Ecological and social influences on cave use and geophagy in the Eastern Chimpanzees (*Pan troglodytes schweinfurthii*) of Bugoma Forest, Uganda



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Declaration

I declare that all work presented in this thesis is primarily my own, unless stated otherwise, and represents my own research.

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Dedication

I would like to dedicate this thesis to my field assistant,

Gerald Mayanja Shaban.

May he rest in peace and his contributions to chimpanzee research continue to protect the animals he loved so dearly for long after he is gone.

Abstract

Caves have been affiliated with human ancestors for millions of years, yet it is a rare behaviour in the closest living relatives of the genus *Homo*, the great apes (Homindae). To date, cave use has only been thoroughly described for the savannah-dwelling Fongoli, chimpanzees in Senegal and no other great apes. However, researchers recently discovered habitual cave use, and the ingestion of the soils therein (geophagy), in the chimpanzees of the Bugoma Forest Reserve in Uganda. This cave is composed of soil and tree roots and set into a large hill and provides an exceptional opportunity to test hypotheses driving cave use and geophagy in one of human's closest living relatives.

This thesis aims to explore hypotheses on the purpose of cave use and geophagy in nonhuman primates by 1) describing the diet and environmental influences surrounding the Bugoma chimpanzees (Chapter 2), 2) conducting comprehensive assays on the characteristics and compositions of the geophagic soils and compare them to nearby control soils (Chapter 3), 3) using proxy variables to determine if the cave is used as a form of shelter, thermoregulatory relief, an access point for water, or a source of nutrients or other health benefits derived from the soil (Chapters 4 & 5), and 4) using the available data to determine if the chimpanzees consume the soil of the cave for its protective properties against toxins, diseases, or gastrointestinal upset (Chapter 4), or if they engage in geophagy to access supplementary nutrients lacking in their everyday diet (Chapter 5). To test these hypotheses, data were collected on weather (rainfall and temperature), diet, soil composition, water levels, and the frequency and duration of geophagic behaviours within the cave. And video data were analysed and each chimpanzee was allocated a sex-age class to determine any influences of sex, life stage, or reproductive status that may impact their decision to enter the cave or engage in geophagy.

Results were not entirely conclusive but do suggest that cave use is most likely driven by access to water or soil. The geophagic soils were found to offer few, if any, nutritional benefits over the controls as the controls are higher in most bioavailable minerals, but the chimpanzees may seek out the higher clay content. Lastly, this thesis concludes that further research into culture and the chimpanzee community dynamics in Bugoma Forest are imperative to fully understanding this rare phenomenon.

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1. Introduction

1.1 The chimpanzees of Bugoma Forest

1.1.1 Chimpanzee ecology and behaviour

Chimpanzees are members of the taxonomic family Hominidae, or the great apes, alongside orangutans (*Pongo* spp.), gorillas (*Gorilla* spp.), and humans (*Homo* spp., though not often referred to as great apes outside of academic contexts) and share their genus with the bonobo (*Pan paniscus*). There are currently 4 subspecies of chimpanzee recognised by the IUCN: the Central Chimpanzee (*Pan troglodytes troglodytes*), the Western Chimpanzee (*Pan troglodytes verus*), the Eastern Chimpanzee (*Pan troglodytes schweinfurthii*), and the Nigeria-Cameroon Chimpanzee (*Pan troglodytes ellioti*; Humle *et al*. 2016). Much of the early study of wild chimpanzees focused on the Eastern Chimpanzee (*P. t. schweinfurthii*) but have since expanded to include all the subspecies at various research sites.

Long-term studies on chimpanzees span the last 60 years (Wilson, 2012) and have provided valuable insights into chimpanzee ecology and behaviour from habitats across Africa. While there are some differences in study effort amongst subspecies, the chimpanzee in general has been exceptionally well described. Chimpanzees exhibit a fission-fusion social system with a larger multi-male/multi-female community comprised of smaller subgroups (parties) whose composition and size vary across time and space (Chapman & Wrangham, 1993; Le Hellaye *et al*., 2010). These communities typically range in size from 20 to 150 individuals (Lehmann & Boesch, 2004) but can even be as small as 10 (Badihi *et al*., 2022). Chimpanzee home ranges can be between 3.1km^2 and 72.1 km^2 (Herbinger *et al*., 2001; Samsun & Hunt, 2012) with most falling between 10km^2 and 30km^2, especially in Eastern Africa (Herbinger *et al*., 2001; Amsel, 2009).

Chimpanzees are omnivorous frugivores, with fruits constituting most of their diet (Wrangham *et al*., 1998), and are considered ripe fruit specialists (Villioth, 2018). In fact, in every habitat where chimpanzees have been studied, ripe fruit constitutes a majority of their diet (McLennan, 2013) and studies have even linked behavioural patterns to their frugivory, such as decreasing subgroup size in response to fruit scarcity (Wrangham *et al*., 1996).

1.1.2 The Bugoma chimpanzees

The Bugoma Central Forest Reserve is a 400km^2 forest block located in western Uganda along the shores of Lake Albert (1°17'40''N 30°59'50''E) and is inhabited by a population of chimpanzees at a density of 1.24 km^{-2} (Plumptre *et al*., 2010). The environment is typically composed of tropical forest with patches of woodland and while it is threatened by illegal logging and deforestation, the protected areas are largely remaining stable or even seeing marginal gains in forest cover in some areas (Twongyirwe *et al*. 2015). Apart from Eastern chimpanzees (*Pan troglodytes schweinfurthii*), there are four other primate species: olive baboons (*Papio anubis*), Ugandan mangabey (*Lophocebus ugandae*), red-tailed monkeys (*Cercopithecus ascanius*), and black-and-white colobus (*Colobus guereza*; Plumptre *et al*., 2010). While there have been surveys on the forest itself (Twongyirwe *et al*., 2015), study of the primate species of this forest only began in 2016 with the formation of the Bugoma Primate Conservation Project (BPCP) which introduced a chimpanzee habituation programme within the Bugoma Central Forest Reserve. Since 2016, the BPCP team has progressed habituation on one particular community, which was named the Mwera community. During the habituation process, the Bugoma chimpanzees, and the Mwera community, were observed engaging in extensive levels of geophagy (ingesting of earthen materials) within earthen cavity near the BPCP camp. As this level of cavity-based soil eating

behaviour has not been described in the literature, this thesis aims to offer initial descriptions of this unique behaviour.

The Mwera community of chimpanzees have not been thoroughly studied (at the time of writing), but recent research endeavours have introduced some literature on the chimpanzees of Bugoma Forest. This research primarily includes studies on nesting, especially the habitual ground nesting behaviour – a normally rare behaviour – which occurs at higher rates in the Mwera community than any other chimpanzee community studied to date (Hobaiter *et al*., 2023; Romani *et al*., 2023; Hicks *et al*., 2019). Additionally, the Bugoma Forest chimpanzees were featured within recent doctoral dissertations (Romani, 2023), but they were not the sole subject which makes this thesis one of the largest bodies of research conducted on both the Mwera community and the Bugoma chimpanzees.

1.2 Cave use

1.2.1 What is a cave?

Definitions regarding what can be considered a 'cave' have evolved considerably over the past 70 years since one of its earliest iterations as a cavity in rock large enough for a human to enter (Cullingford, 1953). This was then refined to describe caves as a space, and not an object, defined by its boundaries and which could also include water filled spaces (Curl, 1964), however nearly every definition or description includes rock as the constituent material of its defined boundaries – even when considering caves created by animal excavation (Lundquist & Varnedoe Jr., 2006; Mylroie, 2019; Lazaridis, 2022). By the early 21st Century the consensus was that a cave is a natural opening into the earth that is both large enough to permit a human entry and deep

enough to keep the internal environs darker and damper than the external environment, and thus requiring torchlight to navigate, and can also be filled with water (Bögli, 1980; White, 1988; McGrew *et al*., 2003; White & Culver, 2012). However, definitions of a cave can vary depending upon the field in which it is defined and what aspects of a cave are to be studied. For instance, there has been an endeavour for biologists to move toward more ecological definitions of caves with some of the most recent criteria including: 1) it is utilised by mesofauna and/or macrofauna, 2) it has accumulations of biotic substrates (guano, debris from vegetation) and sediments, and 3) it is accessed and used by animals, not only by flying species (bats, insects, etc.) but also by terrestrial species not able to use smaller voids or caverns (Moseley, 2009). These criteria focus more on the biotic aspects of the environment within a cave and what it can offer to living things as a shelter, source of nutrients, or the microhabitat created.

Thus far, all the attempts at defining caves above include rock as a defining character. But this fails to capture some nuances when considering cavities that could constitute caves. If an underwater cavity is formed within layers of coral on a reef and would otherwise fit the ecological or basic definitions of a cave given above but is not composed of rock, should this not be considered a cave? Additionally, if the cavity must be 'human-sized' this shows a strong bias towards human exploration, especially considering '*the human as the measure of all caves*' (from Bögli, 1980) necessarily excludes many cavities that may otherwise exhibit characteristics of a cave. Indeed, Bögli (1980) even suggests that while being human-sized is part of the definition, it is up to the discretion of each researcher to decide if smaller cavities are to be considered caves as necessary to the subject of study. To address these issues, the most recent attempt to redefine the cave by Lazaridis (2022) has expanded this definition to include any void/space bounded by solid states and proposes a new definition for caves as:

'*every non-artificial potentially empty underground space in solid matter that can be formed by constructional and destructional geological and biological processes (such as corrosion, erosion/weathering, deposition, tectonics, mass movement, deformation, animal activities or a combination of them) if the same process is capable of creating openings large enough to be entered by humans*' (from Lazaridis, 2022).

One of the key aspects of the definition given above is that it is a *process-based* definition that allows researchers to consider and account for the continual process of cave formation. For instance, a cavity that is created by erosion would be considered a destructional cave, as would one that is excavated by an animal. It is unknown which forces created the cavity in Bugoma, but by all definitions outlined above, this cavity can be considered a cave and will thus be referenced as a cave for the remainder of this thesis.

1.2.2 Cave use in primates

In primates, the use of caves is typically associated with humans, and predominantly as a form of shelter, but there have been occasional descriptions of nonhuman primates utilising cave structures for other purposes, and the need to explore this behaviour further has been highlighted in the literature (McGrew *et al*., 2003). To date, there have been four main hypotheses for cave use in human and nonhuman primates: 1) access to nutritional benefits (via geophagy, the consumption of earthen materials such as soil, discussed in detail in section *1.3* [Chapter 1, this thesis]), 2) access to water, 3) security/shelter, and 4) thermoregulation, either to escape heat during diurnal or cold during nocturnal hours (McGrew *et al*., 2003). However, there is still very little literature on nonhuman primates (henceforth: primates) using caves, and testing these hypotheses has remained elusive.

The earliest reports of cave use by primates were observed in baboons (*Papio* spp.; Gow, 1973; Wells, 1973; and Brain [1981] as cited in McGrew *et al*., 2003), but over time records of other primates visiting caves were also noted. This includes reports of *Colobus satanas* in Gabon entering caves that had been created by elephants (Harrison & Hladik, 1986), and shortly after further observations of *Colobus guereza* and *Cercopithecus mitis* which also took advantage of caves excavated by elephants at Mt Elgon, Kenya were reported (Redmond & Shoshani, 1987). While they did not test any hypotheses, both of these cases of cave use of elephant excavation sites were hypothesised as possible 'saltlick' opportunities. Only one study in the crowned lemur (*Eulemur coronatus*; Wilson *et al*., 1989), suggested an alternative explanation that the caves served as a source of water for the lemurs, and supported this claim by evidencing that cave use ended when the rains started and this also coincided directly with the use of other 'waterholes' in their range. Since these earlier descriptions, several additional publications have highlighted primate cave use, but few have attempted to empirically test any drivers behind this behaviour and many sources in the literature are merely descriptive.

Most studies indicate cave use in primates operates as a form of shelter, either from the elements or predators, and possibly has additional thermoregulatory benefits, with most available data indicating a preference for use as sleeping sites. Capuchin monkeys (*Sapajus libidinosus*) in Brazil have been observed using caves at night and during the day and these are hypothesised to be for thermoregulation and/or shelter (Haslam *et al*., 2009), but there were no data available to investigate these claims. Similarly, while regular use of caves for sleep and water consumption was found in some ring-tailed lemur troops (*Lemur catta*) and hypothesised to be a thermoregulatory response to deforestation pressure, this was not empirically tested (Sauther *et al*., 2013). One group of primates, the limestone langurs (a subset of *Trachypithecus* spp.), has

been noted to preferentially choose caves or ledges as sleep sites (such as the Cat Ba Island langur [*Trachypithecus poliocephalus*] where caves comprise 17% of sleeping sites [Hendershott, 2017]), and studies have hypothesised this behaviour is primarily a function of predator avoidance as caves are often chosen that are positioned in the middle of vertical cliff faces (Huang *et al*., 2003; Greuter & Wei, 2006; Zhou *et al*., 2007; Lui & Bhumpakphan, 2019). This may also be an adaptation to the environment as trees known as sleeping trees (the sleeping preference of many primates [see Fruth *et al*., 2018]) were reported to be lacking in their limestone habitats. Primates may further utilise caves as an adaptation to their environment, such as may be the case in Japanese macaques where there is indirect evidence that they enter caves during winters with heavy snowfalls (Kashiwagi *et al*., 2012). While these, often initial and untested, reports of cave use are imperative to creating a diverse literature from which to investigate any adaptive benefits of caves, further systematic study on whether the cave serves as shelter or a thermoregulatory buffer would be required to fully investigate the underlying benefits.

While the vast majority of recent study focuses on cave use for shelter and thermoregulation, there is now a growing body of literature indicating primates may also seek out caves to access geophagic materials. For instance, in two species of limestone langurs mentioned above, the François' langur (*Trachypithecus francoisi*) and the white-headed langur (T*rachypithecus leucocephalus*), it was hypothesised that cave access could also provide access to nutrients, not only through access to minerals on the cave walls but possibly even a food source of ants or other insects that inhabit the cave (Li *et al*., 2003; Greuter & Wei, 2006). Cave walls serving as a possible 'lick' opportunity have also been hypothesised for some primate species (McGrew *et al*., 2003; Klaus *et al*., 1998), but only recently have studies attempted to investigate this further. A study in Ecuador described an instance of two arboreal species of monkeys, the red howler monkey

(*Alouatta seniculus*) and the white-fronted spider monkey (*Ateles belzebuth*), descending to the ground to access a 'saltlick' in a small cave to both ingest soil and drink water (Link *et al*., 2011). The cave studied here was also the most frequently visited of all geophagic licks in the study area, but little attention was given to the cave aspect compared to its function as a source of soil, so it is unknown how important the cave was to geophagy in these arboreal monkeys or if it was simply a coincidence of location.

Taken together, there appears to be some support for all four hypotheses for cave use (access to geophagy, water, shelter, or thermoregulatory stress relief), with the strongest support for shelter and thermoregulation, but many of these remain untested.

1.2.3 Cave use in chimpanzees

While there are some instances of cave use by chimpanzees, humans' closest living relatives, these are almost exclusively restricted to dry habitats and likely to related to thermoregulation (Pruetz, 2007; Badji *et al*., 2018; Wessling *et al*., 2018), with only one report of forest chimpanzees using a cave (Nishida, 1980). This instance of cave use was observed in a forest community of chimpanzees in Tanzania, which were observed to enter a small cave along the shore of Lake Tanganyika to lick the rock walls (and therefore possibly functions as access to water or geophagy materials). While the authors also suggested this was a very rare behaviour, even this one case shows the impact the surrounding environment might have on cave use. These possible differences in cave function between communities of the same species living in different environments allows for questions of adaptive significance to be explored. Although the savanna dwelling communities in Senegal may provide an ideal platform for investigating human evolution (Pruetz, 2007), research into why and how forest chimpanzees use caves could allow for the

consideration of hypotheses not exclusively related to heat stress. Although forest chimpanzees are not typically considered to be under thermoregulatory stress, studies have shown chimpanzees in Uganda do change their behaviour in response to sun exposure and temperature (Kosheleff & Anderson, 2009). So, thermoregulation may also influence cave use in forest communities but no studies have previously investigated this behaviour. Forest dwelling chimpanzees with access to cave environments may then provide the ideal candidates for investigating alternative hypotheses of cave use.

1.3 Geophagy

1.3.1 What is geophagy?

Geophagy is the deliberate consumption of earth material and is widespread amongst animal taxa, found across nonhuman animal taxa and human cultures alike (Pebsworth *et al*., 2019b; Young *et al*., 2011). This behaviour includes ingesting earth from various sources that can include soil directly from the forest floor, earth from exposed soil horizons (such as deep soils exposed by erosion), earthen mounds such as insect nests, earthen bricks, other nests made with earth (such as ovenbirds) and includes types of earth such as clays and chalks (Pebsworth *et al*., 2019a; Pebsworth *et al*., 2019b). In addition to direct consumption of earth, many studies also include imbibed water that is saturated with soil or clay, such as from muddy pools of standing water (Reynolds *et al*. 2015; Pebsworth *et al*., 2019a). Similar behaviours such as lithophagy (also called lithophany – the ingestion of stones, as seen in some birds and reptiles; Sokol, 1971; Wings, 2007; Downs *et al*., 2019; Uriona & Farmer, 2019), are often not included within geophagy given the differences regarding possible functions. However, it is important to note that some types of behaviour that could fall under a lithophagy umbrella, such as the licking of stone (e.g. in Mahale chimpanzees; Nishida, 1980), are likely to serve similar purposes to that of geophagy. As such,

some argue that lithophagy should be considered within geophagy (or vice versa) as both forms are likely related to digestion and merely reflect a difference in particle size – a commonly measured variable in geophagic analyses – and are therefore on a continuum and not separate behaviours (Downs *et al*., 2019).

Within the context of this study, geophagy will be limited to ingestion of digestible earth materials (to exclude direct consumption of stones, e.g. grit ingestion in birds) but will be extended to include behaviours where stones are handled such that stone contents may end up within the digestive tract of the individual (i.e. licking or holding rocks in the mouth without swallowing). Though this may seem an arbitrary distinction given that swallowing stones would allow access to contents which could be licked from the surface itself, this is helpful as it helps to distinguish from lithophagy functions that digestible earths are unlikely to serve (such as aiding comminution in digestion and mixing of coarse food in avian species; Degernes *et al*., 2012; or serving as ballast or a means to increase specific gravity in aquatic reptiles; Downs *et al*., 2019; Wings, 2007). While lithophagy as a digestive strategy is certainly fascinating, current research does not indicate this is a behaviour found amongst primates or other mammals, thus it will not be directly investigated in this study. However, it will be explored in further sections regarding geophagic behaviour amongst animals to give equal understanding to earth-eating in all its forms and to acknowledge there may be some overlap in function. If the form of earth eating is not as defined above (that is, digestible) then it will be referred to as lithophagy so readers can distinguish between the two. Additionally, the term gastrolith will be exclusively used to describe stones that have been intentionally ingested, and not to describe medical concretions (sometimes also called stones) from the stomach as can be found in some herbivores, such as camelids, and crustaceans (Wings, 2004). Aside from exceptions such as lithophagy, definitions of geophagy rarely differ and most

studies are relatively comparable. Though it should be noted here that an extension of geophagy definitions can be found in human studies to include processing and/or preparation of earth for consumption, such as by baking or grinding (Young *et al*., 2011) which doesn't occur in other animals.

The early study of geophagy focused on spatially limited loci where often more than one species congregated to consume soil, and typically excluded the consumption of soil at so called 'random' locations, or indeed stated that it didn't occur at all (Klaus & Schmidg, 1998). While this is partially true – individuals do seem to return to the same locations and these locations don't appear to be randomly selected – there also appear to be other specific types of sites that animals can utilise (i.e. mounds of specific species of termites in chimpanzees; Mahaney *et al*., 1996b; or 'mining sites' within specific forest types in African Buffalo; Mahaney & Hancock, 1990) which are not simply based on location. These limited locations have been referred to by many names in the literature, such as salt licks, mineral licks, natural licks, salines, or various combinations of these terms (Klaus & Schmidg, 1998), but many of these include words that associate these locations with certain functions (i.e. places to retrieve salt and/or minerals) which may not actually be the case. Additionally, there is very little information known of the size range of these 'licks', but studies typically suggest they can range from simply 'small' (Klaus & Schmidg, 1998) to around 55,500 m^2 (Klaus *et al*., 1998). These licks can be so small and discrete that they are only discovered by direct observation of geophagic behaviour, or large enough that they are mistaken for villages by cartographers using aerial photography (Klaus & Schmidg, 1998; Ruggiero & Fay, 1994). Thus, in this study on chimpanzees, locations where geophagy occurs will be referred to as 'geophagic sites', 'geophagic locations', 'geophagic points', or similar terminology, to avoid associations with multi-species congregations that use one large site and to also include

locations, such as termite mounds, where geophagy can occur that might be species specific or temporally limited.

1.3.2 Hypotheses for geophagic behaviour

Future sections of this chapter will take an in depth look at geophagy in animals, including trying to identify any possible adaptive benefits of geophagy. As such, this will serve as a brief introduction into the main hypotheses behind geophagy. In general, geophagy is usually considered to be an adaptive behaviour with hypotheses falling mainly in three groups: 1) the protection hypotheses, 2) the supplementation hypotheses, and 3) non-adaptive hypotheses.

The protection hypotheses posit that consuming soil grants protective benefits against health ailments such as parasites and gastrointestinal stress, or to help adsorb/mitigate the effects of toxic compounds (Johns & Duquette, 1991; Pebsworth *et al*., 2019b). Testing these hypotheses require determining the chemical composition of the soil and investigating interactions that suggest whether soil compounds bind with toxins or other harmful compounds (Krishnamani & Mahaney, 2000). Also often attributed to the protection hypothesis are the kaolin or related clay minerals which act as anti-diarrhoeal, antacids, and reduce toxicity, which implies consumption of these compounds prevents further ailments of these kinds (Limpitlaw, 2010). Of particular note amongst the protection hypotheses is the suggested role in binding and reducing the effects of harmful Plant Secondary Compounds (henceforth: PSCs; also sometimes called Plant Secondary Metabolites; DeGabriel *et al*., 2009; Pebsworth *et al*., 2019b). While some PSCs can be beneficial, especially if refined and applied in medical uses (Singh *et al*., 2003), they can also cause a number of gastrointestinal problems, such as nausea and diarrhoea, and are especially high in the plant parts of some dietary items in primates (Young *et al*., 2011; Pebsworth *et al*., 2019a).

The supplementation hypotheses states that geophagy occurs to supply nutrients that might otherwise be lacking in the normal diet. The most common examples of nutrients proposed to fulfil the supplementation hypothesis are iron, calcium, zinc, or salts, with emphasis on iron for humans but salt, usually sodium, for animals (Young *et al*., 2008; Limpitlaw, 2010). Nutrient supplementation is considered to be an especially important response for individuals of certain life stages that result in higher nutritional demands, such as when females are pregnant or lactating or young individuals that are still growing (Young *et al*., 2008). This has been given particular attention in human populations, where geophagy (sometimes called 'pica' in historical human literature) is more commonly found in women and children in many cultures (Abrahams, 2009). However, it should be noted that the protection and supplemental hypotheses are not necessarily mutually exclusive, and support may be found for more than one hypothesis even within one study (Pebsworth *et al*., 2019a).

The last hypotheses asserts that geophagy is nonadaptive and that soils are ingested for hedonic sensations, to satisfy hunger, or as part of a behavioural tradition (Krishnamani & Mahaney 2000; Young *et al*., 2011). Studies tend to use this category when there is a lack of evidence for protection and supplementation hypotheses, and it is often treated as a sort of null hypothesis. While the term 'nonadaptive hypotheses' will be retained here as the final category to align with the literature, it is important to note that this tends to be used as a category that encompasses everything not covered under protection and supplementation. In fact, it could be argued that following 'behavioural traditions' may indeed be an adaptive explanation for geophagy, as cultural/behavioural traditions themselves likely serve adaptive functions (Laland & Janik 2006). Therefore, in future discussions, equal weight will be given to this third category of 'nonadaptive

hypotheses', and specifically traditions or cultures will be considered alongside protection and supplementation as none of these three categories of hypotheses are mutually exclusive. Indeed, it would be beneficial to the study of geophagy if future research discontinued the use of 'nonadaptive hypotheses' and/or expanded to contain more categories of hypotheses.

1.3.3 Geophagy in non-primate animals

Nonhuman animal (henceforth 'animal' unless otherwise noted) studies on geophagy span the past 50 years and describe geophagy among invertebrates, birds, reptiles, ungulates, bats, and even fish amongst others (Sokol, 1971; Whittle & Everhart, 1993; Klaus *et al*., 1998; Klaus & Schmidg, 1998; Krishnamani & Mahaney, 2000; Brightsmith & Muñoz-Najar, 2004; Young *et al*., 2011; Abrahams, 2012a; Hasan, 2012; Mahandran *et al*., 2016) and has even been proposed for some extinct species (non-avian dinosaurs) where it is suggested swallowed stones were ingested for mineral acquisition instead of gastrolith function (Wings, 2004). While the behaviour is widespread, the specific types of geophagy and purposes for doing so can vary greatly. As such, this section will investigate geophagy in non-primate animals by taxa and attempt to explore which behaviours are observed, by which individuals, and if there are any possible evolutionary explanations for said behaviour. Additionally, taxa where soil or earth constitutes a major part of the diet will not be explored (i.e. many microfauna or soil living animals; Potapov *et al*., 2022) as geophagy is usually investigated as supplementary feeding apart from a main diet.

Invertebrates

Though there are over 1.5 million species of invertebrates, representing a vast majority of animals, they account for very few recorded cases of geophagy (IUCN, 2022). In fact, to date, geophagy has only been formally described in butterflies, and mentioned in isopods (Jones & Hanson 1985;

Diamond 1999). Additionally, as noted above, there are many invertebrates that are classified as soil consumers (Potapov *et al*. 2022) and therefore wouldn't be described as exhibiting geophagy. This suggests that for many invertebrates it may be more likely they are soil consumers than exhibiting occasional geophagic behaviour.

Another interesting observation that can be made from the literature is that there are some behaviours seen in invertebrates that are not classified as geophagy even though there are elements of geophagy present. One such example is 'puddling' behaviour, found in *Lepidoptera* and some other insects, which originated from the common observation of butterflies congregating to drink water from muddy puddles, and is usually hypothesised as a means of sodium acquisition (Arms *et al*., 1974; Smedley & Eisner, 1996; Shen *et al*., 2009). It is important to note that this term can also be applied to any drinking behaviours associated with faeces and/or carrion, which are clearly not geophagy, and has been expanded to include feeding on dried materials (which is mixed with substance from the proboscis to be ingested as a liquid; Molleman *et al*., 2005) as well as feeding on the eyes, nose, or blood of vertebrates (Molleman, 2010). As such, while not all puddling could be considered geophagy, some instances would clearly involve the imbibing of soil saturated waters (or proboscis fluids) which would be classified as geophagy.

Amongst invertebrates, geophagy is most commonly cited within *Lepidoptera*, butterflies and moths, and the behaviour has been well documented through publications on puddling (Pivnick & McNeil, 1987; Boggs & Jackson, 1991; Boggs & Dau, 2004; Otis *et al*., 2006; Molleman, 2010). Within moths and butterflies, geophagy through puddling is widespread, but not ubiquitous, and seems to occur more often in tropical species than temperate ones (Beck *et al*., 1999). Puddling in *Lepidoptera* most often involves an aggregate of individuals feeding on a moist soil source, and

of these individuals the vast majority are male and often young (Boggs & Jackson, 1991; Otis *et al*., 2006). In the rare instances when females are observed puddling or feeding on soil, they appear to feed singly instead of in groups and females are more likely to be old and appear worn (Otis *et al*., 2006; Scriber, 2002). There are a few hypotheses for puddling behaviour: 1) to obtain water, 2) reduce body temperature, and 3) intake of substances in the water such as sodium, other nutrients, and nitrogenous compounds (Shen *et al*., 2009).

While it is difficult to differentiate between these suggested explanations in wild conditions, experimental settings as well as analyses of wild samples have suggested that sodium intake is the most likely function of puddling (Arms *et al*., 1974; Pivnick & McNiel, 1987; Smedley & Eisner, 1996; Shen *et al*., 2009). The most cited explanation for this male-biased behaviour is the need of sodium for nuptial gifts, resources transferred while mating, because males of *Lepidoptera* transfer complex spermataphores, which include sodium and other nutrients, to the females upon mating (Pivnick & McNeil, 1987; Bissoondath & Wiklund, 1995). One study also found a higher association with polyandry and not with male size suggesting it does play a direct role in nuptial gifts (Molleman *et al*., 2005). Sodium, a necessary and crucial micronutrient for all animals, is rarely found in plants and thus a scarce resource amongst herbivores (Peterson *et al*., 2021), and this combined with the need for a nuptial gift may drive male *Lepidoptera* to seek whatever form of sodium (or other nutrients) they can find, whether in soils or beyond.

Apart from *Lepidoptera*, geophagy has been mentioned in isopods and leafhoppers. Though many publications cite isopods as showing geophagic behaviour (Diamond, 1991; Abrahams, 2012a; Hasan, 2021), searching the literature yields only a single study on one species, *Hemilepistus reaumuri* (Shachak *et al*., 1976), in which it is noted that soil was a large component of the diet,

and therefore probably not geophagy as considered here. Additionally, though a few leafhopper species have been noted as showing geophagic activity (mud-puddling has been described in: *Arrugada affinis* [Rakitov *et al*. 2005], *Luheria constrictal*, *A. linnavuorii* and *A. rugosa* [Zahniser & Dietrich, 2013]), no descriptions nor publications of the behaviour could be found and therefore the reasons for geophagy in these species remain unknown. Therefore, it can be concluded that the adaptive benefits of geophagy within invertebrates would likely fall under the supplementation hypotheses.

Reptiles

Geophagic behaviour in reptiles (excluding modern birds which are discussed below) is often described as lithophagy and usually includes the consumption of nondigestible materials such as stones, and most records appear to be in extinct species whose remains have been found with gastroliths (Whittle & Everhart, 1993). Lithophagy as a phenomenon in reptiles has been present since prescientific times in the form of myths and observations in African crocodiles and was first suggested to play a role in digestion in the 1600s (Wings, 2007). In addition to many dinosaurs, it has been described in a number of extant groups. Sokol (1971) alone cited habitual lithophagy in the following: *Tupinambis rufescens* (Red Tegu), *Tiliqua occipitalis* (Western Blue-tongued Lizard), *Gopherus agassizii* (Desert Tortoise), *Testudo hermanni* (Hermann's Tortoise), *Sceloporus undulatus* (Eastern Fence Lizard), *Sceloporus magister* (desert spiny tail lizard), *Aspidoscelis tigris* (western whiptail), *Crodylus niloticus* (Nile crocodile), *Iguana iguana* (green iguanas), *Ctenosaura pectinate* (Western spiny-tailed iguana), and *Chelonoidus niger* (Galápagos Tortoise). This large group mostly encompasses lizards and tortoises for which lithophagy is mostly hypothesised as a form of trituration, a way to reduce particle size or homogenise a mixture (Sokol, 1971), and other studies on diet and lithophagy also support the hypothesis that this behaviour could function as

a digestive aid through trituration (Wings, 2007; DeBoer *et al*., 2018). Additionally, some argue it is plausible that lithophagy in reptiles might be a means of mineral supplementation, as the mechanical digestion of food using gastroliths would undoubtedly release minerals for further digestion (Sokol, 1971; Wings, 2007), however there is little direct evidence of this in the literature.

Other than trituration and possible mineral supplementation, there are few hypotheses for lithophagy in reptiles and many reptiles that don't fall under these categories may be ingesting stones or earth accidentally. However, one significant hypothesis that has been tested in recent years is the use of stones as ballast in *Alligator mississippiensis* (American Alligator). Though mathematical and computational models concluded that stones could not be used as ballast unless 6% of the individual's body weight was consumed (Henderson, 2003), studies in captivity have found that ingesting stones equal to 2.5% body weight prolonged the dive time in these alligators by 88% (Uriona *et al*., 2019). While this provides a convincing case for the use of stones as ballast, further studies would be needed to fully understand this function of lithophagy.

Therefore, in reptiles, a vast majority of geophagic behaviours derive from lithophagy and its functions, with some hypotheses suggesting mineral supplementation as a secondary effect. Studies into lithophagy are still relatively rare, and further research would be needed to fully understand this behaviour. However, for the purposes of this study, it will be concluded that reptiles do not exhibit deliberate geophagy.

Birds

Geophagy and lithophagy are both widespread amongst extant avian taxa. Initially, lithophagy was considered the main form of earth-eating, much like was hypothesised for non-avian reptiles

above, and many explanations focused on the mechanical and digestive benefits of eating soil or stones. For instance, it was found that ostriches that did not have access to gastroliths to aid in the break down and mixing of food items within the stomach were seen to suffer from complications of constipation, which even included death (Wings, 2004). And within temperate bird species soil and stones, in the form of 'grit', were found to be important as a mechanical digestive aid (Gionfriddo & Best, 1999; Burroughs, 2020).

However, most modern studies have shifted their focus to geophagy found in bird species functioning not as a mechanical aid, but as a form of dietary supplementation and a digestion aid (Lee *et al*., 2014) which would make the current hypotheses behind geophagy similar to those investigated within this thesis. This has been particularly well studied in neotropical birds, such as parrots and other herbivorous birds in the western Amazon Basin, which also constituted the first detailed studies of the behaviour combined with analyses into the content of the geophagic materials. One of these first studies looked into the soil composition of a clay lick in the Tambopata National Reserve, Peru known to be frequented by many bird species, and found that potassium, sodium, and lithium were the only minerals that were significantly higher in the preferred sites when compared to non-preferred sites (Gilardi *et al*., 1999). And more recent studies in Tambopata also confirmed these findings suggesting nutrients, and especially access to sodium, were a core driver of geophagy in 14 different parrot species (Brightsmith *et al*., 2008; Brightsmith *et al*., 2018). Nutrient supplementation is further supported in the case of the Jungle Babbler (*Turdoides striata*) in India which was found to ingest soil for its high calcium content (Jena *et al*., 2023).

Originally, it was thought that this behaviour was more common in tropical bird species (especially those in the Americas and parts of Asia [Diamond *et al*., 1999; Diamond, 1999; Montenegro, 2004]) but there is now evidence it can also be found in temperate species that feed on fruit (Downs *et al*., 2019). For instance, in British Columbia there are reports of clay based geophagy in the Northern Flicker (*Colaptes auratus*) that also predominantly feed on fruit such as elderberry and wild cherry (Rea, 2017). This provides an interesting and compelling parallel for geophagy in primates, as many primates are also dependent upon fruit and other plant parts in their diet (Fleming & Kress, 2011) and often must contend with the effects of toxins and PSCs found within them. These toxins can cause gastrointestinal distress, such as nausea and diarrhoea, and inhibit protein access from other dietary items (DeGabriel *et al*., 2009; Pebsworth *et al*., 2019a) and can be found in various temperate berry species (MacKinnon *et al*., 1992), thus some authors within the avian literature are suggesting a similar role in some bird species. Another study which described geophagy on the earth-based nests built by rufous hornero (*Furnarius rufus*) by three bird species in Brazil also suggested that a diet high in toxins could be a possible driver for these cases (Willrich, 2022).

However, even with this newest evidence that could contribute support to the protection hypotheses via toxin adsorption, nutrient supplementation remains the best supported hypothesis for geophagy in birds – though amongst many temperate species the use of soil as grit is still widely supported.

Non-primate mammals

Non-primate mammals (from here: mammals) constitute some of the earliest observations of geophagy outside of humans, with the one such record from 1792 observing buffalo and deer in

North America ‘licking’ the earth (Jakle, 1968). Indeed, most early observations of geophagy and salt licks derive from observations of large mammals, and these early publications are also where the strong association with sodium chloride supplementation also began (von Bunge, 1873). Interestingly, very little has changed regarding hypotheses behind geophagy in most large mammals, with the consensus that nutrient supplementation is still the predominant explanation behind the use of these licks – especially within ungulates – which remained the prevailing theory for at least 100 years (Jones & Hansen, 1985). Indeed, even the most recent studies on ungulates, such as the red brocket deer, show higher levels of nearly all beneficial minerals (calcium, sodium, magnesium, potassium, copper, zinc, and manganese) in geophagic soils compared to control samples (Griffiths *et al*., 2024a). At the time of writing, supplementation is still considered the most likely reason for geophagy in ungulates with only three species (moose [*Alces alces*], southern giraffe [*Giraffa camelopardalis*] and the babirusa [*Babyrousa babyrussa*]) of the 68 included in a recent meta-analysis showing any evidence supporting the protection hypotheses (Young *et al*., 2011).

Even when considering mammals outside ungulates, most studies show overwhelming support of the supplementation hypotheses with few instances of geophagy as protection and select examples supporting the ‘nonadaptive’ hypotheses (Beyer *et al*., 1994; Klaus & Schmidg, 1998; Houston *et al*., 2001; Mills & Milewski, 2007; Worker *et al*., 2015; Griffiths *et al*., 2024b). As with birds, the most common shared attribute amongst those species falling within the protection hypotheses appears to be a diet consisting of at least partly fruit or leaves high in harmful PSCs (Young *et al*., 2011). One such example can be found amongst an omnivorous bat species (*Carollia perspicillata*) in the Amazon where more frugivorous individuals of the same species were captured at higher rates at mineral licks than their more insectivorous counterparts which were

more common at random control sites (tested using $\delta^{15}N$ derived from biopsies; Voigt *et al*., 2008). This is an especially interesting result, as it is the only study that could be found where individual characteristics, such as dietary preferences and/or variation, were considered, and suggests geophagy could be an individual response to PSCs or toxins in some species.

Lastly, elephants provide a unique comparison for geophagy in primates as well. Studies have supported not only both the supplementation (Høldo *et al*., 2002) and protection hypotheses (Houston *et al*., 2001) in elephants, but also the 'nonadaptive' group as well. Elephants have been shown to extract resources from the same location even if that location has fewer benefits than alternative areas nearby, and it has been hypothesised that this could be a result of self-reinforcing behavioural traditions (Fishlock *et al*., 2016). As chimpanzees also exhibit 'behavioural traditions' (or culture; Whiten *et al*., 1999), this could provide a particularly interesting point of comparison for geophagy in the Bugoma chimpanzees.

Overall, geophagy amongst non-primate mammals shows overwhelming support for nutrient supplementation explanations for geophagy, with isolated examples of protection occurring in species that may require relief from the effects of PSCs, and even nonadaptive hypotheses such as behavioural traditions.

1.3.4 Geophagy in primates

When considering other animals, it is clear that nutrient supplementation offers the most common driver of geophagy, but some ecological factors, such as diet, may make benefits from toxin adsorption especially appealing in some cases. Primates, on the other hand, provide a much more varied and complex presentation. A recent meta-analysis conducted on geophagy in 136

primate species (Pebsworth *et al*., 2019b) provided an exceptional overview of the present knowledge of this behaviour. Findings indicated an overwhelming support for adaptive hypotheses over nonadaptive (however, as noted earlier, this distinction may be arbitrary) and show that while supplementation is supported in a number of species, the protection hypotheses may be a marginally more likely basis for geophagy in many primates.

Nutrient supplementation has been found to play an important role in many primates and has been indicated as a primary function in at least one species in every major primate group. For instance, in strepsirrhines nutrient access was shown to impact geophagy in some troops of Milne-Edwards' sifaka (*Propithecus edwardsi* [Baden *et al*., as cited in Pebsworth *et al*., 2019b]) and studies also described cases of ring-tailed (*Lemur catta*) and brown lemurs (*Eulemur fulvus*) seeking supplemental sodium (Ganzhorn, 1987). Similarly, Montenegro (2004) also described habitual use of geophagic areas by both howler monkeys and spider monkeys in the Peruvian Amazon which were sources of sodium, calcium, and magnesium amongst others. A number of recent studies have provided further support for the use of geophagy as a source of nutrient supplementation in several other primates such as macaques, langurs, colobus monkeys, tamarins, baboons, and bonobos (Fashing *et al*., 2007; Ferrari *et al*., 2008; Rawson & Tuong Bach, 2011; Pebsworth *et al*., 2012; Mastuda *et al*., 2015; Nautiyal, 2015; Monaco *et al*., 2019; Toda & Fasbender, 2024), suggesting that some primate species do seek out geophagic areas as forms of dietary supplementation.

Despite the large amount of literature supporting nutrient supplementation, there is relatively equal support for the protection hypotheses, especially the role of clays in adsorption of PSCs. In the Milne-Edwards' sifaka (*Propithecus edwardsi*) mentioned above, geophagy was also found to

be highly correlated with the ingestion of fruits and seed within the diet (Semel *et al*., 2019), and this has been further confirmed in other primate species (Pebsworth *et al*., 2019b). One of the main resources that soils may provide are clays, which are particularly good at binding PSCs, and high levels of clay are common in many geophagic soils consumed by primates (Reynolds *et al*., 2015; Pebsworth *et al*., 2019b). Chimpanzees were seen to seek out clay rich soils in Budongo Forest, Uganda that were likely eaten to protect from gastrointestinal stress (Pebsworth *et al*., 2019a). Further, in black and white colobus monkeys in Uganda, experiments showed that the monkeys only ingested the clay rich soils presented to them, even descending from the safety of the trees to seek these out, which further supports the role of clays in binding PSCs (Pebsworth *et al*., 2021).

In chimpanzees, there is generally equal support for both the supplementation and protection hypotheses and studies over the past 50 years have found support for nearly every suggested benefit of soil including to aid digestion, adsorb toxins, supplement dietary minerals, as antibacterials and other medicinal compounds, buffer pH in the stomach, and nonadaptive hypotheses such as texture and sensation (see Pebsworth *et al*., 2019b). In fact, one of the only hypotheses that has not yet been suggested is the occurrence of geophagy as a behavioural tradition, as has been proposed for geophagy in elephants as mentioned in section *1.3.3* (Chapter 1, this thesis).

Interestingly, though nonhuman primates show many differences in strategy, humans appear to be most likely to ingest soil as a form of protection against ailments such as parasites or pathogens and possibly dietary toxins (Young *et al*., 2011). However, recent studies have called for

a more complete study of human geophagy, including cultural and psychosocial factors studied in tandem with biological (Young & Miller, 2019).

1.3.5 Current consensus on the role of geophagy

As evidenced here, geophagy is found in a wide range of taxa and support for all three major groups of hypotheses – protection, supplementation, and nonadaptive – has been reported within the current literature, along with some hypotheses unique to certain groups (such as the need for nuptial gifts in *Lepidoptera*, or ballast in *Alligator mississipiensis*). However, nutrient supplementation is overwhelmingly represented and, when lithophagy is excluded, is found to be the most likely driver in invertebrates, birds, and non-primate mammals, but it is important to note the presence of historical biases, as can be demonstrated in the use of names such as 'saltlicks'. A growing body of research has also established support for protection hypotheses, especially as a response to dietary items high in PSCs, and this may especially be the case in tropical species relying on leaves and fruit. A such, the protection hypotheses may be particularly important to some nonhuman primate species. Additionally, though cases are still rare, there is limited evidence for geophagy as a form of behavioural tradition, which may be especially relevant for cultural animals such as chimpanzees.

1.4 The Bugoma cave

Within the Mwera home range, near its northwestern edge, is an earthen cavity exhibiting pronounced levels of geophagy and associated tool use in the form of leaf sponges. The cavity itself is set into the side of a large hill underneath the root systems of two trees (*Alstonia boonei* and *Cynometra alexandrii*) and is composed entirely of earth and tree roots. The cavity is around 135cm at its tallest and extends 5m into the hillside at its deepest. The inside of the cavity is

generally sheltered from external influences resulting in a dark environment that is, at minimum, damp for most of the year and often has pools of water that form over periods of rainfall.

Despite not containing any rock as is typically envisaged in 'cave' environments (Mylroie, 2019), the cavity in Bugoma Forest does meet the most often listed characteristics of caves found within the literature; namely, it is a natural opening into the earth that is both large enough to permit a human entry and deep enough to keep the internal environs darker and damper than the external environment, and thus requiring torchlight to navigate (Bögli 1980; White 1988; McGrew *et al*. 2003; White & Culver 2012). All of these would suggest that the cavity in the Mwera range is very similar to a cave but doesn't match the material definition perfectly. However, with the newest process-based definition outlined in section *1.2.1* (Chapter 1, this thesis) expanding the definition to include any space whose boundaries are composed of solid matter (Lazaridis, 2022), it can be concluded that the hillside cavity explored within the remit of this research falls within this geological definition of caves. As such, it will henceforth be referred to as a cave within the scope of this research.

The cave at Bugoma Forest is comprised of three semi-separated chambers (see Figure 1.1 for a detailed drawing of the cave) and has a main entrance that is around 80cm tall and 120cm wide, as well as a smaller entrance in the ceiling of the cave ('ceiling entrance') where chimpanzees can enter from above (measuring around 60cm by 50cm). Additionally, there is a third, much smaller, entrance opposite the main entrance that can be used by smaller members of the Mwera community (40cm by 55cm at its widest; called the 'back entrance'), and a fourth entrance ('left entrance') that only leads into the left-most chamber ('left chamber') which was never used by

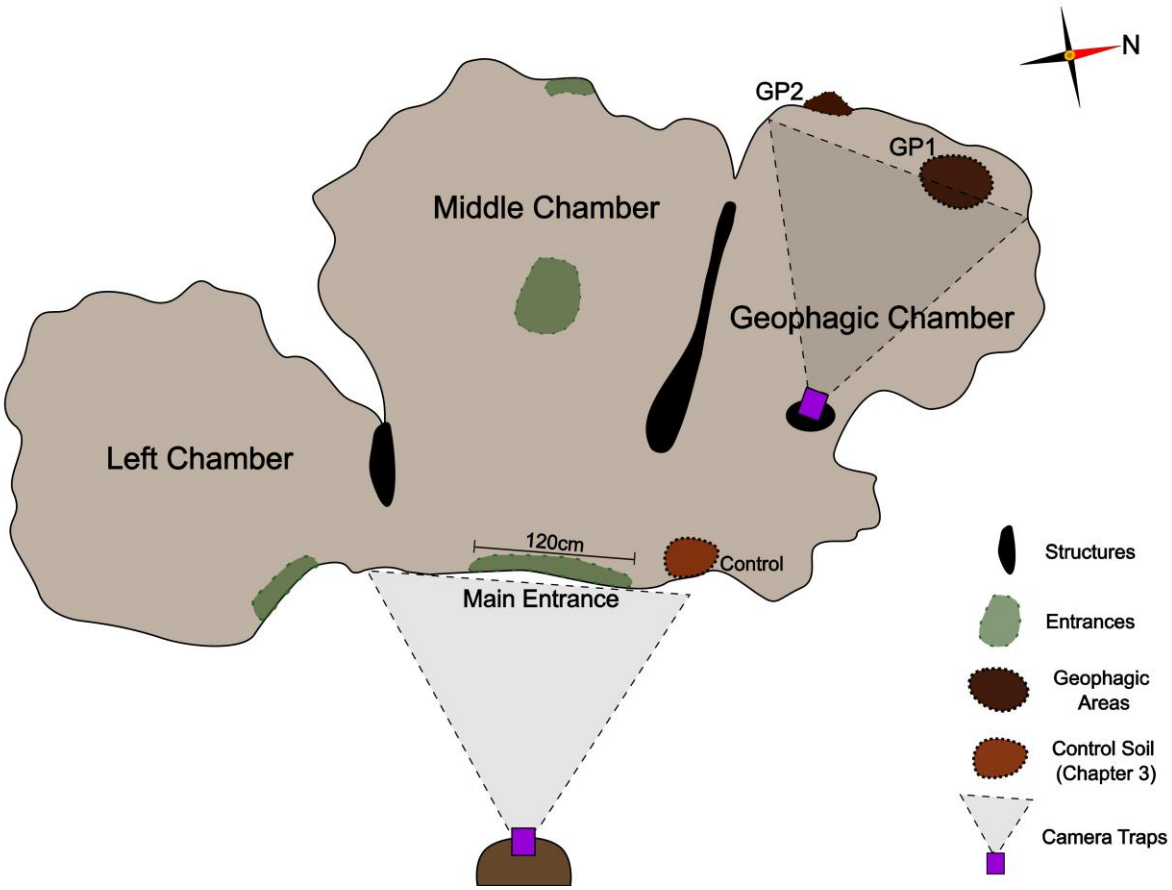


Figure 1.1 A map showing the rough layout of the cave within Bugoma Forest as seen from above. The three chambers detailed in the text are indicated as are the permanent camera trap locations (pink rectangles), and rough field of view of these cameras (dotted grey triangles), that are described in future chapters. The green dotted rounded shapes show the possible entrances, the two dark brown circular shapes show areas where geophagic activity is observed, and the light brown dotted circle near the entrance shows where control soils were collected (see Chapter 3, this thesis). This representation is a recreation from photographs and some limited measurements and is not to scale.

any primate during the duration of this study. The left chamber is the shortest in height (at time of study around 80-90cm from ceiling to floor) and darkest chamber which is also occasionally host to bats that roost within the roots during the day and can also be seen hunting within the cavity at night via remote camera traps. This left chamber has a more irregular shape that is difficult to

measure but roughly extends about 3 meters from the main entrance. There has been no evidence that the chimpanzees enter or use this chamber.

Next to this is the middle chamber, which is the chamber accessed by all entrances except the left entrance, which encompasses the largest, most spacious, area of the cave (roughly 4 meters long and 3 meters wide) and allows access to both other chambers. This chamber is roughly 120cm in height at its tallest and has the most available light within the cave. While evidence of chimpanzees moving through this chamber have been captured by video and evidenced by footprints, there is currently no indication that the chimpanzees utilise this chamber for more than travel.

The last, right-most, chamber contains all locations where geophagy has occurred and where all discarded tools have been found and is referred to here as the geophagic chamber. There is no evidence of outside materials being brought into this chamber (other than sponges made by the chimpanzees), and no faecal remains have ever been found. This chamber reaches almost 5 meters back into the hillside, terminating under the bole of one of the large trees constructing the cave (the *Alstonia boonei* tree), and the distance from floor to ceiling is usually around 100cm. Within the back area are two locations where all chimpanzee geophagy occurs, which are referred to within this thesis as Geophagic Point 1 (GP1) and Geophagic Point 2 (GP2). GP1 is a large area on the floor of the geophagic chamber near the back wall that is approximately 60cm by 50cm and is prone to accumulating standing water after consistent rain. GP2 is a small, thin, cavity on the back wall ,and to the left of GP1, that reaches back into the cave wall. GP2 also accumulates water, but in much larger quantities, and is where most of the discarded leaf sponges have been

found. Together, these two geophagic areas (GP1 and GP2) constitute the basis for all studies of geophagy presented in this thesis.

1.5 Project aims

The goal of this project is to provide initial descriptions of the ecology of Bugoma Forest and to investigate cave use and geophagic activities within the chimpanzees that reside there over the study period starting in September 2019 and ending in December 2020 (for all except weather data, which was extended to December 2021, and soil samples which could not be collected until June 2020 and extended to May 2021). To begin, Chapter 2 of this thesis will compile data on rainfall, temperature, and diet within Bugoma and define a preliminary home range for the Mwera community using GPS data collected during the study period. To investigate diet, this study seeks to create a novel 'dietary stress score' to quantify the quality of the dietary items consumed by the chimpanzees on a daily basis and these will then be used to conduct statistical analyses to investigate whether dietary stress varies significantly by season. This will provide a background understanding of the environmental effects that might impact cave use and geophagy and will be used to calculate seasons at Bugoma which will be needed to test predictions for every following chapter.

Once a general description of these influences has been created, Chapter 3 will then seek to further expand this comprehension of the Bugoma environment by constructing an exhaustive profile of the characteristics and composition of the geophagic soils within the cave. This will involve collecting monthly soil samples from three geophagic areas at the back of the Bugoma cave and comparing them to a control area at the entrance of the cave. This control area was carefully selected to fulfil several criteria suggested within the literature such as: 1) it comprises

an area where the chimpanzees have never been observed to consume soil, 2) the soil is readily accessible to the chimpanzees, yet the chimpanzees choose not to consume it (the chimpanzees must pass by the entrance to reach the geophagic soils inside), 3) the soils of the control seem to be from a relatively similar soil horizon (thus, the soils were collected from the ground and on the inside of the cave to mimic the conditions of the geophagic areas), and 4) as near to the geophagic soils as possible while still maintaining certainty that the chimpanzees do not consume soil from this location (Mahaney & Krishnamani, 2003; Pebsworth *et al*., 2019b). All three geophagic soils and the control soils from each month will then be subjected to an array of laboratory analyses to determine carbon content, pH, conductivity, cation exchange coefficient (CEC), elemental geochemistry (using X-ray fluorescence), mineralogical composition (using X-ray diffraction), clay content, and bioavailability of key elements, which are then statistically compared to determine if there are significant differences between the geophagic and control soils as well as to determine any effects of seasonality on the soils themselves.

Once the climate, diet, and soil contents have been analysed, Chapters 4 and 5 will seek to utilise these data to investigate hypotheses behind cave use and geophagy at Bugoma and describe the possible social influences observed. This project will especially aim to make an initial attempt to empirically investigate these hypotheses using available data to create proxy variables. First, Chapter 4 will investigate environmental variables to test sets of hypotheses for cave use and geophagy. For cave use, each hypothesis will be tested using environmental data from Bugoma and the cave to test cave use as: 1) a means to access supplemental nutrition or health benefits (via geophagy), 2) a source of hydration (water access), 3) a means to shelter from the elements (or predators, but this will not be tested here), and 4) relief from thermoregulatory stress (tested here as heat stress). The proxy variables selected for these (in respect to the order given above)

are: 1) proportion of time in the cave spent engaging in geophagy, 2) water levels within the cave (as a categorical variable) as derived from camera footage, 3) total daily rainfall (as there is no evidence that chimpanzees over-night in the cave, only shelter from the elements is considered), and 4) daily maximum temperature (to ascertain if chimpanzees prefer to use the caves on the hottest days when thermoregulatory needs are highest). Chapter 4 will also aim to test two aspects of the protection hypotheses of geophagy: 1) that high levels of toxins or PSCs in the diet drive geophagy, or 2) that infectious diseases, especially parasites that affect the gastrointestinal tract, lead chimpanzees to ingest soil to treat and alleviate symptoms. Data to directly test these were difficult to obtain from the semi- and unhabituated chimpanzees at Bugoma, so, instead, these will be tested using 1) the daily dietary stress score calculated earlier in this thesis (Chapter 2), and 2) a moving average of the previous 30-day daily rainfall totals (this is used to replicate parasite prevalence in chimpanzees as this has been shown to be significantly correlated with monthly rainfall [Gillespie *et al*., 2010]).

Chapter 5 will continue investigations into drivers of cave use and geophagy by exploring if group composition, measured as majority sex-age class, influences when and for how long the Bugoma chimpanzees engage with the cave and in geophagy. This chapter will assign a group composition of either majority male, majority female, majority juvenile, or mixed composition based on which sex-age classes comprise at least 75% of the total group for three conditions: 1) the external condition which consists of all individuals that could access the cave (representing all individuals that come within 5 meters of the cave entrance but may or may not enter) and will operate as a 'null composition', 2) the internal condition showing the group composition of visitors that choose to enter the cave (regardless of if they choose to then engage in geophagy), and 3) the geophagic condition which includes only those individuals that enter the cave and engage in geophagy. These

will be used to investigate if group composition changes, or remains the same, when comparing the internal and geophagic conditions to the external ('null') composition.

Lastly, Chapter 6 will seek to collate and explore the results of all data chapters (Chapters 2, 3, 4, and 5) to ascertain which environmental and social factors may, or may not, influence cave use and geophagy in the chimpanzees of Bugoma Forest, Uganda.

2. *Ecology of the Mwera chimpanzees (*Pan troglodytes schweinfurthii*) at Bugoma Forest, Uganda*

Abstract

To understand an animal's behaviour, it is imperative to begin by understanding their environment. As such, this chapter seeks to lay the groundwork to provide a preliminary description of the ecological background of the Mwera community of chimpanzees in the Bugoma Central Forest Reserve, Uganda. This chapter pursues three main goals to this end. First, the seasonality of the Bugoma Central Forest Reserve is defined using a pentad approach which examines rainfall in groups of 5 days to establish dry and rain seasons within the forest. Second, temperatures are analysed and statistically tested according to the same pentad approach to establish any seasonal temperature variations. And lastly, preliminary observations on feeding behaviour collected by directly observing the Mwera community are used to establish periods of dietary stress using a novel 'dietary stress score' which are statistically tested using the pentads to ascertain any seasonality within diet. Additionally, this chapter seeks to place the Mwera community within the Bugoma Forest using a basic estimation of their home range. Results show there is a roughly bimodal seasonal pattern, with rain seasons between March-June and September-December each year, and similar annual rainfall and average temperatures to those observed at other Ugandan field sites. While there is some statistical support that dietary stress is higher in the dry season, temperatures were found to not significantly vary by season - though this is likely due to missing data. Given the preliminary nature of these data, future research is necessary to determine the broader ecological context of Bugoma Forest but results from this chapter do provide a seasonal and dietary baseline for future chapters to explore aspects of the Mwera chimpanzee community's behaviour.

2.1 Introduction

Ecology, which examines interactions between organisms and their environment (Relyea & Ricklefs, 2018), has always been an important aspect of understanding animal behaviour and evolution. Indeed, it could be argued that without understanding the environmental conditions in which a species evolved it is almost impossible to fully comprehend its behaviour, both past and present, and how it may or may not change in the future. Behaviour and evolution occur in conjunction with the specific ecology of any given species, and it is thus a fundamental aspect of investigating behavioural or evolutionary questions – particularly those that involve studying a species in natural habitats. As climate change causes unprecedented shifts in the abiotic – and by extension biotic – environment, understanding and monitoring the natural ecology for endangered species, such as the common chimpanzee (*Pan troglodytes*), becomes increasingly important (Caro & Sherman, 2011). Though there is some indication that the behavioural flexibility of chimpanzees may help buffer them against the worst effects (Gruber *et al*., 20196; Kalan *et al*., 2020), it is crucial that research continuously documents changes and trends in the natural environment for this species, and indeed for any species. Not only is it important to determine the environmental conditions at the exact time of study, and thus avoid relying solely on historical data, but it is also imperative to incorporate these past studies, when possible, to understand how the ecology and environment may be changing and what other changes may be seen in the future. In accordance with these views, this chapter will work to establish the ecological background of the Mwera community, a group of wild eastern chimpanzees (*Pan troglodytes schweinfurthii*) living in the Bugoma Central Forest Reserve, Uganda, with the purpose of understanding how the general ecology might influence and inform the behaviours in subsequent chapters. Since the Mwera community has not been studied extensively at the time of this research, this chapter will also serve to create an initial assessment of the abiotic environment for the community and aim

to provide preliminary descriptions of their home range and explore some basic dietary parameters.

Chimpanzee communities are made of large multi-male/multi-female groups ranging from 20 – 150 individuals (Lehmann & Boesch, 2004) and employ fission-fusion social dynamics resulting in smaller parties that can change across time (Chapman & Wrangham, 1993; Le Hellaye *et al*., 2010). These communities inhabit extents between 3.1km^2 and 72.1 km^2 in area (Herbinger *et al*., 2001; Samsun & Hunt, 2012) with most home ranges occupying a 10km^2 to 30km^2 area, particularly those of the eastern chimpanzees (Herbinger *et al*., 2001). Chimpanzees are considered mainly frugivores with ripe fruit comprising most of their diet (McLennan, 2013). Though chimpanzees supplement their frugivory with many other plant parts, insects, and occasionally other mammals (Wrangham *et al*., 1998; Villioth, 2018), studies maintain the importance of fruit within their diet, linking fruit scarcity to party size and ranging behaviour (Wrangham *et al*., 1996).

The Mwera community of chimpanzees has not been extensively studied and little is known about their ecology, thus this study is reliant upon comparing the Mwera community to their well-studied neighbours in Budongo and Kibale. Bugoma is almost directly between these two forests, with Budongo around 80km to the northeast and Kibale just under 100km to the southwest. Budongo Forest is a semi-deciduous tropical forest at a medium altitude (Reynolds, 2005) that receives around 1600mm of rainfall per annum and has a mean temperature of 22.3°C (Sonnweber *et al*., 2018). The Sonso community of Budongo has been continuously studied since 1993 (Lowe *et al*., 2020) and their diet is typical of other chimpanzee communities but also includes regular crop raiding during periods when high-quality foods are scarce (Villioth, 2018). The home range of

Sonso is estimated as 7km^2 (Newton-Fisher, 2003) which is smaller than ranges found in other communities and more recent studies indicate it is now even smaller at only 5.33km^2 (Badihi *et al*., 2022). Research on the Kanyawara community in Kibale Forest began a few years earlier in 1987 (Thompson *et al*., 2020) and longitudinal data on their feeding behaviour show a reliance on ripe fruit, especially figs, with all ripe fruit accounting for 60-80% of their diet, but there is also evidence that this may have decreased to closer to 60% since the original data collection (Gruber *et al*., 2012). Kanyawara has a home range of 32km^2 (Stumpf *et al*., 2009) that lies along the northwestern edge of the forest and this community has also been observed to engage in crop raiding (Thompson *et al*., 2020). Diem *et al*. (2014) report that rainfall in the Kibale area is only 858mm per year, much lower than what was observed in Budongo. However, the Kibale National Park experiences considerable climactic variation from north to south, with Kanyawara's home range experiencing higher rainfall (around 1500mm) and higher temperatures (around 23.3°C) than reported elsewhere (Naughton-Treves *et al*., 1998). The elevation in Kanyawara is estimated to be around 1500m (Naughton-Treves *et al*., 1998) which is higher than 1100m estimated at Budongo (Reynolds, 2005). In general, Bugoma Forest is a tropical rainforest and would be expected to have two rain seasons and two dry seasons as seen in both Budongo and Kibale (Newton-Fisher, 1999; Reynolds, 2005). However, considering there are some differences between Kibale and Budongo it would be difficult to glean any further ecological insights into Bugoma without adequate data, which this study will aim to remedy.

To understand the intersection of ecology and behaviour within the Mwera community of Bugoma, it is important to explore which plants, and plant parts, are consumed within the study period. Many plant parts eaten within the chimpanzee diet contain Plant Secondary Compounds (PSCs) and other chemicals which have been hypothesised to drive behaviours such as geophagy

(Aufreiter *et al*., 2001). As the subsequent chapters focus on geophagy and the adaptive drivers behind it, it is important to understand how PSCs might contribute to the Mwera diet. In general, ripe fruit are considered high-quality food for chimpanzees but they do not contain much protein and their availability can vary drastically throughout the year (Villioth, 2018). Other plant parts and food items can be used to fill these nutritional gaps, in particular young leaves and unripe fruit, but they may carry more risks in the form of harmful compounds like PSCs and may also be more difficult to digest (Lambert & Rothman, 2015). It has also been suggested that PSCs in primate diets vary by season (Pebsworth *et al*., 2019b). Since ingesting soil has been hypothesised to adsorb PSCs and therefore alleviate gastrointestinal distress (Pebsworth *et al*., 2019a), this study will aim to look at dietary stress caused by PSCs and other difficult-to-digest food items. This will allow future comparisons with geophagic behaviours found in later chapters.

The goals of this chapter will be to: 1) give some context for Mwera's position with the Bugoma Forest and visualise an estimate of the chimpanzees' home range, 2) to describe rainfall and temperature trends in Bugoma and discern any seasonality that may be present, 3) to describe Mwera's diet in the form of dietary stress, 4) to statistically compare dietary stress and temperature to any seasons discovered herein, and 5) to set the ecological context for future chapters.

2.2 Methods

2.2.1 The field site

This study was conducted in the Bugoma Central Forest Reserve in the Hoima District of Western Uganda (1°17'40''N 30°59'50''E) with the permission of the Ugandan Wildlife Authority (UWA) and the National Forestry Authority (henceforth: NFA). The reserve, which lies within the Albertine Rift

Forest System, consists of 400km^2 of tropical forest and has an elevation of around 1100m as measured at the field site (Plumptre *et al*., 2010; see Figure 2.1). While the forest is still under threat from illegal logging, analyses using imaging technology indicate that forest cover loss in the central area of the Bugoma Central Forest Reserve has stabilised after 2010 (Twongyirwe *et al*., 2015). This could also be the result of a more selective logging strategy as the presence of the Ugandan NFA may discourage the more obvious clear-cutting seen in the past. Ultimately, as the most recent study is nearly a decade old, it is difficult to know the exact state of the reserve during the time of this study, but recent political developments have made the previous stabilisation of Bugoma more precarious (T. Gruber, personal communication). Surveys in 2010 found evidence that the Bugoma Forest supports a large diversity of animal species compared to the surrounding forest fragments, in addition to the chimpanzee population studied here (Plumptre *et al*., 2010). Amongst the other mammals found within Bugoma during these surveys were elephants (*Loxodonta* spp.), olive baboons (*Papio anubis*), black-and-white colobus (*Colobus guereza*), red-tailed monkeys (*Cercopithecus ascanius*), and the endemic Ugandan mangabey (*Lophocebus ugandae*).

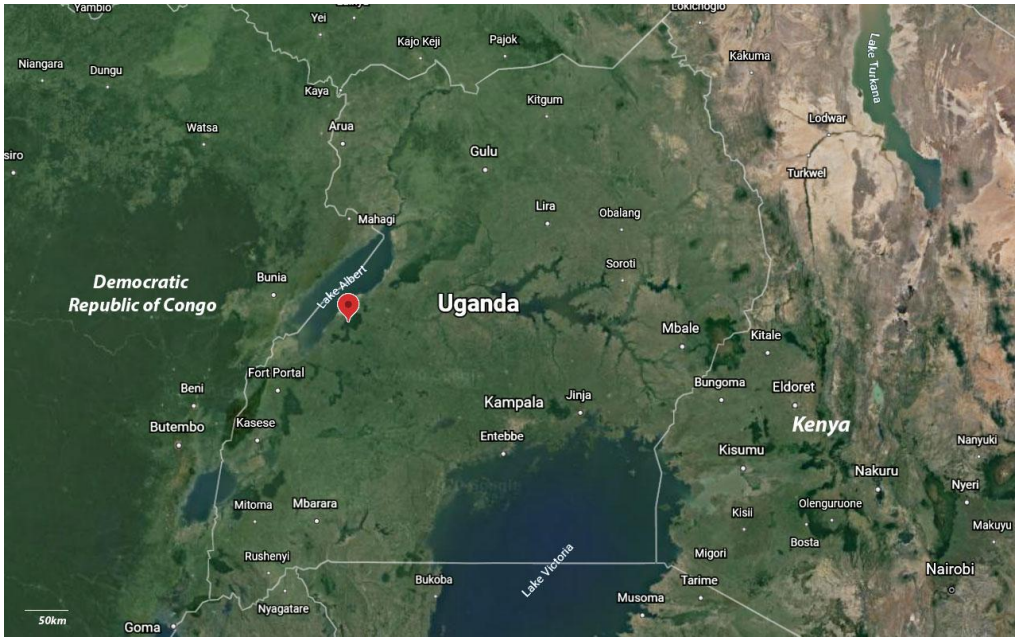


Figure 2.1. Location of Bugoma Forest, indicated with a red marker, near the western border of Uganda and Lake Albert. ©google

2.2.2 Data Collection

Behavioural Data

Initial data collection started in February 2019; however, only data from September 2019 onward will be examined in this chapter. Habituation of the Mwera community began in 2016 after the creation of the Bugoma Primate Conservation Project (from here: 'BPCP'; https://bugomaprimates.com/), and the community was semi-habituated (described in more detail below) by the period of this study (2019-2020). For the duration of this study, the BPCP research team consisted of 7-9 research assistants hired mostly from the local area. They collected the bulk of the project's long-term data and also assisted any researchers present at the field site, with the goal that on any given day there would be at least two teams consisting of two people to locate and follow the chimpanzees. By February 2019, the chimpanzees could be located with some regularity – the teams were able to record some observations for around half of the days when research was attempted – and the chimpanzees would tolerate the presence of researchers as long as a distance of at least 20m was maintained. By the end of the study period in December 2020 (nearly two years later), chimpanzees were considerably more accustomed to the presence of observers, allowing researchers much closer than the previous 20m, and were located and recorded on most days when research was conducted. For the health and safety of the Mwera community, surgical masks were donned when the first chimpanzee was spotted, regardless of distance, and kept on until the group was considered 'lost', when researchers had no visual on any member of the group for more than 30 minutes, and the researchers had moved at least 100m away from the location of the last sighting. No researchers were permitted to enter the forest if they showed signs of infectious disease and additional precautions were used during the COVID-19 pandemic, when research was suspended by the local authorities for a time and only daily follows of Mwera for health monitoring were permitted.

When a research team went into the field to collect data on the chimpanzees, they would attempt to find a party (subgroup) of the Mwera community as early as possible (preferably from the previous day's sleeping location if known) and follow them for as long as they were able, or as long as the chimpanzees allowed. Once chimpanzees were located, the researchers would find and sit in one location an appropriate distance away (appropriate being determined by factors such as visibility or any apparent distress) where they could see the group and would only move if they lost visual of the chimpanzees. This allowed the chimpanzees to come as close to the researchers as they preferred and removed stress that might be caused by the researchers' movements. Once visual on the party was lost (usually when the party instigated travel behaviour), the researchers would attempt to follow at a distance. It was at this point that parties were most often lost. As time progressed, the chimpanzees came to ignore the researchers and eventually would come into close proximity with them, occasionally as close as 5m. As the BPCP recommended minimum distance is 7m, researchers began standing to facilitate slowly moving away if the chimpanzees came too close.

Upon finding members of the Mwera community, the researchers would commence scan data collection which included taking behavioural data at 15-minute intervals. A scan would include searching for each member of the current group and writing down their behaviour at that exact moment, specifically recording any food items being eaten by noting the plant part (also noting the condition of a plant part, such as ripe vs unripe) and species. As the community was not fully habituated and individuals could not be assigned unique IDs, the researchers would note behaviour by sex-age class only and not with individual IDs. For instance, if a scan began at 07:00 and the group consisted of three adult males, two adult females, and one juvenile female (total of six), it would be noted as 'two adult males feeding, one on ripe fruit of species X and one on young

leaves of species Y, one adult male and one adult female resting, one adult female grooming, one juvenile female being groomed.' If an individual was not visible for a scan, this would also be noted. If the sex was unknown the individual would be referenced as an unknown adult or juvenile (which also applies for an unknown age class). Scan data were collected directly in the field, then transcribed into Excel and compiled and cleaned to extract feeding behaviour.

Each individual scan is also associated with a GPS coordinate (waypoint) and general ranging behaviour could be compiled from these GPS data. A Garmin GPSMAP® 64s or 66s device was used for collection of waypoint data.

Weather Data

Data on rain and temperature were collected at the BPCP field camp, a rehabilitated NFA forest station, which is located within the Mwera home range. A simple ground-based rain gauge was installed in an open area near the main building to ensure accurate collection. Additionally, an electronic thermohygrometer was installed in a shade covered location on the outside of the main building and measured current temperature and humidity as well as the high and low for temperature and humidity in the previous 24-hour period. Measurements from both the rain gauge and the thermohygrometer were collected daily at 18:00, or as soon as teams returned from the forest if they returned after 18:00. This time was chosen as it would allow for consistent time measurements, as the teams don't always leave for the forest at a uniform time, but teams did usually return between 17:00 and 18:00. Measurements began in October 2019, however due to some equipment failures, only weather data starting from November 2019 were used, however, collection of weather data continued well beyond the original study period. This study examines weather data collected between November 2019 and December 2021.

2.2.3 Data processing and analyses

Home Range Estimation

To estimate the home range between February 2019 and December 2020, all scan waypoints were mapped using QGIS and all outliers removed. The coordinates of the edges of the estimated home range were estimated using a modified minimum convex polygon method (for minimum convex polygon method see Powell, 2000), which discarded any waypoints suspected of including neighbouring communities and those that were over 250 meters removed from any other waypoint. These were then mapped into Google Earth Pro (©google; Google Earth, 2020) to produce an image of the home range and create a polygon to allow home range perimeter and area estimation. This did not provide a statistical estimation of the Mwera home range and was merely an attempt to map the physical space explored within this study. Therefore, this range estimation should not be considered an accurate estimation but merely considered a visual aid for conceptualising possible space utilised by the Mwera community.

Pentad Creation

To understand the seasonality of Bugoma Forest, a pentad approach was employed. Pentads have been used to determine meteorological patterns in past studies (for instance, Kousky *et al.*,1988; Kousky & Cavalcanti, 1988), including to determine dry seasons (Di Giuseppe *et al.*, 2013; Fu *et al.*, 2013). While using daily rainfall and temperature data is ideal for understanding the local climate of a study area, the pentad approach offers a compromise between monthly and daily measurements allowing for fine scale investigations of weather trends while also allowing some leeway for missing data (Di Giuseppe *et al.*, 2013). The relatively new nature of the field site (though the project started in 2016, the current data collection methods were not fully established until 2019) combined with the COVID-19 Pandemic in 2020 resulted in patches of missing data

throughout the study period. Thus, the pentad approach was chosen to allow for dry season determination using averages and weighted averages to account for missing days of data. This method was expanded beyond rain measurements to include temperature and dietary stress. This allowed for direct comparisons between these data and data in future chapters and will additionally allow for simple direct comparison for future studies, as pentads remain constant through the years.

Pentads were determined by selecting 5 consecutive, non-overlapping, days starting on 1 January 2019. Thus Pentad 1 consisted of the dates 1 January 2019 to 5 January 2019, and Pentad 2 consisted of 6 January 2019 to 10 January 2019, and so on until all 73 pentads of the year were filled. The same procedure was utilised for the year 2020, with Pentad 1 of 2020 also receiving an ID of 'Pentad 74' to allow complete differentiation. The year 2020 was a leap year. As such, the date 29 February was assigned to Pentad 12 of 2020 (Pentad 85) resulting in a six-day pentad (to ensure Pentad 12 is always 25 February to 1 March regardless of year). Pentads were weighted according to how many days of data were successfully collected.

Weather Analyses

Creating pentad data for rainfall included taking the rainfall measurements for the days of the respective pentad and adding them together to create a value for total rainfall. This was then divided by the number of days with data (i.e. not always by 5) and the number of days with data were noted as 'Observation Days' to be used to create weighted averages. The annual weighted average for rainfall was then calculated. The dry season was then determined by comparing the average rainfall per pentad and finding those with less than half the annual average (for instance, if the annual weighted rainfall average was 2mm of rain, then the pentad would need to be less

than 1mm on average) and investigating the next 8 consecutive pentads. If the identified pentad (that is, a pentad with less than half the annual average) was followed by 8 or more consecutive pentads where at least 5 pentads had an average of less than 1mm of rain, then this period would be defined as a dry season. The dry season began on the first day of the identified pentad that had less than half the annual average, and ended on the last day of the last pentad with an average of less than 1mm. In addition to this, minor dry seasons were identified using the same method above but using less than the average instead of using less than half of the average. With the example given above, that would mean the annual weighted average of 2mm of rain was used as an initial minor dry season starting threshold instead of less than 1mm. This was done to reflect that other Ugandan field sites report a strong dry season and a weak dry season (Reynolds, 2005). Average rainfall per pentad was then plotted and the determined dry seasons highlighted.

In addition to using pentad data, annual rainfall was estimated for 2020 and 2021 and the total and average rain for each month was plotted.

For temperature data, the average high temperature and low temperature for each day were recorded. The temperatures were averaged to create a pentad value for both high and low temperatures. Seasons for the temperature were not determined as it was not possible to use less than half the average in most cases. The temperature highs and lows for each pentad were then plotted with highlighted areas to compare to the dry seasons.

Determining Dietary Stress

To understand possible dietary stresses of the Mwera community, feeding data from direct observations were analysed. Any feeding observations from a period with at least 2 consecutive

scans were isolated and converted into feeding bouts. A feeding bout included any confirmed ingestion of any feeding materials of a certain species (most often plant parts, but also occasionally soil or insects) and all subsequent instances of feeding on that specific part of that species until the chimpanzees left the area. Within this study, if plant parts were in a different condition, such as ripe or unripe, this was considered a *separate plant part* as these often contain different nutritional components (i.e. an unripe fruit and ripe fruit are technically the same plant part but unripe fruits typically contain more PSCs and ripe fruits usually contain more beneficial nutrients, therefore these are considered separate plant parts from here). Observational data were inconsistent and dependent heavily on degree of habituation at the time of data collection, as such, feeding time could not be used as a reliable indicator for analysing diet. Instead, bouts were recorded as an instance of feeding which were all treated equally. While not ideal, treating the bouts in this way helps to avoid bias arising from the community being more or less habituated when data were collected.

Once bouts by plant part and species were determined, they were compiled by day such that each day showed each instance of feeding behaviour on all possible feeding items (by species). As an example, 13 September 2019 had three recorded feeding bouts: 1) a bout feeding on ripe fruit of the species *Celtis durandii*/*C. gomphophylla* (from here: CDU), 2) a bout of feeding on unripe fruit of the species CDU, and 3) a bout of feeding on unripe fruit of *Ficus mucuso* (from here: FM). Note that these would all count as separate bouts. Though bout 1 and bout 2 were both feeding on CDU, they were feeding on different plant parts, and while bout 2 and bout 3 were both feeding on unripe fruit, they were from separate species.

As laid out in Chapter 1 (this thesis), one key explanation for soil eating behaviour is that it aids in digestion and adsorption of Plant Secondary Compounds or Plant Secondary Metabolites (from here referred to as PSCs), which are typically harmful and have adverse effects (Johns & Duquette, 1991). Therefore, dietary items were ranked according to insoluble fibre and PSC content (Lambert & Rothman, 2015; Table 2.1). They were then assigned a score based on their stress ranking, with 5 denoting the highest likely stress and assigned to Bark/Wood, which is very difficult to digest with limited nutritional content, and zero denoting the lowest stress and assigned to ripe fruit as the preferred food type for chimpanzees as it is both easily digestible and low in PSCs. Since chimpanzees are frugivores, the stress value of zero is assigned to fruit to represent the null state of their diet. While all chimpanzees consume more than merely fruits to meet their dietary needs, it is unlikely that eating fruit would cause the chimpanzees any unusual dietary stress. While the PSC content of the edible plant parts discussed here can vary from species to species, forest to forest, and even plant to plant, rough content estimates provide a valuable option in the absence of direct sampling and content analyses of the local flora populations. There was only one recorded observation of the chimpanzees eating a non-plant part, which was a soil eating observation on 28 May 2020. So, for the purposes of this study, soil was assigned a stress value of zero as it was not only a rare dietary item, but it is also the main focus of future chapters.

Plant Part	Insoluble Fiber	PSCs	Stress Value
Bark (Wood)	Very High	Low	5
Mature Leaves	High	High	4
Resin	Low	High	3
Unripe Fruit	Moderate	Moderate	2
Young Leaves (Seeds)	Moderate	Moderate	2
Flowers	Moderate	Low	1
Ripe Fruit	Low	Low	0

Table 2.1. General estimates of insoluble fibre and Plant Secondary Compounds (PSCs) for each dietary item recorded (as reported in Lambert & Rothman, 2015) as well as the associated 'stress value' 2 used for analyses.

Once each plant part was given a score, the scores were applied to the daily feeding data creating a dietary stress total for each day based on which plant parts were consumed and how often. These days were then totalled for each pentad and then divided by the number of days observed to create a pentad average, after which a weighted average was taken for the entire year. To mirror the pentad determination of a dry season, the weighted average was then doubled to create a 'high stress threshold' such that any value above this represents a pentad with unusually high dietary stress. Then the pentad data were investigated for seasons using the same approach as for rainfall, thus any period of 8 weeks where at least 5 of those weeks were above the high stress threshold was considered a stressful dietary season. The average pentad dietary stress value was then plotted with dry seasons highlighted.

After creating the pentad data, a 5-day moving average starting on 1 September 2019 and ending with 31 December 2021 was calculated to compare to the pentad approach. This was done to investigate whether a pentad approach or moving average approach might capture different diet stress trends - in particular because the small sample size of the dry season pentads may make it difficult to test for differences in the data. The moving averages were then plotted with the dry seasons highlighted, as in previous plots, and used to test for dietary differences.

2.2.4 Statistical analyses

Once pentad values were determined for diet, rainfall, and temperature, statistical analyses were used to test whether dietary stress and/or temperature varies by rainfall season. The hypotheses for dietary stress are as follows: Hd_0 – there is no difference in dietary stress between the rain and dry seasons, Hd_1 – dietary stress values are higher in the dry season than the rain season, and Hd_2 – dietary stress is lower in the dry season than the rain season. The hypotheses for average high

temperatures are as follows: Hh_0 – temperature daily maximum did not vary by season, Hh_1 – the maximum daily temperature was higher in the dry season than the rain season, Hh_2 – the maximum daily temperature was lower in the dry season than the rain. And for average low temperatures: Hl_0 – there are no differences in minimum temperatures in the rain and dry seasons, Hl_1 – the minimum daily temperature was lower in the rain season than the dry season, and Hl_2 – the minimum daily temperature was lower in the dry season. While the goal would be to differentiate between all of these hypotheses, current data is only sufficient to test whether to reject the null hypotheses (Hd_0, Hh_0, and Hl_0) and not differentiate between the two alternative hypotheses (Hd_1 versus Hd_2, Hh_1 versus Hh_2, and Hl_1 versus Hl_2) To test these, the data were first visualised and tested for a normal distribution using a Shapiro-Wilk Test. As none of the data were normally distributed, a Mann-Whitney U Test was used. This was performed on Dietary Stress and Season, Temperature High and Season, as well as Temperature Low and Season.

Since pentads are not normally used to describe diet, testing for differences was done using the moving averages determined above. This allowed a more complete look at trends in the dry season, as a strict cutoff such as that found in the pentad approach may miss important aspects of feeding behaviour. Only 5-day moving average values that were fully comprised of dates that fall within a dry season were included as a dry season. As an example, if a dry season begins 1 December and ends 1 February, the dry season for moving averages would be calculated as beginning with the first 5-day moving average period where 1 December appears (which would include 1-5 December) and ending after the last 5-day moving average where 1 February appears (which would be the moving average from 28 January to 1 February). All other moving average values were grouped into the rain season. The data were tested for normality as above and then

analysed using a Mann-Whitney U Test with the last three pentads removed as these represented an incomplete season.

2.3 Results

2.3.1 Home range estimation

The estimated home range of the Mwera community (Figure 2.2) comprises a large area of the more contiguous northern forest of the reserve. However, it is also worth noting that one road bisects the Mwera range in a general north-south direction and this road eventually meets with another near the southern end of the known range. So, while this community is more centrally located within the forest reserve, they likely have some significant interactions with the local human populations via road crossing in addition to illegal human activities in the forest facilitated by the presence of the road. The home range seen in the 2019-2020 data has been estimated to have an overall area of roughly 9.8km^2 and a perimeter of 12.3km.

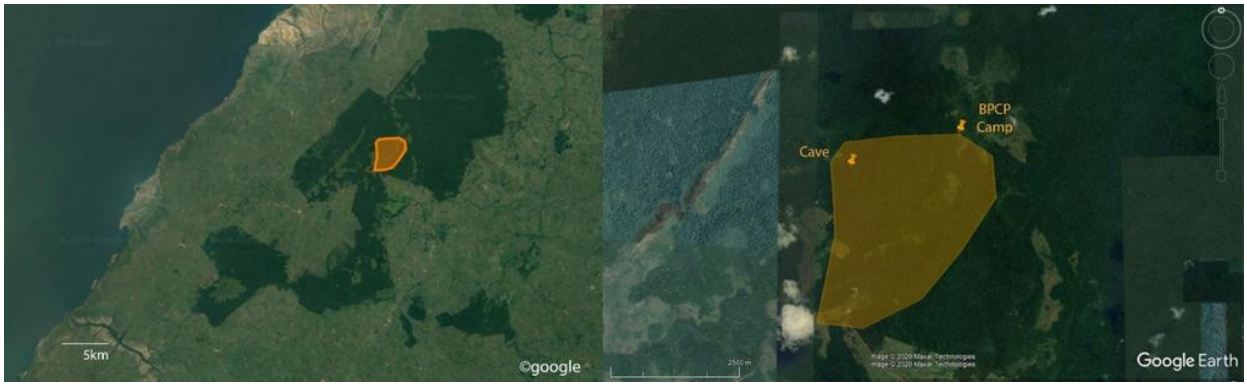


Figure 2.2. *a*. (left) Bugoma Forest Reserve. An estimate of the 9.8km^2 Mwera home range (in orange) created using GPS waypoint data from 2019-2020 collected by Bugoma Primate Conservation Project (BPCP) and B. Boughan. Only the core area where chimpanzees are frequently contacted and known to be Mwera community were used - outliers were discarded so the true range may be larger. ***b.*** (right) The cave and the BPCP camp are used to define the northern range of Mwera until more comprehensive studies on home range overlap in this area can be conducted. The cave constitutes the location of geophagic behaviour examined in the remaining chapters. ©google

2.3.2 Weather

Rainfall

Annual rainfall was measured at 1120.6mm in 2020 and 958mm in 2021. The monthly rainfall generally follows a bimodal pattern (Figures 2.3 *a*&*b*) exhibiting the highest rainfall in May and October of 2020 and March and October in 2021, and the lowest rainfall in January and July of 2020 and February and June in 2021. However, due to the emerging COVID-19 pandemic in 2020, there is a significant amount of rainfall data missing for late February and most of March 2020 (the last 8 days of February 2020 and 17 of 31 days in March could not be measured). By comparing the daily average to the total, it is possible to see that March 2020 was trending toward high levels of rainfall and that the total is probably heavily underestimated for this month. The annual average rainfall was 4.6mm per pentad for 2020 and 4.92mm in 2021.

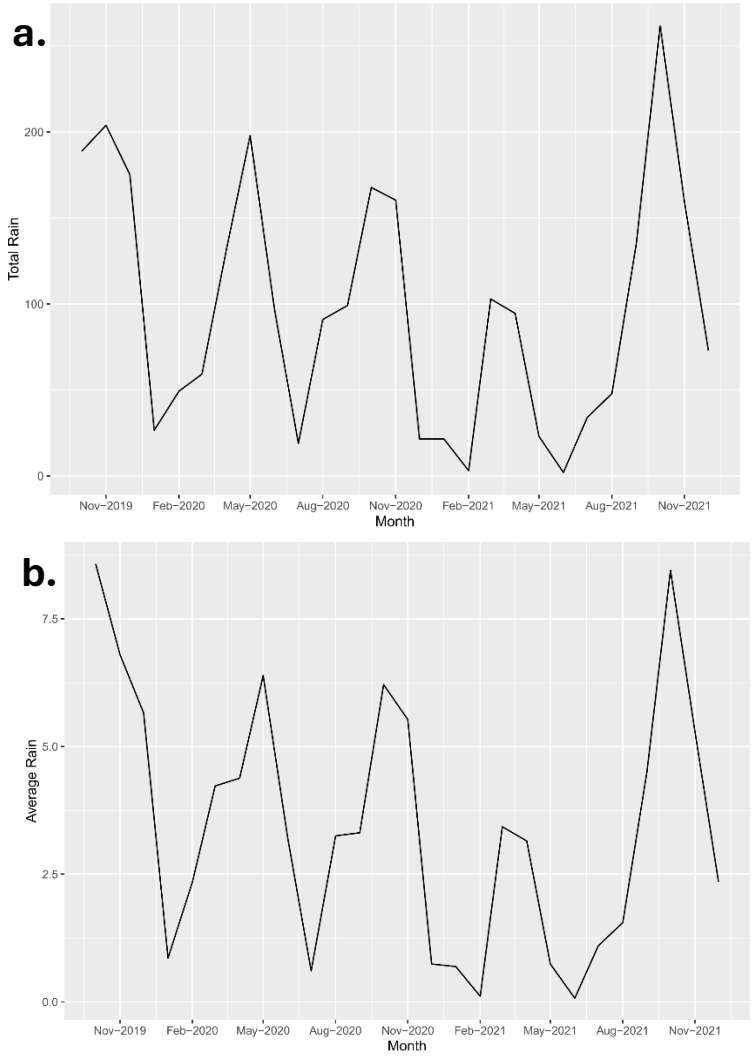


Figure 2.3. *a*. (top) the total rainfall data collected for each month during the study period (November 2019 to December 2021), clearly indicating the bimodal season distribution at Bugoma Forest. And ***b*.** (bottom) shows the daily rainfall averages for each month calculated by taking the monthly total and dividing it my number of days data were collected. February and March 2020 had the most missing data.

Using the pentad approach outlined above, there were three dry seasons identified between November 2019 and December 2021. One dry season was identified from pentad 73 to pentad 82 indicating a dry season starting on 27 December 2019 and ending on 14 February 2020, the next occurred from pentad 143 to 158 (12 December 2020 to 1 March 2021), and the last from pentads 173 to 185 (11 May 2021 to 14 June 2021). Additionally, though it did not meet the definition of a dry season, there was another dry period from 15 June 2020 to 3 August 2020. In this case, there were a number of pentads with over 1mm of rain within the consecutive 8 pentads; however, most (7 out of 10 pentads) did not surpass the annual average and half of the pentads (5 of 10 pentads) had no measurable rainfall. Most of the rain during this period was concentrated on two separate days, 22 June 2020 and 27 June 2020, which accounted for 96% (22mm of the total 22.8mm) in pentad 35, and 81% (38mm of 46.8mm) in pentad 36 of the total pentad rainfall, respectively. As such, this will be referred to as a minor dry season while the dry season from December 2019 to February 2020, December 2020 to March 2021, and May to June 2021 will be referred to as major dry seasons (see Figure 2.4).

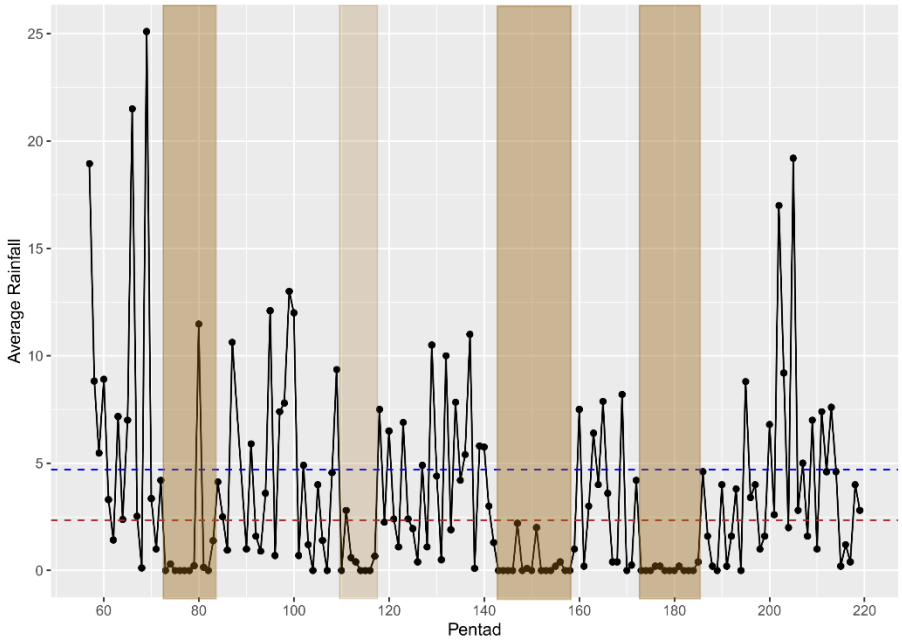


Figure 2.4. Average rain per pentad with the dry seasons highlighted in orange. The darker orange beginning around pentads 73, 143, and 173 indicate the major dry seasons, the lighter orange in the middle near pentad 110 indicates the minor dry season from June to August 2020. The top (blue) horizontal dotted line indicates the annual weighted rainfall average, and the bottom (red) dotted line indicates the threshold for a dry season (less than half the average).

Temperature

For temperature, the average recorded high and low temperatures were averaged separately per pentad (Figure 2.5). It was not possible to use the season determination as used with rainfall, so instead temperatures will be referred to as above or below the average high/low. The annual average high was 31.1°C for 2020 and 31.9°C for 2021, and the annual average low was 21.1°C for 2020 and 24.5°C for 2021. There is some concern about the measurements from the last six pentads recorded in 2020, as these temperatures were considerably lower than any temperatures previously recorded and may constitute either measurement error or abnormal weather patterns. These brought the annual average down by an entire degree on for both the high and low temperatures. When removed, the annual temperature high becomes 30.6°C and the annual temperature low becomes 19.6°C. These six pentads were removed from future statistical analyses as they fall mostly within a possible dry season and may skew results.

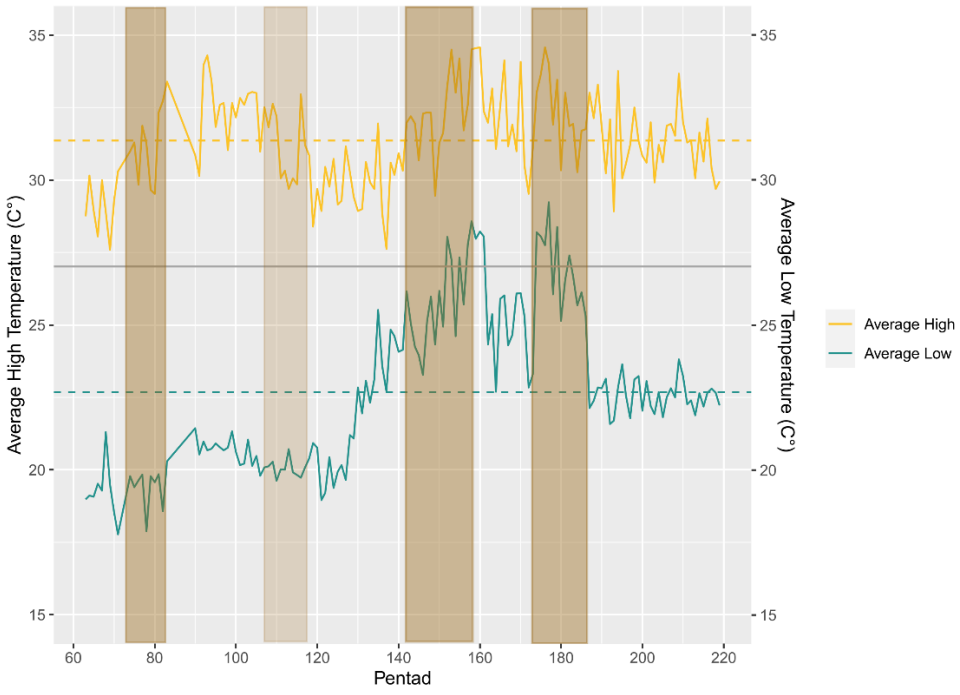


Figure 2.5. Plot showing average high and low per pentad (both y axes show the same scale). The top solid line (yellow) shows the average high per pentad with the average high for the entire study period (31.4°C) marked with the dotted horizontal line. The bottom line (green) shows the average low with the average low (22.7°C) for the entire study period represented by the dotted horizontal line. The three major dry seasons are indicated by the dark orange boxes and the minor dry season is indicated by the lighter orange box.

2.3.3 Dietary stress

Feeding behaviour was observed on 283 of the 488-day dietary study period. As expected, dietary stress data were predictably much noisier than the rainfall data and, as with the temperature results, no 'high dietary stress' seasons could be determined using the same method as for rainfall (Figure 2.6). Using the dietary stress scores outlined above, the annual weighted average dietary stress was 4.3 (note: a pentad needed at least 2 feeding days to be included in the analyses). Of the total 89 pentads recorded, 55 pentads (61.8%) fell below this average, and 22 of 89 total pentads (24.7%) had a dietary stress score of zero (i.e. only ripe fruit were observed to be eaten during these pentads). There were only 5 pentads from the study period where chimpanzees were not observed eating fruit: pentads 49, 88 (pentad 15 of 2020), 105 (pentad 32 of 2020), 129 (pentad 56 of 2020) and 133 (pentad 60 of 2020). Of these, pentads 49, 88, and 129 each only had one day with feeding observations, and 105 only had two days with feeding observations. Pentad 133 was the only pentad with significant feeding observations (feeding observed on 4 of 5 days) where ripe fruit were not observed to be eaten and the chimpanzees instead relied upon unripe fruit and seeds during this pentad.

There were 12 pentads (13.5% of total observed pentads) with dietary stress scores (in the following paragraph referred to as 'score') that were more than twice the average (the corresponding value to the dry season threshold for the rainfall data): pentad 53 (score = 18), pentad 66 (score = 10), pentad 69 (score = 12), pentad 70 (score = 14), pentad 75 (pentad 2 of 2020;score = 10), pentad 76 (pentad 3 of 2020; score = 10), pentad 77 (pentad 4 of 2020; score = 21), pentad 99 (pentad 26 of 2020; score = 10), 103 pentad (pentad 30 of 2020; score = 11), 108 pentad (pentad 35 of 2020; score = 12), pentad 138 (pentad 65 of 2020; score = 17), pentad 142 (pentad 69 of 2020; score = 16). Pentad 77 was the highest observed score and fell within a major

dry season, and the only other pentads with high stress scores (or twice the average) within the major dry seasons were pentads 75 (score = 10) and 76 (score = 10). Pentads 75 and 76 also happened to precede the highest stress score in pentad 77 and these three should likely be considered as a period of 15 days with particularly high dietary stress instead of three individual periods. Pentad 108 was the only high scoring pentad within the minor dry season. Additionally, 21 pentads of the total 89 (23.6%) were between the average and twice the average. Thus, 24.7% of pentads had no dietary stress, 38.1% had below average dietary stress, 23.6% had above average dietary stress, and 13.5% were considered high dietary stress.

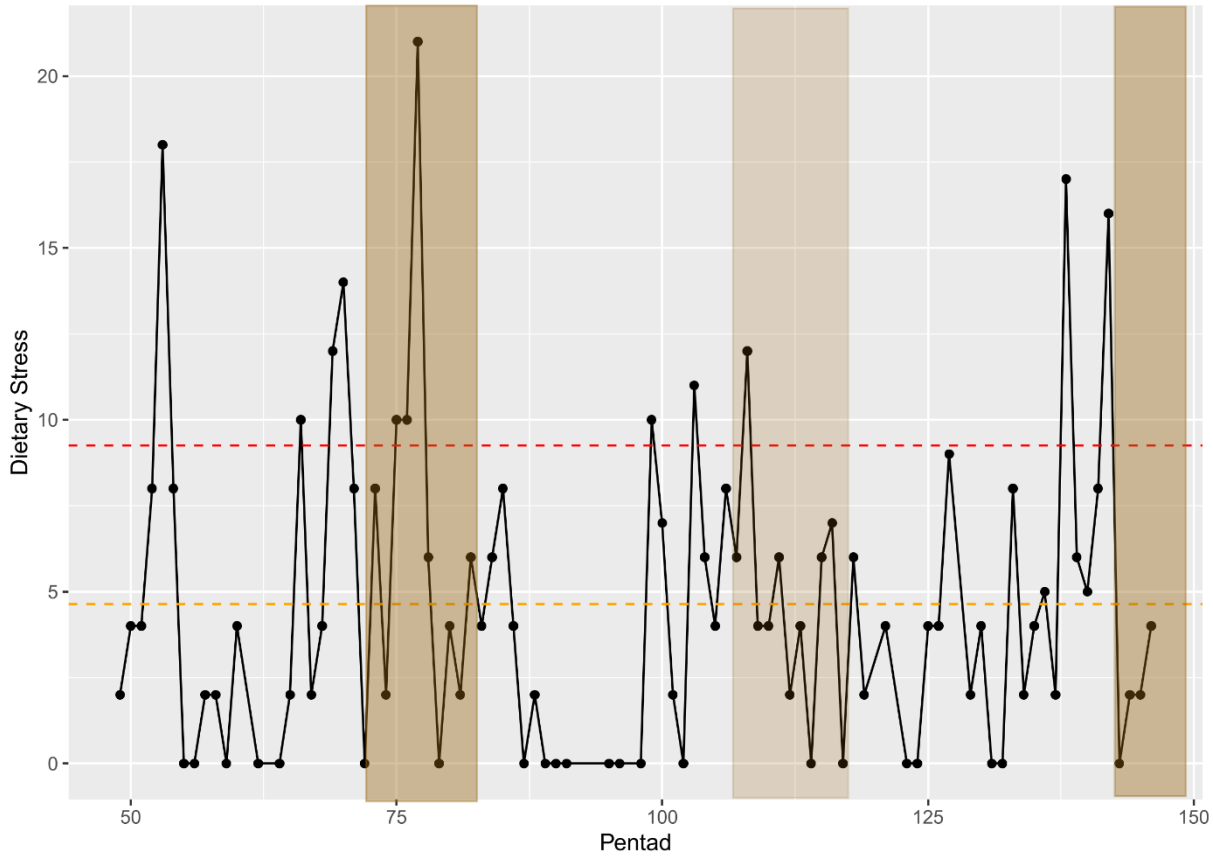


Figure 2.6. Dietary stress scores per pentad. The bottom horizontal dotted line (yellow) represents the annual weighted average stress score while the top dotted line (red) represents the dietary stress threshold (more than twice the average). No high dietary stress seasons could be identified using the same approach as for rainfall, so instead the dry season has been indicated (the orange boxes) in the same fashion as previous figures: the left dark orange box shows the major dry season, the lighter middle box shows the minor dry season, and the light orange box on the right indicates the beginning of the next major dry season on pentad 143.

To investigate whether the pentad method might return biased results, a 5-day moving average was applied to all available data (Figure 2.7). While the moving average does seem to represent the data better, it does not seem to vary much from the pentad results. The exception being a very strong spike around moving average group 250 (which corresponds roughly to pentad 99 [pentad 26 of 2020; 5-10 May 2020]) which is likely due to missing data. The annual daily average dietary stress was found to be 1.47, with the dry season average being 1.85 and the rain season average being 1.37.

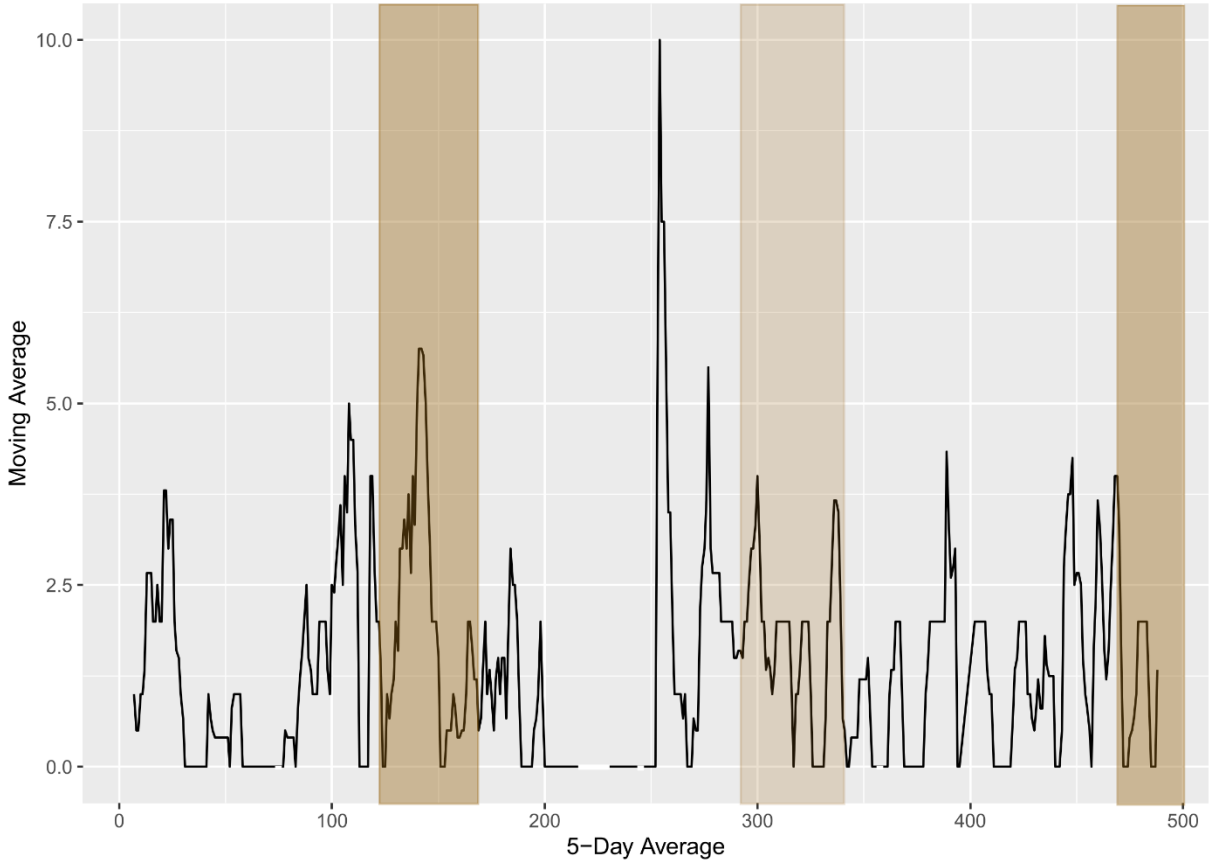


Figure 2.7. The 5-day moving average of daily dietary stress values throughout the study period, also showing time periods when data were missing (around the group 70 which corresponds to 3-13 November 2019, group 225 which was 26 March to 16 April 2020 and due to the COVID-19 pandemic, group 240 from 23 April to 1 May 2020, and group 350 which was 17-24 August 2020). The dry seasons are again highlighted in orange with the major dry season (left orange area), the minor dry season (middle orange area), and the possible beginning of the next major dry season (far right orange area).

2.3.4 Statistical comparisons between season, temperature, and diet

A Mann-Whitney U-Test revealed that average daily high/low temperature during the dry season was not significantly different from that during the rain season ($p=0.118$ and $p=0.928$, respectively). The results for dietary stress were mixed. When using the pentad approach, variation in dietary stress was not significant ($p=0.096$). However, when analysing the moving averages, there were significant differences in dietary stress between the rain and dry seasons ($p<0.001$) and dietary stress is higher in the dry season by 0.50 per day.

2.4 Discussion

2.4.1 Home range estimation

The methods typically used to estimate chimpanzee home ranges were not feasible given the constraints of the current study, but instead the range reported merely represents where the chimpanzees were found during the study period. However, when comparing to more sophisticated home range estimation techniques employed in other studies, the Mwera community's ranging behaviour estimated here, covering approximately 9.8km^2 in 2019-2020, seems to be in line with other East African chimpanzee communities (for a true comparison, the Mwera community size would be necessary and this is, unfortunately, currently unknown). The Kanyawara community in Kibale Forest, Uganda, were found to have a home range between 7.8-14.9km^2 (Chapman & Wrangham, 1993), the home range of the Sonso community in Budongo was estimated to be around 7km^2 (Newton-Fisher, 2003), and the home ranges of the Kakombe (Gombe), Kasakela (Gombe), and K (Mahale) communities in Tanzania are 9km^2, 12km^2, and 10km^2 respectively (as cited in Herbinger *et al*., 2001). The estimates reported in this study cannot be directly compared but are meant as an initial step toward mapping the home range of the

Mwera community before it is fully habituated, and to give context for their general location within Bugoma Forest. Future studies should aim to do a systematic evaluation of the Mwera home range using established methods to produce results that are comparable to those available at other field sites.

2.4.2 Weather

Rainfall

The 2020 annual rainfall in Bugoma of 1120.60mm was slightly below what is typically found in both Kibale (1479mm; Watts *et al*., 2012) and Budongo (mean of 1600mm, range of 1240–2187mm; Reynolds, 2005), but the 2021 rainfall of 958mm was far below what would be expected. However, at least for 2020 there were significant periods of missing data, with 34 days of rainfall data missing overall, with 30 of these missing days falling during the rain season. Therefore, the total was likely higher as nearly an entire month of rain data is unavailable but still may be lower than other field sites. A study looking at rainfall data in Hoima District (Bulindi), where the Bugoma Central Forest Reserve is located (though much of it also lies within the Kikuube Distict), has shown a general downward trend for annual rainfall which has fallen from around 1700mm in the early 1980s to 1270mm in 2012 (Mubiru *et al*., 2015). As there were only two full years of rainfall data for Bugoma, it is impossible to know if the comparatively low rainfall during 2020 and 2021 may be indicative of the same trends found in other parts of Hoima or simply an effect of missing data. When discussing the rainfall for 2021 with BPCP staff (personal communication), it was remarked that 2021 was an exceptionally dry year and it could be that this year represents an anomaly.

In 2020 and 2021, the monthly rainfall follows a bimodal pattern with one major and one minor dry season in 2020 and two major dry seasons in 2021 (falling between December-February and June-August, and December-March and May-July, respectively), which is nearly identical to seasonality described at other Ugandan field sites (Ashford *et al*., 2000; Reynolds, 2005). In 2019-2020 the peak of the major dry season at Bugoma fell in January, with a period of 22 days with no measured rainfall and for 2021 the dry season peaked in February with 30 days with only 3mm of rainfall total (which fell on 3 separate days) during this time. The minor dry season of 2020 experienced no rain for 17 consecutive days in mid-late July, and 2021 peaked in June with 18 days with no rain and 36 days (from June-July) where only 1mm of rain fell in total. The rain seasons peaked in October/November 2019, May 2020, November 2020, March-April 2021, and October 2021 which is also in line with studies at Budongo (Newton-Fisher, 1999). Thus, the pentad approach seems to produce results in line with other similar field sites in the region, even with less than two years of data. However, 2021 showed very different rainfall trends, with a pronounced 'minor' dry season that was just as strong as the major dry seasons. As mentioned earlier, this could represent a particularly dry year, or a long-term shift in rainfall patterns that requires more long-term data to fully understand.

Temperature

Hoima District is one of the hottest areas in Uganda with an annual average high of over 30°C in 2012 and high temperatures trending hotter with time (Mubiru *et al*., 2015). This is reflected in the average high temperatures (2020 = 31.1°C; 2021 = 31.9°C) in Bugoma Forest during the 2019-2021 study period but is even more evident in the average low temperatures (2020 = 21.1°C; 2021= 24.5°C). In 2012, the average yearly low in Bulindi Forest (Hoima District) was estimated to be 11.5°C (Mubiru *et al*., 2015) which is much lower than what was observed at Bugoma in 2020. In

this same study, the annual average high temperature at Bulindi was predicted to increase after 2012 but the annual average low temperatures were expected to stay constant. Without historical longitudinal data on temperature for Bugoma it is not possible to know if there might be long-term changes in temperatures that diverge from trends seen in other forests in Hoima, but data from the nearby Budongo Forest show a different trend from Bulindi. A study on the Sonso community in Budongo Forest, Uganda shows that the average monthly temperature was typically between 20-25°C between 1994 and 1995 (Newton-Fisher, 1999), and a few years later the average maximum and minimum temperatures were reported as 32°C and 19°C (Reynolds, 2005). A more recent study in the Budongo Forest spanning 2006 to 2009 supports a relatively stable trend, with monthly averages ranging from 21.2–24.1°C and the average of all months as 22.3°C (Sonnweber *et al*., 2018) putting these temperatures slightly warmer but very similar to the temperatures in 1994-1995. Additionally, this would match fairly well with the average of all high and low temperatures at Bugoma (26.1°C), albeit slightly warmer. The most likely scenario is that Bugoma Forest has features that keep the forest warmer overall and that higher lows are more typical there than Bulindi and Budongo. Indeed, this could be a consequence of the extensive deforestation in the Bulindi area and a devastating loss of forest cover rather than climactic shifts (McLennan & Hill, 2013). If the average low temperature has increased it is more likely a small increase as exhibited in Budongo, and not the large jump seen between Bulindi and Bugoma. As with rainfall, future studies should aim to expand the temperature data presented here as long-term trends cannot be determined from only 26 months of data.

Temperature and Season

All investigations into whether temperature varies by season returned non-significant results. Therefore, hypotheses Hh_1, Hh_2, Hl_1, and Hl_2, cannot be accepted and the null hypotheses Hh_0 and

H_{l_0}, which state maximum and minimum temperatures did not vary significantly between the rain and dry seasons, cannot be rejected. Given the short duration of this study and the missing data, this is not entirely surprising. Though there were multiple measurements available for both seasons, the 26 months of data were comprised of only 3 dry and 4 rain seasons and thus might not provide enough context to inform statistical results. Though it is worth noting that early studies at Budongo showed relatively constant average temperatures throughout the seasons (Newton-Fisher, 1999), so there is a chance that the results here may be representative of temperature in Bugoma. As such, it is ideal to wait until further studies can compile years-long datasets to properly investigate the relationship between temperatures and season at Bugoma.

2.4.3 Dietary stress

The pentad approach for diet stress was a relatively successful method for conceptualising diet at Bugoma. Assigning a dietary stress score to each pentad to directly compare to the season pentads proved advantageous and would also allow for easy direct comparison in future studies. Pentads were relatively forgiving of missing data, allowing a relatively fine-grain examination of trends without relying solely on daily diet data, which was particularly important for a partially habituated community. Results also seemed fairly representative of what one might expect from a chimpanzee diet, with a preponderance of ripe fruit throughout the year supplemented mostly by young leaves and unripe fruit and occasionally other food items. For instance, the average dietary stress score of 5.02 roughly represents eating unripe fruit or young leaves 2 to 3 times per pentad, or bark/mature leaves once per pentad, and otherwise relying solely on ripe fruit. With nearly a quarter of pentads exhibiting no dietary stress (i.e. eating only ripe fruit) and only one pentad with sufficient observations where ripe fruit was not eaten, ripe fruit clearly seems to constitute the base diet of Mwera when available. However, this also raises the important point

that true dietary stress could be driven by many different factors (what plant parts were available, variation in feeding species in different forest patches, food preference, etc.), all of which cannot be captured with the currently available data on the Mwera community. Therefore, this provides a decent preliminary look into Mwera's diet and feeding behaviour but lacks the specificity to tackle more complicated hypotheses.

For all the advantages the pentad approach has to offer, moving averages may still provide the best framework for understanding the dietary stress data represented here. When comparing the plots of the dietary stress per pentad to that of the 5-day moving average (Figure 2.6 & Figure 2.7), the data appear less messy with moving averages. Additionally, in the pentad approach there are often large shifts happening from pentad to pentad while the moving averages capture the gradual increase and decline of dietary stress peaks and troughs. The moving average also allows a more defined look at dates, allowing periods where stress can be examined as one date to another instead of one pentad to another, where the pentads can enforce harsh cutoffs between two dates that may actually be very similar. For instance, imagine there are two days with high dietary stress values of 6 each that fall in neighbouring pentads (pentad A and pentad B) as opposed to in the same pentad and that pentad A has values (in date order) of 0, 0, 0, 3, 6 (total score of 9) and pentad B of 6, 2, 1, 1, 0 (total score of 10) and the pentad preceding A and following B have values of 1, 0, 1, 0, 0 and 0, 0, 0, 1, 0, respectively. Instead of seeing a gradual dietary stress peak, as would be seen in a moving average, there would instead be a jump from a near zero value to the 9 and 10 values showing an abrupt and small plateau followed by another jump down to near zero. While this could be of some interest when looking over large datasets to pinpoint large jumps, it could be misrepresentative of how the chimpanzees are actually experiencing this dietary stress by making it appear more extreme (i.e. instead of slowly needing to rely on more stressful dietary

components there is a sudden increase in stress from one day to the next which insinuates a sudden extreme level of stress). This is illustrated in the major dry seasons on both plots (see left dark orange box in both Figure 2.6 and Figure 2.7) where the pentad data tends to jump between high and low values whereas the moving average shows a generally smooth incline followed by a smooth decline in dietary stress. Especially in the context of such a short study on an unhabituated community of chimpanzees, the pentad approach lacks the ability to examine gradual increases and decreases in dietary stress in finer details that are otherwise captured within the moving averages. However, phenology data of the available food sources in the Mwera diet could help determine if one, or both, methods capture a true picture by examining if decreases in available plant parts with low dietary stress are sudden, gradual, or a combination of the two.

Diet and Season

It is difficult to know which hypothesis for dietary stress to accept or reject based on the conflicting results. However, given the higher specificity of the moving average approach and its ability to capture daily scales better than the pentad totals, these methods might provide the best estimations of dietary stress until data from longer study periods become available. Therefore, Hd_1 , which states that the chimpanzees experience higher dietary stress in the dry season, can be tentatively regarded as worth consideration in future studies, but Hd_0 cannot be completely rejected with the current data available. Additionally, Hd_2, that dietary stress is lower in the dry season, can likely be rejected given the results from this data. An estimated 0.5 increase in daily dietary stress during the dry season may not seem like a large value when compared to pentad totals but would in reality constitute 1/3 of the value of an average daily diet since the annual daily average dietary stress across all seasons measures only 1.47. This suggests there could be some

effect of season on diet and future studies incorporating more longitudinal data on the Mwera community should endeavour to explore this futher. In particular, this only suggests a seasonal dietary difference specifically in the consumption of plant parts that are relevant for geophagic behaviour and does not necessarily represent other factors such as phenological availability, accessibility influenced by ranging behaviour, or any dietary stress not based on digestibility and PSC content. These results are generally consistent with other primatological research suggesting PSCs are higher in the dry season (Pebsworth *et al.*, 2019b).

2.5 Conclusions

The results of this chapter have outlined some elements of the abiotic factors of the Mwera community of eastern chimpanzees in the Bugoma Central Forest Reserve and made a preliminary attempt to describe their diet. Though this study did not systematically investigate home range, the rough estimate from Mwera community sightings of 9.8km was similar to ranges found in other eastern chimpanzees. The rainfall data and average temperatures were found to be generally in line with results from other field sites East African field sites and general trends for Hoima District. This includes the onset and duration of both the major and minor dry season at Bugoma, with the major dry season occurring from December-February and the minor dry season from June-August. However, there is no statistically significant difference in temperature between the rain and dry season. Using daily dietary stress values, it was estimated that Mwera's diet was based mostly on ripe fruit, with regular supplementations in the form of unripe fruit and young leaves and rare consumption of more dietarily stressful plant parts. Additionally, there is some statistical evidence that dietary stress does vary between the rain and dry seasons, but only when examined on a daily scale and not when using pentad values.

Relevance for future chapters

Results reported here contribute to hypothesis testing in all of the following chapter. For example, if access to water is a driver of cave use in Bugoma, there would likely be a strong peak in cave use observed in the early- to mid- dry season (most likely in the first weeks of January 2020) as this would be when water becomes scarce but before it dries up completely from the cave (which is likely to happen during the complete lack of rainfall in mid-late January). And there may be an additional, smaller, peak in cave use during the minor dry season starting in June 2020 – particularly the last two weeks of June. If temperature drives cave use, there will probably be no season trends and cave use will instead be confined to days when the recorded maximum was exceptionally high. For geophagy, if adsorption of PSCs or other digestive aid informs soil eating, then geophagy would be expected in the cave when PSCs (measured as a dietary stress score) are high in the dry season. Alternatively, if geophagy is higher during the rain season this could indicate that geophagy is a response to a seasonal increase in infections often found during the rain season (Pebsworth *et al*., 2019b). Both seasonal hypotheses would provide general support for the protection hypothesis of geophagy. If there are no seasonal trends in geophagy, this would likely indicate that hypotheses should investigate mineral supplementation, soil composition, and whether certain sex-age-reproductive classes engage in geophagy more often than others.

3. *Analyses of geophagic materials ingested by chimpanzees at Bugoma Forest, Uganda*

Abstract

Geophagy, the consumption of soil, is found throughout the animal kingdom, including humans and nonhuman primates. While this behaviour is well-studied, it is still unclear which adaptive mechanisms may be driving it. There are three main hypotheses: 1) soil supplements the necessary dietary nutrients, 2) it protects against toxins and illness, and 3) it is a result of famine, the pleasant taste/texture of the soil, or behavioural tradition (the 'nonadaptive' hypotheses). This study aims to explore geophagy in a unique environment never systematically investigated in chimpanzees – an earthen cave set within a hillside in the Bugoma Forest Reserve, Uganda. For this, 12 samples each from 3 geophagic points were collected and compared to 12 control samples collected at the mouth of the cave where the chimpanzees do not eat soil. These soil samples were analysed for colour, texture, carbon content, pH, conductivity, CEC, geochemical elemental content, mineralogical composition, clay mineral content, and bioavailability of nutrients then statistically compared to investigate composition variation between seasons, as well as any differences between all four soil sampling locations. Results showed seasonal variation only in tin content, but the locations were found to vary statistically on many parameters. Most notably, the control contained more bioavailable nutrients than the geophagic area, and the geophagic points contained higher relative abundance of 1:1 and 2:1 clay minerals. It was concluded that nutrient supplementation was an unlikely explanation, however clays are known to both help adsorption of toxins and relieve gastrointestinal stress which provides support for the protection hypothesis.

3.1 Introduction

Geophagy is the purposeful ingestion of earth materials from sources such as directly from the forest floor, exposed soil horizons, earthen mounds (e.g. termites and ants), or even nests made from earth (Wilson, 2003; Pebsworth *et al*., 2019b, Panichev *et al*., 2013). This behaviour is described throughout the animal kingdom (Sokol, 1971; Klaus & Schmidg, 1998; Klaus *et al*., 1998; Brightsmith & Muñoz-Najar, 2004; Ghanem *et al*., 2013; Mahandran *et al*., 2016) and is particularly common amongst human and nonhuman primates (NHPs), with at least 136 NHPs species known to engage in geophagy (Krishnamani & Mahaney, 2000; Young *et al*., 2011; Pebsworth *et al*., 2019b). In both humans and NHPs, geophagy is found on every continent in which they occur but is more commonly observed in tropical environments – though for NHPs this could be a function of their predominantly tropical distribution (Pebsworth *et al*., 2019b; Young & Miller, 2019). Though geophagy has been well-studied in NHPs and humans, there is still some uncertainty in the possible adaptive benefits of geophagy and modern studies consider geophagy to have many possible, and not mutually exclusive, benefits. These fall into three main groups of hypotheses for geophagic behaviour, the nutrient supplementation hypotheses, the protection hypotheses, and the 'nonadaptive' hypotheses.

The nutrient supplementation hypotheses encompass all explanations that suggest soil provides essential nutrients and elements or is used to treat nutrient deficiencies (Kreulen, 1985; Krishnamani & Mahaney, 2000). This also includes the culture-nutrition hypothesis which states that nutritional stress leads to a behavioural response (geophagy) in humans and then becomes an institutionalised cultural behaviour and was one of the earliest proposed hypotheses for geophagy in humans (Hunter, 1973). The most often cited nutrients supplemented by geophagy are iron, calcium, zinc, or salts (Young *et al*., 2008; Limpitlaw 2010). These hypotheses are

typically driven by the conditions or states of the individual consumer, especially sex-age class. For instance, in humans, a pregnant or lactating female would typically have higher nutrient demands during this time (Njiru *et al*., 2011) and may seek to supplement these additional needs.

The protection hypotheses cover a number of similar explanations related to possible protective health benefits (Young *et al*., 2011). These include benefits such as direct adsorption of toxins by the soil, antacid functions by pH buffering, an antidiarheoal, to directly counteract infections by parasites, and relieving gastrointestinal stress by coating the intestines (Krishnamani & Mahaney, 2000). These groups of hypotheses are more likely a response to external environmental forces, for instance seasonal parasite infections, increased exposure to toxins via diet or other means, or to counteract gastrointestinal symptoms caused by viral or bacterial infections. However, it can also be used to treat symptoms of pregnancy in humans such as nausea and diarrhoea (Young *et al*., 2011).

The 'nonadaptive' hypotheses cover all other explanations of geophagy such as hedonistic/tactile sensations, behavioural tradition/culture, or a response to famine or hunger when no other food is available (Krishnamani & Mahaney, 2000; Pebsworth *et al*., 2019b). While behavioural traditions and culture have been grouped into this category, it is important to note that culture can provide an adaptive benefit (Laland, 2008). Similarly, seeking out alternative food sources in response to the food scarcity referenced by 'hunger hypothesis' could also have adaptive benefits if it does not actively harm health – however, there is some evidence that soil ingestion can lead to nutrient deficiencies (Abrahams, 2012b). Therefore, while this category is considered 'nonadaptive' within the literature, this should not be taken to mean that it serves no adaptive benefit, but that the contents or composition of the soil do not provide the benefits directly.

In chimpanzees, there is evidence for all three hypotheses with geophagy as a means to supplement nutrient (Mahaney *et al*., 1997; Reynolds *et al*., 2015), aid digestion (Mahaney *et al*., 1996b; Tweheyo *et al*., 2006), adsorb toxins (Aufreiter *et al*., 2001; Reynolds *et al*., 2015), work as an antacid by buffering stomach pH (Mahaney *et al*., 1999), serve as an antibacterial agent (Ketch *et al*., 2001), and even to bioactivate certain medicinal plants (Klein *et al*., 2008) all mentioned within the literature. The most common source of soil in chimpanzees was typically termite or other insect mounds (Mahaney *et al*., 1996b; Aufreiter *et al*., 2001; Hedges & McGrew, 2012; Pebsworth *et al*., 2019b), but other sources have included under the bole or at the base of a tree (Reynolds *et al*., 2015; Pebsworth *et al*., 2019a), and even licked directly from rock surfaces (Nishida, 1980; Nishida & Uehara, 1983). However, while most studies on chimpanzee geophagy have completed at least some form of soil analysis, only more recent studies are investigating a wide range of soil parameters – especially bioavailability (Pebsworth *et al*., 2019b).

To test these hypotheses, it is imperative to explore both the soil composition and contents as well as the geophagic behaviour itself. Particularly, a systematic and comprehensive approach to collecting and analysing geophagic soils, in addition to choosing an appropriate control, is necessary to inform any behavioural variations observed but is relatively lacking within previous research (Mahaney & Krishnamani, 2003; Young *et al*., 2008; Pebsworth *et al*., 2019b). This includes selecting a control sample as near as possible to the geophagic area of interest that is readily available but not known to be consumed, ascertaining the full elemental, mineral, clay, and carbon content of the soils, collecting descriptive data on soil qualities such as colour and texture to inform on any visual and mouthfeel contributions to soil choice, and measuring bioavailability of elements since some elements may be bound on forms that cannot be digested.

This study aims to investigate geophagic behaviour of the Mwera community of eastern chimpanzees (*Pan troglodytes schweinfurthii*) in the Bugoma Central Forest Reserve in western Uganda. Near the northwestern edge of the Mwera range is an earthen cavity set into the side of a large hill with evidence of geophagy that was subsequently confirmed with remote camera trap footage. Though the cavity is made of earth instead of stone, it meets the general criteria described for caves within the literature (Bögli 1980; White 1988; McGrew *et al*. 2003; White & Culver 2012) and functionally operates as a cave being not only dark and damp, but also sheltered enough to create an isolated microhabitat. Since this cavity could serve similar ecological functions as a cave, this underground cavity will henceforth be called a cave.

As the cave seems to lie on the northern edge of the Mwera home range, it is believed that the neighbouring community of chimpanzees to the north may also visit this area and may even possibly consume soil there. Since encounters between different chimpanzee communities can be fatal (Wilson & Wrangham, 2003; Watts *et al*., 2006; Pruetz *et al*., 2017; Martínez-Íñigo *et al*., 2021), engaging in geophagy in this area could prove especially costly – particularly given the confined nature of the cave. Additionally, the general area is frequented by poachers hunting with dogs that could also pose a threat, though they do not actively hunt chimpanzees. Therefore, there are three potential costs to consuming soil within the cave: 1) the cave is not within the central Mwera range and thus may require additional time and energy to reach, 2) the potential to encounter members from a different chimpanzee community with fatal results, 3) risking contact with hunting dogs and humans that could be distressing or even fatal under certain circumstances. Despite this, the chimpanzees continue to visit the cave and consume soil from within from one specific spot. Thus, this scenario provides a unique opportunity to not only investigate why chimpanzees engage in geophagy within an underground cave, but to also

understand possible benefits of consuming soil that outweigh the potential costs outlined above. To this end, a comprehensive analysis on soil qualities, composition, and contents was conducted to understand what nutrients or other health benefits the soil might offer the chimpanzees. Additionally, the sweeping analyses in this study can serve as a baseline for future studies investigating geophagy within Bugoma as well as in other studies investigating cave geophagy.

3.2 Methods

3.2.1 Field site and sampling locations

Field site

The Bugoma Central Forest Reserve is a 400km2 tropical forest reserve at 1100m elevation near Lake Albert in Uganda (1°17'40''N 30°59'50''E; Plumptre *et al*. 2010) which is home to the Mwera community of chimpanzees. Ecological observations and the habituation of the Mwera community started in 2016 and were conducted by the Bugoma Primate Conservation Project (from here: 'BPCP'). Bugoma Forest receives around 1000mm of rain per year and has two dry seasons, a more severe dry season between December and February, and a weaker dry season from June to August (Chapter 2, this thesis). The Mwera community of Budongo Forest has been continuously studied since 2016, but all soil samples for this study were collected over a 12-month period from June 2020 to May 2021 by BPCP staff and PhD student Kelly R. Mannion.



Figure 3.1. *a*. (top left) photo taken from the entrance of the cave at the end of the rain season looking back toward the 'right chamber' where geophagy by chimpanzees and other primates occurs. The entrance is generally well lit; however, the deeper sections of the chamber remain largely dark (photo taken near the end of the dry season 11 December 2019). ***b*.** (top right) photo taken from the entrance of the cave looking back toward the 'right chamber' (with flash) at the end of the dry season during the pilot study (9 April 2019). ***c*.** (bottom) panorama photo taken from the cave entrance (using flash). The right, geophagic, chamber begins from the central pillar and extends to the right, while the middle chamber can be partially seen to the left of the pillar. The left chamber cannot be clearly seen from the entrance and is not pictured.

Sampling locations

The cave within the Mwera range consists of three chambers that are all accessible to each other, but semi-separated by aggregations of soil and roots, and is made entirely of earth and roots from two large trees (*Alstonia boonei* and *Cynometra alexandrii*) that appear to add structural integrity. Though the cave is generally buffered against external conditions, there is still considerable seasonal variation with water collecting over the rain seasons and then slowly drying through the dry seasons (Figures 3.1 *a*&*b*). Geophagy has only been observed in the right-most chamber (Figure 3.1 *c*) and there is no evidence that geophagy occurs in either of the other chambers.

After observing geophagic behaviour via camera trap footage, it was determined that the chimpanzees only consume soil from one location within this chamber – a small area of approximately 0.6m2 at the deepest part of the chamber, roughly near the base of the Alstonia boonei tree. All geophagic samples were collected from this identified area, specifically from three geophagic points: the left side of the geophagic area near a large root (from here: GP1), the right side of the geophagic area which is slightly more open to the rest of the chamber (from here: GP2), and lastly the back middle section of the geophagic area which is in the deepest part of the cave and nearest the back earth wall (from here: GP3). Though most samples were collected consistently throughout the study, there was a small discrepancy in which two samples for GP3 (October 2020 and January 2021) were collected from the back wall and not strictly from the geophagic area. As the difference in location is negligible, these were included in the analyses as part of GP3, however, there may still be differences between the two. All GP samples are confirmed to have been ingested by chimpanzees at least once, with the possible exception of the GP3 samples noted above as this area is difficult to observe via camera trap footage.

Control samples for geophagic soil analyses can be difficult to obtain and many studies do not use controls at all (Pebsworth *et al*., 2019a; Pebsworth *et al*., 2019b). The current consensus appears to be that control samples should be: 1) as near as possible to the location where geophagy occurs, 2) is not ingested by the species in question, and 3) from the same soil horizon (Mahaney & Krishnamani, 2003; Pebsworth *et al*., 2019b). Therefore, the chimpanzees need to have access to the control sample location with no extra effort needed to obtain it, and yet still prefer the established geophagic area. Thus, for this study, the best available control was determined to be from the internal wall of the main cave entrance where the soil is known to be of a similar (though not identical) colour, type, and horizon and where chimpanzees have yet to be

observed collecting or ingesting soil even though it is readily accessible and would not require entering the cave to collect (i.e. would require less effort as most individuals walk by this area to access the geophagic area within). This area was also specifically chosen as it is continuously observed by external camera traps that monitor the entrances to the cave, thus if the chimpanzees had chosen to ingest soil at the entrance this should have been previously recorded.

3.2.2 Laboratory analyses

Sample collection and processing

Soil samples were collected from all four locations (GP1, GP2, GP3, and Control) at the beginning of each month from June 2020 to May 2021, apart from May 2021 when GP3 was not collected, which resulted in 12 samples each for GP1, GP2, and Control points and 11 samples for GP3. Additionally, soil at each location was inspected in situ for soil colour using Munsell charts and soil texture using a ribbon test. These soil qualities were collected only once in 2022, which was after all soil samples had been collected and sent for laboratory analyses. At the beginning of each month, approximately 100g of soil were collected from all four sampling locations using sterile tools and gloves then placed in a plastic bag and labelled. These were then sealed, and the soil dried in the sun before being transported to Switzerland for laboratory analyses. Once received, soil samples were dried under mild conditions of 29°C for 48 hours to preserve crystalline integrity for clay analyses, then weighed and lightly mixed to break up any clumps. Subsamples of the soils were collected and sieved (<63 microns) for clay mineral analyses. Soils were analysed for a number of properties including carbon content, pH, conductivity, cation exchange coefficient (CEC), elemental geochemistry (using X-ray fluorescence), mineralogical composition (using X-ray diffraction), clay content, and bioavailability of key elements.

Soil analyses

Soil analyses were supervised by Prof. Montserrat Filella, from the University of Geneva. Carbon content analyses were carried out at the Swiss Federal Institute of Aquatic Science and Technology (EAWAG) employing DIN 19539 (Investigation of solids - Temperature-dependent differentiation of total carbon (TOC400, ROC, TIC900)) methods and using a soliTOC® cube (Elementar Analysensysteme GmbH, Germany) for sample determination. This allowed for measurement of Total Organic Carbon content released up to 400°C (TOC400; henceforth: TOC), Total Inorganic Carbon released up to 900°C (TIC900; henceforth: TIC), and Residual Oxidisable Carbon (ROC) as percentage of sample weight, as well as investigations into Total Carbon content (TC).

All analyses on pH, soil conductivity, and Cation Exchange Capacity (CEC) were conducted by the Bibliothèque nationale du Luxembourg, (BnL, Luxembourg) in accordance with ISO 10390:2021 (Soil, treated biowaste and sludge – Determination of pH) and ISO23470:2018. (Determination of effective cation exchange capacity (CEC) and exchangeable cations using a hexamminecobalt(III)chloride solution). To measure pH and soil conductivity, a subsample of soil was suspended in a 1:5 (volume fraction) suspension of water and 5mL was subsequently mixed using a mechanical shaker for 60 minutes (±10 minutes). Samples were left to rest for 1-3 hours after which they were measured using a glass electrode. Conductivity is given in µS/cm-1. For CEC, a solution of soil is prepared with 50mL hexamminecobalt(III)chloride and 10mL is then added to water in a distilling flask for a final volume of 200mL, after which anti-bumping granules were added along with 10mL sodium hydroxide solution and a few drops of phenolphthalein. This was then distilled into a recovery flask with 40mL of Boric acid solution and some Tashiro indicator (a

mixture of methyl red and methylene blue 95% ethanol) until 150mL of distillate is produced within 30 minutes. The solution is then titrated, and the CEC calculated as cmol (+)/kg.

X-ray fluorescence analyses (XRF) were conducted to determine elemental composition of the soil. These were completed at the University of Plymouth using a Niton™ XL3t XRF spectrometer calibrated in soil mode (Thermo Scientific™, USA). X-ray diffraction analyses were conducted by the University of Jena with the aim of identifying crystalline (clay) structures. Samples were x-rayed, results recorded, and the subsequent graphs were then analysed for mineral content. A further preliminary investigation into the relative abundance of clay minerals was also conducted using 16 of the 48 total samples: samples from all four locations in July and January to represent the dry season, and samples from all four locations in October and April to represent the rain season. As these analyses were time and cost intensive, only this subset could be analysed. For clay mineral analyses, an aqueous suspension of soil was prepared, and a mild vacuum was used to create oriented mounts on ceramic filters to ensure preferred orientation and minimal sedimentation leading to sorting by particle size. These were scanned from 5-90 degrees 2θ with a D8 ADVANCE with DAVINCI design (Bruker, USA), with Cu Kα radiation, Ni filter, and Lynxeye 1D detector. A step size of 0.02 °2Θ was used with 0.25 seconds per step. They were first scanned in their natural state but were then scanned again after saturation procedures (using desiccators with ethylene glycol).

Though the general elemental composition is described in the XRF results, it is necessary to understand if these elements are available to the chimpanzees under normal digestive conditions. To facilitate this, the soils were treated under artificial digestive conditions according to Method 1340 (United States Environmental Protection Agency). This involved simulated

digestion of soil samples with 0.4M glycine (pH 1.5) at 37°C under rotation for 1 hour. After simulated digestion, elemental concentrations were then assessed using ICP-MS or ICP-OES.

3.2.3 Statistical analyses

To investigate any influences on the protection hypotheses of geophagy in chimpanzees, an analysis investigating seasonality of soil composition was conducted. Firstly, all samples from geophagic points (GP1, GP2, and GP3) were pooled for each parameter measured (e.g. TOC, or CEC, or for XRF analyses grouped by element) and subsequently divided between rain and dry season based on their date of collection. These datasets were then tested for normality. As all datasets were small and/or not normally distributed, a Mann-Whitney U Test was applied apart from TIC analyses which had a binomial distribution and was instead analysed using a Fischer's Exact Test. This exact procedure was then reproduced and applied to the control soil samples to investigate if seasonality differs between soils that are eaten (GPs) and those that are not eaten (Control). However, XRD and clay mineral control samples were not analysed as the sample size was too small. To help control and account for multiple testing in such a large analysis, the Benjamini-Hochberg correction was applied.

Nutrient supplementation hypotheses were also investigated by analysing soil composition by location. For these analyses, the values for each GP were treated separately and a Kruskal-Wallis test was used to find determine which locations, if any, differed from each other. If differences were found, a Wilcoxon Ranked Sum test was then applied to investigate where differences occur. As with soil seasonality analyses, a Benjamini-Hochberg correction for multiple testing was applied.

To investigate mineral and clay content, all GP samples were pooled due to the small sample size of soils analysed and the two GP3 samples (October 2020 and January 2021) collected from a different sample location were removed. These were then compared to the control samples values using a Mann-Whitney U test due to small sample sizes.

3.3 Results

3.3.1 Soil qualities and composition

The soils within the cave (all GPs) were consistently of a strong brown colour according to Munsell Charts (Hue: 7.5YR, Value: 5, Chroma: 6), whereas the Control was found to be brown (Hue: 7.5YR, Value: 5, Chroma: 4). However, the GP3 samples (October 2020 and January 2021) that were collected from a slightly different location were found to be more similar in colour to the control than any of the other GPs and was also designated as brown (Hue: 7.5YR, Value: 5, Chroma: 4). Additionally, the same patterns hold for soil texture and estimated clay content with GP1, GP2, and the majority of GP3 exhibiting a light clay texture and 35-40% clay content, while the Control as well as the GP3 outliers both show a clay-loam texture and 30-35% clay content. However, all GP samples and control samples were generally classified as silty clay loams. The pH of all soils sampled ranged from 5.0 to 6.1, a majority of which were between 5.5 and 6.0 (32 out of 42; see Figure 3.2 *a*). Conductivity of geophagic soils generally exhibited larger ranges than the control samples (Figure 3.2 *b*), however GP3 had a somewhat narrower range of conductivity (GP1 range = 5.5 – 9.84 µS/cm-1; GP2 = 3.7 – 9.6 µS/cm-1; GP3 = 5.2 – 8.9 µS/cm-1). Carbon content indicated roughly 1% organic content for the GPs but a somewhat higher organic content of 1.5-2% for the control samples (Figure 3.2 *c*).

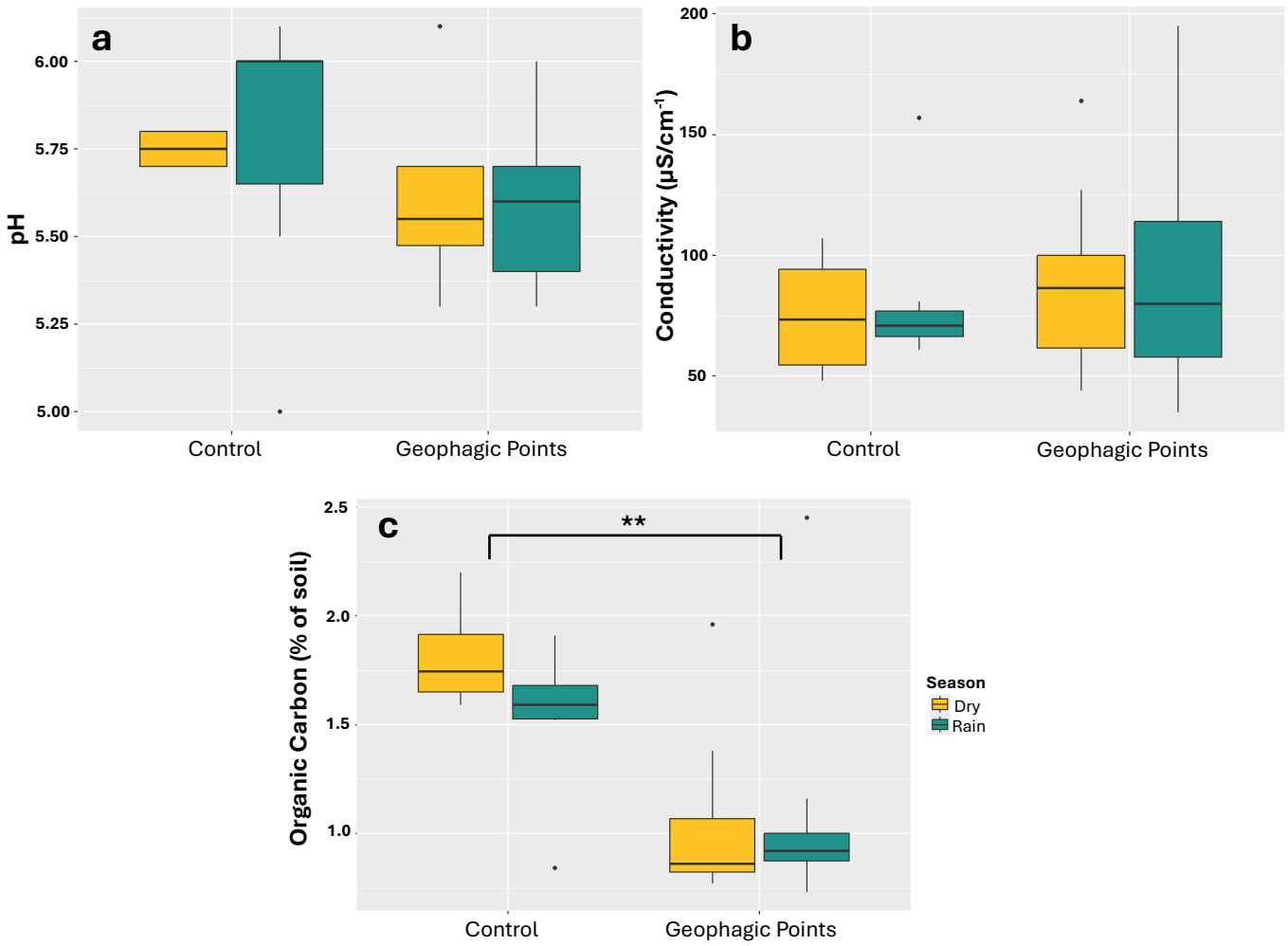


Figure 3.2. boxplots showing ***a.*** (top left) pH, ***b.*** (top right) soil conductivity, and ***c.*** (bottom) organic carbon content by season for the sample location on the x axes (control site and combined geophagic points). The rain season is depicted in green and the dry season in yellow. pH does not vary between sample locations, Conductivity does not vary between GPs and control but GP3 has significantly lower conductivity than all other samples (not shown), and organic carbon content is significantly higher in the control samples.

3.3.2 Seasonality

Only one parameter analysed, tin content, showed a significant difference between soils collected during the dry and rain seasons (Table 3.1). Additionally, tin content only showed seasonal trends within the geophagic area whereas the control samples showed no seasonality of tin content. Results show that tin content is higher in the dry season. The carbon content, pH,

soil conductivity, CEC, elemental composition (apart from tin), mineralogical composition, clay content, and bioavailability of elements did not vary by season.

Soil Analyses (unit)	Original p-value	Corrected p-value (BH)	Seasonality?
Total Carbon (%)	0.670	0.899	None
Organic Carbon (%)	0.655	0.899	None
Soil pH	0.951	1	None
Conductivity ($\mu S/cm^{-1}$)	0.903	1	None
CEC ($cmol_c\ kg^{-1}$)	0.590	0.899	None
As - Arsenic (ppm)	0.154	0.899	None
Ba - Barium (ppm)	0.305	0.899	None
Ca - Calcium (ppm)	0.482	0.899	None
Cr - Chromium (ppm)	0.504	0.899	None
Fe - Iron (ppm)	0.668	0.899	None
K - Potassium (ppm)	0.400	0.899	None
Mn - Manganese (ppm)	0.668	0.899	None
Mo - Molybdenum (ppm)	1	1	None
Pb - Lead (ppm)	0.932	1	None
Rb - Rubidium (ppm)	0.694	0.899	None
Sn - Tin (ppm)	**< 0.001**	**0.016**	**Dry > Rain**
Sr - Strontium (ppm)	0.281	0.899	None
Th - Thorium (ppm)	0.555	0.899	None
Bioavailability			
As - Arsenic (µg/kg)	0.185	0.899	None
Ca - Calcium (µg/kg)	0.878	1	None
Co - Cobalt (µg/kg)	0.986	1	None
Cu - Copper (µg/kg)	0.702	0.899	None
Fe - Iron (µg/kg)	0.498	0.899	None
K - Potassium (µg/kg)	0.440	0.899	None
Mn - Manganese (µg/kg)	0.878	1	None
Pb - Lead (µg/kg)	0.578	0.899	None
Zn - Zinc (µg/kg)	0.698	0.899	None

Table 3.1. Shows a selection of results for seasonality analyses with both the original and Benjamini-Hochberg corrected p-values. Significant values have been bolded. Though only one parameter showed a significant seasonal difference (tin content – which was higher in the dry season), many of the other included parameters showed significant differences between GPs and Control for the location analyses (see Table 3.2).

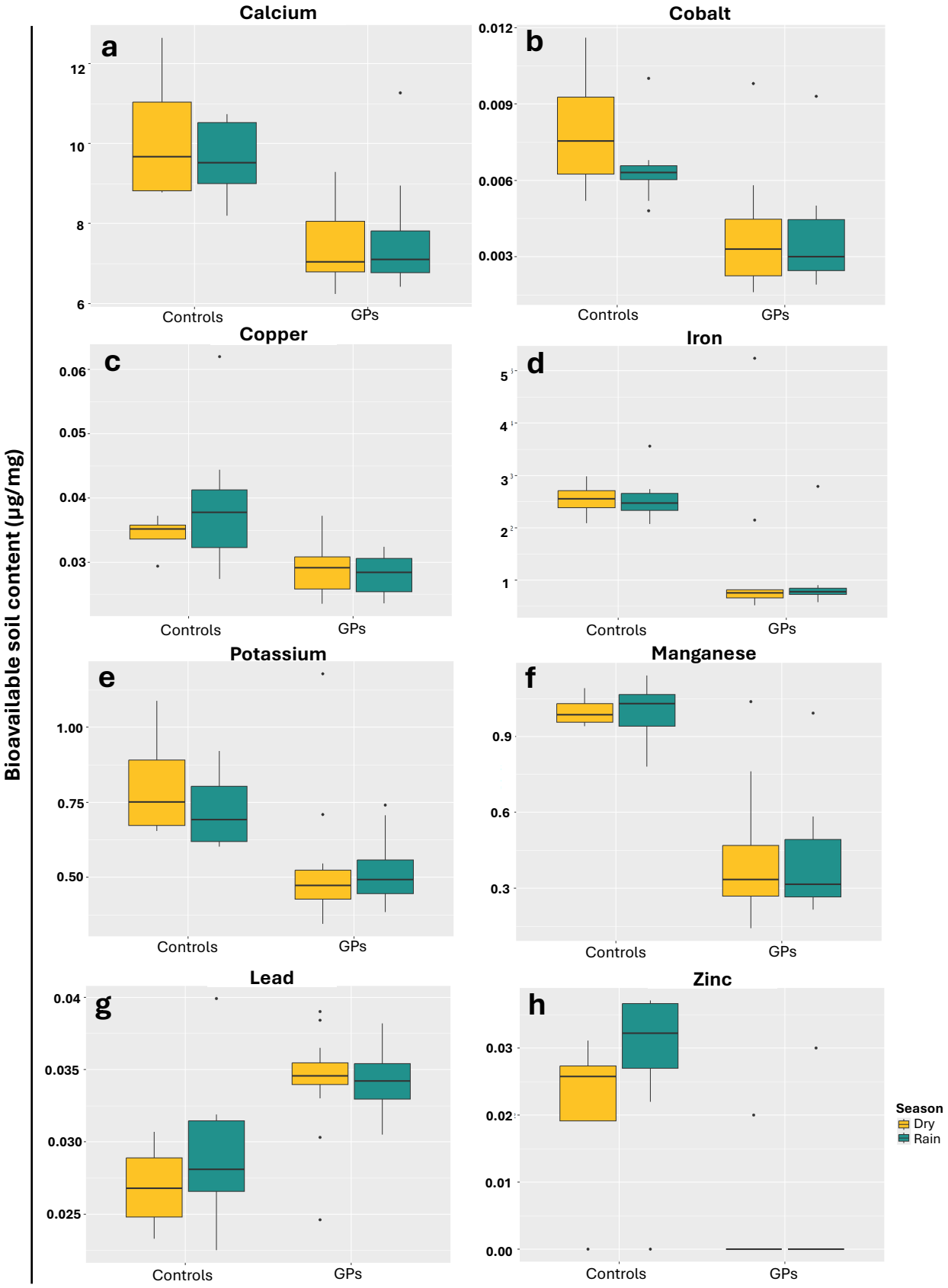


Figure 3.3. shows the bioavailable soil content in the control samples and all GP samples by season for ***a.*** calcium, ***b.*** cobalt, ***c.*** copper, ***d.*** iron, ***e.*** potassium, ***f.*** manganese, ***g.*** lead, and ***h.*** zinc. Of particular note is that the control samples are higher in beneficial content (calcium, cobalt, copper, iron, potassium, manganese, zinc) and low in lead, a highly toxic metal.

General trends indicate that the control samples have higher levels of organic carbon, calcium, manganese, bioavailable calcium, bioavailable cobalt, bioavailable copper, bioavailable iron, bioavailable potassium, bioavailable manganese, and bioavailable zinc, and lower levels of chromium, iron, potassium, rubidium, thorium (though there is overlap with GP2), and bioavailable lead (Table 3.2). The control samples also have significantly lower levels of molybdenum than GP1 ($p=0.013$), but the molybdenum levels in GP2 and GP3 fall between, and overlap with, GP1 and the control. This is also the case for total lead levels, where control samples have less lead than GP1 and GP2 but overlap with GP3 (which also overlaps with GP1 and GP2). GP2 has significantly higher barium levels than the control samples ($p=0.043$) but overlaps with GP1 and GP3 which have intermediate values between the two. GP3 has the lowest soil conductivity of all the sites sampled, and GP3 and the control samples have lower Arsenic than the other GPs. Across all locations, silver ($p=0.251$), cadmium ($p=0.114$), caesium ($p=0.133$), copper ($p=0.0524$), mercury ($p=0.542$), antimony ($p=0.654$), scandium ($p=0.781$), tin ($p=0.799$), tellurium ($p=0.408$), titanium ($p=0.249$), uranium ($p=0.827$), zinc ($p=0.22$), zirconium ($p=0.890$), and bioavailable chromium ($p=0.544$) content did not differ.

Soil Analyses (unit)	Original p-value	Corrected p-value (BH)	*C – GP1*	*C – GP2*	*C – GP3*	*GP1 – GP2*	*GP1 – GP3*	*GP2 – GP3*
Total Carbon (%)	< 0.001	< 0.001	✓	✓	✗	✓	✗	✗
Organic Carbon (%)	< 0.001	0.001	✓	✓	✗	✓	✗	✗
Soil pH	0.048	0.077	✗	✗	✗	✗	✗	✗
Conductivity ($\mu S/cm^{-1}$)	< 0.001	0.001	✗	✗	✓	✗	✓	✓
CEC ($cmol_c\ kg^{-1}$)	0.501	0.601	✗	✗	✗	✗	✗	✗
As - Arsenic (ppm)	0.001	0.003	✓	✗	✗	✗	✓	✗
Ba - Barium (ppm)	0.025	0.044	✗	✓	✗	✗	✗	✗
Ca - Calcium (ppm)	< 0.001	< 0.001	✓	✓	✓	✗	✗	✗
Cr - Chromium (ppm)	< 0.001	0.001	✓	✓	✓	✗	✗	✗
Fe - Iron (ppm)	< 0.001	< 0.001	✓	✓	✓	✓	✓	✓
K - Potassium (ppm)	< 0.001	< 0.001	✓	✓	✓	✓	✓	✗
Mn - Manganese (ppm)	< 0.001	< 0.001	✓	✓	✓	✗	✗	✗
Mo - Molybdenum (ppm)	0.007	0.014	✓	✗	✗	✗	✗	✗
Pb - Lead (ppm)	< 0.001	0.001	✓	✓	✗	✗	✗	✗
Rb - Rubidium (ppm)	< 0.001	< 0.001	✓	✓	✓	✗	✗	✗
Sn - Tin (ppm)	0.799	0.839	✗	✗	✗	✗	✗	✗
Sr - Strontium (ppm)	0.021	0.038	✗	✗	✗	✗	✗	✓
Th - Thorium (ppm)	0.004	0.007	✓	✗	✓	✗	✗	✗
Bioavailability								
As - Arsenic (µg/kg)	0.032	0.053	✗	✗	✗	✗	✗	✗
Ca - Calcium (µg/kg)	< 0.001	< 0.001	✓	✓	✓	✓	✓	✗
Co - Cobalt (µg/kg)	< 0.001	< 0.001	✓	✓	✓	✓	✗	✓
Cu - Copper (µg/kg)	< 0.001	< 0.001	✓	✓	✓	✓	✓	✗
Fe - Iron (µg/kg)	< 0.001	< 0.001	✓	✓	✓	✗	✗	✗
K - Potassium (µg/kg)	< 0.001	< 0.001	✓	✓	✓	✗	✗	✗
Mn - Manganese (µg/kg)	< 0.001	< 0.001	✓	✓	✓	✓	✗	✓
Pb - Lead (µg/kg)	< 0.001	0.002	✓	✓	✓	✗	✗	✗
Zn - Zinc (µg/kg)	< 0.001	< 0.001	✓	✓	✓	✗	✗	✗

Table 3.2. Shown are all parameters with significant differences in soil content by location as well as some key characteristics without significant differences. On the left the original p-values and Benjamini-Hochberg corrected p-values are listed. The columns on the right show which locations differ from each other in soil composition (denoted with a blue check mark) and which do not differ significantly (denoted by a black 'x'). For instance, the three middle columns show where the Control soil ('C') differs from the Geophagic Points (GPs 1-3) and the right three columns indicate which of the GPs differ significantly from each other. Of particular interest are the bioavailability results which show that if there is a significant difference between the Control and one GP, there will also be significant differences with the other two GPs.

3.3.3 Mineral and clay analyses

The control samples were found to contain significantly more quartz than the geophagic points (p=0.002), whereas the geophagic points contain significantly more kaolinite (p=0.002), illite (p=0.006), and goethite (p=0.02; Figure 3.4 *a*). However, when comparing relative clay abundance as a subset of the mineral content, there were no significant differences between the proportions of the types of clay present (Figure 3.4 *b*).

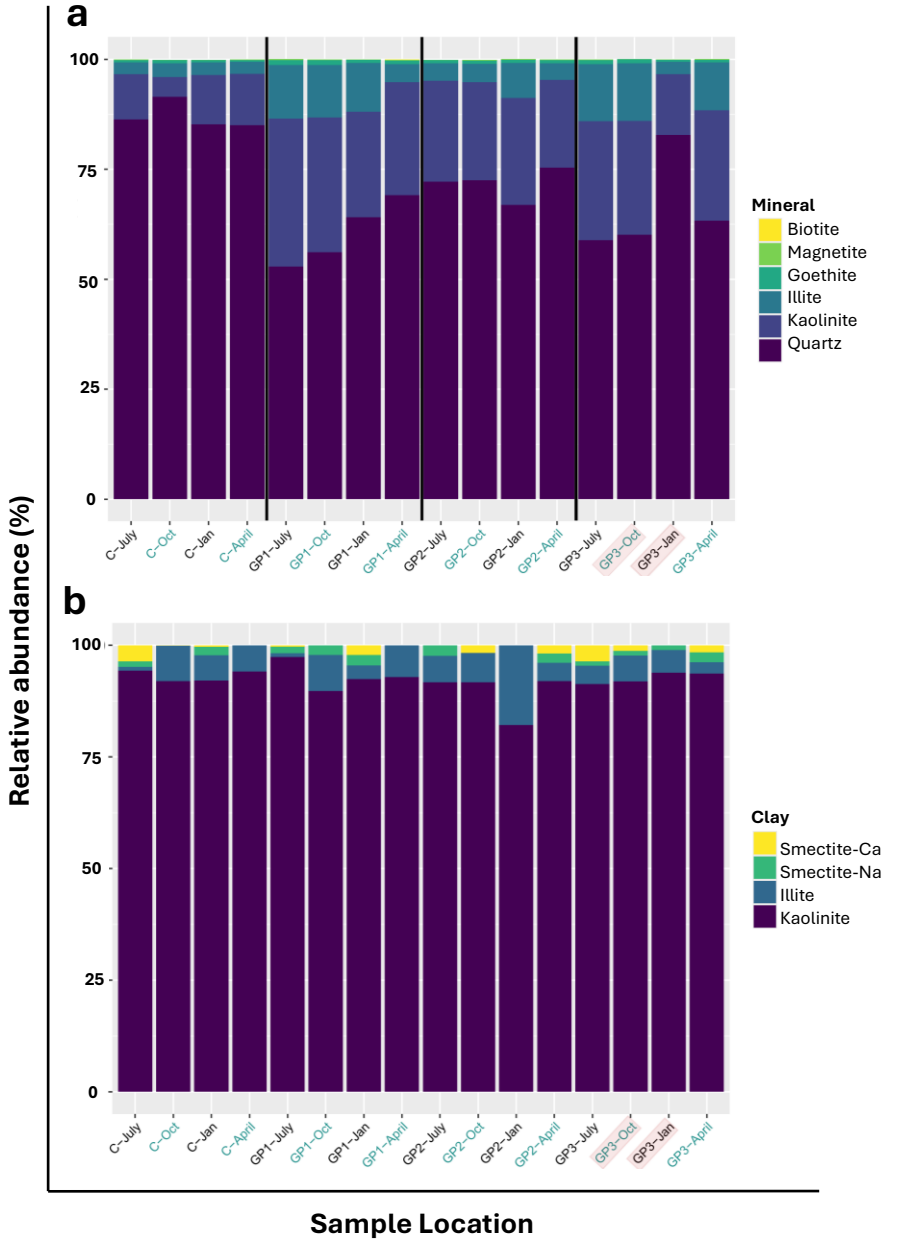


Figure 3.4. ***a.*** (top) shows mineral content for the control samples and the geophagic points. This shows the relatively high quartz content of the control compared to the GPs and the resulting high levels of kaolinite, illite, and goethite. There was no difference in magnetite content and biotite was only found in one sample (GP1 in April). The text of the soil samples representing a rain season are shown in blue, with the dry season in black text. GP3 October and January were taken from slightly different locations and are thus highlighted. ***b.*** (bottom) the relative clay mineral content of geophagic points and the control samples. Rain seasons are shown in blue text while the dry season is shown in black and the GP3 samples taken from a different location are again highlighted.

3.4 Discussion

3.4.1 Soil qualities

In general, the soil colour and texture were as expected, however the difference between the two GP3 samples from a slightly different location and the other GPs was somewhat surprising as these locations are all very close to each other. One consideration is the relative position of these soils. The October and January GP3 soils were taken from very near the back wall of the cave, and the control samples which most resembled these were also along a wall but near an entrance. As these walls are vertical and tend to round toward the floor of the cave, this may indicate that the two GP3 soils and the control soils might be collected from a marginally different soil horizon as the depth of the cave away from the wall is slightly lower. This could also affect the organic carbon content of the soils, as the controls were found to be significantly higher than GP1 and GP2, but not significantly different from GP3.

Organic carbon content was found to be much higher in the control than the GPs. There could be a number of reasons for this pattern. Firstly, the control sample is collected in an area with much greater contact with the external environment as the entrance is more likely to accumulate organic matter similar to that found on the forest floor. This could include not only decaying plant matter but also any bacteria or fungi that are better suited to the external environment. Similar studies in apes have suggested these organic compounds could be anything from harmful pathogens, beneficial anti-bacterials, or simply neutral agents (Ketch *et al*., 2001; Mahaney *et al*., 2016). Additionally, organic matter in soils can also provide a source of nitrogen (Ngole *et al*., 2010). Without further analyses into microbial content, it is difficult to know exactly how these may contribute to soil choice, however, the increased organic content could alter the taste of the soil enough to encourage the chimpanzees to choose an alternative (Mahaney *et al*., 2016).

3.4.2 Seasonality

The only parameter tested that showed seasonal variation in the geophagic points was the total tin content (chemical element Sn, Group 14, atomic number 50), which was higher in the dry season than the rain season. Interestingly, this seasonal variation did not occur in the control site. There could be a few reasons for this result: 1) the control location is near the entrance of the cave and may be subject to influence from the external environment which prevents buildup of tin during the dry season or otherwise removes seasonal effects, or 2) the sample size of the control samples (dry=4; rain=8) in combination with many zero values may not be robust enough to detect seasonal variation. Interestingly, when inspecting the raw data, it appears that tin content was either near zero (below level of detection) or between 18.04 – 23.67ppm in both the control and the GPs suggesting tin was either present in similar levels, or not present. Additionally, all control samples where tin was present (June 2020, September 2020, and October 2020; range=18.6 – 20.03ppm) were collected in the rain season which seems to indicate that tin was present only in the rain season at the entrance of the cave but was only found at the GPs within the cave during the dry season. However, there are too few control samples to know if this trend can be generalised to the control site. The dry season trend of tin presence within the geophagic areas was found in most samples (8/12 samples; tin was not found in July 2020 for GP1 and GP3, January 2021 for GP1, and February 2021 for GP1) with tin only found once in the rainy season (November 2020 at GP1).

Organic tin is not typically found naturally in soils and is a human produced pollutant (Ostrakhovitch 2022) and thus unlikely to be the tin represented in these samples. However, inorganic tin occurs naturally in the environment and is not necessarily toxic, but can become toxic under some natural conditions (e.g. methylation of tin(II) by methyl donors; Craig &

Rapsomanikis, 1985). Studies investigating tin methylation reactions in aqueous solutions simulating aquatic environments have found that salinity (higher salinity increases methylation under aerobic conditions; Chen *et al*., 2007) and pH (highest methylation occurs around 5 pH; Chen *et al*., 2012) are both important factors for this process. Since the cave can retain water well into the dry season, it is possible that the pH conditions with accumulated water could lead to some methylation of tin(II), however it is impossible to know for certain without further analyses.

The non-zero tin content of 18.04 – 23.67ppm found within GPs during the dry season was generally much higher than 3-6ppm found in other soils (Tsangaris, 1992 as cited in Blunden & Wallace, 2003), but does fall within the range found in rainwater (2.2-41ppm; Cardarelli, 1988). Therefore, the rainwater accumulation on the soil before sample collection and subsequent drying could influence tin levels, however, this would not adequately explain why tin levels are lower in the rain season which also exhibits regular accumulation of rainwater. Additionally, the seasonal differences of tin content may have no impact on geophagic behaviour due to the very low level of tin. In humans and other animals, the first symptoms of tin toxicity (typically gastrointestinal stress) from inorganic tin don't typically occur until at least 700ppm (Benoy *et al*., 1971; Blunden & Wallace, 2003) which is well above even the highest tin level of 23.67ppm found within this study. Indeed, if 23.67ppm tin was enough to cause harmful effects, then it is likely that these effects would also occur when ingesting rainwater. Therefore, it is very unlikely that tin seasonality, or soil seasonality of any kind, influences geophagy in the Mwera chimpanzees.

3.4.3 Soil variation by location

Several differences between sample locations (GP1, GP2, GP3, and control) were found in elemental composition and bioavailability. In general, the control samples were more often

significantly different than the GPs while the GPs were similar to each other – which is the case for calcium, chromium, manganese, rubidium, bioavailable iron, bioavailable potassium, bioavailable lead, and bioavailable zinc – or most of the sites all differed from each other, as was the case for iron, potassium, bioavailable calcium, bioavailable cobalt, bioavailable copper, and bioavailable manganese (e.g. iron where GP1, GP2, and GP3 differed from each other and each also differed from the control; see Table 3.2). This indicates that in most cases the control soils are generally quite different to the soils in the geophagic area, but when this isn't the case it is due to the soil content being inconsistent and varying between all sites. As such, if elemental composition or bioavailability of nutrients drives geophagic behaviour, it would likely be due to those parameters where the control samples have a statistically different composition and discussion will mostly be limited to these examples.

Iron was found to differ significantly between all sites (controls and all geophagic points), however the control had drastically lower overall iron levels while the iron at the control site appears to be more bioavailable (Figure 3.3 *d*). This is also the case with potassium where most locations differ from each other (except GP2 and GP3 whose values overlap) but bioavailable potassium is higher in the control site. This trend continues with other contents, such as copper and zinc where content did not differ between sites, but bioavailable copper and bioavailable zinc were significantly higher in the control. This could suggest that while the overall content of some elements might be similar, the forms in which these elements exist in the control are different, and more bioavailable, than in the GPs. In fact, for many of the beneficial bioavailable contents tested such as calcium, cobalt, copper, iron, potassium, manganese, and zinc there is a much higher bioavailability in the control and the control sample also shows lower levels of lead (a known chronic toxin, especially in young children; Chandran & Cataldo, 2010). This is contrary to what

might be expected and indicates that if the chimpanzees needed to supplement certain nutrients that are otherwise hard to obtain, they would likely receive more benefits (and fewer negative effects) if they ingested the control samples instead of the GPs. However, this would depend on the needs of the chimpanzees compared to what is available such that if the chimpanzees need iron and the bioavailable iron found in the GPs is sufficient to meet these needs then they may choose the GPs over the control for other reasons. For instance, a study on sheep in New Zealand described an ailment called 'bush-sickness', a deficiency in cobalt, and found even <20g of soil/week could offer protection against the disease (Rigg & Askew, 1934). This could be the case for a number of the elements examined here. To further understand this relationship, more detailed information on the Mwera chimpanzees' diet and nutrient intake would be required.

3.4.4 Minerals and clay

The control samples exhibited a higher relative abundance of quartz compared to all GPs – even when including the October and January GP3 samples taken from the back wall instead of the geophagic area. On average, the control samples contained 87.9% quartz while all GPs average 66.2% (GP1=60.6%, GP2=71.8%, GP3=66.3%). Additionally, when the amount of kaolinite, goethite, and illite increased in the GPs as quartz decreased, the relative proportions of clay minerals stayed constant (i.e. the percentage of kaolinite may increase, but illite and goethite also increase in proportion). The amount of quartz present in the control samples may make the soil there less desirable as this could change the mouthfeel or taste of the control or it may indicate that the increased clay mineral content of the geophagic soil is sought after. Clays are generally known to relieve gastrointestinal ailments by protecting intestinal mucin (González *et al*., 2004), neutralising or adsorbing antigens, PSCs, or other harmful compounds (Papaioannou *et al*., 2005; Ta *et al*., 2017; Pebsworth *et al*., 2019a). If clays are rare within the Mwera community's

environment, the 5-10% (as measured with soil texture) or 20% (as measured by XRD) higher clay content in the GP samples compared to the control samples could provide sufficient reason for bypassing the more accessible soils at the entrance. Studies have found that geophagic soils contain clay and often at higher levels than control soils, particularly in humans where clay soils accounted for 98% of reviewed geophagy studies (Young *et al*., 2011; Pebsworth *et al*., 2019b).

All the soils, including the control, were similar in clay mineral abundance and were comprised of kaolinite, illite, and two types of smectite (Na-smectite and Ca-smectite). Clay minerals fall into two main types based on their layer structure: the 1:1 clays in which each layer is composed of one tetrahedral sheet and one octahedral sheet, and the 2:1 clays which has one octahedral sheet with two tetrahedral sheets (one on each side of the octahedral) which is more reactive than 1:1 (Kumari & Mohan, 2021). These clay types exhibit different reaction properties and can behave differently when ingested. For instance, 1:1 clays like kaolinite tend to treat gastric illnesses, such as diarrhoea, more efficiently while the 2:1 clays like illite and smectite tend to be better at adsorption of tanins and organic molecules (Slamova *et al*., 2011). While the abundance of clays compared to each other doesn't change, as noted above, both 2:1 and 1:1 clays relative abundance increase compared to quartz, and thus the chimpanzees may choose to consume the soil at the geophagic area to take advantage of the benefits of both the 1:1 and 2:1 clay minerals.

3.5 Conclusion

This study investigated whether soil content drives geophagic behaviour in the Mwera community of chimpanzees in Uganda. The most likely explanation for the Mwera chimpanzees to ingest the soil from within the cave (the geophagic points) instead of the control appears to relate to either the clay content or the taste and mouthfeel of the soil. Interestingly, there is an inverse relationship

found in the element composition and bioavailability, such as in the case of potassium and iron. This could indicate that the elemental composition contributes to the taste by making the soil taste as if it has a certain nutrient, but the nutrient is not actually bioavailable. It could also be that instead of taste, the chimpanzees choose soil based on mouthfeel and texture, which may be altered by elemental composition as well. In particular, the lower organic carbon content of the geophagic materials may make it more pleasant to eat as it may have less decomposing organic matter or bacteria. Additionally, as there was no difference in soil content between the rain and dry seasons, it is likely that any hypotheses regarding seasonal geophagy in the Mwera community are related to the behaviour of the chimpanzees or the local environment since the soils retain a consistent composition through the year. Lastly, the higher clay content of the geophagic soils could be preferred over the control samples because they can help treat diseases or gastrointestinal problems, to bind toxins, or adsorb PSCs. In particular, the high kaolinite content may be of interest, as this has been used to protect the gastrointestinal tract and as an anti-diarrhoeal, amongst many other uses, in humans (Carretero, 2002). However, it is worth noting that while the bioavailability extractions used here are common within soil analyses, there are better ways to simulate animal digestion such as the in vitro Caco-2 model (Glahn, 2009). While these analyses are expensive and time consuming, they better simulate digestion and are thus considered to return a more accurate estimation of bioavailability (Pebsworth *et al.*, 2019b). Thus, future studies into geophagy within the cave in Bugoma forest should aim to 1) use Caco-2 models to better estimate bioavailability, 2) identify any bacterial or fungal agents present in the geophagic soils that might act as harmful antigens or beneficial antibacterials, 3) investigate behavioural components of geophagy such as sex-age class, health status, and frequency, and 4) attempt to investigate the role of taste of soil selection in geophagy, if possible.

Relevance

The results presented in this chapter have directly tested parts of the supplementation and the protection hypotheses. The soil composition suggests that geophagy is unlikely to be driven by nutrient supplementation in the Mwera community. But this may not be true if individuals of specific sex-age classes are more likely to engage in geophagy, such as for pregnant or lactating females with heightened nutrient demands where any amount of supplementation is helpful. If there are no seasonal trends in geophagy, but there are sex-age class trends, this might suggest that only certain individuals need to seek out the soils. As there were essentially no seasonal trends in soil composition, it is likely that any seasonal variation in geophagic behaviour found in subsequent chapters is related to direct effects on the chimpanzee (for instance, diet, pathogens, or water availability) and not on any particular quality of the soil. Lastly, the protection hypothesis was partially supported by the results in this chapter due to the increased clay content found in the GPs vs the control, but future chapters will need to investigate if there are behavioural trends in season to support these conclusions. In particular, as the predominant type of clay, kaolinite, is particularly well suited to treating gastrointestinal stress, geophagy would be expected to be higher in the rain season when infection rates are higher. However, it is also possible that illite content, while comprising less of the clay, may be sufficient to adsorb plant secondary compounds that are typically higher in the dry season.

4. Environmental effects on geophagy and cave use in chimpanzees at Bugoma Forest, Uganda

Abstract

Cave use and geophagy (ingestion of soil) within caves are rare behaviours amongst primates, with attempts to systematically test hypotheses behind these behaviours even rarer within the literature. Possible explanations for cave use include as: 1) shelter, 2) thermoregulators, 3) water access, and 4) access to supplemental nutrition via geophagy. Likewise, there are a three main groups of hypotheses to explain geophagy such as 1) nutrient supplementation, 2) protection from disease or to help digest plant toxins, and 3) 'nonadaptive' explanations such as seeking soil to assuage hunger, for the textural sensations, or as a behavioural tradition. This study seeks to understand cave use and geophagy in the Bugoma chimpanzees, which engage in cave-based geophagy, by using proxy variables to test possible drivers of both. First, time within the cave is modelled using GLMs and proxy variables including rainfall (as shelter), daily max temperature (for thermoregulation), water levels within the cave (as water access) and proportion of time chimpanzees spent ingesting soil (for geophagy). Geophagy hypotheses were then tested by modelling time spent ingesting soil using variables such as previous rainfall (as 30-day rainfall is associated with parasitic infections), and dietary stress scores (see Chapter 2) to provide an estimate of difficult-to-digest food items. Lastly, descriptions of the type and durations of different behaviours within the cave are provided. Results showed that proportion of time spent engaging in geophagy and water levels were the only significant predictors for cave use, and geophagy models showed a similar importance of water within the predictions as previous rainfall was the only significant predictor. This suggests that water access and geophagy may be important drivers for cave use in Bugoma, and soil as a means to alleviate parasitic infections (protection hypotheses) provides a possible explanation for geophagy.

4.1 Introduction

Remains of humans and human ancestors have been associated with caves for millions of years, yet amongst nonhuman primates (henceforth: primates) cave use is a relatively uncommon phenomenon (McGrew *et al*., 2003; Dirks & Berger, 2013). Apart from some langur species (*Trachypithecus* spp.), most primates do not make habitual use of caves (Zhou *et al*., 2007; Hendershott, 2017; Lui & Bhumpakphan, 2019). Of those primates that have been reported using caves, such as some baboons (*Papio* spp.), lemurs (*Lemur* and *Eulemur*), Japanese macaques (*Macaca fuscata*), and capuchin monkeys (*Sapajus libidinosus*), they seem to be more an exception than a rule and there is a surprising absence of great apes (Homindae), the species most closely related to humans, amongst them (McGrew *et al*., 2003; Barrett *et al*., 2004; Haslam, 2009; Kashiwagi *et al*., 2012). Within great apes, only chimpanzees (*Pan troglodytes*) have been observed using caves and only at two field sites, Fongoli, Senegal (Pruetz, 2007) and Mahale, Tanzania (Nishida, 1980).

Cave use was long believed to function predominantly as a means of shelter or as a thermoregulatory aid, however recent literature has now identified four main hypotheses to be considered in cave use: 1) as shelter from the elements or predators, 2) for thermoregulation, 3) as a form of water access, and 4) as access to nutrients through behaviours such as geophagy – the ingesting of earth or soil materials (McGrew *et al*., 2003). While there is ample support for the first two of these hypotheses, there is a growing amount of evidence in favour of caves as an access point to hydration and nutrients via geophagy. For instance, crowned lemurs (*Eulemur coronatus*) were observed entering caves to drink water during periods of water scarcity (Wilson, 1989), and a number of species have been observed licking the walls of caves in a possible form of geophagy (Redmond & Shoshani, 1987; Li *et al*., 2003; Greuter & Wei, 2006). This stone-licking

behaviour was also witnessed in the Mahale chimpanzees in Tanzania that seemed to enter small riverine caves to access water or nutrients from cave walls (Nishida, 1980) – however this behaviour has not been reported since these initial sightings.

Other than the one case of forest chimpanzees accessing caves in Tanzania, the only other evidence amongst human's closest relatives is found in the savannah-dwelling chimpanzees of West Africa (Pruetz, 2007). Since this cave use in the Fongoli chimpanzees was discovered, there have been a number of studies attempting to understand any potential drivers behind it, and most indicate it is used for the thermoregulatory benefits of shade and thermal buffering that these caves can provide (Boyer-Ontl & Pruetz, 2020). Given how rare cave use appears to be in chimpanzees, the Mwera community of semi-habituated eastern chimpanzees (*Pan troglodytes schweinfurthii*) of the Bugoma Central Forest Reserve offer a unique opportunity to test hypotheses of cave use in forest-dwelling chimpanzees.

Study of the Mwera community of chimpanzees began in 2016 when the Bugoma Primate Conservation Project (henceforth: BPCP) began collecting indirect data on the chimpanzees of Bugoma Forest in conjunction with the initiation of a habituation program to acquaint the community with the presence of researchers and facilitate long-term research. While habituating this community, researchers documented the chimpanzees entering an earthen hillside cave on the border of the Mwera territory. Upon placing camera traps in the area, this behaviour was officially confirmed and constant video surveillance of the cave commenced. Additionally, not only were the chimpanzees in Bugoma forest observed entering the cave, there was also evidence of extensive levels of geophagy occurring, which constituted one of the first instances of cave-based geophagy in chimpanzees.

This study seeks to take advantage of this rare behaviour by investigating when and why the chimpanzees choose to enter the cave to test the four hypotheses of cave use outlined above. To do so, data on rainfall, temperature, season, water levels within the cave, and rates of cave-based geophagy were collected and used to model the hypotheses of caves as a form of shelter, thermoregulatory relief, access to water, and access to nutrients via geophagy. In addition to data on cave use, this study will also investigate two possible 'protection' hypotheses behind geophagy, 1) that soil ingestion can help mitigate the effects of harmful toxins in the diet and 2) that soil is used to protect against infections that affect the gastrointestinal tract (see Chapter 1 of this thesis for an overview of the protection hypotheses). To model and test these two hypotheses, proxy variables were utilised including: 1) a dietary stress score (as calculated in Chapter 2, this thesis) to approximate the levels of toxins and difficult-to-digest dietary items within the Mwera diet, 2) a moving average for the previous 30 days of rainfall, which was used to simulate the possibility of parasitic infections that were shown to be significantly higher during months with more rainfall (Gillespie *et al*., 2010). By modelling which of these proxy variables best predicts time spent within the cave, or engaging in geophagy, this study aims to test the hypotheses driving both of these behaviours in the Bugoma chimpanzees.

4.2 Methods

4.2.1 Data collection

Field site

Data were collected from eastern chimpanzee (*Pan troglodytes schweinfurthii*) groups residing in the Bugoma Central Forest Reserve in Western Uganda (1°17'40''N 30°59'50''E). While habituation for long-term data collection was underway during the time of this study, it was not yet at a level where chimpanzees could be consistently identified nor observed continuously.

Instead, researchers sought out chimpanzee groups each day and followed them, collecting data on behaviour and diet in accordance with the tolerance of the chimpanzees being followed. By the end of the study period, chimpanzees were observed nearly daily but often only for a few hours at a time. Since the chimpanzees within the study area could not be observed engaging with the cave directly, remote activated camera traps were used to collect most behavioural data for the purposes of this research. In addition to a lack of direct behavioural observations at the cave, the inability to identify individuals within the semi-habituated Mwera community meant that it was not possible to know if the chimpanzees recorded were from the same or different communities. As the location of the cave is near the northwestern edge of the Mwera range (see estimated home range map, Chapter 2, this thesis), it is possible that one or more additional chimpanzee communities also access this resource. Since community differentiation was not possible at the time of this study, variables related to specific communities could not be explored and community itself could not be considered as a variable or grouping level. Instead, variables related to dietary stress, weather, water access, and exposure to gastrointestinal infections were considered to be similar across communities. Data collection for this study occurred between 23 September 2019 and 31 December 2020.

Within the cave are three connected, semi-open, chambers, one of which with confirmed geophagic activity. Within this chamber there are two areas which were the primary focus of chimpanzee geophagy: Geophagic Point 1 (henceforth: GP1) and Geophagic Point 2 (henceforth: GP2). GP1 is a roughly 0.6m^2 section of floor at the back of the cave roughly 5 metres from the entrance. This point collects standing water after rainfall but eventually dries in the dry season. GP2 is a small cavity that extends into the ground/wall of the cave and is just large enough to

permit the limbs of one or two chimpanzees. GP2 is around 1 metre to the left (when viewing from the entrance) from GP1 and tends to collect substantial amounts of water.

Video data

As mentioned above, direct observation of interactions at or within the cave were not possible and motion activated camera traps (Bushnell 119777, Bushnell HD 119876C) were used to collect data on cave use and geophagy instead. Cameras were placed such that the geophagic areas and the main entrance to the cave were continuously monitored and batteries were replaced at least once every 14 days to avoid power failure. When available, additional cameras were placed at other locations to monitor activity in and around the cave. This provided year-long coverage of the area, however, environmental conditions (e.g. rain and humidity) did cause cameras to malfunction on occasion. Only dates when there were at least one internal and one external camera recording data were included in the study. Data that could be collected from these videos included 1) times when chimpanzees were seen on screen or engaging in geophagy, 2) water levels within the cave, and the 3) behaviour of each chimpanzee observed (such as: 'feeding', 'resting', 'moving', and 'grooming'). These data were mostly used as the basis for the response variable (time spent engaging with the cave/in geophagy) analysed within the two models investigating possible hypotheses behind cave use and geophagy. Geophagy was not only investigated as the response variable in its own model, but was also included as a predictor for cave engagement as one of the postulated hypotheses for transient cave use in primates (McGrew *et al*., 2003).

At the time of this study, video recognition software could not be used to automate the video analysis processes, and instead all video processing was completed manually by the author. This involved watching each individual video in which a chimpanzee appears and assigning individual

IDs for each chimpanzee and time stamps for every individual and activity (i.e. feeding, resting, moving etc.) present by hand. An inter-observer reliability test was not used within this study given the time intensive nature of manually processing roughly 8000 photos and 2000 videos.

Aside from time spent in the cave and engaging in geophagy, water access was also measured from video data. This was assessed by watching all videos recorded within the cave, including videos with chimpanzees, other animals, or no animals at all, and recording whether moisture can be seen accumulating around the cave – especially at GP1, on the floor of the cave, where most geophagy took place. These observations were placed into three categories: 0) no moisture/water (null state – no visible moisture accumulation and dry powder-like soils), 1) muddy/some water available (the soil within the cave consists of mud that can cling to itself and contains some water), and 2) abundant water or evidence of water saturated soils (this category includes when water is seen pooling on the floor of the cave and/or dripping from the walls). Originally, a binary variable of present/absent was used for water in the cave, but there were several cases where this was too ambiguous to determine from video data alone, so a middle category was created to capture this uncertainty.

Engagement time for the cave and geophagy models was measured per day and totalled for all chimpanzees recorded for that day (with number of chimpanzees observed used to control for group size). This represented a means to measure the importance of soil and/or the cave as a resource. For instance, if the cave were to be used for thermoregulation purposes to cool down on hot days, the amount of total time would be a good measure of time needed to reduce body temperature on a particular day. Likewise, if geophagy is used to offset the effects of PSCs, toxins, or GI disease, then the time spent engaging in geophagy could be a proxy for how much soil is

consumed as there is no way to know the actual volume. In other words, if soil is used to treat GI stress and/or disease and there is also a high burden of disease or toxins within the population, more chimpanzees would likely spend more time ingesting soil to meet their needs. While this is not a perfect measure of the soil as a resource, it does set a basis for comparing these variables and to understand which aspects contribute most to the chimpanzees' choice to ingest soil or enter the cave.

Cave engagement started the moment an individual chimpanzee entered the cave and ended the moment they left the cave or recorded area. If a chimpanzee were to leave and then re-enter, then these two instances were added together and the period when they were not in the cave (or on camera) was deducted from their total time. For engagement in geophagy, time was totalled for each chimpanzee beginning when they first approached the geophagic area and ending when they departed. An 'approach' was considered any time a chimpanzee moved/re-oriented themselves toward GP1 or GP2 with intent clearly focused on the geophagic area (indicated with body orientation and where their eyes/focus are directed). This could include entering the cave and walking directly to the geophagic point without pause, or entering the cave and sitting near a GP for a few seconds before turning both body and eyes toward the geophagic point. In the latter case, the re-direction of attention would begin engagement time and the time while sitting near the geophagic location would be excluded. Similarly, geophagy was considered concluded if a chimpanzee moved away from the point or shifted their body/attention away from the point with the exception of visible soil in their hand or mouth, in which case they were still considered engaging in geophagy until they either left the recorded area or there were no mouth movements nor soil visible. If a chimpanzee's face could not be seen after moving away from the GP, this was always considered a disengagement. Since the chimpanzees always engaged in geophagy within

the cave in one of two locations that can be captured on one video camera, it was possible to catch nearly all instances of geophagy throughout the period of study. As with the cave engagement above, the time was totalled per chimpanzee with any gaps between geophagy excluded. For both cave and geophagy engagement, all the individuals' total time was then combined for each day to create a 'cumulative time', which was then controlled for using group size. All analyses in this chapter investigate the cumulative time spent in a condition. For example, if three chimpanzees that entered the cave simultaneously at 09:00 and left together at 09:15, this would be a cumulative cave time of 45 minutes, and if these three individuals each engaged in 2 minutes of geophagy this would be 6 minutes of cumulative geophagy time.

Environmental data

Temperature and rainfall data were collected using a weather station set up at the BPCP field site. This included a simple rain gauge that was placed in an open area within the researchers' camp that was unobstructed by buildings or foliage, and a thermohygrometer to record maximum, minimum, and current temperature as well as humidity was placed nearby. All weather data collection occurred daily at 18:00 (or as soon as possible thereafter), which represented the end to the current day when the chimpanzees were likely to have ended their daytime activity. While there is some evidence of chimpanzees engaging in other nocturnal activities at other field sites (Tagg *et al.*, 2018), the latest recorded geophagic activity ended by 18:33 and primate cave activity after 18:00 was exceptionally rare. Thus, this collection protocol allowed for data to be collected in standard 24-hour intervals that generally aligned with an end to possible cave activities.

Due to issues and local travel restrictions in place during the COVID-19 pandemic, it was not possible to record some days of weather data in 2020 which led to gaps within the data. To account

for this, moving and weighted averages were used during season calculations for Bugoma Forest (using a pentad approach; see Chapter 2, this thesis) and moving averages were again utilised within this chapter.

Behavioural data

The direct behavioural data used within this study was limited to observations on diet. These data were collected by following the chimpanzees, when possible, and recording all instances of feeding. Then, using these data, a 'dietary stress score' was calculated for each day using a combination of features such as nutritional quality, level of PSCs, and insoluble fibre for each plant part per plant species consumed per day (for more details see Chapter 2, this thesis). Since chimpanzees are ripe fruit specialists (Chapman *et al*., 1995; Tweheyo *et al*., 2004) any instance of feeding on ripe fruit was scored with a stress score of zero, but bark, which is much more difficult to digest and offers little direct nutrition (Lambert & Rothman, 2015), would be scored as the highest possible stress score of 5. The other scored items were mature leaves (stress score 4), resin (stress score 3), young leaves (stress score 2), unripe fruit (stress score 2), and flowers (stress score 1). During this study, there were no direct observations of hunting nor insectivory, so these dietary options were not included. These scores were applied per day and any day without direct feeding observations was excluded from analyses.

Though the main goal of this chapter is to test hypotheses regarding cave use and the protective features of geophagy, sex-age class was also assigned to each individual (please note: an individual may visit more than once, and if this individual was not recognised on re-entering the frame it would be assigned a new ID, therefore the use of 'visitor' typically overrepresents the true number of chimpanzees present) as a preliminary exploration of individual characteristics. This

included assigning a sex based on sexual characteristics as either male or female, and an age class of adult or juvenile; infants were excluded from this study except to indicate if a female was lactating. Individuals were classed as adults if they were of adult size with fully developed sexual characteristics, juveniles were those chimpanzees that were well below adult size, retain some infant visual characteristics (such as posterior white tufts of hair and lighter faces), and were generally still found travelling with their mother. A subadult class was also used for individuals that fell between adult and juvenile categories, however, there were very few individuals falling within this class and all that did were close to adult size, without juvenile colorization, and appeared to have at least some sexual development. Given the small number of individuals and large similarity to adults, these individuals were grouped with the adults for the purpose of this chapter. Furthermore, adult females were subdivided by reproductive status as lactating or in oestrous. Females were classed as lactating if they had an entirely dependent infant (requiring constant carrying by the mother) and in oestrous when there were visible signs of a sexual swelling indicating a female is receptive to reproductive advances.

Lastly, to help control for the instances of one individual accounting for more than one 'daily ID' which can happen if the individual leaves the screen and is not recognised upon re-entry, a 'maximum group size' was calculated to apply a conservative estimate of group size. This was determined by calculating two numbers: 1) the highest number of chimpanzees seen on screen at any given time, and 2) the number of unique sex-age-reproductive classes present. As an example, if there were 10 'visitors' recorded for one day but 1) a maximum of 3 chimpanzees on screen at one time (excluding infants) and 2) there were 4 adult males, 1 adult female, 1 lactating female, and 4 juveniles of unknown sex for a total of 4 unique classes (excluding the infant), then a group size of 4 would be used. This is the 'group size' variable used in all models.

4.2.2 Statistical analyses

Descriptive statistics

Descriptive statistics were used to explore the underlying structure of when and why chimpanzees might engage in geophagy or the cave. Every interaction with the cave by each chimpanzee was assigned a length of interaction, behaviour, and length of each behaviour for the cave analyses (which included geophagy). These behaviours within the cave included: 1) moving (anytime an individual was on their feet, including standing or moving in any direction), 2) resting (anytime an individual was sitting with no other activity apparent), 3) feeding (when there is clear mouth movement and/or something in the hand – within the confines of the cave this was found to only include soil), 4) a behaviour termed investigate (where a chimpanzee clearly walks towards and looks at a GP or an individual at a GP but does not initiate an interaction [do note that this is not considered an approach because there is not a movement of the body toward the GP to interact]), 5) grooming (includes auto grooming, all parties involved would be considered 'grooming' whether they were grooming or being groomed), and 6) an 'other' category which encompassed all other discernible activities (these were limited to social interactions, apart from one occasion of solitary tool creation/manipulation). Any behaviour that could not be categorised, usually because an individual was turned away from the camera or only partially visible, were categorised as 'unknown' behaviours. The time spent in each behaviour was then totalled per chimpanzee, per day, to create a general profile of how the chimpanzees spend their time within the cave. Since it was more difficult to identify individual chimpanzees between different days than throughout a single day, the unique ID assigned to each chimpanzee was reset every evening.

For geophagy, there were no behaviours recorded as with cave use above, but the durations and averages of time spent interacting with each geophagic area (GP1 and GP2) were explored instead.

Once these durations were calculated, the total and average times spent at each geophagic point per chimpanzee were examined. For both cave visits and geophagy engagement, the time of day of the visit was also extracted. Any individual with interactions beginning before 12:00 (12:00pm) was considered to visit in the morning and any chimpanzee beginning their interaction on or after 12:00 were considered afternoon visitors.

Modelling cave engagement

To investigate which factors influence cave use behaviour in the Bugoma chimpanzees, a generalised linear model with negative binomial distribution (glm.nb function from R package MASS [Venables & Ripley, 2002]) with an offset to account for group size was created. The variables used to predict duration of time spent inside the cave were water levels (as a categorical variable of 3 levels: 0 as no water/dry, 1 as some water/muddy, 2 as abundant/standing water; see Figures 3.1 *a-c* in Chapter 3 of this thesis for examples of dry and saturated soils), total rainfall, maximum temperature recorded, and geophagy (as an arcsin transformed proportion of time spent engaging in geophagy out of total time spent in cave). All variables were measured per day and any day for when one of the variables was unavailable (for instance, if there was a gap in data collection due to COVID restrictions or camera malfunction) was removed from the analyses. Each variable is used to represent a possible hypothesis behind cave use. Water levels represented access to water, rainfall represented protection/shelter from the elements, maximum temperature represented thermoregulation, and geophagy represented access to soil/nutrition.

A maximal model with all variables was created (formula: glm.nb(time spent inside the cave ~ water level + daily rainfall + maximum temperature + proportion of geophagy + offset(log(group size), link = log)) then each variable was dropped from the maximal model one at a time and

statistically compared to the maximal model using ANOVA to determine its significance within the model. Nonsignificant predictors were dropped from the final model (Engqvist, 2005) and effect size was calculated using the report package of R (calculated with standardised beta coefficients; Makowski *et al*., 2023).

Modelling geophagy engagement

Geophagy engagement was also investigated using generalised linear models with a negative binomial distribution and offset for group size. The hypotheses that were investigated in this model were both related to the protection hypotheses of geophagy: 1) since monthly rain as a percentage of days with rainfall has previously been shown as significantly associated with parasite infections (Gillespie *et al*., 2010), a moving average of previous 30-day rainfall was used as a proxy for disease stress, and 2) dietary stress was used to represent how diet (especially plant parts hard to digest or high in PSCs) influenced how long chimpanzees spent engaging in geophagy. Additionally, since the 30-day scale for rainfall was relatively arbitrary, two other rainfall scales were also explored. Firstly, a 5-day average was applied to represent diseases or parasites with shorter symptomatic onset and to coincide with the pentad approach previously used (see Chapter 2, this thesis), and a 15-day moving average was also used to serve as a middle option between these two.

As with cave use, a maximal model with all variables was created (formula: glm.nb(time spent engaging in geophagy ~ 5/15/30-day rainfall average + dietary stress score + offset(log(group size))) and each variable was dropped and then compared to the maximal model to ascertain significance as described above. Days for which there was not a value for dietary stress or rainfall were removed from analyses. For the maximal geophagy models, a different model was made using each of the 5- and 15-day average rainfall adjustments to compare to the chosen model of 30-day averages and AICs were compared to see how time scale influenced model fit. As above in

the cave use models, nonsignificant predictors were dropped from the final model and effect size was calculated using the report package of R (Makowski *et al*., 2023).

All analyses for cave and geophagy models were implemented using R Statistical language (version 4.4.1; R CoreTeam, 2024) and RStudio (version 2024.9.0.375 'Cranberry Hibiscus'; Posit Team, 2024) on Windows 11 x64 (build 26100) and the following R packages were utilised: dplyr (version 1.1.4; Wickham *et al*., 2023), forcats (version 1.0.0; Wickham, 2023b), ggplot2 (version 3.5.1; Wickham, 2016), lubridate (version1.9.3; Grolemund & Wickham, 2011), MASS (version 7.3.61; Venables & Ripley, 2002), purrr (version 1.0.2; Wickham & Henry, 2023), readr (version 2.1.5; Wickham *et al*., 2024), report (version 0.5.9; Makowski *et al*., 2023), sjPlot (version 2.8.16; Lüdecke, 2024), stringr (version 1.5.1; Wickham, 2023b), tibble (version 3.2.1; Müller & Wickham, 2023), tidyverse (version 2.0.0; Wickham *et al.*, 2019), and tidyr (version 1.3.1; Wickham *et al*., 2024).

4.3 Results

4.3.1 Descriptive statistics

Cave use

There were 809 visitors from the Bugoma chimpanzee communities that entered the cave on 130 days between 23 September 2019 and 31 December 2020, spending a cumulative 57.78 hours (57h47m00s [hours:minutes:seconds format]), or an average of 4 minutes 44 seconds per visitor with a median of 1 minute 08 seconds, within the cave. Of these 809 visitors, 245 were of an unknown sex and/or age class, leaving 564 visitors with positively identified sex-age classes.

Within the cave, geophagic behaviours constituted a vast majority of activity within the cave (43.95 hours, 76.1%, of cumulative time within the cave was spent engaging in geophagy; see Table 4.1 for overview) and all other behaviours only accounted for 14.25 hours (14h14m58s) combined, or 24.7% of total accumulated cave time. Of these non-geophagic behaviours, the most common was unknown (09:03:48; 15.7% of total time spent in cave), followed by resting (02h46m30s or 4.8% of total cave time), and moving (01h38m38s; or 2.9%) while all other behaviours constituted only 1.6% of total accumulated cave time. Very little time was spent grooming (00h00m50s totalled over all individuals) or engaging in social/other behaviours (00h00m27s total), and investigation (00h01m08s total) was done exclusively by juveniles who either approached the GPs to watch adults ingest soil or engaged with the GPs by looking at/inspecting the soil but did not otherwise interact. Of the 809 individual visitors observed within the cave, 626 engaged in geophagy (77.6%) and all feeding behaviour observed in the cave was exclusively on soil.

Condition	Rain (%)	Total time (hours)	Visit length (average)	Visit length (median)
Cave	89.3	57.78	4 min 44s	1 min 08s
Geophagy	89.0	43.95	4 min 12s	0 min 55s

Table 4.1 A summary of time spent engaging in the cave and geophagy including the percentage of interactions in the rain season, the total hours spent inside the cave and engaging in geophagy, and the average and median lengths of each visit inside the cave and engaging in geophagy. This particularly indicates a large discrepancy between median and average suggesting a few individuals are skewing the average time upward.

By number of chimpanzees that engage in a behaviour (discounting geophagy, which is explored further below and unknown behaviours), moving was the most common (Table 4.2) – though this is essentially necessary as chimpanzees must move from the entrance to the GPs to engage in geophagy. However, there were 189 of the 809 total individuals that visited where movement was detected but the chimpanzee had already started engaging in a different behaviour by the time the camera activated. There were also cases in which a chimpanzee may have been moving near the camera such that its full body could not be seen which could be coded as an 'unknown' behaviour instead of movement. If an individual was considered as moving, the average time they spent moving was around 9 seconds, while the median was lower at 5 seconds. Following moving behaviour, the next most common by total chimpanzees that engaged was resting, which was much lower with only 89 of the 809 total visitors showing resting behaviour. Interestingly, chimpanzees only rested in the cave during the rain season with no time observed resting on days during the dry seasons. Of those that did rest, the average time spent resting was 1 minute 51 seconds, however, the median was less than half that time at only 43 seconds indicating there were likely a small number of individuals that rested for particularly prolonged periods. Of the 89 instances of resting recorded, only 9 were over 5 minutes (range: 3 seconds to 12 minutes 8 seconds). Nearly half of these (n = 4) were individuals of unknown age or sex class, 2 were adult males, 1 was an adult female, 1 was a lactating adult female, and 1 was a juvenile.

Only 4 individuals engaged in 'other' actions, 3 of which were social and 1 of which appeared to be tool creation/manipulation and all occurred on 16 May 2020. All three social interactions were agonistic in nature and involved three individuals during the same time period. These events were

Cave behaviour	Visitors (*n*)	Total time (hh:mm:ss)	Duration (average)	Duration (median)
Geophagy	626	43:57:22	4 min 44s	1 min 08s
Moving	620	01:38:38	0 min 9s	0 min 5s
Resting	89	02:46:30	1 min 51s	0 min 43s
Social/tool	4	00:00:27	0 min 7s	0 min 7s
Investigate	3	00:01:08	0 min 23s	0 min 19s
Grooming	2	00:00:50	0 min 25s	0 min 25s

Table 4.2 Distribution of behaviours within the cave by number of individuals and cumulative time spent in each behaviour, as well as the average/median duration. The increased average duration of geophpagy and resting suggest that some individuals spent much more in these activities than others, while also confirming there are fewer of these outliers for moving, social and tool behaviour, investigating geophagic points, and grooming – though this could also be an artefact of low sample sizes.

verified using three camera angles (one internal camera at the entrance of the cave, the permanent camera fixated on the GPs, and the permanent external camera filming the cave entrance) and occurred as follows: 1) a female in oestrous (henceforth: AFo) is ingesting soil from GP2 (note: this is the small cavity within the wall of the cave and is only big enough for a chimpanzee to permit their arm and chimpanzees engaging at this point simultaneously must be very close in proximity) while an adult female (neither lactating nor in oestrous; henceforth AF) is at GP1, 2) AF leaves GP1 and approaches GP2 and AFo, 3) some interaction occurs (unclear as they are both facing away from the camera and partially off screen) and AFo appears to be displaced by AF, 4) AFo appears to stand near GP2 for a few seconds before leaving GP2 and approaching GP1 briefly before returning to rest beside GP2, 5) there is a short gap in camera coverage in which time AFo reapproaches GP2 with AF also nearby – though it is unclear if AF is

interacting with GP2 at this time, 6) approximately 4 minutes later, an adult male (henceforth: AM) enters the cave and approaches AFo, 7) a fight breaks out between AFo and AM, AFo vocalises and runs out of the cave, chased by AM, AF follows, 8) a larger conflict can be heard off camera on the external camera and no chimpanzees enter the cave after. This was the only recorded social interaction within the cave.

The case of tool manipulation involved an adult male bringing leaves into the cave and possibly attempting to make a leaf sponge, however, upon approach to GP1 the male drops the bundled leaves and they were not used to engage with any geophagic point. It was noted that at the time there was little to no water accumulation at GP1, thus it could be that the tool could not be used for its likely intended purpose of accessing water. Additionally, only 3 individuals (all juveniles) engaged in an investigative behaviour and 2 other individuals engaged in grooming, both of which were females self-grooming to remove clay from their hair (one female was lactating).

Excluding individuals with unknown sex/age class, there were 354 visitors entering the cave in the morning (62.8% of total visitors) compared to 210 in the afternoon (37.1%) indicating a trend toward morning visits. Of the morning visitors,124 were females (including 24 lactating females, 17 females in oestrous, and 5 subadult females), 124 were males (5 of which were subadults), and 106 were juveniles. Afternoon visitors included 72 females (including 9 lactating females, 13 females in oestrous, and 2 subadult females), 76 males, and 62 juveniles. Proportions of sex-age class were generally similar between morning and afternoon visits to the cave indicating little difference in tendencies of age or sex and time of day.

Geophagy and geophagic points

Of the 57.78 hours spent in the cave, 43.95 hours (43h57m22s) were spent engaging in geophagy (43h31m30s directly interacting with geophagic points and 00h25m52s consuming soil away from a point) in 865 interactions by 634 individuals. Of these 43.95 hours, 8.38 hours (8h23m19s) were conducted by 131 individuals of unknown sex/age class, which were subsequently removed from the following descriptive statistics, resulting in 35.15 hours (35h09m43s) over 721 interactions made by 503 chimpanzees.

Excluding individuals of unknown sex/age class, the average time spent feeding on soil (including at the GPs and away) was 4 minutes and 12 seconds, with a median of only 55 seconds. When removing time spent ingesting soils away from the GPs, there was only a marginal decrease in average time spent ingesting soil directly from a GP, which drops to 3 minutes and 45 seconds with a similar decrease in the median to 44 seconds, suggesting that most geophagy occurred directly at the GPs. There were many individuals (*n* = 133; 26.4%) who made multiple trips to the GPs to consume soil on the same day, and of these that made multiple trips the average number of trips was 2.6 (median of 2 trips). The average time spent feeding on soil per interaction, including soil taken from a GP and consumed elsewhere within the cave, was 3 minutes and 24 seconds for individuals that made only one visit to a GP (median 38 seconds) and 2 minutes 28 seconds for those that made multiple visits (median 43 seconds). Time spent ingesting soil ranged from 1 second (these instances often involved a chimpanzee quickly collecting soil in their hand or mouth before exiting the cave) to 1 hour and 24 minutes and 34 seconds and most individuals (*n* = 446 of 503 total; 88.7%) spent less than 10 minutes total engaging in geophagy. Of those spending more than 10 minutes on geophagy there were 24 females (42.1% of interactions >10 minutes; of which, 6 of these females were lactating females [25% of females], 0 were females in

oestrous, and 1 was a subadult female [4.2%]), 20 adult males (35.1% of >10 minutes), and 13 juveniles (22.8% of >10 minutes). This is generally similar to the overall proportions found where females constituted 36.2% (*n* = 182), males 38.2% (*n* = 192), and juveniles 25.7% (*n* = 129). However, there were apparent differences in the reproductive state of females, with higher proportions of lactating females represented at longer feeding times (25%) than feeding times for all female interactions (15.9%), and no oestrous females spending longer than 10 minutes ingesting soil even though 15.4% of all females engaging in geophagy were in oestrous.

Geophagy was directly confirmed with video footage at both GP1 and GP2, but the chimpanzees showed a clear preference for the larger geophagic area on the floor of the cave (GP1) over the smaller crevice within the earthen cave wall (GP2). Of the total geophagy time, only 55 minutes 35 seconds (2.6%) occurred at GP2, and only 73 individuals (of 503; 14.5%) chose to interact with GP2. Of those that interacted with GP2, adult females accounted for 33 individuals (19 adult females, 3 lactating females, 7 oestrous females, and 4 subadult females), adult males for 26 (with only one subadult male), and juveniles for 14. At GP2, the average interaction time was 40 seconds and the median 10 seconds, and all but four individuals visited during the rain season (one subadult female and one adult male visited in late July, 6 days apart; and two males visited in mid-late December 2020). Only 19 individuals visited only GP2 and did not approach GP1 (3.8% of all individuals; 26.0% of those that visited GP2), while 54 individuals (10.7% of all individuals; 74.0% of those that visited a GP) visited both GP1 and GP2. GP1 was visited by 484 individuals (with 33 [6.8%] visiting in the dry season and 451 [91.2%] in the rain season), with an average interaction time of 3 minutes 7 seconds (median of 44 seconds).

The Bugoma chimpanzees also showed a slight preference for time of day, with 309 individuals engaging in geophagy in the morning (61.3%) and 195 visiting in the afternoon (38.7%). There was only one individual, a female in oestrous, who visited in both the morning and afternoon on the same day and she is included twice within this total (making the new total for time of day 504 individuals instead of 503). Morning visitors included 111 females total (including 22 lactating females, 14 oestrous females, 5 subadult females), 119 males (of which 5 were subadults), and 78 juveniles, while the afternoon visitors included 71 females (including 7 lactating females, 14 oestrous females, 2 subadult females), 73 males, and 51 juveniles. Overall, proportions of females, males and juveniles engaging in geophagy remained largely constant between morning and afternoon, except for a marginal increase in oestrous females engaging in geophagy in the afternoon.

4.3.2 Model results

Modelling cave engagement

Generalised linear models were created to test variables associated with hypotheses behind cave use: 1) access to soil and/or nutritional benefits measured using geophagy as a proportion of time spent within the cave, 2) water access via accumulated water levels within the cave, 3) shelter from the elements as measured using the daily recorded rainfall, and 4) thermoregulation using the daily maximum temperature. These variables were then used to predict cumulative time spent within the cave using 86 instances where all variables in question were available for that day. Significance values indicate that proportion of geophagy (Pr(Chi) = 0.002; small effect size, Std. beta = 0.34, 95% CI [-0.61, 1.33]) and water levels (Pr(Chi) = <0.001; saturated/pooling water as well as partial water/muddy water both had large effect sizes – with Std. beta = 2.70, 95% CI [1.76, 3.61] and Std. beta = 1.92, 95% CI [0.62, 3.54] respectively) are the only significant predictors for

time spent within the cave of the variables investigated. Water levels show the strongest predictions for cumulative cave time, with roughly 700 seconds (or 00:11:40; range of 00:06:40 – 00:20:00) predicted if the cave is at least muddy (i.e. has some water available within the soil) and roughly 1400 seconds (or 00:23:00; range of 00:15:00 – 00:36:40) if there is available standing water (or soil is water saturated; Figure 4.1 *a*). While proportion of time spent engaged in geophagy was also a significant predictor, these predictions were much smaller than for water level, with the highest predicted value being roughly 275 seconds (or 00:04:35) when 100% of time spent in the cave is spent ingesting soil and predicted values take an exponential shape (see Figure 4.1 *b*). Therefore, model results suggest that as water levels and proportion of time spent ingesting soil increase, so does time spent in the cave, suggesting these factors, of those tested, are linked to drivers behind cave use.

Modelling geophagy within the cave

Investigations into the predictive value of monthly average rainfall and daily dietary stress levels indicated that previous rainfall was a significant predictor of time spent engaging in geophagy (medium effect size with Pr(Chi) = <0.001; Std. beta = 0.58, 95% CI [0.15, 1.09]) but dietary stress was not (Pr(Chi)= 0.178; medium effect size with Std. beta = -0.55, 95% CI [-1.25, 0.28]). When the 30-day moving average was compared to the alternative time scales, the 30-day moving average showed the best fit (AIC = 946.98) compared to the of 5-day (AIC = 962.98) and 15-day (AIC = 951.1) moving averages. This is also reflected in significance values derived from each model, where the

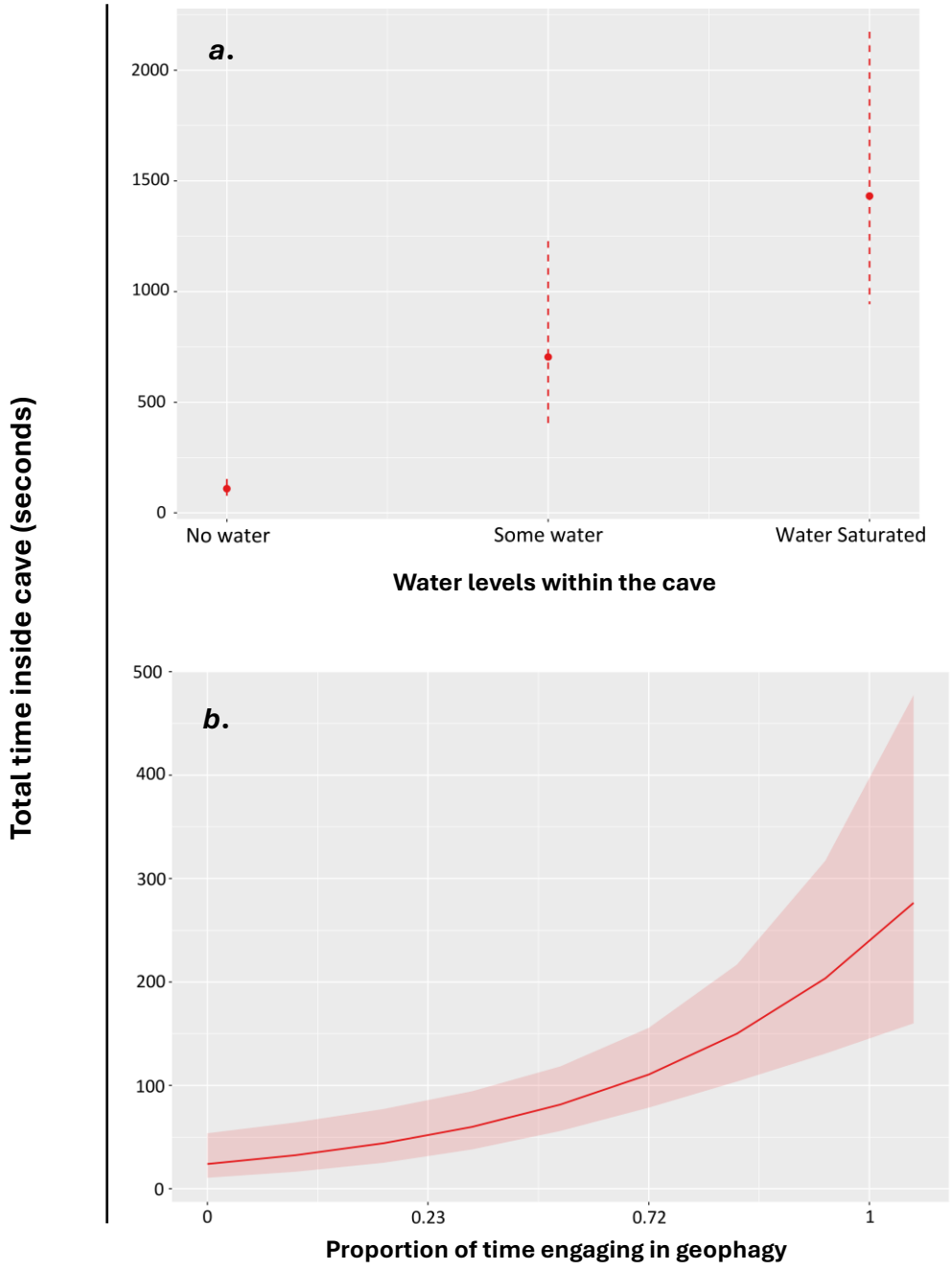


Figure 4.1 Predicted times spent in the cave, in seconds, derived from a generalised linear model for ***a.*** (left) water levels within the cave as categories ranging from no water/moisture present within the cave to some water (in the form of muddy with small accumulations of standing water), and water saturated soils with ample standing water, and ***b.*** (right) the proportion of time spent in the cave that is dedicated to geophagy. Water levels show the stronger effect with the presence of any water resulting in an additional 575 seconds (9 minutes 35 seconds) per day accumulated across all individuals. Proportion of geophagy also showed a significant effect on time spent within the cave but was limited to an increase (from no geophagy) of 150 seconds (2 minutes 30 seconds) if the chimpanzees spent at least 50%, or 250 seconds (4 minutes 10 seconds) at 100%, of their time engaging in geophagy.

5-day moving average results indicate that previous average rainfall does not predict time spent consuming soil (Pr(Chi) = 0.516; effect size very small (Std. beta = 0.03, 95% CI [-0.53, 0.66]), but the 15-day moving average is shown as significant (medium effect size, Std. beta = 0.61, 95% CI [0.17, 1.10]). This suggests that the longer averaged period of 30-days results in an overall better model for predicting geophagy within the cave.

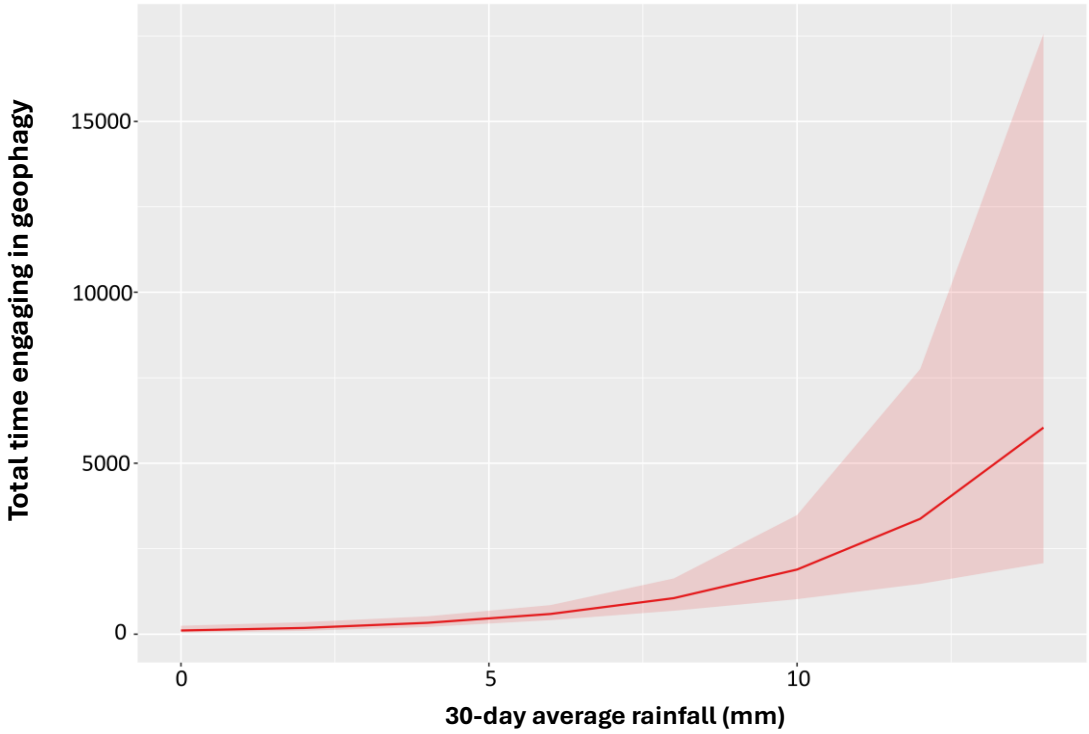


Figure 4.2 Predicted cumulative time engaging in geophagy, in seconds, derived from generalised linear models as predicted by a 30-day moving average daily rainfall measure. An increase in 30-day average rainfall results in an exponential increase in time ingesting soil, from nearly 0 seconds at 0mm average daily rain, to 500 seconds (8 minutes 20 seconds) at 5mm and then increasing further to roughly 2000 seconds (33 minutes 20 seconds) at 10mm daily average. Predictions beyond 10mm of rain have an exceptionally large predictive and should be considered cautiously.

4.4 Discussion

4.4.1 Cave use by the Bugoma chimpanzees

The Bugoma chimpanzees visited the cave regularly over the study period and spent a majority of their time within the cave ingesting soil from one particular location, GP1, instead of engaging in other behaviours. The GP1 location, which was the farthest point from any opening in the cave during this study, could represent a unique opportunity to access freshly unearthed soils from horizons not normally available to the chimpanzees; particularly those of the middle soil layers in horizon B, which has more minerals and clays than the topsoil in Horizon A, and are normally not easily accessible without excavation (Sing & Sing, 2010). It is unknown which soil horizons are represented within the cave, but previous analyses of the cave's soil content show a decrease of organic matter and accumulation of clay content when moving from the entrance of the cave to GP1 (Chapter 3, this thesis). This suggests that the soils at GP1 are possibly upper B horizon or lower A horizon and subjected to some degree of illuviation (the process of accumulation of small soil particles and compaction leading to formation of clays; Sing & Sing, 2010; Bockheim & Hartemink, 2013). However, this does not explain the preference for GP1 over GP2 as these soils were generally found to be similar in content (Chapter 3, this thesis).

While there is a clear preference for geophagy over other activities when examining both number of individuals and time spent engaging in a behaviour, it is possible that this may not be representative of the entire Bugoma population. Given the lack of individual identification available for any of the possible communities, and the inability to know if the same chimpanzees are visiting over different days or weeks, it could be that these interactions are driven by only a few members of the population. Indeed, the large discrepancies seen between average and median times (see Tables 4.1 and 4.2) spent in the cave and engaging in geophagy suggest the overall

interaction times could be driven by a small number of individuals that make multiple trips on the same day – and therefore possibly on other days – and these individuals also spent a large amount of time engaging in geophagy. There is also the possibility that the camera filming locations introduce bias given the limited camera coverage. The small number of available cameras meant that only two cameras were permanently recording the same locations over the entire study period. One permanent camera was filming the external main entrance to the cave, and the other recording the geophagic chamber within the cave, which was only one of three chambers. Both other chambers were routinely checked for evidence of chimpanzee activity, with no evidence of chimpanzees entering the leftmost chamber (a chamber with a very low ceiling filled with roots and stalactites and mostly resident to bats), and little evidence of activity beyond some footprints in the middle chamber which happens to contain a small access point to the cave (though this did grow over the study period due to water erosion). The middle chamber was filmed periodically within a camera rotation and showed no evidence of chimpanzee activity other than movement when chimpanzees were recorded within. However, given these gaps in coverage cannot exclude the possibility that chimpanzees use this chamber differently than they do the geophagic chamber. Therefore, future studies should aim to have permanent coverage of this middle chamber to describe which behaviours, if any, are routinely observed there and integrate these findings with those on the geophagic (rightmost) chamber.

The models investigating predictive variables for time spent within the cave suggest that access to soil materials and water are important for determining the drivers behind cave use in the Bugoma Forest chimpanzee communities. Interestingly, though both water levels and rainfall were included in the full cave use model, an increase in water level indicated an increase in cumulative cave time, whereas rainfall did not. This indicates that previous rain levels may impact the length

of visits to the cave by altering water access, but the cave is likely not used as a shelter from the elements (in this case rain). Additionally, the maximum temperature was also not associated with longer stays within the cave, concluding that thermoregulation, namely avoidance of heat stress, is not a suitable hypothesis for cave use in the Bugoma communities. The only other evidence of cave use in chimpanzees within the literature was found in a community of a different subspecies, western chimpanzees (*Pan troglodytes verus*), at the Fongoli field site in Senegal (Preutz, 2007). Studies of the Fongoli chimpanzees demonstrate that the main purpose of cave use in this community is thermoregulation, especially for lactating females which have higher metabolic and hydration stress (Pruetz *et al*., 2002; Boyer Ontl & Pruetz, 2020). However, this community occupies a mosaic-savanna habitat with a mixture of woodland, gallery forests, and open grassland (Pruetz, 2007), a much different habitat than the tropical forest dwelling Bugoma chimpanzees. Given the likely differences between environmental stresses in sun-exposed savanna dwelling chimpanzee communities compared to forest dwelling communities with ample shade availability, it is unsurprising that cave use may be influenced by different environmental factors. This is additionally supported by the preference for morning visits to the cave in the Bugoma community, which would be the coolest parts of the day and least likely to aid in coping with thermoregulatory stresses. However, since dry season visits were rarer in this study period, and thus have a much smaller sample size, future studies should endeavour to investigate time of day, and length of each visit, by season when more years of data are available to see if these patterns persist.

4.4.2 Geophagy engagement within the cave

As reported above, the average time engaging in geophagy for those that made only one visit was 3 minutes and 24 seconds (median 38 seconds), but this only decreased to 2 minutes 28 seconds

for those that made multiple visits (median 43 seconds). Given this is per interaction with a GP and the median number of interactions for those that made multiple visits was 2 (the average of 2.6 was likely driven upward by only a few individuals), those that did engage with the GPs multiple times a day only accumulated 1 additional minute of interaction time, on average. Therefore, multiple visits only led to a marginal increase in total time spent engaging in geophagy and there is overall little difference between those that make only one trip versus those that make many. Examining the sex and age class of the individuals that engaged in the longest bouts of geophagy (>10 minutes spent ingesting soil) shows no obvious differences in which classes consumed the most soil. However, the sharp increase of lactating females, and complete absence of females in oestrous, represented in the longest feeding times does indicate that reproductive status may play a role in soil consumption. Thus, future studies should aim to systematically analyse reproductive status and its influence on geophagy.

Models predicting time spent engaging in geophagy, and therefore possible factors influencing soil ingestion by the Bugoma chimpanzees, indicated that dietary stress did not impact geophagy but previous monthly rainfall did show strong predictive values. Each additional millimetre of rainfall averaged over the previous 30 days showed an exponential increase in time spent ingesting soil within the cave, indicating a strong relationship between rain and geophagy over the study period. Since there was an observed increase in model fit with the addition of longer rainfall periods (an increase in number of days included in the previous rainfall average from 5 to 15 to 30 days led to a subsequent drop in AIC), a post-hoc analysis investigated the addition of a 60-day rainfall model. This model showed a further decrease in AIC (AIC = 934.51), suggesting that the metric of rainfall could reflect that season is more important than month. Studies generally find that parasite and infectious disease burdens are higher in rain seasons compared to dry within chimpanzee

populations (Huffman *et al.*, 1997), so the Bugoma chimpanzees' geophagy engagement within the cave could relate to the protection hypothesis and alleviations of infections, but results within this study are inconclusive. Future studies should aim to collect accurate readings not only on water levels within the cave, but across the chimpanzees' home ranges to combine with data regarding health status/parasite load of the communities that visit the cave and engage in geophagy. This would allow the exploration of access to water across the habitat to be examined alongside water levels within the cave which could unravel the complex relationships between season, water availability, and the role of infectious diseases in geophagy.

4.5 Conclusions

The aim of this study was to provide a preliminary description of how chimpanzees in Bugoma forest interact with the cave as well as model contributing factors for time spent within the cave or engaging in geophagy therein. Taken together, both the cave use and geophagy models showed a significant influence of water for predicting how long individuals would engage. This could indicate that water levels and 30-average daily rainfall may be related, as we know that water is more likely to accumulate when soils are not especially dry – dry soil is hydrophobic and results in water run off instead of accumulation (Bayad *et al.*, 2020) – however, this would heavily depend on the morphological characteristics of how the cave slopes and where the water would flow. If the soils are particularly dry and the floor of the cave is convex then it is likely to flow outside of the cave, but if it is instead concave this could indicate that for a short period after rainfall there would be some water available as it has nowhere to run. When considering why the chimpanzees choose to engage in geophagy within the cave, it could be that the combination of soil and at least some water is desirable as it provides hydration and any benefits from the soil, especially since the chimpanzees do choose to consume soil in addition to simply drinking from accumulated

water elsewhere in the cave. However, an alternative hypothesis is that the water makes it easier to access the soil. For instance, when chimpanzees ingest soil during the dry season, they often had to pry soil from the hard and compacted earth in the cave. This dried soil can be difficult to chip away from the cave walls and floors and there were some videos that indicate that the amount of soil the chimpanzees could collect when the soil is in this state is minimal. Additionally, the water may add to palatability of the soil as the consistency in the dry season was often hard and/or powdery. This powder-like texture could increase thirst during periods when there is already very little water. The last possibility is that the chimpanzees simply enjoy the taste of the soil, especially when it is damp, or that the combination of water and soil may alleviate hunger when access to food is low (though the latter is less likely given that high dietary stress should also indicate the consumption of low-quality food). In order to understand how the water and soil might interact to encourage, or discourage, geophagy it will be important for future studies to find a way to disentangle these factors, for instance by finding a way to quantify soil amounts meaningfully without using time as a proxy, and by taking more detailed data on diet once the Bugoma chimpanzees are fully habituated.

Geophagy within the cave in Bugoma forest could also present a unique insight into the importance of culture, or behavioural traditions, in how and why these communities engage with the cave in the way they do. Elephants (*Loxodonta africana*) also exhibit routine, seasonal, geophagic behaviour and some studies have shown that the geophagic materials selected did not contain significantly more nutrients than control samples, instead suggesting that this behaviour is a result of cultural resource-use that no longer gives the elephants any nutritional benefit, but could have in the past (Bowell *et al*., 1996; Fishlock *et al*., 2016). Cultural traditions could also be

a possibility within the Bugoma chimpanzees that cannot be entirely discounted at this time and warrants further investigation.

Relevance for future chapters

The large increase of lactating females represented in the longest bouts of geophagy, those over 10 minutes, and complete lack of oestrous females warrants further investigation in future chapters. While this chapter makes a good case for external environmental factors (such as access to water and soil) as the primary influence for utilisation of the cave and its soil within Bugoma, these could still result from individual stresses associated with sex, age, or reproductive status resulting in increased nutrient demands. Future chapters should investigate if certain sex-age classes are more likely to enter the cave and engage in geophagy to further explore the role of individual increases in nutrient demands. If certain sex-age classes are more likely to visit the cave and/or consume soil then it is possible that the nutrient supplementation hypothesis may provide a possible explanation for this behaviour. However, if lactating females are found to enter the cave and engage in geophagy more often than others, as could be suggested by the increased length of geophagic bouts described above, this could also represent the increased hydration and thermoregulatory stresses associated with lactation. If no sex-age class engages with the cave more than others, then this would strengthen the hypothesis that water and soil access drive cave use, that health benefits from consuming soil may drive geophagy, or possibly that long-standing behavioural traditions allow cave use and geophagy to persist with the Bugoma population.

5. Behavioural and ecological factors affecting geophagy and cave use in chimpanzees at Bugoma Forest, Uganda

Abstract

Cave use and geophagy have both been linked to sex-age class specific stressors within primates. Cave use in chimpanzees has been hypothesised to provide thermoregulatory relief to lactating or pregnant females that are under higher heat stress, and studies suggest geophagy may play an important role in nutrient supplementation for those under higher nutrient demands such as growing juveniles and lactating/pregnant females. This chapter investigates the role of sex-age class and reproductive state on cave use and geophagy in the Bugoma Forest chimpanzees using a majority (more than 75%) group composition of three separate conditions: 1) the external condition which provides a baseline composition of individuals observed outside the cave, 2) the internal condition comprised of those that choose to enter the cave, and 3) the geophagic condition consisting of individuals that choose to enter the cave and engage in geophagy. The compositions of the internal and geophagic conditions were then statistically compared to the external compositions (as a null state) as well as modelled to garner further understanding of how sex-age class and reproductive status impact behaviour. Results showed chimpanzees in Bugoma visited the cave more often in the rain season, and that group size was a significant predictor in all conditions. Additionally, mixed-sex groups were more likely to enter the cave and engage in geophagy and there were some seasonal trends in group composition – though more data is needed to fully understand these. Contrary to most hypotheses which indicate females are generally more likely to engage in geophagy, majority-male groups spent significantly more time within the geophagic condition, indicating male presence as a possible driver of geophagy within the Bugoma chimpanzees.

5.1 Introduction

Geophagy, the intentional ingestion of soil or earth, has been described in many different animal taxa across the world (Sokol, 1971; Jones Hanson, 1985; Whittle & Everhart, 1993; Klaus *et al*., 1998; Krishnamani & Mahaney, 2000; Brightsmith & Muñoz-Najar, 2004; Young *et al*., 2011; Abrahams, 2012; Hasan, 2012; Mahandran *et al*., 2016). There are many suggestions as to why any particular animal might choose to engage in geophagy, including as a way to access nutrients, to aid in the mechanical digestion of food (especially in birds and reptiles; Gionfriddo & Best, 1999; Lee *et al*., 2014; Downs *et al*., 2019; Uriona & Farmer, 2019; Burroughs, 2020), to alleviate symptoms of gastrointestinal stress, and to bind harmful dietary toxins (Johns & Duquette, 1991; Krishnamani & Mahaney, 2000; Young *et al*., 2008; Limpitlaw, 2010; Pebsworth *et al*., 2019b). Many of the earliest observations on geophagy in wild animals described what have been termed 'saltlicks', where many different species congregate to lick and/or eat exposed soils, and the earliest hypotheses suggest this may have provided supplemental sodium (Jones & Hanson, 1985). Recent literature has endeavoured to understand the adaptive significance of geophagy further, and though there have been several studies on many species, the true drivers of geophagy are still relatively unknown.

In mammals, there are three main groups of hypotheses for geophagy: 1) the protection hypotheses, 2) the nutrient supplementation hypotheses, and 3) the 'nonadaptive' hypotheses. The protection hypotheses suggest geophagy as a means to protect against or treat certain ailments by binding toxic Plant Secondary Compounds (PSCs), creating a protective film in the intestine, or for alleviating or fighting off digestive infections (Young *et al*., 2011; Pebsworth *et al*., 2019b). Meanwhile, the supplementation hypotheses have posited for hundreds of years that animals engage in geophagy to gain access to nutrients otherwise lacking in their diets, and there

is indeed a significant amount of research to support this in most mammal species (Young *et* al., 2011; Griffiths *et al*., 2024a). Lastly, the 'nonadaptive' hypotheses include consuming soil to satiate hunger in times of famine, for their pleasant taste/textures (hedonistic sensations), or as part of behavioural traditions, however, this is a poorly named grouping as these reasons for geophagy may still provide adaptive benefits (see Chapter 1, this thesis). Amongst nonhuman primates, there is relatively equal support for both the protection and supplementation hypotheses within the literature – though it has also been recognised that these hypotheses are not necessarily mutually exclusive and reasons behind geophagy may be multifaceted (Pebsworth *et al*., 2019b).

In chimpanzees, support for nearly every possible hypothesis of geophagy has been described across their range which indicates that geophagy is likely a response to localised pressures and probably not a species-wide response (Pebsworth *et al*., 2019a). It is thus important to study the ecology and behaviour of the chimpanzee community in question when investigating drivers of geophagy in wild populations. The aim of this chapter will be to build upon the previous chapters to further test these hypotheses, in particular its use as a form of supplemental nutrition, as well as to investigate the observed cave use in the Bugoma chimpanzees, by empirically exploring whether group composition impacts these behaviours. To do so, an 'ID' will be allocated to each individual per day indicating their age (adult or immature) and sex class (male or female) to facilitate the assigning of a 'majority sex-age class' to compare group composition between conditions. The conditions investigated here will be the external condition (representing individuals who come within 5 meters of the cave), the internal condition (individuals that come within 5 meters of the cave and then also choose to enter), and the geophagic condition

(chimpanzees that come within 5 meters of the cave, choose to enter the cave and then also choose to engage in geophagy).

Previous studies have indicated that geophagy may be more common in female chimpanzees, especially if they are pregnant or lactating (Tweheyo *et al*., 2006; Wrangham *et al*., 2014 as cited in Pebsworth *et al*., 2019b; Pebsworth *et al*., 2019a), as these individuals may have higher nutrient demands. Additionally, studies on chimpanzee cave use in Senegal also found a relationship between cave use and lactation (Boyer-Ontl & Pruetz, 2020). Therefore, in addition to investigating sex-age class trends, this chapter will test reproductive status to determine if females that are lactating or in oestrous (i.e. may soon be pregnant) were predictors for longer bouts of geophagy or cave use when compared to the external group compositions.

5.2 Methods

5.2.1 Data Collection

Field site

All data were collected from chimpanzee (*Pan troglodytes schweinfurthii*) communities that visit and consume soil from an earthen cave in the Bugoma Central Forest Reserve in Western Uganda (1°17’40’’N 30°59’50’’E). Previous chapters have established a bimodal rainfall pattern typical of nearby field sites (Newton-Fisher, 1999; Reynolds, 2005) with the rain seasons punctuated by two dry seasons – a stronger one from December to February and a weaker one between June and August. Through the course of the Bugoma Primate Conservation Project’s (from here: ‘BPCP’; https://bugomaprimates.com/) efforts to habituate chimpanzee communities in the reserve, researchers discovered an earthen cave set into a hillside with evidence of geophagic behaviour. This geophagic behaviour was later visually confirmed using motion activated camera traps.

At the time of the study, only one community, the Mwera community, was semi-habituated to researcher presence and the process of assigning individual IDs was ongoing but in early phases. Therefore, individual animals within this study were not able to be accurately identified. Additionally, while chimpanzees could be located and followed on most days, they were only observed for short periods (a few hours or less) at a time and were not comfortable enough with researcher presence to allow for direct observation at the cave. Therefore, observational data were limited to what could be collected from 10-20m distance and without confirmed individual identifications. With these constraints, it was not possible to collect direct observational data on cave use and geophagic behaviour given the confined nature of the cave. There was also evidence (recorded by remote cameras) that the chimpanzees would leave the cave if humans approached the larger area where it was located, thus direct observation was not attempted to avoid unnecessary stress to the animals.

Remote monitoring of cave and geophagic area

To reduce possible stress on the study population, motion activated camera traps (Bushnell 119777, Bushnell HD 119876C) were used to collect data on cave use, geophagy, and group composition outside the cave. To do this, at least two cameras monitored the cave at all times, barring technology failures which occurred due to battery issues or humidity damage. One camera was placed so that it monitored the main entrance to the cave. This camera also showed the only other entrance large enough for adult chimpanzees which is near the main entrance. The other camera monitored the geophagic sites in the back of the cave and allowed a larger view of the area surrounding these geophagic locations. When available, other cameras were placed in and around the cave to monitor activity in other areas, but these cameras rarely showed chimpanzee

activity. Batteries were changed at least once every 14 days to avoid loss of footage to battery depletion. All camera data from this study were collected starting September 2019 until the end of December 2020.

5.2.2 Data processing

Video data

Once the video data were collected from the cameras, it was screened for any animal presence, animal type (bat, feline, rodent, etc.) was recorded with detail on species when available, and all videos with chimpanzees present were extracted and subsequently analysed. The confirmed chimpanzee videos were watched in their entirety with the viewer noting the time the video started and ended, the sex-age class and activity of every chimpanzee on screen, the duration they were on screen, and assigning a unique daily ID to each individual which did not carry over from day to day. While it was generally possible to track the same chimpanzee through most of the internal and external videos, without reliable identification it was not always possible to track individuals if they went off screen. This was especially true for young chimpanzees which have fewer identifying characteristics. Therefore, if a chimpanzee left the recording area for more than 5 seconds, they were considered to have left the location and would be assigned a new ID if they happened to return and were not recognised. Data were collected on an individual level and included: age, sex, reproductive status (lactating or in oestrous), duration of time spent on screen, and duration of time spent consuming soil (when applicable). The viewer also noted the maximum number of chimpanzees on screen per clip.

Bouts

Chimpanzee visits to the cave and surrounding area were often observed to occur once a day, however there were also several occasions where groups were observed visiting two or more times a day with many hours in between visits. Chimpanzee communities do not normally seek out contact with their neighbours, indeed these encounters are often violent and can be fatal. Therefore, it is unlikely that communities overlap at the cave, but still possible multiple communities could visit on the same day. Additionally, without the ability to recognise and assign a unique identifier to each individual, it was not possible to analyse the data by specifying the amount of time each individual spent in or around the cave in a meaningful way. To address this, data were analysed by group 'bouts' to account for the behaviour of the group that was visiting. A bout was defined as the time from the moment the first chimpanzee appeared on screen to when the last chimpanzee left the screen. Since there could be multiple visits on the same day, a group was considered to have ended their bout if a period of over 30 minutes had lapsed since chimpanzees were observed on any camera near the cave. The 30-minute period was originally chosen by examining the pilot data from October 2019 and observing most gaps were either less than 30 minutes or well over an hour, thus 30 minutes was chosen as it would likely be enough time for a group and/or community to vacate the area and a new group to take over.

Group IDs and reproductive status

As mentioned, identification of specific individuals from the Mwera community was limited at the time of the study. This was further complicated by the black-and-white only video footage from the low light setting of the cameras within the cave, making identification even more difficult. As such, it is not only unknown which communities, apart from Mwera, were making use of this cave but it was also not possible to reliably distinguish which groups were from Mwera. To solve the lack

of individual IDs, and attempt to address issues with repeated measures, a system was devised to assign Group IDs by the majority sex-age class present per bout. To do this, each chimpanzee was assigned a sex-age class when they were first seen on screen. Individuals' ages were classified as adult, immature, or unknown, and their sex could be male, female, or unknown. The immature class included all individuals that were not fully mature (and typically consisted of subadults or juveniles), with the exception of infants. Infants, defined here as young chimpanzees that are still carried by their mothers and not yet weaned (weaning typically occurs between 3-5 years; Pusey, 1983; Sugiyama, 2004; Londsdorf *et al*., 2021), were excluded from the analysis because they do not voluntarily interact with the cave, but are instead carried in by their mother, and were not observed to engage in any geophagy throughout the study. This resulted in 7 sex-age classes: 1) adult male, 2) adult female, 3) unknown adult, 4) immature male, 5) immature female, 6) unknown immature, and 7) unknown age and sex.

Once each chimpanzee was assigned one of the above IDs, the number for each sex-age class could be tallied and a majority sex/age determined. To determine this, the percentage of each class was calculated and any group with over 75% of one classed received a classification of that sex-age class. For instance, if there were 76% adult males, 12% adult females, 8% immature, and 4% unknown this would be given a group ID of 'majority male group'. If the group composition did not meet this threshold, it was considered a mixed group and if it contained >25% unknown individuals it was considered an unknown group since it could not meet the 75% threshold. The 75% majority threshold was chosen because it provided a more conservative figure than a simple majority (>50% threshold), while also allowing some leeway for unknown individuals that wouldn't be possible for all-male or all-female Group IDs (i.e. if 100% is required to be anything but a mixed group then the vast majority of bouts would fall within this or the unknown category since it is

common to have at least one unknown individual) This system created 5 possible groups for each bout: 1) majority-male group, 2) majority-female group, 3) mixed group, 4) majority-immature group, or 5) unknown group. If the Group ID was classed as unknown (>25% individuals with unknown sex-age class) it was not used in further statistical analyses.

In addition to Group IDs, each bout was also assessed for the reproductive status of any females present, namely, if females were in oestrous or lactating. Oestrous status was determined by presence or absence of a sexual swelling and represents the reproductive stage where females are receptive to mating and therefore just before or possibly in the earliest stages of pregnancy if conception occurs (Thompson, 2013). It was also important to consider oestrous status because it could affect presence/proportion of males as they seek reproductive opportunities (Matsumoto-Oda, 1999; Hashimoto *et al*., 2001). A female was considered lactating if she was observed carrying a dependent infant, but not if her offspring was classed as a juvenile (immature). This was for two main reasons: 1) if there are two or more adult females present it can be difficult to determine which female may, or may not, be the mother to any accompanying juveniles from camera footage alone (since juveniles were classed according to their independence from their mother, i.e. not carried and moving independently), and 2) even though weaning can take up to 5 years in chimpanzees, the highest energetic costs to the mother are observed in the first two years of life (Thompson *et al*., 2012), and in humans micronutrient supplementation is recommended while lactating (breastfeeding is typically required for 12+ months; Section on Breastfeeding, Eidelman *et al*., 2012), to meet increased nutrient demands (Kominiarek & Rajan, 2016; Marshall *et al*., 2022). Both oestrous and lactation were classed as binary presence/absence variables within this study to avoid overrepresentation of singular individuals as it was difficult to know if the

same female was visiting multiple times per bout, or if there were indeed multiple females in oestrous/lactating.

External, Internal, and Geophagic conditions

To investigate hypotheses for cave use and geophagy, three conditions were defined: 1) External, 2) Internal, and 3) Geophagy. External bouts included data from the external cameras that view the entrance of the cave. Since this camera would capture most chimpanzees within roughly 5 meters of the entrance, this condition includes those chimpanzees that visit the site of the cave and come close enough to enter the cave but may or may not choose to do so. Any time a chimpanzee appears on screen, their unique ID (including sex-age class) is generated and they are followed until they leave the screen. In this way, chimpanzees were counted and group compositions were determined using the majority rules outlined above. An external bout was defined as the exact time the first chimpanzee appears on screen to the time the last chimpanzee exits the recording area – as long as this chimpanzee does not enter the cave. If a chimpanzee entered the cave in the last video of the bout, the end time of the internal bout was taken as the end of the external bout. This could happen if the chimpanzees exited the cave so quickly that the camera triggered too late to capture the chimpanzee. Similar to external bouts, internal bout lengths were defined as the time the first chimpanzee is seen on a camera within the cave, to the time when the last chimpanzee exits, or leaves the recording area. This included all chimpanzees observed in the cave during a bout, regardless of their behaviour, and included chimpanzees engaging in geophagy.

Since geophagic bouts were included within internal bouts (as geophagy is a possible hypothesis for cave use in animals), these bouts were determined using a modified system. Instead,

geophagic bouts were taken as the amount of time when there was at least one chimpanzee engaging in geophagy on screen. If a chimpanzee disengaged and reengaged with the geophagic areas, the time in between these interactions was removed from the total bout length. For instance, consider a chimpanzee enters the cave (becomes visible on camera footage, thus starting an internal bout) at 08:00:00 (written as hour:minute:second) and approaches the geophagic point to consume soil at 08:00:30, then leaves the point at 08:01:00 but stays on camera and continues eating soil they have taken away from the point (this includes soil in hand and mouth movements observed) until 08:01:30, before reapproaching the point at 08:02:00 and ultimately leaving the point at 08:02:30 and exiting the screen at 08:03:00. This would create an internal bout lasting from 08:00:00 to 08:03:00 (3 minutes) but a geophagic bout lasting 1 minute and 30 seconds (from 08:00:30 to 08:01:30 [1 minute] and another from 08:02:00 to 08:02:30 [30 seconds]). However, if this bout included two chimpanzees, with one engaging as outlined above but the new chimpanzee approaching the geophagic point at the same time (08:00:30), leaving the geophagic point at 08:02:30, and exiting at 08:03:00 with the other chimpanzee, then the internal bout remains the same (3 minutes) but the geophagic bout becomes 2 minutes because at least one chimpanzee was engaging in geophagy between 08:00:30 and 08:02:30. This keeps the focus of analyses at a group level, instead of an individual level, to measure how behaviour changes according to group composition and not individual tendencies.

Using this system, the external bout condition acts as a 'null composition' that represents the general composition of groups occupying the area of the cave and allows a baseline composition to which the internal and geophagic bouts can be compared. If the composition is the same between the external and the internal/geophagic conditions, then it is unlikely that sex-age class influences whether chimpanzees choose to enter and stay in the cave or to consume soil, but if

there are differences between different group compositions then this could indicate that certain sex-age classes are more likely to engage in one or more of these behaviours.

In addition to the behavioural conditions of external, internal, and geophagy, each bout was also assigned a seasonal category of rain or dry based on the date of the bout. As exact seasons had been previously calculated for this study period (Chapter 2, this thesis), it was possible to use precise dates for seasonal determination. If a bout fell on a date from 23 September 2019 to 26 December 2019, 15 February 2020 to 14 June 2020, or 4 August 2020 to 11 December 2020 it was classified as belonging to the rain season, while any other bouts were classified as dry season (these occurred between 27 December 2019 and 14 February 2020,15 June 2020 to 3 August 2020, or 12 December 2020 to 31December).

5.2.3. Data Analyses

Descriptive statistics such as mean, median, and general distributions were calculated by bout condition (external, internal, and geophagic) for bout length, group size, reproductive status, and season to create a picture of each condition. Afterward, two separate sets of statistical analyses were conducted to investigate if cave use and/or geophagy vary depending on group composition. The first set of analyses aimed to directly compare parameters in external bouts (null state) to internal bouts (cave use in general) or geophagic bouts (geophagy), while the second set modelled parameters that could affect how long each group spends interacting with one condition.

For the first group of analyses directly comparing conditions, the external, internal, and geophagic bouts were matched to by date and time so that each covered the same period of bout time. This was done by comparing the recorded bout times and comparing between conditions, so if there

was an external bout from 08:00:00 to 10:00:00, an internal bout from 08:30:00 to 09:30:00, and a geophagic bout from 08:40:00 to 09:20:00 on one day then these were grouped under the same overall bout period and group compositions between these were considered comparable. Then each of these bout conditions (external, internal, or geophagic) were assigned their own group composition according to the procedure outlined above. Bouts to be included for these analyses were then determined by removing any combined bouts where the external condition was found to be an 'unknown' group composition. Some visuals were then created to show the flow of group compositions through conditions before this process of eliminating 'unknown' group bouts was then continued for the internal/geophagic conditions. The interest of these analyses was to compare external to internal/geophagic bouts but not to each other, as such the trimming of unknown bouts was only done in pairs of external versus internal, and external versus geophagic. So, if a specific bout was found to have a majority male composition external condition, an unknown composition for the internal condition, and a mixed group composition for the geophagic composition, then this bout would be included to investigate geophagic hypotheses (external male majority group compared to geophagic mixed group) but would not be included in the cave (internal) conditions. Additionally, any dates/bouts where a camera malfunctioned and resulted in no data for any of the three conditions were excluded.

After these bouts were determined, each condition comparison was conducted using a Fisher's exact test when possible or a chi-squared test if not possible. Chi-squared analyses were used to compare age and sex categories separately by condition. A chi-square was used as the possible compositions had three categories (male, female, and mixed for available sex classes, and adult, immature, or mixed for age classes). In this way the external group composition was compared to the internal or geophagic composition. Season and reproductive status were also examined, but

a Fisher's exact test was used in place of chi-squared tests since these variables resulted in 2x2 tables. A visit (bout) was classed as either occurring in the dry or rain season and belonging to either the external or internal condition (or geophagic condition for geophagy analyses), and the reproductive statuses of 'lactating' or 'in oestrous' were analysed separately and as simply present or absent for each bout. Effect sizes were calculated using an adjusted Cramer's V as calculated using the report package in R (version 0.5.9; Makowski *et al.*, 2023).

All above analyses treated each bout as a singular occurrence regardless of length, such that a bout of 1 minute would be treated equally to a bout of 10 minutes, while further analyses described below investigate factors that influence how long the chimpanzees spend in any condition. To do this, linear models were created for each of the external, internal and geophagic conditions, and residuals were plotted against the fitted values to confirm validity. Bout length was used as described previously, that is external/internal bouts were defined as the period from the appearance of the first chimpanzee to the disappearance of the last, and geophagic bouts being altered from these to represent the time when at least one chimpanzee was engaging in geophagy. In all cases, three maximal models were produced that predicted time spent in the specified condition (external, internal, or geophagy) using 1) group ID (using method described above with 5 possible classes [majority male, majority female, mixed sex, majority immature, and unknown], bouts with unknown ID were excluded), 2) group size (as the total number of chimpanzees observed – this could possibly include repeated individuals), 3) season (as a category of either rain season or dry season), 4) lactating females present, and 5) oestrous females present (both lactation and oestrous were binary presence/absence variables). To calculate significance for each variable, the maximal model (formula: log(Bout Length) ~ Group Composition * Season * Group Size + Lactating females + Oestrous females; interactions were

included as season is likely to influence both group composition and size and group size would also likely influence composition [Doran, 1997]) was compared to the model without that variable to understand which variables significantly impact the time spent in each condition. The nonsignificant predictors were then dropped to investigate the influence of the significant predictors (according to Engqvist, 2005), creating the final models. This procedure was used for all three conditions and effect sizes were calculated using Cohen's (1988) recommendations and the report package in R.

All data visualisations and statistical analyses were conducted using R Statistical language (version 4.4.1; R Core Team, 2024) on Windows 11 x64 (build 22631) and the following packages: dplyr (version 1.1.4; Wickham *et al*., 2023), ggplot2 (version 3.5.1; Wickham, 2016), ggsankey (version 0.0.99999; Sjoberg, 2024), report (version 0.5.9; Makowski *et al*., 2023), and sjPlot (version 2.8.16; Lüdecke, 2024).

5.3 Results

5.3.1 Descriptive results

In every bout condition, there were more observed bouts during the rain season than the dry, with the internal and geophagy conditions showing even higher proportions in the rain seasons (external: 97 of 126 bouts [77%], internal: 128 of 147 bouts [87.1%], and geophagy: 141 of 161 bouts [87.6%]). Group size between composition appeared to be relatively constant, with a mean between 2.6-2.8 chimpanzees per bout, and a median of 2 individuals, in all three conditions. Additionally, all conditions showed similar mean and median bout lengths, with the exception of the mean geophagic bout length which was noticeably shorter (8 minutes and 46 seconds compared to an external mean of 12 minutes 29 seconds, and an internal of 13 minutes and 9

seconds; medians were external: 1 minute and 10 seconds; internal: 1 minute and 23 seconds; geophagy: 1 minute and 9 seconds). Similarly, there are slight differences between conditions with oestrous females and lactating females. Around 17.5% of external bouts having at least one lactating female, but this drops to 15.0% for the internal and 14.2% for the geophagic conditions, inversely 11.9% of external bouts had at least one oestrous female and the internal and geophagic bouts increased to 15% and 15.5%, respectively. However, these numbers are similar and likely to be within an expected range of variation. Altogether, most differences in mean and percentage of bouts are small and suggest there is little difference between these conditions.

Since these conditions represent a flow of individuals from one to another (from external to internal to geophagic), this was visualised using Sankey diagrams to get an initial understanding of how group composition might progress (Figures 5.1 *a*-*c*; Figures 5.2 *a*-*c*; Figures 5.3 *a*-*c*; Figures 5.4 *a*-*c*). For instance, in order for an individual to engage with the geophagic area (the 'geophagy condition') they must enter the cave (internal condition), and to enter the cave they must be within roughly 5 meters of the entrance (external condition). Therefore, if the external group composition could be considered the baseline composition (as this represents all individuals with immediate access to the cave), it could be predicted that the internal and geographic conditions would be a subset of the external composition with the null expectation that group composition does not change between these conditions.

For the majority sex class for each bout (Figure 5.1 *a*) the most common categorisation for the external condition was an unknown majority sex (n = 61), followed by majority female groups which occur at similar levels (n = 53), while majority male and mixed sex groups only account for 29 external bouts total (majority male n = 20; mixed sex n = 9). However, when the seasons are plotted separately, the most frequent external group composition during the rain season was majority

female (n = 48), closely followed by unknown majority (n = 40) , then male majority (n = 13) and mixed sex (n = 7; see Figure 5.1 *b*), while the most frequent group in the dry seasons were unknown majority (n = 18), followed by male majority (n = 7), female majority (n = 5), and only 2 external mixed sex bouts. In particular, this seems to indicate that a large portion of majority male visits happen in the dry season while majority female groups seem to visit less often in the dry season as a proportion of total bouts.

In the internal bouts for all seasons (Figure 5.1 *a*), there appear to be similar trends to the external group composition with majority female being the most common (n = 33), followed by unknown majority (n = 28), male majority (n = 19), and mixed sex (n = 10). However, the new most common scenario becomes 'none' (or 'no group'; n = 53), resulting in 36% of bouts where the chimpanzees do visit the area of the cave but choose not to enter (thus do not enter the internal condition resulting in 'none'). This tendency does not, however, continue when viewing seasonal trends. In fact, when viewing the rain and dry seasons separately, it becomes apparent that the dry season accounts for nearly as many (n = 20) instances of no group entering as the rain season (n = 32), despite the dry season making up only 22% of total bouts (internal dry season bouts n = 32 of total n = 140). The other noticeable difference in sex class between seasons is the much lower proportion of majority female groups entering the cave in the dry seasons (n = 2; 17% of dry season bouts excluding 'no group') compared to the rain seasons (n = 30; 39% of rain bouts excluding 'no group'). Though it is important to note that there are large discrepancies in sample size between these seasons, and the low sample size of dry season bouts might not create a representative picture.

Lastly, there are few, if any, differences between the internal and geophagic conditions, with the only noteworthy observation being that if a majority female group enters the cave during the dry season then they also always engage in geophagy. Similarly, in any season, it is rare for a group to enter the cave but not engage in geophagy. However, the sample size caveat described above also applies to these geophagic bouts.

When examining age class (Figures 5.2 *a*-*c*), there are similar overall seasonal trends to those found in sex class, as expected given these data come from nearly identical bouts. In all conditions, majority immature groups are rare, which is also unsurprising given that juvenile chimpanzees, which make up most of the classified immature individuals in this study, tend to spend most of their time (possibly up to 88-100% depending on age and sex; Pusey, 1990) with their mothers. In general, there are higher proportions of majority adult groups in all categories, but it is hard to draw conclusions due to missing demographic information on the underlying age structure of the populations in Bugoma forest. However, the overall number of majority immature IDs does increase through each condition from 2 immature groups outside the cave, to 8 immature groups inside the cave, to 9 immature groups engaging in geophagy, and these extra immature groups derive roughly equally from adult and mixed age external groups (n = 4 and n = 3, respectively).

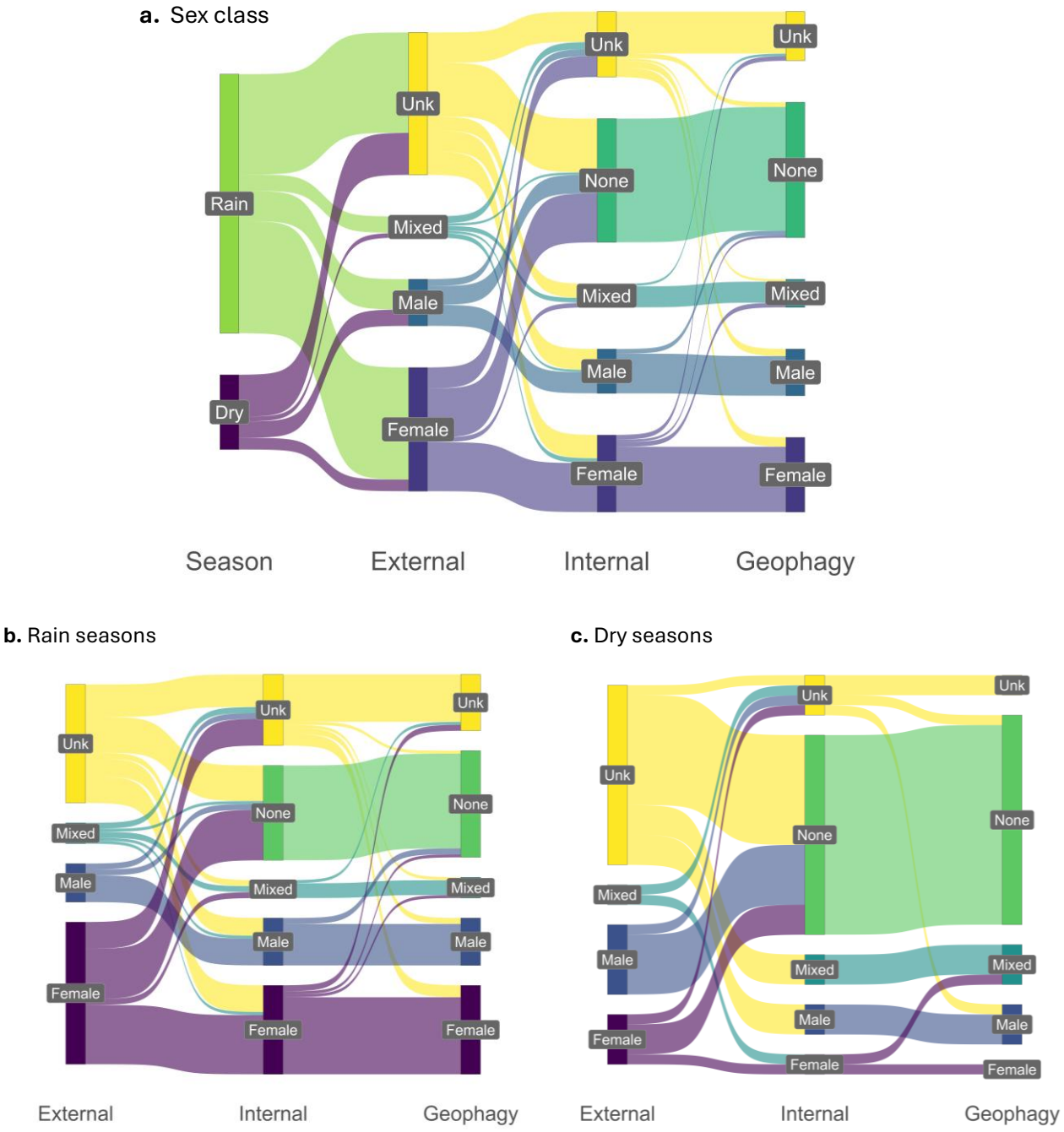


Figure 5.1 Sankey diagrams representing changes in majority sex (defined as: a male majority if >75% males, a female majority if >75% female individuals, a mixed group if there is no sex majority, unknown if >25% of individuals are of unknown sex, and none indicates cases when there were chimpanzees captured on external cameras that do not enter the cave or they enter the cave but do not engage in geophagy) flowing between each External, Internal, and Geophagy nested conditions where ***a.*** (top) shows the breakdown of majority sex starting with season to show the general groups proportions in the rain versus dry, ***b.*** (bottom left) shows the only bouts from the rain season, and ***c.*** (bottom right) shows only bouts from the dry season. These emphasise the change in proportion of majority male and female groups between the rain (***b***) and dry (***c***) seasons – especially for the external bouts – as well as show that there was a higher proportion of 'none' cases in the dry season suggesting they may be less likely to enter the cave and/or engage in geophagy during these periods. However, this could be a result of small sample size (rain season *n* = 108, dry season *n* = 32).

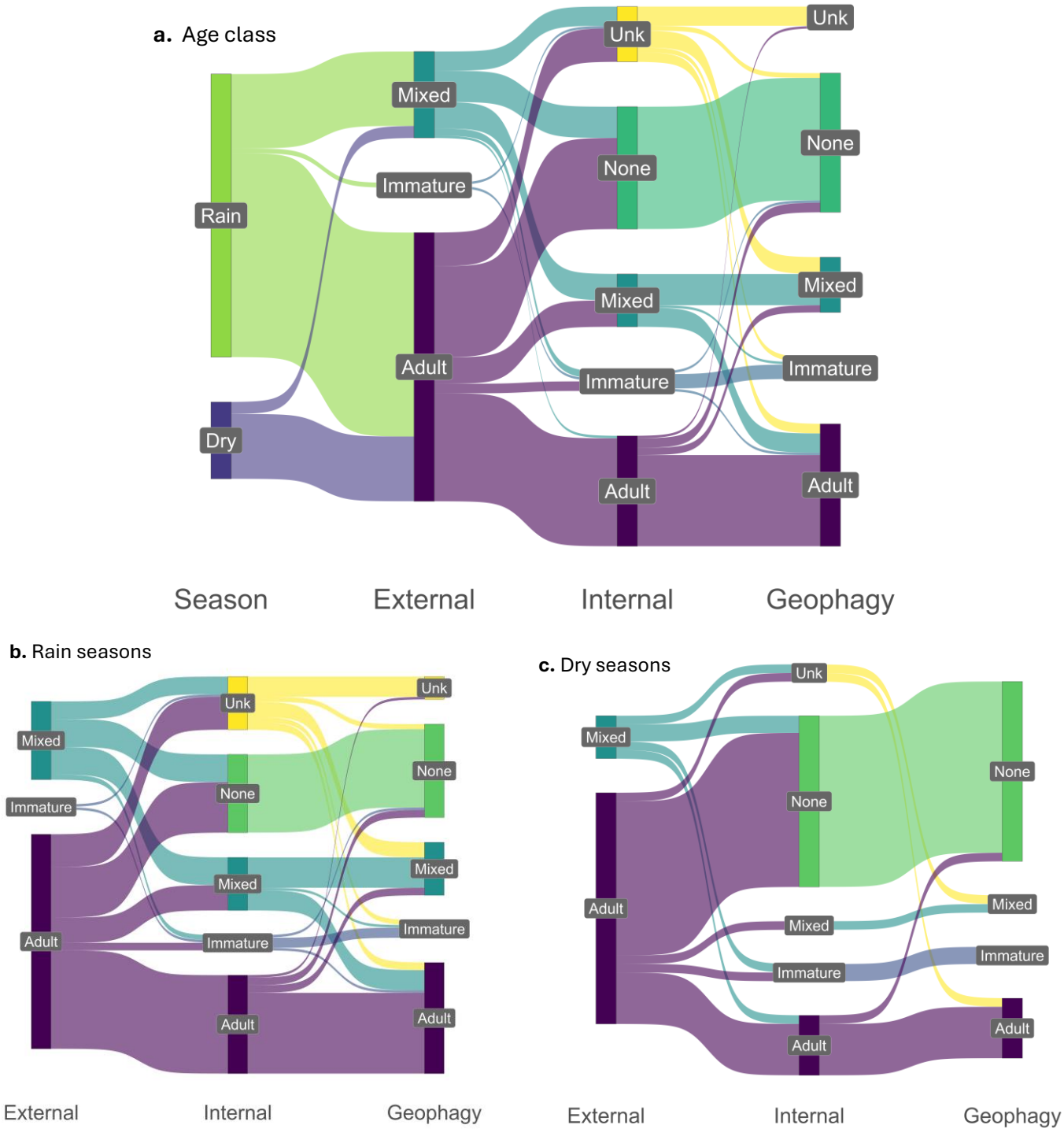


Figure 5.2 Diagrams of majority age groups (defined similarly to sex classes: >75% adult/immature individuals, mixed when no age majority, and unknown if the age of >25% of individuals is unknown) flowing between each External, Internal, and Geophagy conditions. ***a.*** (top) shows the age majority for each bout beginning in the rain or dry season to indicates the proportion of bouts in the rain and dry seasons, ***b.*** (bottom left) shows the only those bouts occurring in the rain season, and ***c.*** (bottom right) shows bouts from the dry season. Of particular note is the increased proportion of mixed age groups seen in the rain season (***b***) compared to the dry (***c***). Similarly to the majority sex class (Fig. 5.1) there also seem to be more cases in the dry season when chimpanzees choose not to enter the cave or consume soil. This could be an effect of sample size (rain $n = 118$; dry $n = 32$).

When considering reproductive status, there seems to be little difference between female groups with at least one lactating female and those without lactating females (Figure 5.3 *a*) with both showing similar proportions of opting against entering the cave (56% of groups with nonlactating females [55/98 bouts] and 58% of groups with at least one lactating female [15/26 bouts]). The same was true for oestrous females as well (Figure 5.4 *a*) with 25 of the 61 groups (41%) without oestrous females and 10 of 28 groups (36%) with oestrous females choosing not to enter the cave. All groups with at least one lactating female that entered the cave also engaged in geophagy (see Figure 5.3 *a*), but 22% of these groups switch from a lactating internal group ID to a nonlactating geophagic group (2/9 bouts) indicating there are cases when lactating females enter the cave and do not go on to engage in geophagy. There were no substantial differences between the rain and dry seasons when examining lactation. Conversely, when considering groups with females in oestrous, there are clear seasonal trends apparent in proportions (Figure 5.4 *b*&*c*) but these are specific to the external condition alone. In the rain season, 24% of external bouts had oestrous females present (18 of 75) but this rises to 71% of dry season bouts (10 of 14). This difference is not observed in the internal or geophagic conditions where the dry season bouts with oestrous females drops to 29% (4 of 14). However, if instances where a group chooses not to enter are discarded (i.e. 'none') then 4 of the 5 observed bouts inside the cave and 3 of 4 geophagic bouts included at least one female in oestrous. These trends were not observed in the rain season which stayed relatively constant.

Overall, descriptive results suggest that there may be some differences in sex class and reproductive status between the baseline group composition (external condition) and the cave use and geophagy conditions, while age class was generally inconclusive.

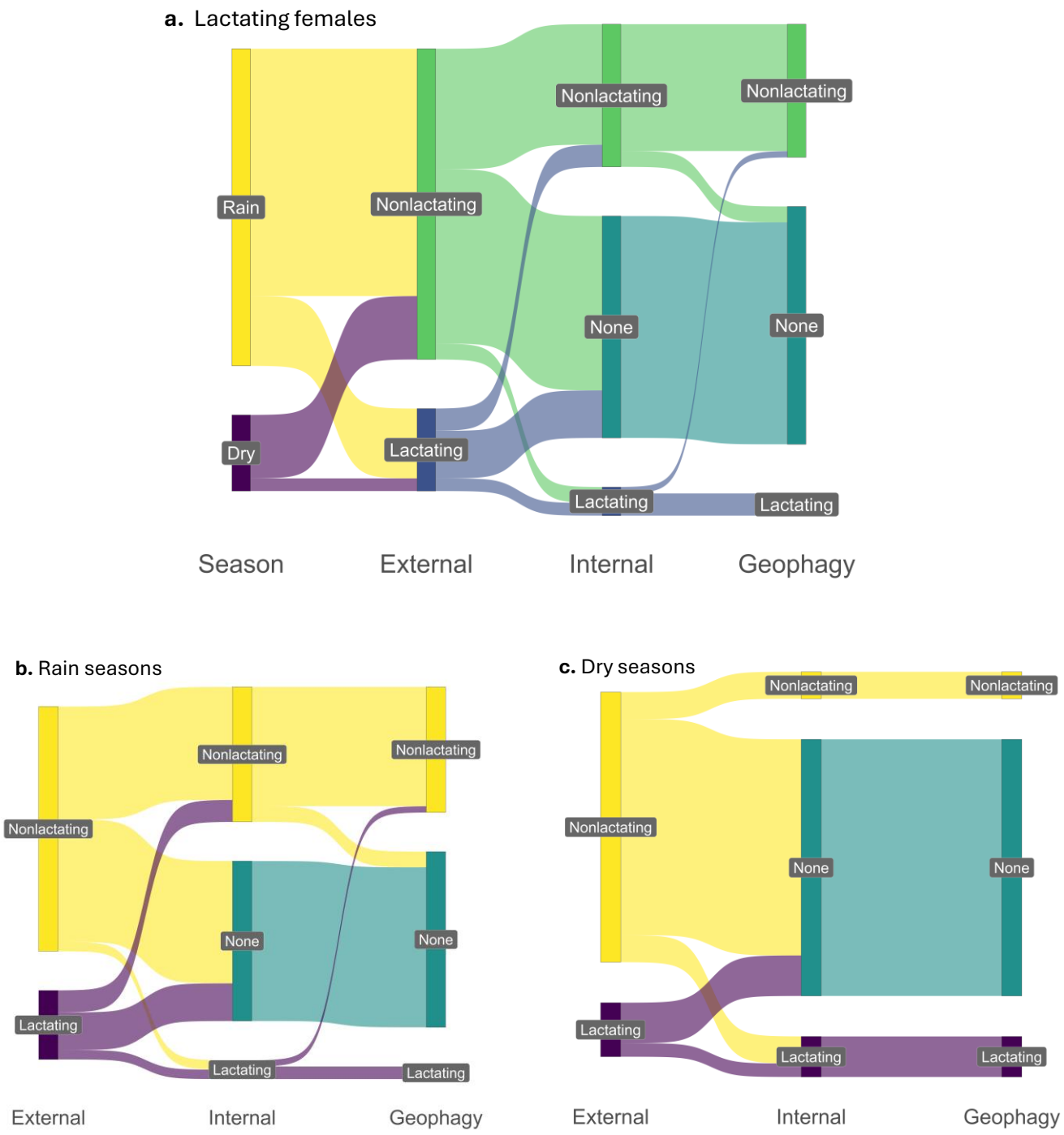


Figure 5.3 Diagrams showing external, internal, and geophagic bouts where at least one lactating female was present. All bouts without at least one female (lactating or not) were excluded such that these diagrams only compare groups with at least one nonlactating female to all groups with at least one lactating female for ***a.*** (top) all bouts flowing from season to external, to internal, to geophagy, ***b.*** (bottom left) only bouts occurring in the rain seasons, and **c.** (bottom right) dry season bouts only. The differences in ***b.*** and **c.** indicate that nonlactating females may be less likely to enter the cave and engage in geophagy in the dry season compared to the rain.

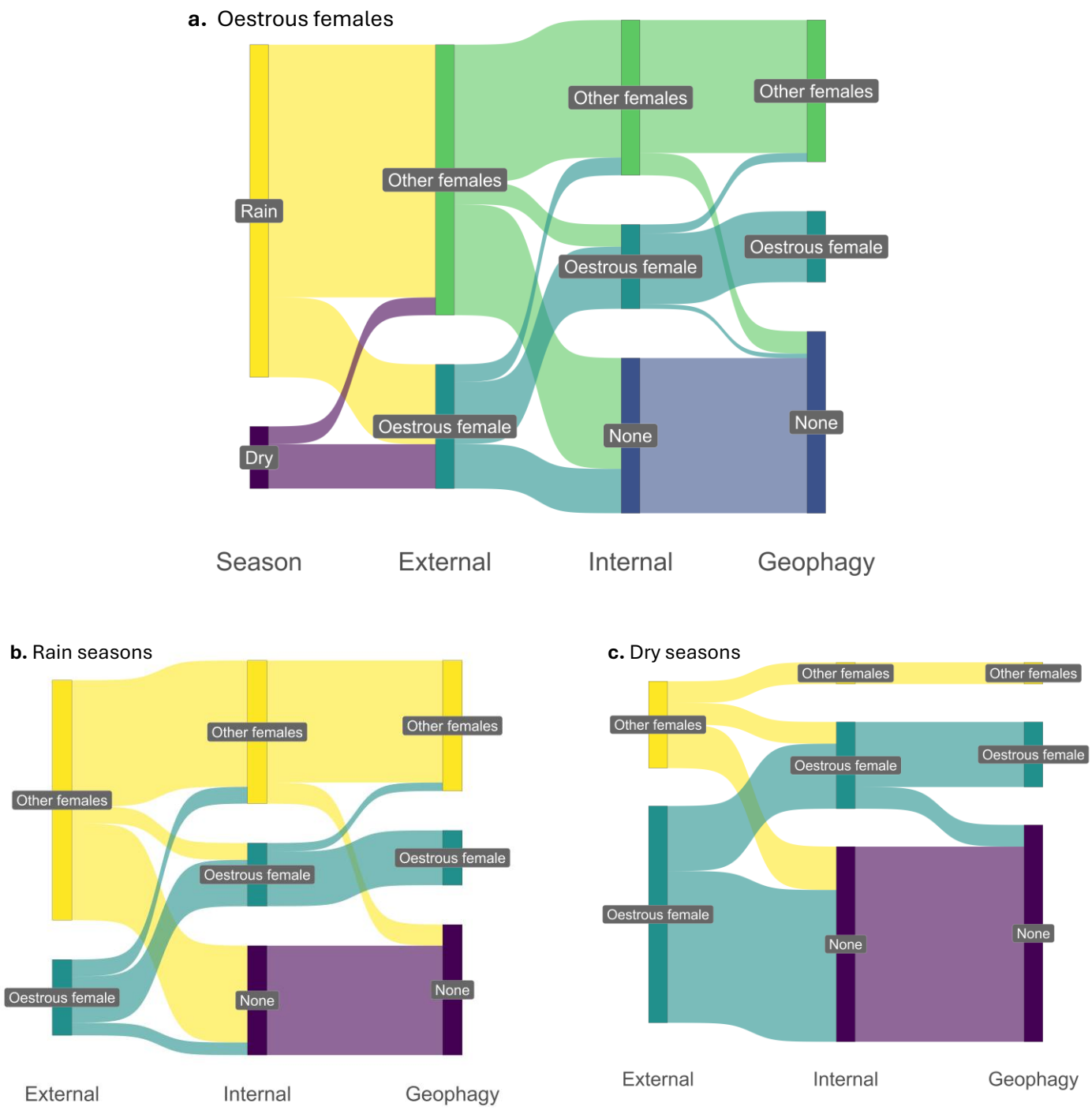


Figure 5.4 Sankey diagrams demonstrating the change in proportion of external, internal, and geophagic bouts with one or more females in oestrous were present. Only bouts with at least one female (regardless of reproductive status) were included to only allow comparison between groups where it was possible for there to be a female in oestrous (i.e. all male groups or all immature groups were disregarded). These show ***a.*** (top) all bouts by season and external, to internal, to geophagy conditions, ***b.*** (bottom left) rain seasons bouts, and ***c.*** (bottom right) dry season bouts. Note that there is a marked difference in proportion in groups with at least one oestrous female between the rain season (*b*) and dry season (*c*) with $n = 75$ and $n = 14$, respectively.

5.3.2 Statistical results

Statistical investigations comparing the internal and geophagic bouts to the external bouts as a baseline composition were conducted to test whether certain sex/age classes or individuals with specific reproductive statuses are more likely to enter the cave or engage in geophagy. The aims of these were to test hypotheses related to individual stresses or nutrient demands from reproductive statuses that might lead certain individuals to use the cave and/or consume soil.

Comparing the external bouts to the internal bouts, that is, testing whether group composition or reproductive status of individuals that enter the cave is significantly different than the groups outside, determined that sex class, lactating females, and oestrous females are not significantly different (sex class: $p = 0.078$ with a small effect size [adjusted Cramer's $V = 0.11$]; lactation: $p = 0.361$ with a tiny effect size [adjusted Cramer's $V = 0.01$]; oestrous: $p = 0.747$ with a tiny effect size [adjusted Cramer's $V = 0.01$]) but season ($p = 0.013$, small effect size [adjusted Cramer's $V = 0.14$]) and age class ($p = 0.031$, small effect size [adjusted Cramer's $V = 0.11$]) were. For season analyses, results showed that there were more internal bouts than expected in the rain season and more external bouts in the dry season. For age class, immature groups appear to be overrepresented in the internal condition as there were over twice as many majority immature groups seen in the cave ($n = 16$) than outside ($n = 7$) even though the sample size for internal conditions ($n = 173$) was considerably smaller than the external ($n = 220$).

Comparisons of geophagic bouts to the external bouts showed significant results for sex class (chi-square $p = 0.025$ with small effect size [adjusted Cramer's $V = 0.14$]) and season (chi-square $p = 0.041$ with small effect size [adjusted Cramer's $V = 0.11$]). The seasonal results for geophagy mimicked the results seen in the internal condition, with fewer bouts in the dry season and more

in the rain season than expected. Interestingly, the sex class analyses indicated more majority male groups engaging in the geophagic condition than the external condition which is contrary to the internal condition (which was not significant, see above). However, when visually comparing the sex age class data for all three conditions, the numbers of each group composition category showed only 28 majority male groups in the external condition, while there were 51 in the internal and 53 in the geophagic conditions. Given these values, it is very possible that this could be a result of missing sex ID data in the internal condition (as it was sometimes harder to correctly identify those individuals that did not approach the GPs) resulting in missing group composition values. This could also indicate the internal condition is on the cusp of significance and more data may be needed to determine if sex class is a significant factor for the internal as well as the geophagic condition.

Age class (chi-square p = 0.05 with very small effect size [adjusted Cramer's V = 0.08]), lactating females (chi-square p = 0.447 [effect size: adjusted Cramer's V= < 0.001]), and oestrous females (chi-square p = 0.999 [effect size: adjusted Cramer's V = <0.001]) did not significantly differ between geophagic and external conditions suggesting these did not impact if individuals choose to enter the cave when they readily have access to it.

Linear models examining the influence of sex-age class (group ID), season, presence of lactating or oestrous females, and group size on external bout length investigated if attending the general area of the cave might already be biased toward these attributes. Significance values extracted from the external models found the only influence on bout length was group size (p = <0.001; small effect size, Std. beta = 0.33, [95% CI 0.27, 0.38]) while group ID (p = 0.230), season (p = 0.629), lactating females (p = 0.601), and oestrous females (p = 0.549) did not play a significant

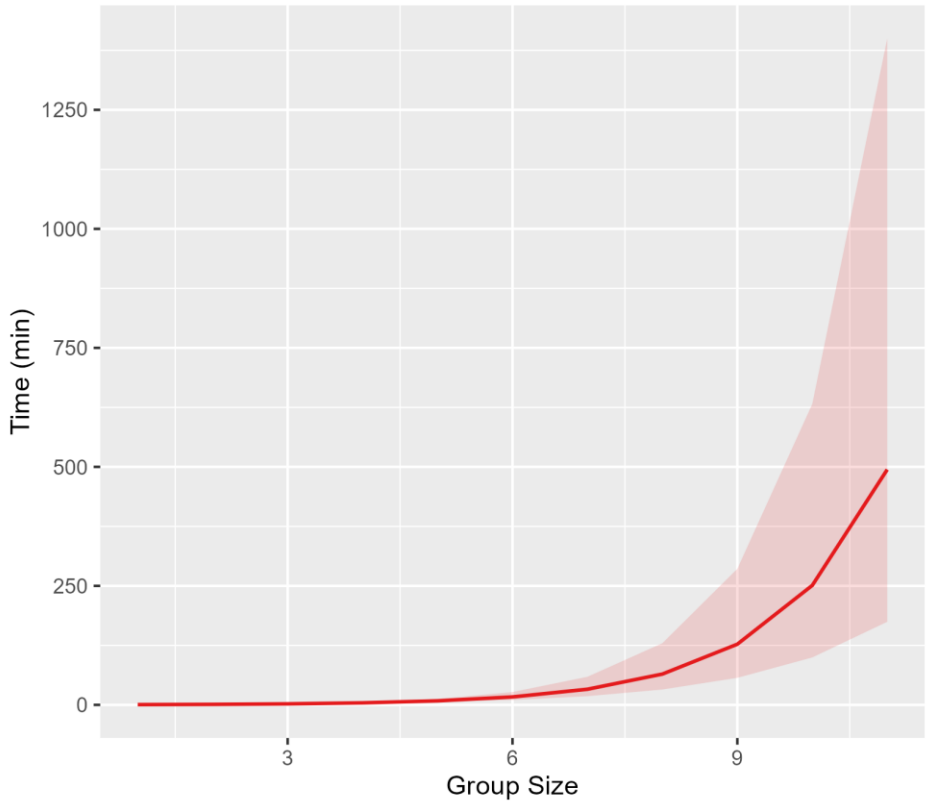


Figure 5.5 Expected external bout length (in minutes) by group size (number of individuals seen on screen throughout entire bout length) as predicted by linear models. This is generally as expected since the largest group size recorded was 11 which also had the longest bout length at 161 min (or around 2 hours and 40 minutes), and 58 of 126 bouts (46%) were below 60 seconds with 42 of these 58 bouts consisting of only one individual.

role. In particular, there seems to be an exponential increase in time observed within the predicted values (Figure 5.5). This is not particularly surprising, as nearly half of all external bouts lasted less than one minute (n = 58 bouts of 126 total) and 48 bouts consisted of only one individual resulting in smaller group sizes and shorter bout lengths being over-represented and very few long bouts and even fewer larger groups represented.

For internal bout length, group ID (p = <0.001) was also found to be significant in addition to group size (p = <0.001) and season (p = 0.333), lactating females (p = 0.487), and oestrous females (p = 0.4) were again insignificant. The interaction between group size and the mixed group ID was significant (p = <0.001) but had a very small effect size (Std. beta = -0.20, 95% CI [-0.37, -0.02]). When breaking down group ID to investigate each majority, all group compositions except female majority (p = 0.586; very small effect size, Std. beta = -0.06, 95% CI [-0.17, 0.04]) were significant (male majority: p = <0.001, small effect size, Std. beta = 0.27, 95% CI [0.19, 0.36]; mixed group: p = <0.001, small effect size, Std. beta = 0.35, 95% CI [0.18, 0.51]), but effect size for majority immature groups was very small (p = 0.002, Std. beta = -0.08, 95% CI [-0.27, 0.10]). The main group composition effect within this model appears to be driven by mixed groups spending much more time within the cave (Figure 5.6). Like the external bouts, this could reflect the relatively low prevalence of mixed groups (n = 17 bouts of 147 total) with all but one of these mixed groups spending over 1 minute inside the cave (n = 48 bouts <60 seconds [43%]).

Geophagic models were very similar to the internal models with only group size (p = <0.001 Std. beta = 0.43, 95% CI [0.28, 0.57]) and group ID (p = <0.001) significantly influencing bout length (season: p = 0.312, lactating females: p=0.482, and oestrous females: p = 0.479). Again, all group IDs apart from majority female came back significant, however all of these had very small effect sizes except for majority male (p = <0.001; Std. beta = 0.23, 95% CI [0.16, 0.31]). Predicted geophagic bout lengths were generally much shorter than predicted internal lengths, which is most likely due to the considerable decrease in predicted mixed sex bout lengths.

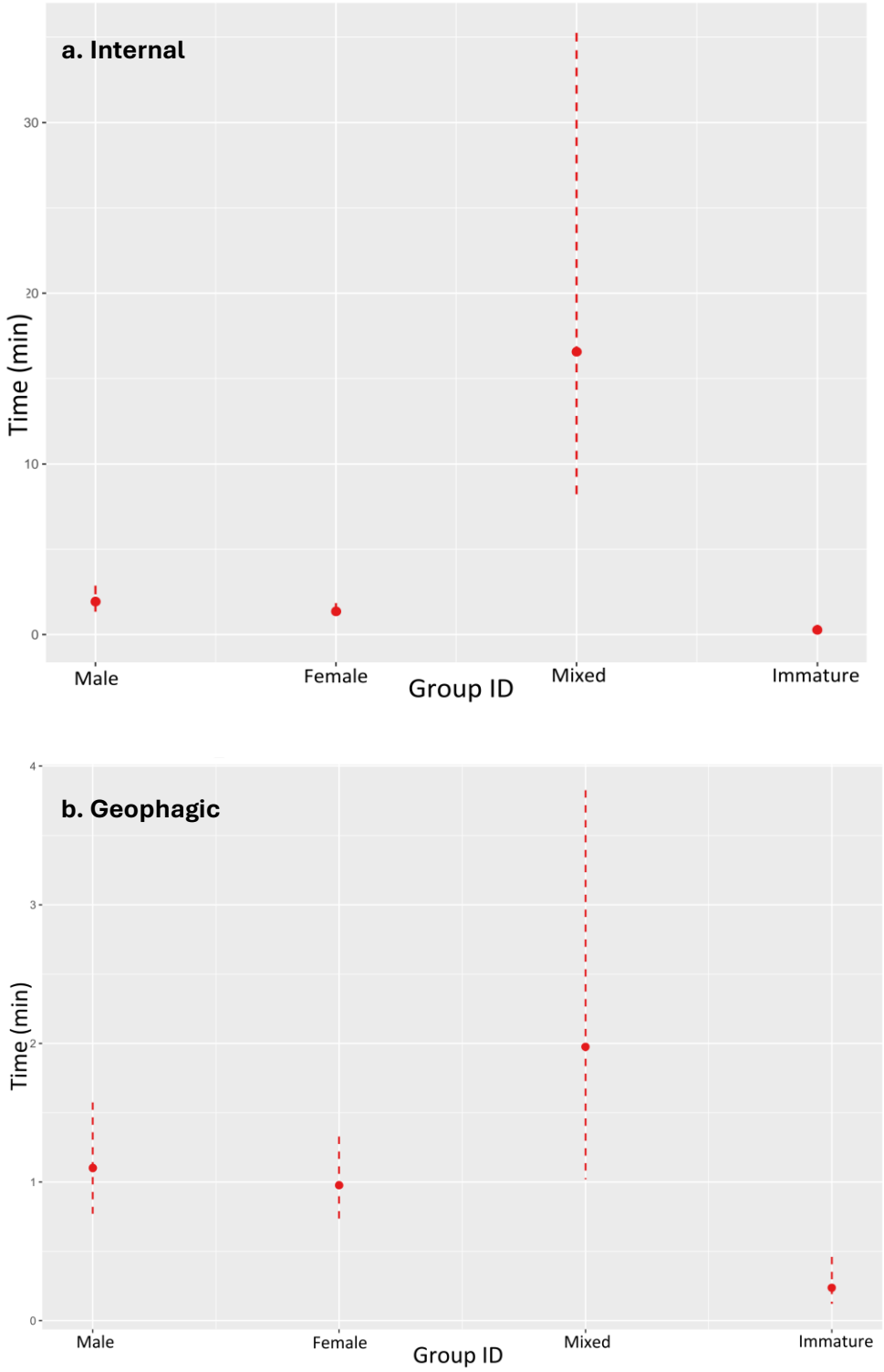


Figure 5.6 Depicts the predicted bout lengths by majority (>75%) sex-age class where male is majority adult male, female is majority adult female, mixed is an adult group with no majority, and immature represents any group where juveniles and/or subadults comprise >75% of individuals. ***a.*** (top) shows internal bout length as predicted by group ID (or composition). This especially shows which groups are likely to spend the most time inside the cave and includes any time spend consuming soil and emphasises the increase in time associated with mixed sex groups. ***b.*** (bottom) shows the predicted geophagic bout length (i.e. time spent consuming soil within the cave) by group composition. While the geophagic bouts still show a marked effect of mixed groups predicted to have longer bouts, the differences are much smaller than those found within the internal conditions.

5.4 Discussion

5.4.1 External condition

The external data within this research operates as a baseline for which to compare cave use and geophagic behaviour. As such, it helps build a concept for group structure of communities that visit the cave without these data being readily available given the habituation levels at the time of study. The descriptive results suggest that while the chimpanzees do visit this area throughout the year, there were many more bouts near the cave in the rain seasons than the dry. This could be because they have a true preference for visiting in the rain season, however it is likely influenced by an asymmetry in lengths of seasons. During this study period of 466 days, the rain season accounted for 341 (73.2%) and the dry for 125 (26.8%) days, and the external bouts included a maximum of 149 days with 118 (79.8%) in the rain and 32 (21.5%) in the dry seasons. Since the percentage of observation days (79.8% rain and 21.5% dry) generally matches the distribution of total rain and dry season days within the study period (73.2% rain and 26.8% dry), it can be concluded that, while there might be marginally more rain season external bouts, the bouts are roughly proportional by season (this was confirmed with a post hoc chi-square goodness-of-fit test [$p = 0.118$]). Thus, the external seasonal data matches what would be expected if season does not impact when they choose to visit this area of their territory and forms a good baseline of comparison.

When examining the group structure of individuals that visit the external condition, the most common group structure was unknown, but followed by majority female. Unknown majority sex groups were particularly common because undergrowth around the cave can hide most of an individual's body and successful IDs were generally not possible when a chimpanzee travelled through the area swiftly. However, since age could usually be determined using body size or other

obvious features (young chimpanzees have posteriorly positioned white tufts that can stand out against the foliage) this was not seen within the majority age classifications, which showed that majority adult groups were the most common grouping by far. Reproductive status of the external bouts is hard to interpret in isolation, as the demographic structure of the Bugoma chimpanzees was unknown at this time, but there were proportionally more groups with oestrous females observed in the dry season (note this only includes groups with 1+ females). One explanation for this could be due to seasonality in breeding behaviour. Though chimpanzees are not considered seasonal breeders, there is evidence of seasonal influences within their reproductive cycles – particularly that oestrous in postpartum females is more commonly observed in the late dry season (Goodall, 1986; Nishida *et al*., 1990). This has been linked to food abundance, temperature, and rainfall in the previous two months in chimpanzees in Taï National Park (Anderson *et al*., 2006), and it is unknown if there are similar trends in Mwera or the surrounding communities. If rainfall does influence oestrous timing in the Bugoma chimpanzees, this may explain the increased proportion of oestrous females observed in the dry season within this study, even at the baseline (external) level.

5.4.2 Cave use: the internal condition

To explore questions regarding when and why the chimpanzees choose to enter the cave, the internal condition was considered and compared to the external conditions outlined above. This internal condition represented those chimpanzees that not only enter the external condition, allowing them immediate access to the cave, but subsequently also choose to enter the cave itself. As previously stated, it would be expected that if entering the cave is beneficial to chimpanzees of a certain sex, age, or reproductive class, but not to others, then these group compositions within the cave should be different to the compositions observed outside the cave.

Of particular interest is an examination of those groups that choose not to enter the cave at all, since 'no group present' was found to constitute the largest total number of observed internal bouts by sex and age class. The high proportion of bouts where chimpanzees are seen at the cave entrance but do not enter indicates that there is some variable influencing the decision to enter the cave (see Figures 5.1-5.4 *a*-*c*), and it is especially noteworthy that this scenario is more commonly seen in the dry season. If there were no decision process to enter the cave, then the expectation would be either a very high or very low proportion of internal visits because the chimpanzees would either a) nearly always, or b) almost never enter the cave any time they are observed at the entrance. The relatively intermediate value of 36% of external bouts that do not subsequently result in an internal bout shows neither of these scenarios, nor is there a strictly 50/50 chance to enter the cave, and thus some other factor influences when they enter. These intermediate values also occur when examining bouts by season, however, it becomes apparent that the dry and rain season diverge with the chimpanzees choosing not to enter the cave in 63% of dry season bouts, but only 25% of rain season bouts. While both of these still fall generally into the middle of the spectrum, this does suggest it is very possible that season, or something related to season, plays a part in this decision process.

Group composition does not appear to change drastically, at least proportionally, between the external and internal conditions overall (see Figure 5.1 *a* and Figure 5.2 *a*), but there are some differences observed between seasons. For sex class, there was a marked increase in the proportion of majority female groups in the dry season compared to the rain season, however this is likely due to the small sample size of internal dry season bouts, as only 5 were recorded. Additionally, the chi-square tests did not show a significant difference in sex class when

comparing the external to internal conditions, further indicating this is unlikely to be a significant factor but rather an effect of sample size.

While age classes don't show major changes, there do appear to be slightly more majority immature groups entering the cave in the dry season (Figure 5.2 *c*). This is corroborated by the chi-square tests for age class which show more immature groups enter the cave than expected given the external bout values. It is not known if this indicates any particular benefit, especially since age class was not a significant factor for geophagic bouts, but it could be that immature groups are more likely to seek out some other function of the cave. However, the majority immature groups increasing from external to internal, and also to geophagy, could be due to juveniles more frequently leaving the screen and returning, artificially inflating the number of immature individuals recorded and thus increasingly the likelihood of a false group ID. One particular internal bout appears subject to these biases where there were nearly 20 juveniles recorded but only two adult females. The most likely scenario in this case would be that these 20 juveniles were actually only a few juveniles that moved on and off the screen repeatedly, erroneously raising the immature counts because they could not be successfully re-identified when they re-entered the frame. Juveniles were much more difficult to recognise between videos compared to adults, who often had unique characteristics (such as scars, injuries, or distinct morphology) that were uncommon amongst younger chimpanzees. This likely led to more adults positively re-identified later in a bout even after they left the recording areas. Overall, for age class, sample sizes were too small for the immature groups to make meaningful conclusions regarding nutrient supplementation for growing individuals, but this should be re-examined in any future studies, preferably with access to individual identification of juveniles and their mothers to counteract these counting biases.

For reproductive status, the presence of lactating females generally didn't differ when comparing total or seasonal bouts between the external and internal conditions. For oestrous females, though the dry season does appear heavily biased toward oestrous females entering the cave, as noted previously, this is likely due to a small sample size. This is further confirmed by analyses showing there is no difference in oestrous female group occurrence between the external and internal conditions, and this is also confirmed within the same analyses for the groups with lactating females. Taken together, it is unlikely that reproductive status impacts whether or not a female enters the cave.

Lastly, visits by season were found to be significantly different between external and internal conditions with more external bouts in the dry season and internal bouts in the rain season than expected. This does suggest that when the chimpanzees visit the area, they are more likely to enter the cave in the rain season and/or less likely to enter in the dry season.

5.4.3 Geophagy

The main difference between the internal and geophagic bouts was seen in the age structure and sex classes observed, which further supports the assumption that reproductive status does not affect cave use or geophagic behaviour. As with the external and internal bouts, the combined seasonal data shows little difference between the internal and geophagic bouts, but some seasonal differences are apparent. Visually inspecting the data shows that the dry season has more majority female groups in the geophagic than the external condition, however this does not appear to differ from proportions seen in the internal condition. Overall, the Sankey diagrams for sex and age class seem to indicate little to no difference between these two conditions. However,

statistical analyses indicate that while there were more immature groups than expected in the internal condition, this was not true for the geophagic analyses. Instead, sex class is shown to differ between geophagic and external bouts, with more majority male groups in the geophagic condition than expected given the external values. Lastly, the seasonal trends of more bouts in the rain and/or fewer bouts in the dry seasons was also observed in the geophagic condition. This further suggests not only that season plays a large part in when the chimpanzees choose to engage in these behaviours, but also emphasises the similarities between the internal and geophagic conditions.

5.4.4. Predictors of cave use and geophagy

Linear models indicated that the only significant predictor of how long a group would spend within the area of the cave (regardless of whether or not they enter) was groups size. As would be expected considering the external condition as a basis for further conditions, groups size was also a significant predictor for the cave use (internal), and geophagic models as well. In addition to group size, group ID (composition) was a significant factor for both internal and geophagic bout length and this was mostly driven by mixed groups for both, plus majority male groups for geophagy. Since the cave is believed to lay on border of 2 or more different chimpanzee communities, and intergroup interactions between communities are often violent and/or fatal (Watts & Mitani, 2001; Wilson & Wrangham, 2003), this could suggest that chimpanzees spend very little time in this area without the safety of numbers (group size) or the presence of males (mixed groups and majority male groups). If this is the case, these models may actually be a measure of safety, measuring how long chimpanzees are willing to spend in the area given the risk, and not how they engage with the cave in general or how long they spend consuming soil. This issue of safety could also offer an explanation for some seasonal differences observed in previous

sections, as pressure from neighbouring communities has been shown to be lower in the rain season (Lemoine *et al*., 2020).

In addition to issues of safety outlined above, it is possible that geophagy is underestimated within this study, as there were a number of occasions where an individual removed soil from the geophagic point but left the cave before consuming it. While the external camera did capture video evidence of the chimpanzees removing soil and consuming it at the entrance, it is not possible to estimate how often this occurs given the possibility that the chimpanzees may take the soil off screen before consuming it. Considering this and the possible safety factors, results from these linear regressions should be considered cautiously. However, these models do still represent some form of access to the cave and/or soil based on individual characteristics of sex-age class, though instead of individual nutrition needs driving time spent in a condition it may instead represent a trade-off of risks and benefits given the possible dangers that come with entering a confined space with few ways to escape.

Another possible issue within this study is the chance of missing individuals within one bout which leads to artificial group composition changes between conditions, and this is especially important when considering reproductive status which operates on a presence or absence basis with little nuance. Presence or absence of lactating females shows some nonlactating groups becoming lactating groups in subsequent conditions on more than one occasion – suggesting that there were at least some instances when individuals were missed on external cameras. This could be for a number of reasons which have already been noted, such as low visibility in the undergrowth (especially if individuals are moving quickly), but this could also indicate that there is an entrance that was not adequately monitored. Since the cave is made of soil and not rock, the environment

within and around it is constantly changing due to water erosion, chimpanzee activity, and possibly the influence of other species. Thus, it is possible that later during the study period one of the smaller holes leading into the cave could have expanded enough to permit chimpanzees alternative access pathways. Any future studies should employ more cameras, as well as individual IDs, to properly quantify which individuals are using the cave and why.

5.5 Conclusions

This study offers few conclusions about cave use and geophagy in sex, age, and reproductive class, but does show strong seasonal trends and indicates a strong bias of group size that warrants further study. To investigate characteristics that might influence whether an individual enters the cave or engages in geophagy, it is imperative that future studies are able to positively identify individuals from at least one of the visiting communities to allow for stronger nested models. As there has been positive habituation and identification progress since the study period described here, it is possible future studies may be able to answer questions regarding if lactating/oestrous females or juveniles are supplementing their nutrition by visiting the cave and engaging in geophagy. Since the lack of habituation and the inability to track individuals away from the cave made it impossible to consider the condition or diet of each individual chimpanzee within this research, future studies should consider this and health statuses such as illness, injuries, or apparent gastrointestinal stress of each individual as possible predictors of geophagy – especially given the preponderance of majority male groups which indicates something other than growth or female reproductive status may drive cave visits and geophagy in Bugoma.

6. Discussion

6.1 Thesis findings: a synopsis

The overarching goal of this thesis is to provide a preliminary description of social and environmental influences (such as abiotic and dietary factors) and test hypotheses on cave use and geophagic behaviours observed in the Mwera chimpanzees of the Bugoma Central Forest Reserve, Uganda. To meet this goal, this thesis delivered a general introduction to geophagy and cave use in nonhuman animals, chimpanzee behaviour and ecology, and the Mwera community (Chapter 1: Introduction) before then producing a preliminary study on seasonal patterns of rainfall and temperature and creating a novel 'dietary stress score' to quantify the Mwera diet for future analyses (Chapter 2). Chapter 2 established a baseline of ecology in the form of seasonality and diet from which to explore the unique cave use and geophagic practices of the Bugoma chimpanzees. This resulted in the defining of previously undefined seasons within Bugoma Forest with seasonal precision down to the exact days for the entire study period, and a rudimentary, but functional, daily record of diet within the Mwera community. These newly defined seasons were used in every subsequent chapter and provided necessary environmental context with which to explore the hypotheses laid out in this thesis.

Chapter 3 set out to describe the chemistry and composition of the geophagic soils found within the cave by conducting monthly sampling of three geophagic points and comparing them to a control area at the main entrance of the cave. The characteristics of the soils were then defined and the elemental composition tested for differences in total content, bioavailability, and seasonality. Defining the soil compositions in the Bugoma cave were imperative to gaining insight into any supplemental nutrition or other health benefits the soil might confer upon those ingesting

it. The next two chapters (Chapters 4 and 5) explored how the chimpanzees engaged with the cave, and the geophagic soils therein, and tested hypotheses to understand what drives these behaviours. Cave use hypotheses included the cave as 1) an access point for nutrients via soil, 2) water access, 3) shelter from the elements (or predators, not tested here), and 4) thermoregulatory stress relief (McGrew *et al*., 2003). These were tested by modelling time spent in the cave as predicted by variables operating as proxies for each of these hypotheses (Chapter 4) which were 1) proportion of time spent engaging in geophagy, 2) water levels within the cave, 3) daily rainfall, and 4) daily maximum temperature, to deduce which of these significantly predict time spent inside the cave.

The hypotheses behind geophagy, however, presented a more complex puzzle. The two main sets of hypotheses for geophagy within the literature are 1) the protection hypotheses, that state that geophagy offers protective benefits against gastrointestinal intestinal issues and/or help neutralising Plant Secondary Compounds (henceforth: PSCs; Limpitlaw, 2010), and 2) the nutrient supplementation hypotheses which indicate geophagy as a means to treat deficiencies or gain access to nutrients otherwise lacking in the diet (Young *et al*., 2008). To disentangle these sets of hypotheses for geophagy, both Chapters 4 and 5 sought to investigate them separately. Chapter 4 built upon the cave models above to test aspects of the protection hypotheses using dietary stress scores (calculated in Chapter 2) to simulate the role of PSCs or difficult-to-digest dietary items, as well as rainfall over the previous 30-day period to represent possible parasitic infections (as monthly rainfall has been shown to be significantly correlated with parasite prevalence in Gombe chimpanzees; Gillespie *et al*., 2010). Since exposure to parasitic infections and dietary stress were likely similar amongst individuals, and, more importantly, dependent on individual qualities such

as overall health status, these were treated as having relatively equal exposure for all sex-age classes.

To address nutrient supplementation, Chapter 5 sought to investigate the different pressures that might impact an individual chimpanzee's decision to enter the cave and/or engage geophagy by examining patterns in the sex-age class distribution of groups that visit the cave. Nutrient supplementation has long been hypothesised to especially benefit females who are either lactating or pregnant, and growing juveniles, as these individuals are subjected to higher nutrient demands (in nonhuman primates: Zhao *et al*., 2008; Pebsworth *et al*., 2012; Pebsworth *et al*., 2019b; see Black *et al*. [2013] for effects of nutrient deficiencies in humans). Therefore, the final data chapter (Chapter 5) built upon the methods introduced in the previous chapter to model the effects of group composition, season, and group size on time spent in three conditions: 1) outside the cave (external, a null composition), 2) within the cave (internal), and 3) engaging with geophagic areas (geophagic). These group compositions were compared for both the internal (cave use) and geophagic test conditions against the external condition (individuals close enough to the cave to enter who nonetheless chose not to). Additional models predicting time spent for all three conditions were also generated and compared. Together, these chapters sought to create a wholistic perspective for why the chimpanzees in Bugoma enter the cave and engage in geophagy.

6.2 Novel cave using behaviour in Ugandan chimpanzees

The Bugoma Cave

In addition to testing hypotheses for cave use in forest-dwelling chimpanzees, this thesis also offers an initial description of the earthen cave in the Bugoma Central Forest Reserve. Since the

Bugoma cave is comprised solely of earth and held together with the root systems of two living trees, it is particularly unique amongst geophagic caves. Studies of primates thus far have described few cases of cave use in nonhuman primates (*General cave use*: Wilson *et al*., 1989; Preutz *et al*., 2001; Rogers, 2002; McGrew *et al*., 2003; Barrett *et al*., 2004; Preutz, 2007; Haslam *et al*., 2009; Kashiwagi *et al*., 2012; Hendershott, 2017; see Chapter 1 for further discussions), and even fewer cases of cave use with evidence of geophagy (Nishida, 1980; Harrison & Hladik, 1986; Redmond & Shoshani 1987; Sauther *et al*., 2013).

Therefore, a general description of the cave is provided here for future research (see Chapter 3). In short, the Bugoma cave is an open cavity constructed of hardened earth and tree roots set into a hillside, and composed of three chambers utilised by various species, including, in addition to the chimpanzees studied here: rodents, bats, felids, baboons, and the arboreal colobus and red-tailed monkeys. Indirect video observations indicate that one chamber (the 'left' chamber) is not entered by any larger mammals, and also show little evidence of chimpanzees using the 'middle' chamber for activities other than travel/movement within the cave. The last right chamber, or 'geophagic' chamber, is where most chimpanzee activity occurs and the only place where geophagy has been observed. Additionally, geophagy within the cave was only recorded in two confined areas for chimpanzees, and though the other primates (such as the olive baboon [*Papio anubis*] red-tailed monkey [*Cercopithecus ascanius*] and black-and-white colobus [*Colobus guereza*]) that visited also ingested soil, they did so indiscriminately within the geophagic chamber, taking soil from most available surfaces.

Cave use in Bugoma

Results indicated that thermoregulation or shelter from the elements are unlikely to serve the primary function of cave use in Bugoma. Additionally, since chimpanzees are capable of arboreal locomotion, it is improbable they would use the cave as a safe place to escape predators, even if there was evidence of predation in Bugoma. While thermoregulatory stress has been described in forest-dwelling chimpanzees, in the form of heat stress, there is evidence that spending time on the ground is sufficient to alleviate this burden (Kosheleff & Anderson, 2009). Thus, it is equally unlikely that the cave offers any additional benefits in regard to thermoregulation and the data presented here supports these conclusions. Even though resting was the second most common behaviour observed within the cave, it only represented 4.8% of the cumulative time that individuals spent inside, and resting behaviour was never witnessed outside of the rain seasons. Since resting and grooming are also correlated with terrestriality, and therefore with higher temperatures, (Kosheleff & Anderson, 2009), the little time spent engaging in both of these activities in the cave further suggests this is not the main purpose behind cave use in Bugoma – especially since more visits occurred in the morning, when the external temperatures were likely to be cooler, than in the afternoon when heat stress was likely to be highest. Similarly, the chimpanzees were unlikely to seek out the cave solely to shelter from the weather, as water would often permeate and drip through the roof of the cave during heavy rainfall. This leaves access to water and/or nutrients via geophagy to be the most likely drivers of cave use in Bugoma. However, these two resources are difficult to disentangle as not only could the presence of water soften the soil, making it easier to access, but standing water could absorb nutrients from the soil itself, allowing the chimpanzees to access some benefits simply by imbibing the water. Future studies will need to investigate these interactions further, but some evidence within the current study suggests that access to water is not the main contributing factor.

In the conclusion section of Chapter 2, it was hypothesised that water as the driving resource behind cave use, and possibly geophagy, would likely result in peaks of cave activities in the early weeks of any given dry season. This represents a time when water has not yet evaporated from within the cave (or filtered into the lower soil horizons) allowing some access to water, but when water availability outside the cave is also low. Thus, using the seasons calculated in this project (Chapter 2), peaks in cave activity were expected to occur in early January and late June of 2020. While there were some camera outages during both of these periods, cave use was indeed observed at both of these predicted times. However, these cave bouts were 1) shorter in duration than the average over the study period (average total time for predicted peak activity days was only 2 minutes 42 seconds [median 1 minute 40 seconds], but total cave time average was 26 minutes 53 seconds [median 2 minutes 48 seconds]), 2) did not comprise any of the longest bouts (no interactions during the predicted peaks were in the top 25% of interactions times), and 3) did not contain any period with the most consistent visits as measured by consecutive days. Instead, cave use, and geophagy, were concentrated in the rain seasons when there should be ample external water availability. While this is still not conclusive, it provides a strong argument that even if water access does impact cave use, it is certainly not the only driver.

6.3 Cave-based geophagy in the Bugoma Central Forest Reserve

Building upon the results highlighting the importance of water levels and geophagy in instigating cave use at Bugoma, this study attempted to reach conclusions about which hypotheses might compel the geophagic activities within this cave. Again, the results from this project were not entirely conclusive; however, after integrating the results of Chapters 3, 4 and 5, there is little evidence in support of the nutrient supplementation hypotheses of geophagy. If nutrient

supplementation drove geophagy in Bugoma, it would be expected that those under the most nutritional stress would consume the most soil. Previous studies have concluded that those individuals with the highest nutritional stress, and therefore engaging in more geophagy, would be pregnant and lactating females, and field studies have confirmed this association (pregnant females engaging in more geophagy: Pebsworth *et al.*, 2012; Pebsworth *et al*., 2016b; lactating females: Ampeng *et al*., 2016; Pebsworth *et al*., 2016a). However, to date, only one previous study in Japanese macaques has found increased rates of geophagy in juvenile individuals (Wakibara *et al*., 2001). Interestingly, this is contrary to all results found within this thesis. At Bugoma, not only were lactation and/or pregnancy in females not a significant predictor of geophagic bout length, but majority male or mixed groups were predicted to have the most impact on time spent engaging in geophagy and majority immature groups were the only groups observed more often than would be expected.

When considering this in conjunction with the soil results, the support for nutrient supplementation becomes even more precarious. The results from Chapter 3 indicated that the geophagic soils (GP1, GP2, and GP3) did not offer significantly more nutrients than the controls taken a few metres away. On the contrary, the control samples were significantly higher in most bioavailable micronutrients that would be considered beneficial (in particular, the following bioavailable micronutrients: calcium, cobalt, copper, manganese, and zinc) and lower in toxic metals such as tin. Therefore, if the chimpanzees were seeking micronutrients and wanted to maximise the bioavailable content from the soil while minimising the intake of toxins, the best location to do so would be at the more easily accessible soils located at the entrance of the cave and not at any of the possible geophagic areas.

It is, however, still possible that nutrient supplementation may impact the chimpanzees' decision to engage in geophagy. Studies on domesticated sheep (*Ovis aries*) in New Zealand found what was believed to be a cobalt deficiency (possibly linked to B_{12} synthesis [Brebner *et al*., 1985]), termed 'bush-sickness', and determined that even 20g, or less, of soil per week was sufficient to prevent disease (Rigg & Askew, 1934). This was later confirmed in studies that found even those soils with as little as 0.008 mg Co/kg offered protection against this cobalt deficiency (MacPherson *et al*., 1978). Hypothetically, if chimpanzees were to have similar needs to these sheep, then, in theory, the concentrations of bioavailable cobalt found in GP3 in October 2020 (0.009 mg Co/kg) and January 2021 (0.01 mg Co/kg) would be sufficient to meet these demands. For other micronutrients, another study on sheep found that there were no benefits beyond a certain level of supplementary selenium (Grace, 2006) and this could also be the case for other available micronutrients. Therefore, micronutrients from geophagy might be sufficient even at very low quantities and may also only be beneficial up to a certain amount, which would not sufficiently encourage the selection of soils with higher concentrations. However, without comprehensive studies on the nutrient availability and, crucially, the demand experienced by the Bugoma chimpanzees, it is impossible to know to what degree this might be true.

Lastly, one aspect currently lacking within the literature is the possibility of male nutrient supplementation. In humans, there have been studies investigating the impacts of male nutritional supplementation on fertility (Mora-Esteves & Shin, 2013) and supplementing a human male diet with additional zinc and selenium led to higher sperm concentrations, motility, and advanced morphology (Salas-Huetos *et al*., 2018). However, at the time of writing, no studies have investigated a relationship between fitness and nutrient supplementation in male nonhuman primates. It is recommended that future studies find a way to consider this possibility, since males

were a strong predictor of geophagy at Bugoma, and it may be possible to do so by measuring male fitness in conjunction with how often that male engages in geophagy.

The minimal support for nutrient supplementation outlined above taken together with results from the predictive geophagy models and clay content within the geophagic soils, advocate for the protection hypotheses providing the best explanation for cave-based geophagy in Bugoma. Geophagy has been described in humans for thousands of years (Young *et al*., 2011), but historical records indicate many cases of geophagic activity in humans sought soils specifically for their clay content (Macgregor, 2013). Even within the current literature, the inert and binding qualities of clays, such as kaolinite, are still used as active pharmaceutical ingredients to treat ailments including diarrhoea, indigestion, and stomach pain or as antibacterials, detoxificants, and even topical agents for wound healing (Awad *et al*., 2017). One key conclusion derived from the soil analyses of the geophagic soils at Bugoma (Chapter 3) shows that the geophagic soils had higher percentages of clay when compared to the control samples. Therefore, one hypothesis to be considered is that the higher clay content found in the deeper areas of the cave may make the geophagic areas more desirable as it provides access to contents of particular soil horizons not available at the entrance.

Soil layers are normally categorised into horizons A, B, and C where C represents the lowest, bedrock layers providing source materials, horizon B the middle layers containing the most minerogenic materials and clay minerals, and horizon A which constitutes the top soil with the most biogenic materials (Sing & Sing, 2010). As soil horizon A accrues and expands over time, it facilitates the slow downward filtration of smaller soil particles to soil layers below resulting in the formation of the B horizons and the accumulation of clays therein (a process known as illuviation,

Chesworth, 2008). Though the soil horizons were not identified in this study, there is some evidence that the geophagic soils may fall within the B horizon. When comparing the clay content between horizons A and B, it is expected that horizon B would contain between 3-8% more clay than horizon A depending on the overall clay content of horizon A, with 3% more clay in horizon B if clay content is below 15% in horizon A and 8% more clay in B if A is above 40% (Bockheim & Hartemink, 2013). Since the clay content in the control samples estimated *in situ* was between 30-35% and the geophagic points were between 35-40% clay, it is possible that the soil at the geophagic areas constitutes a B horizon which would be otherwise inaccessible to the chimpanzees. Ideally, future studies can confirm or deny these suspicions, but the geophagic soils do appear to give at least marginally higher clay content.

Additionally, the clay mineral analyses conducted within this thesis (Chapter 3) also provide insight into which functions of the protective hypotheses might be pursued within Bugoma. Clays are typically categorised by their structure as either 1:1 clays or 2:1 clays and these different structures allow different types of chemical interactions and therefore fulfil different functions (Barton, 2002). Studies on nonhuman animals have found that the less chemically active 1:1 clays – kaolinite – are more suited to treat gastrointestinal upset and diarrhoea (Vermeer & Farrell, 1985; Davies & Baillie, 1988; Mahaney *et al.*, 1996a) while the more active 2:1 clays – illite and smectite – are able to react and bind PSCs more efficiently as well as act as a gastric buffer (Davies & Baillie, 1988; Slamova *et al.*, 2011; Ta *et al.*, 2018; Pebsworth *et al.*, 2019a). Soil analyses at Bugoma have indicated that there are significantly more clays (and significantly less quartz) relatively abundant in the geophagic soils compared to the control soils, and these increases are especially apparent in the abundance of kaolinite (a 1:1 clay) and illite (a 2:1 clay). While both of these are significantly higher in the geophagic soils than the controls, their relative abundance to each other does not

change and there are, therefore, two main possibilities regarding clay mineral acquisition. The most likely of the two is that the chimpanzees seek both benefits of the 1:1 and 2:1 clays which are both relatively higher in geophagic soils, resulting in complementary effects. The 2:1 illite may help adsorb toxins within the gastrointestinal tract, relieving the cause of the symptoms and protecting against future symptoms, while the 1:1 kaolinite may treat the immediate gastrointestinal symptoms directly. However, given the large excess of 1:1 clays compared to 2:1 clays (e.g. kaolinite makes up 90% or more of the clays in every sample collected; see Chapter 3), an alternative possibility is that the chimpanzees seek the clay to treat symptoms of any diseases and toxins alike and any benefit of 2:1 clays is secondary to this main desired effect. This would be especially true if the amount of 2:1 clays present were not sufficient to create a therapeutic effect, thus future studies at Bugoma should seek to use the methods provided in Pebsworth *et al*. (2019a) by testing soils collected from the geophagic sites in laboratory based trials to see their effectiveness of adsorbing PSCs or other toxins.

Lastly, while the significant effects of rainfall found in the 30-day period preceding geophagy could suggest the soil might offer protection against parasites, the even stronger effects seen when considering the rainfall over the previous 60-days could indicate this rainfall metric was measuring effects of season – which could or could not include heightened levels of parasitic infections. Thankfully, determining if health status does impact geophagy should be an easy problem to solve given the continued progress on habituation at Bugoma, as it should soon be possible to measure individual health through faecal sampling and direct observations once formal IDs are established and link this directly with geophagic behaviour. However, at this time, the effect of infections on geophagy are still uncertain.

6.4 Conclusions and future directions

Solving the riddle of why the Bugoma chimpanzees decide to enter the cave and engage in geophagy within – a behaviour previously unexamined in forest dwelling chimpanzees – is a complex undertaking requiring the synthesis of data on animal behaviour and physiology, climate, soil chemistry, and plant biology. As such, while this thesis has aspired to reach scientific conclusions, the complexity of studying this question in free-ranging chimpanzees raises just as many questions as answers.

When examining the environmental influences on the cave-based geophagy studied here, it becomes clear that season has a considerable impact on when and why chimpanzees enter the cave. Not only was there a significant effect showing more chimpanzees entering the cave, and engaging in geophagy, in the rain season (Chapter 5), but water level within the cave and rainfall over the previous 30 days were both significant predictors of time spent inside the cave and engaging in geophagy (respectively, Chapter 4). Most importantly, seasonality of soil composition or content can be ruled out as an explanation for this discrepancy, as there was no significant seasonal effect observed within the soil (apart from tin content, but as discussed in Chapter 3, the tin content is likely too low to impact the health of the chimpanzees). So, while effects of rain and season seem apparent for other aspects of geophagy, it is unlikely this derives from the soil itself.

One last point to consider is the effects subgroup composition might contribute toward patterns of cave use and geophagy in Bugoma. Though the presence of females, pregnant, lactating or otherwise, were not a significant predictor of how long the chimpanzees would stay in the cave or consume soil, this may actually be due to which individuals are determining how long the group

spends in the general area via instigation of travel behaviour. Since the cave is a border area between groups, it might be that the presence of males is an important safety factor for females. Recent studies looking at social mammals have determined that while lactating females may strongly influence travel behaviour for resources (Fischhoff *et al*., 2007; Sueur & Deneubourg, 2011), they are more risk averse than males who tend to lead instances of intergroup conflict (Smith *et al*., 2022). Therefore, even if the females benefit most from the effects of soil consumption, it might be that they are not the determining factor for how long the groups stay in the area of the cave, as the males might be making their own decisions given the possibility of intergroup conflict which influence the safety of the females. Therefore, instead of the length of a bout determining the relative importance of soil for that group composition (Chapter 5), it may actually represent the level of risk involved, biasing longer bout length toward groups with more males.

There is ample water accumulation on the floor of the cave near the main entrance that should, in theory, provide equal access to water and similar access to clays and micronutrients in the soil. Yet, the communities choose to bypass this source of soil and water, and instead seek out one of two very specific, and relatively small, locations near the back of the cave. This takes the chimpanzees at least 2 metres from the nearest exit, which could increase the possibility of dangerous confrontations, or even being trapped within the cave and surrounded by another community, if the chimpanzee cannot exit quickly. Despite the danger and effort involved, these chimpanzees sought out these two isolated geophagic areas and consumed soil so consistently from them that they appeared to be changing the morphology of the cave itself over time. This is evident as videos show this part of the cave has continually changed in shape and depth over the study period. Interestingly, this contrasts with the other nonhuman primates engaging in geophagy

within the cave, which often ingested soil from many different locations and did not appear selective in their preference. This suggests that whatever benefits the soils may contain are apparently available at other points in the cave, and thus, it might not necessarily be the current compositions of clays or nutrients themselves that the chimpanzees seek. Consider, for instance, the case of elephants (*Loxodonta spp.*), another species of animal with sophisticated cognitive abilities that shape their environment through activities such as geophagy (Bowell *et al*. 1996) and also participate in extensive behavioural traditions or cultures (Fishlock *et al*., 2016). A recent study investigating geophagic behaviour in such elephants found that the soils do not offer nutritional or health benefits, and it has since been hypothesised it is indeed these behavioural traditions that play an important role in their choice of area for resource extraction (Fishlock *et al*., 2016).

Culture (behavioural patterns typical of a group and shared by members of the community that rely on information that is socially learned and transmitted; Laland & Janik, 2006), is considered widespread amongst chimpanzees and is increasingly found in a variety of animal taxa (Whiten *et al.,* 1999; Luncz *et al*., 2012; Whiten *et al*., 2022). Considering the role culture can play in the lives of animals, the modern geophagy witnessed within the Bugoma cave could feasibly result from an older cultural practice established an unknown amount of time before the present study. Since the cave has been extending further back into the hill over time, it is possible that, if this is a cultural tradition, this behaviour began when the back wall of the cave was much closer to the main entrance, and thus the more nutrient rich soils of the entrance. While this is merely speculative, it is worth considering the possibility that this is a tradition that started within the Bugoma chimpanzees to acquire the richer nutrient contents at the entrance but, over time as the tradition continued, the selected geophagic areas were pushed deeper and deeper into the cave,

away from the original area that contained more bioavailable nutrients. At this point, it is impossible to know how, when, or why the Bugoma cave formed, but as the research stands, even though water levels do appear to play a significant role in geophagy and cave use at Bugoma, the importance of these hypothetical cultural traditions cannot be excluded without further study.

Lastly, the cave may represent a time sensitive resource. Since the cave is made of earth and soil, it is much more susceptible to erosion than other caves that are made of stone, and this erosion could eventually lead to a collapse of the Bugoma cave. Indeed, one such collapse has already been witnessed within the middle chamber. During the pilot study for this thesis from January to April 2019, there were only two possible entrances to the cave: a small entrance that led to the left chamber which was not used by any chimpanzee throughout the entire study period, and the main entrance described within this thesis that gives access to the middle and geophagic chambers. However, at some later point in 2019, there was a small collapse in the roof of the middle chamber leading to a hole large enough for smaller chimpanzees to access, and the opening of an even smaller hole in the back wall of the middle chamber. Both of these openings widened over the study period, to the point that chimpanzees were frequently seen entering the cave through the ceiling of the middle chamber by the end of the study, and this gap even permitted significant amounts of light to permeate the back reaches of the cave (Figure 6.1). Therefore, it is possible that the cave is only a temporary resource and that studying this transitory phenomenon before it disappears should be prioritised.



Figure 6.1 a photo taken from the main entrance of the cave in March 2020 looking into the middle chamber. This photo illustrates the continued erosion seen several months after the collapse of the ceiling in the middle chamber. The dark area to the right leads into the geophagic chamber, which still retains very little access to light even after the collapse.

The cave at Bugoma represents a unique resource for forest chimpanzees – potentially allowing access to water and health benefits derived from the soil that are otherwise difficult to acquire. With this context, if there are multiple communities bordering the Bugoma cave, as has been suggested by the Bugoma Primate Conservation Project's team (henceforth: BPCP; personal communication), it could be that the communities must find ways to safely procure this resource – or to maintain and guard their access to it. Contacts between typical chimpanzee communities are generally rare, occurring on only 1.9% of follow days in the nearby Kibale chimpanzees, and when they did occur, were most often restricted to acoustic interactions via vocalisations (Wilson *et al.*, 2012). However, when chimpanzees from different communities do come in close contact

with each other, these interactions are often aggressive and can be fatal (Goodall, 1976; Kutsukake & Matsusaka, 2002; Watts *et al*., 2006; Wrangham & Glowacki, 2012; Wilson, 2013). Chimpanzees are also well known to engage in boundary patrolling when groups of, usually male, chimpanzees quietly walk the boundary of their home range (Bates & Byrne, 2009) which makes the situation of the Bugoma cave especially hazardous for visitors.

The Bugoma cave was visited frequently over the study period from September 2019 to December 2020. Chimpanzees were regularly seen on 2+ consecutive days and, at the peak of cave use in 2019, there were visitors on all but 6 days of October and all but 5 days in November. There was even a period during November 2019 when at least one chimpanzee entered the cave on 11 consecutive days. Considering the amount of time the chimpanzees have spent inside the cave it would be expected that some agonistic interactions would have been captured on video footage. However, there has only been one aggressive interaction witnessed in the direct vicinity of the cave (see Chapter 5), and this interaction was likely between members of the same community given that the two females did not react when the male entered the cave but only when he physically approached the female in oestrous. The location at which all observed geophagy has occurred within the cave also comprises the least safe area to occupy if there is threat of violence as it is the farthest point from any available exit. This is especially surprising if this resource is shared amongst communities and future research should consider how multiple communities can manage their access to the cave without coming into direct contact or risking significant aggression. It is also imperative to collect data on home range boundaries and usage in the Bugoma communities and investigate if there is any evidence of boundary patrolling around the cave by any community.

Recent endeavours have also been made by the BPCP team to identify individual members of the Mwera community, and significant progress has been made on identification of males with the study community such that most males are now able to be identified (at the time of submission for this thesis, T. Gruber, personal communication). Therefore, it should soon be possible to reexamine the video data used in this study to determine which communities have visited on which days. There have also been promising advances in understanding the community structure at Bugoma. Since the studies within this thesis were conducted, developments in and around Bugoma Forest, and especially around the chimpanzee communities studied there, have occurred that might make re-examination of these data with updated knowledge worth pursuing. Not only are there now two Mwera communities (Mwera North and Mwera South) that both have access to the cave, but there are some indications that there are other groups involved or possibly even shifting dynamics within and between these communities (unpublished, personal communication with T. Gruber). Though it is difficult to say at this time, especially without published research on these communities, tracking these inter- and intra-community dynamics could provide valuable information on the relative importance of the cave as a resource.

While there is certainly more research needed on the Bugoma cave, this thesis comes to the preliminary conclusion that the main purpose behind cave use at Bugoma is most likely the acquisition of clay rich soils, and possibly water, that help treat health ailments such as gastrointestinal symptoms and alleviate the burden of these infections on the chimpanzees. Though these chapters constitute preliminary investigations on semi-habituated chimpanzees – thus making consistent data collection and subsequent analyses difficult – taken together, this thesis provides a comprehensive and thorough account of geophagic and cave use behaviours in the chimpanzees of the Bugoma Central Forest Reserve and supplies future researchers with an

exceptional baseline from which to study these behaviours further. This thesis also highlights the need for further research on the chimpanzee communities of Bugoma, especially the establishment of individual identification, statistical descriptions of food availability, a need for longitudinal data on geophagy in the cave and elsewhere, and a better understanding of community structures, home ranges, and general dynamics of the communities that may share this resource.

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