebrechtsiana*	Nkombe									x		1,2
Marantaceae	*Hypselodelphys*	sp.	Bomomongo									x		1
Marantaceae	*Marantochloa*	*leucantha*							x					2
Marantaceae	*Megaphrynium*	*macrostachhyum*	Nkombe									x		1,2
Marantaceae	*Sarcophizynium*	*schweinfurthianum*	Nkokoloko						x	x		x		1
Maranthaceae	*Hypselodelphys*	sp.	Inkuma											
Melastomataceae	*Dissotis*	*brazzeana*					x	x	x					2
Melastomataceae	*Memecylon*	*calophyllum*	Iseke kodzi					x						1
Melastomataceae	*Ochthocharis*	*ancellandroides*	Lolemba					x						1,2
Melastomataceae	*Ochthocharis*	*dicellandroides*	Lolemba				x	x						1,2
Melastomataceae	*Tristemma*	*mauritianum*	Lolemba					x						1,2
Meliaceae	*Carapa*	*procera*	Bonkonga											
Meliaceae	*Entandophragma*	*angolense*	Paki ya kodzi							x				1
Meliaceae	*Guarea*	*cedrata*	Bonsasa											

Family	Genus	Species	Local name	ar	sb	ca	fl	le	me	se	pe	pi	ro	Ref*
Meliaceae	*Guarea*	*glomerulata*	Botemankoso (Wambo)											
Meliaceae	*Guarea*	*laurentii*							x	x				2
Meliaceae	*Trichilia*	*heudelotii*	Eonge	x						x				1
Menispermaceae	*Albertisia*	*villosa*	Lomaloma ya pembe						x	x				1
Menispermaceae	*Triclisia*	*dictyophylla*	Lomaloma ya moindo						x	x				1
Menispermaceae			Botutuankwa											
Mimosaceae	*Pentaclethra*	*eetveldeana*	Bosimpango											
Mimosaceae	*Pentaclethra*	*macrophylla*	Boala				x			x				1,2
Mimosaceae	*Piptadeniastrum*	*africanum*	Bokungu							x				1,2
Mimosaceae	*Tetrapleura*	*tetraptera*	Bolese											
Mimosaceae			Bokakango											
Moraceae	*Ficus*	*cyathistipula*	Ficus					x	x	x				1,2
Moraceae	*Ficus*	*exasperata*	Ficus						x	x				1,2
Moraceae	*Ficus*	spp.	Ficus						x	x				1,2
Moraceae	*Morus*	*nigrum*							x	x				2
Moraceae	*Treculia*	*africana*	Boimbo						x	x				1,2
Myristicaceae	*Pycnanthus*	*angolensis*		x										2
Myristicaceae	*Pycnanthus*	*marchalianus*	Boondo	x						x				1
Myristicaceae	*Staudtia*	*kamerunensis*	Bokolombe	x						x				1,2
Nympheaceae	*Nymphaea*	*lotus*	Bototoko					x				x	x	1,2
Ochnaceae	*Camphylospermum*	*vogelii*	Imonankoso (butterflower)											

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Ochnaceae	*Campylospermum*	*lecomptei* (?)												
Ochnaceae	*Rhabdophyllum* or *Campylospermum* (?)	sp.	Impandza edza											
Olacaceae	*Olax*	sp.	Loyenze											
Olacaceae	*Onkogea*	*gore*	Boleko						x	x				1
Olacaceae	*Strombosia*	*grandfolia*	Bongondo											
Olacaceae	*Strombosiopsis*	sp.	Botaka						x	x				1,2
Olacaceae	*Strombosiopsis*	*tetranda* (?)	Bokolomposo											
Olacaceae	*Strombosiopsis*	*zenkeri*	Bongondo						x	x				1
Piperaceae	*Piper*	*guineense*	Boleleko						x	x				1
Rhamnaceae	*Lasiodiscus*	*fasciculifloris*	Bomote											
Rhamnaceae	*Lasiodiscus*	*manii*	Ikomi losi				x	x	x					1
Rhamnaceae	*Maesopsis*	*eminii*	Bongombili											
Rubiaceae	*Aidia*	*micrantha*	Enkendu						x	x				1
Rubiaceae	*Canthium*	*orthancantha*	Lilala											
Rubiaceae	*Canthium*	*pulchrum* (?)	Enkundu ya mai											
Rubiaceae	*Cephaelis*	sp.	Mpitschima ya pembe											
Rubiaceae	*Geophila*	sp.	Imbaka											
Rubiaceae	*Leptactina*	*pynaertii*	Kelengenye											
Rubiaceae	*Leptactina* (?)	*benguelensis*	Bolowaalongo											
Rubiaceae	*Massularia*	*acuminata*	Vielo											
Rubiaceae	*Mitragyna*	*stipulosa*	Bodzuwa					x	x	x				1,2
Rubiaceae	*Morinda*	*morindoides*	Kongo bololo					x						1
Rubiaceae	*Nauclea*	sp.							x	x				1

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Rubiaceae	*Oxyanthus*	*formosus* (?)	Losema a sombo											
Rubiaceae	*Rothmannia*	*octomera*	Lolemi ya nkoi											
Rubiaceae	*Tricalysia*	*welwitschii* (?)	Bokakalaka											
Rubiaceae			Unknown Rubiaceae						x	x				1
Rubiaceae			Bonkwala											
Rubiaceae			Ememo											
Rutaceae	*Fagara or Zanthoxythum*	sp.	Paalango											
Sapindaceae	*Blighia*	*welwitschii*	Booso	x										1,2
Sapindaceae	*Chytranthus*	*macrobotrys*	Bonsemi					x	x	x				1,2
Sapindaceae	*Eriocoelum*	*microspermum*	Boembe							x				1,2
Sapindaceae	*Haplocoelum*	*congolanum*	Ipadzi					x	x	x				1
Sapindaceae	*Pancovia*	*belesii*	Mpanda						x	x				1
Sapindaceae	*Pancovia*	*laurentii*	Botende						x	x				1,2
Sapindaceae	*Pancovia* (?)	sp.	Tende a loco											
Sapindaceae	*Placodiscus*	*paniculatus*						x	x	x				2
Sapindaceae	*Placodiscus*	*pynaertii*	Etende nkema						x	x				1
Sapindaceae			Indole											
Sapotaceae	*Autranella*	*congolensis*	Bonianga											
Sapotaceae	*Gambeya*	*lacourtiana*	Bopambu						x	x				1,2
Sapotaceae	*Manilkara*	*malcoleus*	Boonya						x	x				1,2
Sapotaceae	*Manilkara*	*obovata*							x	x				2
Sapotaceae	*Manilkara*	sp.							x	x				2
Sapotaceae	*Manilkara*	*yangambiensis*	Ilonge pambu						x	x				1,2

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Sapotaceae	*Omphalocarpum*	sp.	Bosanga											
Sapotaceae	*Pachystela*	*bequaertii*							x					2
Sapotaceae	*Synsepalum*	*longecuneatum*	Bopfunga					x	x	x				1,2
Sapotaceae	*Synsepalum*	*subcordatum*	Bopfunga tootodzu						x	x				1
Sapotaceae	*Zeyherella*	*longepedicellata*							x	x				2
Sapotaceae			Boiliki											
Sapotaceae			Boyoko											
Simaroubaceae	*Quassia*	*africana*	Bomengia											
Sterculiaceae	*Cola*	*bruneelii*	Bontula					x	x	x				1,2
Sterculiaceae	*Cola*	*clamydantha*	Bosekantete					x	x	x				1,2
Sterculiaceae	*Cola*	*diversifolia*	Lomama					x						1,2
Sterculiaceae	*Cola*	*gigantea*	Bofu					x	x	x				1,2
Sterculiaceae	*Cola*	*louisii*	Bonkasa ya moindo					x						1
Sterculiaceae	*Cola*	sp.	Lopetu ya bonkoko											
Sterculiaceae	*Cola*	spp.	Bonkasa ya mai					x	x	x				1,2
Sterculiaceae	*Cola*	spp.	Bontula					x	x	x				1,2
Sterculiaceae	*Cola*	*urceolata*	Mpome					x						1,2
Thymeleaceae	*Dicranolepis*	*soyauxii*	Bontole ba dzumba					x						1
Tiliaceae	*Desplatsia*	*subericarpa* (?)	Bokomi											
Tiliaceae	*Grewia*	*pinnatifida*	Bopfumo arbuste						x	x				1,2

Family	Genus	Species	Local name	ar	sb	ca	fl	le	me	se	pe	pi	ro	Ref*
Tiliaceae	*Grewia*	spp.	Bopfumo			x		x	x	x				1,2
Ulmaceae	*Celtis*	*brieyi*	Boeko						x	x				1
Vabanaceae	*Clerodendron*	*speciosum*	Imonankoso											
Verbenaceae	*Vitex*	sp.	Londaa						x	x				1,2
Violaceae	*Rinorea*	sp.	Longe ya dzamba											
Vitaceae	*Cissus*	*dinklagei*	Botaatata						x	x				1,2
Zingiberaceae	*Aframomum*	sp.	Bayoyo				x					x	x	1,2
Zingiberaceae	*Aframomum*	sp.	Nsonsombo				x		x	x		x		1,2
Zingiberaceae	*Renealmia*	*africana*							x	x				2
			Bankuala									x		1
			Imbedzi						x	x				1
			Imbedzi ya moindo					x						1
			Yolama (fern epiphyte 1)									x		1
			Yolama (fern epiphyte 2)									x		1
			Matiti I									x		1
			Matiti III									x		1
			Balelenge									x		1
			Mpandzo					x						1
			Orangenia						x					1
			Bokan											
			Bokanga											

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			Bokeke											
			Bosembele											
			Botoo											
			Bwaka											
			Felele											
			Ikuliotomba											
			Iombi											
			Ipale											
			Lukumba											
			Ndola											
			Nkangate											
			Nkeledza											
			Nkoko											
			Singa ya bapa											