Title

Chimpanzee and Macrotermes Interactions: Investigating the Dynamic Relationship Between Chimpanzees and their Preferred Termite Prey in a Savanna Woodland Ecosystem with Implications for Early Hominin Foraging Ecology

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CHIMPANZEE AND MACROTERMES INTERACTIONS: INVESTIGATING THE DYNAMIC RELATIONSHIP BETWEEN CHIMPANZEES AND THEIR PREFERRED TERMITE PREY IN A SAVANNA WOODLAND ECOSYSTEM WITH IMPLICATIONS FOR EARLY HOMININ FORAGING ECOLOGY

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By

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Abstract

Chimpanzee and Macrotermes Interactions: Investigating the Dynamic Relationship Between Chimpanzees and Their Preferred Termite Prey in a Savanna Woodland Ecosystem with Implications for Early Hominin Foraging Ecology

Seth Phillips

This doctoral dissertation examines the stable isotope and reproductive ecology of termites of the genus *Macrotermes* in conjunction with the foraging behavior of termite-fishing chimpanzees (*Pan troglodytes schweinfurthii*) in order to evaluate hypotheses on hominin foraging behavior associated with termites, as well as to gain novel insight into the cognitive abilities associated with termite-fishing behavior in extant chimpanzee communities. In chapter 2 I expanded the search for evidence that Plio-Pleistocene hominins may have consumed termites similar to what we can observe in many extant chimpanzee societies as isotope data from some hominin taxa seem to suggest. We add to this conversation by investigating the isotopic ecology of a candidate termite prey, *Macrotermes spp.*, occupying modern savanna-woodland habitats. In Chapter 3 I investigated and described the seasonal constraints on termite-fishing tool use at a savanna-woodland site in western Tanzania related to the reproductive ecology of the chimpanzees preferred termite prey, *Macrotermes spp*. I observed how the ephemeral nature of this embedded resource imposes a considerable challenge to foraging primates. Chapter 4 explores how a community of chimpanzees in the Issa Valley of Tanzania intelligently navigates this temporal obstacle through insight into their environment. The

convergence of these studies highlights how the interaction between chimpanzee and termite prey presents the opportunity to test various hypotheses on hominin dietary ecology, seasonal factors affecting great ape tool-use variation, and primate foraging cognition.

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Chapter 1

Introduction

Wild chimpanzees create and manipulate tools from their natural environment as means to extract otherwise difficult to access food sources. The first report of wild chimpanzee tool use came from Jane Goodall who reported that chimpanzees from the Gombe Stream in Tanzania inserted thin, flexible probes made from vegetation into the open passageways of termite mounds (van Lawick-Goodall, 1968). The soldier caste of termite colonies bite and hold onto the chimpanzee's tool to defend their nest long enough for the ape to retract the tool and consume this termite prey. Evidence of "termite-fishing", as this behavior is referred to, revolutionized our conceptions not only of chimpanzees but also of humankind and our relation to the animal kingdom. "Now we must redefine 'tool', redefine 'man', or accept chimpanzees as humans!" Louis Leakey famously responded to Goodall's report of tool using chimpanzees (Peterson, 2008, p. 212). This discovery inspired generations of anthropologists and primatologists to study the intricacies of this termite-fishing behavior such as how chimpanzees position their bodies, hands, or modify tools when termite-fishing (Boesch et al., 2020), as well as the many other subsequently discovered tool-use behaviors in chimpanzee communities across equatorial Africa (McGrew, 1992; Sanz & Morgan, 2013; Whiten et al., 1999). Yet there is another important agent in this species interaction that is regularly overlooked in studies on chimpanzee termite-fishing, namely the targeted termites themselves. *Macrotermes* *spp.* are the genus of termites consisting of largest termites in the world and happen to be the strongly preferred genus of termites for chimpanzee exploitation (Lesnik, 2014). There is a need to account for observed variations in the timing and intensity of termite-fishing behavior between chimpanzee communities as well as to understand how and when chimpanzees decide to construct tools and attempt to termite-fish. By studying the behavior and ecology of *Macrotermes* in addition to the chimpanzee's ability to problem-solve to forage for them, we can expand our understanding of wild chimpanzee foraging cognition in addition to critically evaluate hypotheses on hominin exploitation of this eusocial insect.

Despite Leakey's clever retort to the discovery of termite-fishing, we certainly have not accepted chimpanzees as human. Anthropologists and primatologists should exercise caution when drawing comparisons between chimpanzees and hominins as there are millions of years independent evolution to often different environmental pressures separating our hominin lineage from those of our great ape cousins (White, Asfaw, et al., 2009). Yet, there remain compelling reasons to study chimpanzee termite-fishing from an anthropological perspective (Lesnik, 2014; Moore, 1996). Some Plio-Pleistocene hominins exhibit carbon isotopic signatures suggesting a strong contribution of C_4-savanna grasses in the diet, that have led researchers to hypothesize several potential food sources associated with C_4-plant consumption, including termites, as possible sources for this C_4-signal (Lesnik, 2014; Sponheimer et al., 2005, 2006). There is experimental evidence to suggest that hominins may have similarly foraged for termites living in large above-ground mounds. Use-wear analysis of bone tool replicas of Swartkrans material found that experimental bone tools used for digging or perforating into termite produced striations similar to archaeological material (d'Errico & Backwell, 2009; Gardner, 2019; Lesnik, 2011).

Though by now several primate and non-primates have been observed to use tools in the wild, examples of chimpanzee tool use are more diverse, more frequent, and arguably more complex than any non-hominin species (McGrew, 1992, 2010). Chimpanzee tool-use variation and the ecological challenges prompting it, particularly in highly seasonal landscapes, are the most insightful analogies available in the animal kingdom to anthropologists interested in the origin and diversification of hominin tool use. Given the hypothesized importance of seasonal mosaic landscapes to hominin evolution (Foley, 1993; White, Ambrose, et al., 2009), I selected to conduct my research on a community of wild chimpanzees and the nearby *Macrotermes* mounds in western Tanzania at a highly seasonal mosaic site known as the Issa Valley. The data in the chapters to follow have relevance to both wild chimpanzees foraging in a naturalistic context as well as to hypotheses on hominin foraging behaviors.

The data collection efforts in this dissertation are motivated by a need to address important and previously undervalued considerations of invertebrate prey ecology and its role in shaping primate and potentially hominin tool use behavior. Chapter 2 reports the carbon and nitrogen isotope values of *Macrotermes* termites from six savanna woodland sites in chimpanzee field sites in Africa. By analyzing the stable isotope ecology of these termites, we attempt to find evidence that termite consumption is traceable in hominins as well as unhabituated communities of chimpanzees. The effort to identify the carbon isotope signature of *Macrotermes* adds valuable context to discussions to both the primatological and paleoarchaeological literature on dietary ecology for species and communities of species for which we don't have access to direct behavioral observations.

Chapter 3 discusses a multi-year research effort in which we monitored year-round surface activity of *Macrotermes* at their mounds. We utilized experimental termite-fishing methods to observe difference in seasonal availability of *Macrotermes* prey. This chapter will also report instances of vertebrate predators feeding on the reproductive caste of *Macrotermes* during their rare dispersal flights for which there exists little previously reported data (but see Dial & Vaughan, 1987). By focusing research effort towards the mound-building termites themselves, I aim to characterize the dynamic obstacle this prey species plays in this seasonal species-interaction. Additionally, I attempt to close the loop on some of the first overlooked and still unquantified observations made by Jane Goodall that termite reproductive ecology is connected to chimpanzees' ability to fish for termites (van Lawick-Goodall, 1968).

Chapter 4 investigates how Issa chimpanzees effectively forage for this seasonally constrained resource. In this study, I analyzed four years of motion-sensor camera footage of chimpanzees at or near to termite mounds. In particular, I recorded the frequency, timing, and durations of inspection behaviors at termite mounds as well as record instances when chimpanzees bypass mounds without stopping to inspect. Chapters 3 and 4 taken together establish an ecological obstacle that may select for tool-use intelligence in addition to identifying a cognitive strategy that may have been similarly available to hominins foraging for seasonally accessible termites.

Chapter 5 concludes the dissertation by exploring potential future avenues of research. It is my hope that the research presented here will inspire future researchers and conservationists. Studying chimpanzee-termite interactions is important when evaluating habitat health and conservation strategies for chimpanzee communities. For instance, rainfall patterns are projected to change substantially in East Africa over the next few decades (Dunning et al., 2018; Palmer et al., 2023) which may alter *Macrotermes* seasonal activity with downstream effects on chimpanzee foraging strategies and tool use traditions. The methodologies utilized throughout this dissertation are minimally invasive and can be employed to study both habituated and unhabituated communities of chimpanzees as well as the mound-building termites in their environment.

The three main chapters of this dissertation have either been published in (chapter 2 and 3) or submitted to (chapter 4) peer-reviewed journals (Phillips et al., n.d., 2021, 2023). In the following, these papers have been reformatted from their original to fulfill the dissertation formatting requirements at the University of California Santa Cruz Graduate Division.

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Chapter 2

Limited evidence of C_4 plant consumption in mound building *Macrotermes* termites from savanna woodland chimpanzee sites

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Abstract

Stable isotope analysis is an increasingly used molecular tool to reconstruct the diet and ecology of elusive primates such as unhabituated chimpanzees. The consumption of C_4 plant feeding termites by chimpanzees may partly explain the relatively high carbon isotope values reported for some chimpanzee communities. However, the modest availability of termite isotope data as well as the diversity and cryptic ecology of termites potentially consumed by chimpanzees obscures our ability to assess the plausibility of these termites as a C_4 resource. Here we report the carbon and nitrogen isotope values from 79 *Macrotermes* termite samples from six savanna woodland chimpanzee research sites across equatorial Africa. Using mixing models, we estimated the proportion of *Macrotermes* C_4 plant consumption across savanna woodland sites. Additionally, we tested for isotopic differences between termite ecolony in a subset of 47 samples from 12 mounds. We found that *Macrotermes* carbon isotope values were indistinguishable from those of C_3 plants. Only 5 to 15% of *Macrotermes* diets were comprised of C_4 plants across sites, suggesting that they cannot be considered a C_4 food resource substantially influencing the isotope signatures of consumers. In the *Macrotermes* subsample, vegetation type and caste were significantly correlated with termite carbon values, but not with nitrogen isotope values. Large *Macrotermes* soldiers, preferentially consumed by chimpanzees, had comparably low carbon isotope values relative to other termite castes. We conclude

that *Macrotermes* consumption is unlikely to result in high carbon isotope values in either extant chimpanzees or fossil hominins.

2.1 Introduction

Our understanding of wild chimpanzee (*Pan troglodytes*) feeding ecology has been primarily informed by direct observations of feeding behavior within the limited number of chimpanzee communities consistently monitored by long-term research projects (e.g.[1,2]). This bias towards a small number of chimpanzee communities has been tempered by the increasing use of indirect methods, such as stable isotope analysis, which enable large-scale cross site comparisons of the various feeding behaviors of both habituated and unhabituated chimpanzee communities [3–10]. Insights obtained from such studies are only as good as our understanding of the various stable isotope ratios, such as carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), of consumed organisms by chimpanzees at different locales [3–5,11–13]. Chimpanzee $\delta^{13}C$ values thus far indicate that chimpanzees primarily feed on vegetation relying on the C_3 photosynthetic pathway as well as organisms consuming such vegetation [3–8,14–17]. Still, small variation in $\delta^{13}C$ values within the observed range of chimpanzee values may be representative of differences in feeding behavior across communities or across demographic classifications within communities [3–5,8,14]. For example, some savanna woodland chimpanzee communities exhibit $\delta^{13}C$ values that are higher than baseline C_3 vegetation even if they are still consistent with a predominately C_3 based diet [3,4,8,15].The consumption of C_4 plant reliant termites has been posited as a potential contributor to relatively high $\delta^{13}C$ values in some savanna woodland chimpanzee communities [3].

Termites contribute key nutrients to primate diets [18,19] and even termite soil is particularly rich in nutrients [20–23]. Across Africa many, yet far from all, chimpanzee populations have been observed to forage for termites [24] with a common preference for the large, mound-building and fungus-growing termites of the genus *Macrotermes* (*Macrotermitinae*) (summarized in [25–29]). The ecology of *Macrotermes,* and termites in general, is often cryptic and difficult to observe under natural conditions [30]. Additionally, many behaviors within the genus *Macrotermes*[27,31–33] may vary significantly intraspecifically depending on the ecological context. *Macrotermes* is a genus of termites found exclusively in the Old World tropics. Some species, such as *M. bellicosus and M. subhylinus,* are broadly distributed across the African continent [31,34]. In general, *Macrotermes* spp. build large epigeal mounds and spend most of their time in the subterranean chambers and galleries throughout the year. *Macrotermes* workers forage for cellulosic debris such as leaf litter, dead grass, woody litter, and wood [35] including live crop plants [36]. Foraged items are returned to the nest as nutritional substrate for the growth of *Termitiomyces* fungus combs which consists of fungal biomass and partly decayed plant matter [37–40]. Old workers feed primarily on the fungus combs and the soldier castes rely on the workers to feed them directly with pieces of these fungal combs. By contrast, young workers may subsist primarily on foraged plant matter as well as on the protein-rich *Termitiomyces* nodules to some extent [37]. Though primatologists have begun to account for the specific isotopic values of chimpanzee plant foods within their environments [3–5,11–13] in cross site comparisons, there remains a need for complimentary data on insects food sources, such as *Macrotermes*, given their relevance to the diets of several chimpanzee communities (summarized in [25]).

The isotopic values of *Macrotermes* within savanna woodland ecosystems may be of specific utility in elucidating the source of comparably high $\delta^{13}C$ values, particularly found in some savanna chimpanzee communities. In the unhabituated eastern chimpanzees (*P. t. schweinfurthii*) at the field site of Issa, Tanzania, van Casteren and colleagues [8] reported higher $\delta^{13}C$ values than cannot be explained by the consumption of the sampled C_3 plant foods alone. Relatively high $\delta^{13}C$ fractionation factors ($\Delta^{13}C$) between chimpanzees and a selection of C_3 plants suggested that open canopy plant foods or C_4 plants could be potential contributors to $\delta^{13}C$ values. However, given that termite consumption is well documented in this population [41], the authors also posited that C_4 plant harvesting termites may contribute to $\delta^{13}C$ value enrichment in the chimpanzees. Wessling and colleagues [4] reported even higher $\Delta^{13}C$ values in five western chimpanzee (*P. t. verus*) communities at the very edge of the species range in Senegal [4]. Four of the five communities in that study are unhabituated and the source of these high $\Delta^{13}C$ values remained largely unclear. In the habituated community of Fongoli in Senegal however, chimpanzees have been observed to occasionally consume C_4 plants [42]. Additionally, Fongoli chimpanzees are exceptional with regards to the frequency and intensity that they consume *Macrotermes* [43]. This consistent consumption of an organism which may rely on C_4 vegetation is another possible explanation for the relatively high $\delta^{13}C$ values observed in Senegalese chimpanzees [3,4]. The extent to which, if at all, unhabituated chimpanzees in Senegal consume *Macrotermes* is not yet known. Only in the Kayan chimpanzees were termites from the genus *Macrotermes* identified in feces [3]. Still, the degree to which termite consumption could contribute towards high $\delta^{13}C$ values in savanna chimpanzees is not clear due to the limited dataset available on the stable isotope ratios of this termite genus within sub Saharan Africa.

Analysis of the isotopic signature of *Macrotermes* from a range of sub Saharan African sites may also have implications for paleodietary analysis of hominins. The genus *Macrotermes* diversified 6-23 million years ago as savannas spread across the African continent and remained relatively unchanged today despite climactic shifts [44,45]. Thus, it is highly likely that *Macrotermes* and early hominins coexisted in the African savanna landscapes. We can further hypothesize that these termites would have had similar diets as we see in extant *Macrotermes* diets from African savanna woodlands today. Hominins in east, south, and central Africa began to consume foods enriched in ^{13}C approximately 3.5 million years ago [46–57]. In relation to the high $\delta^{13}C$ signatures observed in *Paranthropus robustus* and *Australopithecus africanu*s specimen from Sterkfontein, modern $\delta^{13}C$ values of termites and sedges from nearby Kruger National Park were analyzed to investigate the hypothesis that either sedges or termites may be account for the observed high $\delta^{13}C$ values in hominins [52]. Accordingly, termite taxa across the park had an average $\delta^{13}C$ value of -20.1‰, with a mean of -15.3‰ (n=10) for open environment termites and a mean of -21.7‰ (n=30) for termites from closed environments. Based on these results, the authors concluded that termites could reveal a C_4 plant dependent isotopic signature, yet their consumption could not solely account for the enriched $\delta^{13}C$ values detected in the compared hominins. However, this study did not report the taxonomic classification of the termite specimen sampled (excepting a brief reference to the harvester termite genera *Trinervitermes* and *Hodotermes*) which would provide insights into whether these species could have been subject to hominin predation, nor was further ecological information on sampling locations provided.

There are a handful of other studies examining the relative contributions of C_3 and C_4 resources to termite diets in sub-Saharan Africa. The first systematic study of termite foraging ecology utilizing stable isotope analysis investigated the relative dependence of *M. michaelseni* on herbaceous (C_4) vegetation versus woody (C_3) vegetation by sampling from the termite head tissue and using a mixed modeling approach [58]. Both woody and herbaceous food sources contributed to the diets of *M. michaelseni* at two Kenyan savanna grassland sites but varied in their relative contributions. The dietary contribution from herbaceous vegetation utilizing the C_4 photosynthetic pathway was estimated to be 70% at one site and 36% at another site. These results indicate that termites of the same species can vary significantly with regard to $\delta^{13}C$ values in two, ecologically similar yet geographically distinct, environments. Additionally, this study provides some preliminary support for the hypothesis of *Macrotermes* as a partial C_4 resource for chimpanzees as well as hominins.

A similar termite isotope study at a humid savanna site (defined as grass savanna, shrub savanna and semideciduous plateau forest) in Côte d'Ivoire also found that the relative contributions of C_3 versus C_4 plants of four sympatric termite species within the termite subfamily *Macrotermitinae* was considerably varied even among the same species depending on habitat type and seasonality of sampling effort [59]. More recently, Vesala and colleagues [40] investigated the isotopic values between termite castes within four *Macrotermes* colonies located in southern Kenya. While three of the four colonies exhibited $\delta^{13}C$ values in range with herbaceous vegetation, one mound with more abundant grass surrounding had relatively enriched $\delta^{13}C$ values. Additionally, the authors reported significant differences in the $\delta^{13}C$ and $\delta^{15}N$ values between castes of the same colony suggesting that the nutritional contribution of fungal symbiont (*Termitomyces*) varies between castes within the same colony [40]. These studies demonstrate the utility of adding to the African termite isotope database while distinguishing between taxa, habitat type, seasonality, as well as caste (see also [60–62]).

Termite-fishing broadly describes a behavior in which a chimpanzee inserts a vegetative tool into a passageway at the surface of a termite mound and subsequently eats the soldier termites that bite onto the tool. Some chimpanzees preferentially forage on *Macrotermes* during the onset of the rainy season which may be due to an increase in accessibility during the colony’s reproductive cycle [63]. By contrast, other communities reliably consume *Macrotermes* year-round which may be attributable to more sophisticated tool-sets [18,64–66] or a dependence on the termites as a source of protein [43].

In the present study we analyze *Macrotermes* spp. samples collected at six chimpanzee savanna woodland field sites across equatorial Africa in the interest of further elucidating possible contributions of this genus of termites in chimpanzee isotope signatures. We focus here on *Macrotermes* spp. termites due to their status as the most commonly consumed genera among chimpanzees that termite-fish [24–27]. However, it is worth noting here that some populations do not termite-fish despite the presence of mound building termites, such as at the site of Gashaka, in Nigeria, which we report on here [67]. With this study we seek to address the following two questions:

- Do we find evidence for substantial C_4 plant consumption by *Macrotermes* across chimpanzee field sites via stable isotope analysis?
- Do we find intra-specific (between colonies) and intra-colony (between castes) isotopic variation in *Macrotermes* from the same field site?

2.2 Material and Methods

2.2.1 Sample collection and isotope analysis

In this study we collected *Macrotermes* from six savanna woodland sites across Africa, which represent relatively dry and open environments inhabited by chimpanzees today. Savanna woodland habitats are more likely to have substantial amounts of C_4 vegetation termites may rely on, as compared to forest habitats. We opportunistically collected 39 termite samples from fungus-growing mound builders at five savanna woodland chimpanzee field sites in West Africa (see Table 2.1 for further details and season of sampling), following a standardized sample and data collection protocol within the framework of the "Pan African Program - The Cultured Chimpanzee" project [68–70]. Permissions to conduct research were issued under the research permits N^o NPL/GEN/378/V/504 (*ministère de l'ecologie et de la protection de la nature, direction des eaux, forêts, chasses et de la conservation des sols,* Nigeria), N^o 078/2015/OGIPAR/MEEF/Ck (*ministère de l'environment, eaux et forêts, office Guinéen des parks et reserves,* Guinea), N^o 01316/DEF/DFG (Senegal) and N^o 219/MESRS/DGRSIT/TM (*ministère de l'enseignement superior et de lla recherche scientifique, direction generale de la recherche scientifique de de l'inovation technologique,* Côte d'Ivoire). At all field sites, except for Gashaka in Nigeria, chimpanzees termite fish [24,67]. We collected termites, predominantly of the major soldier caste, directly from mounds and recorded data on the habitat type surrounding the mound location following the protocol of the Pan African Programme [71,72]. Assigned habitat categories broadly describe the immediate surrounding vegetation at termite mounds in terms of the dominant vegetation type and sometimes the density of canopies as well as understories (e.g. "forest-mixed, closed understory” or “savanna-wooded”). Given our interest in termites as a potential food source for chimpanzees, we decided to combine multiple individual termites of the same caste and the same colony in one measurement.

Table 2.1 *Macrotermes spp.* samples from six chimpanzee field sites. Habitat abbreviations are as follows: CU= forest-mixed, closed understory, F= fallow, FB= forest on bamboo, FC= forest-colonizing, FR= forest on rock, GF= gallery forest, OU= forest-mixed, open understory, SH= savanna-herbs, SW= savanna-wooded. The site of Comoé GEPRENAF is here abbreviated as Comoé.

Country	**Location**	**Genus**	**Species**	**n**	**Habitat**	**Month**	**Year**	**Season**	**Seasons defined after**
Guinea	Bakoun	*Macrotermes*	*spp.*	7	SW (n=6); GF (n=1)	February, April	2014, 2015	Dry	[83]
Nigeria	Gashaka	*Macrotermes*	*spp.*	8	SW (n=5); CU (n=2); OU (n=1)	January, July, September	2012, 2013	Wet & Dry	[67]
Guinea	Sobeya	*Macrotermes*	*spp.*	3	SW	April, August	2013	Dry	[83]
Senegal	Kayan	*Macrotermes*	*spp.*	4	FB (n=3); FR (n=1)	May, June, July	2013	Wet	[4]
Côte d'Ivoire	Comoé	*Macrotermes*	*spp.*	9	SH (n=4); OU (n=2); SW(n=1); SR (n=1); FC" (n=1)	June, July	2014	Wet	[84]
Tanzania	Issa Valley	*Macrotermes*	*subhyalinus*	*47*	GF (n=28); SW (n=12); FC (n=6)	May, November, December	2017, 2018	Wet & Dry	[77]

For stable isotope analysis, we initially submerged the termites in ethanol and then stored them dry on silica in 15 or 50ml tubes. From each termite colony we obtained a second sample stored in ethanol for subsequent taxonomic identification by R.S., which revealed that all samples indeed contained *Macrotermes* termites of undetermined species.

While sample storage of termites between the field and the lab is essential for taxonomic identification, it may also cause slight shift in isotope ratios. In tissue samples of fish $\delta^{13}C$ values have been reported to become enriched by ~0.5 to 1.5‰, whereas $\delta^{15}N$ values increased by 0.5 to ~1‰ when fish samples were stored in 80% ethanol[73]. However, Arrington and Winemiller (2011) found a similar trend in fish samples yet concluded that these shifts are so small that they should not have considerable consequences for the use of preserved specimens in ecological research[74]. For insects, such as ants, crickets and flies, ethanol preservation did not affect $\delta^{15}N$ values, but $\delta^{13}C$ values shifted by ~0.6 to 1.5‰. However, one should note that non-chemically preserved samples (frozen, freeze-dried, fresh) also appeared to differed in their isotope values, suggesting that there may some influence of inter-individual variation that may be just as large as a preservation bias. In support, other isotopic work on ground beetles and aquatic consumers did not suggest that storage in ethanol significantly affects values $\delta^{13}C$ significantly (reviewed in [75,76]). Despite the highly contradictory evidence in the literature, we are not concerned with potential small-scale shifts in $\delta^{13}C$ values in our samples as our goal is to identify much larger isotopic differences between C_3 and C_4 food resources in termites, which will exceed 10‰ in tropical habitats. We thus follow the recommendation by Arrington and Winemiller (2002), suggesting that the tradeoff between specimen taxonomic preservation and isotopic integrity is sufficient to address the ecological questions raised in this study.

We rinsed all isotope samples thoroughly with ethanol, dried them down and then homogenized them to a fine powder in a pebble mill (Retsch MM400). Subsequently, we weighed 500μg of homogenized termite sample into tin capsules for stable isotope analysis performed in parallel to IAEA standards and several internal standard materials in a FLASH HT Plus coupled to a MAT 253 Isotope Ratio Mass Spectrometer (both by Thermo Scientific, Waltham, MA, USA) at the commercial laboratory IsoDetect GmbH in Leipzig, Germany. Stable isotope ratios of carbon and nitrogen are here expressed as the ratio of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ using the delta (δ) notation in parts per thousand or permil (‰) relative to the international standard materials Vienna PeeDee Belemite (vPDB) and atmospheric N_2 (AIR), respectively. Analytical error calculated from repetitive measurements of international and lab-internal standard materials in each run is lower than 0.2‰ (2σ) for $\delta^{15}N$ and $\delta^{13}C$.

We collected 47 additional *M. subhyalinus* samples from 12 different mounds at the Issa Valley chimpanzee field site in western Tanzania between November 2017 and May 2018 under the research permit Nº 2017-336-NA-2017-341 (Tanzanian commission for science and technology, Tanzania). These samples were identified to species level by S.P. with the support of R.S. [34]. At Issa, we primarily collected samples at mounds known to be used by chimpanzees as well as at two active mounds not observed to be used by chimpanzees but located within the chimpanzee home range [77].We recorded habitat types surrounding each mound sampled following the same protocol as mentioned above [71]. The Issa samples included termites from three separate castes (major soldiers, minor soldiers, and workers - major and minor workers not differentiated here), which we analyzed separately in order to detect potential isotopic differences within the same termite colony. We transported the samples in 85% ethanol, then dried them down and homogenized them into a fine powder using a pebble mill (Retsch MM400). We weighed between 500µg and 800µg of this powder into tin capsules for stable isotope analysis at the University of California, Santa Cruz Stable Isotope Laboratory. Isotopic and elemental composition was determined by Dumas combustion using a Carlo Erba 1108 elemental analyzer coupled to a ThermoFinnigan Delta Plus XP isotope ratio mass spectrometer and corrected towards the same international standard materials as specified above. Analytical precision of internationally calibrated in-house standards was better than 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$.

2.2.2 Mixing models to estimate C_4 plant proportions

We performed all statistical data analyses in R, version 3.4.4 (R Core Team, 2018). To determine the proportion of C_3 versus C_4 plants in the diets of *Macrotermes* termite colonies, we employed a Mixing Model based on Gaussian likelihood running Markov Chain Monte Carlo (MCMC) analyses in the R-package “siar” version 4.2 [78] on the normally distributed variables $\delta^{13}C$ and $\delta^{15}N$ values we measured in termites. We determined the mean $\delta^{13}C$ and $\delta^{15}N$ values and their standard deviations for non-reproductive parts of C_3 plant sources of various species of trees and shrubs (leaves, bark) we measured from the three of the six field sites presented here. We limited the plant data to plants found in the same habitat types from which the termite samples were derived (as shown in Tables 1 and 2). We refer here to 13 samples from Issa [8], 14 from Kayan [3,4] and five new isotope datapoints from Comoé GEPRENAF (see Appendix 2.3). Given the environmental similarities between sites, these C_3 plants resulted in remarkably similar δ^{13}C values for all three sites (mean δ^{13}C -28.6 ±1.3‰, mean δ^{15}N 2.3 ±2.3‰). Although all sites are considered savanna woodland sites and grasses, presumably C4 grasses, are abundant in the landscape, no systematic taxonomic survey of these plants was conducted. As a result, our plant reference sample contained hardly any C_4 plants we refer to the commonly accepted average δ^{13}C value for African savanna C_4 plants of -12.5±1‰ [58,59,79,80]. Given the lack of published δ^{15}N values for savanna woodland C_4 plants, we assigned the same mean δ^{15}N value for the C_4 plants as we had calculated for C_3 plants.

Table 2.2 *Macrotermes* δ^{13}C and δ^{15}N values from all sites in this study other than Issa Valley. Habitat abbreviations are as follows: CU= forest-mixed, closed understory, F= fallow, FB= forest on bamboo, FC= forest-colonizing, FR= forest on rock, GF= gallery forest, OU= forest-mixed, open understory, SH= savanna-herbs, SW= savanna-wooded. The site of Comoé GEPRENAF is here abbreviated as Comoé.

Location	**Habitat**	**δ^{13}C**	**δ^{13}C Mean**	**SD (1σ)**	**Min**	**Max**	**δ^{15}N**	**δ^{15}N Mean**	**SD (1σ)**	**Min**	**Max**
Bakoun			**-23.8**	**1.2**	**-24.6**	**-21.2**		**-0.2**	**1.3**	**-1.4**	**1.9**
Bakoun	SW	-24.5					-0.7				
Bakoun	SW	-24.1					-0.5				
Bakoun	SW	-24.1					1.3				
Bakoun	SW	-24.6					-1.1				
Bakoun	GF	-24.5					-1.2				
Bakoun	SW	-21.2					1.9				
Bakoun	SW	-23.6					-1.4				
Gashaka			**-24.7**	**1.6**	**-26.9**	**-21.6**		**-1.5**	**0.8**	**-2.3**	**0**
Gashaka	SW	-25.3					-1.8				
Gashaka	SW	-25.5					-1.4				
Gashaka	FM, OU	-24.6					-2.2				
Gashaka	FM, CU	-24.4					-2.2				
Gashaka	FM, CU	-26.9					0				
Gashaka	SW	-25.8					-2.3				
Gashaka	SW	-21.6					-0.6				
Gashaka	SW	-23.5					-1.8				
Sobeya			**-24.2**	**0.9**	**-25.1**	**-23.2**		**-0.8**	**0.7**	**-1.6**	**-0.2**
Sobeya	SW	-23.2					-0.2				
Sobeya	SW	-25.1					-0.4				
Sobeya	F	-24.3					-1.6				
Kayan			**-25.9**	**0.5**	**-26.3**	**-25.2**		**0.2**	**0.9**	**-0.5**	**1.5**
Kayan	FR	-25.2					-0.5				
Kayan	FB	-26.3					1.5				
Kayan	FB	-26.2					0.1				
Kayan	FB	-25.7					-0.4				
Comoé*			**-24.1**	**2.1**	**-27.1**	**-20.7**		**4**	**2.2**	**1.4**	**8**
Comoé	SH	-21.7					5				
Comoé	FM, OU	-25.4					8				
Comoé	SR	-25.2					1.4				
Comoé	SH	-23.3					4.3				
Comoé	SH	-20.7					1.4				
Comoé	SH	-22.6					3.7				
Comoé	FM, OU	-27.1					6.2				
Comoé	FC	-26.4					2.7				
Comoé	SW	-24.2					2.9				

To correct the mixing model for isotopic fractionation (Δ) between diet and body tissue measured, we included a trophic enrichment factor of 2.3‰ for $\delta^{13}C$ and 0.3‰ in $\delta^{15}N$ following the only published Δ-data available, at the time of analysis, for fungi-cultivating African termites [61]. We did not alter the trophic enrichment factor based on caste nor did we record the age-class of termites, although these parameters affect differential consumption of plant matter, fungus combs, and *Termitomyces* nodules [37,39] that ultimately lead to differences in $\delta^{13}C$ fractionation [40,62]. Termites are known to associate with N-recycling bacteria and contain a rich diversity of microbes in their gut aiding digestion resulting in fractionation factors that can range from –1.6‰ to + 8.8‰ in $\delta^{15}N$ and from –2.2‰ to + 3.0‰ in $\delta^{13}C$ in different termites species with different dietary specializations within the same forest [61].

2.2.3 Assessing the effects of habitat and termite caste

We ran two linear mixed models (LMM) with Gaussian error structure using the lmer-function [81]. Our models tested the effect of the predictor's habitat type and termite caste on the responses $\delta^{13}C$ and $\delta^{15}N$ values measured in individual termites. By including the random effect of individual termite mounds, we accounted for multiple measurements per termite mound [82]. We obtained p-values by conducting likelihood ratio tests comparing each full model with a null model excluding the fixed effects. We tested the variance inflation factors (vif) for each model and consistently obtained values around one. Finally, we inspected the normality and homogeneity of the residuals shown in a histogram, a qq-plot, and residuals plotted against fitted values and found no violation of model assumptions.

2.3 Results

We measured the $\delta^{13}C$ and $\delta^{15}N$ values in 79 specimens of *Macrotermes* termites from six savanna woodland sites (Table 2.1, Fig 2.1) and present the raw data as well as site averages in Table 2.2. Our mixing model estimates the relative amount of C_4 plant resources in termite diets are consistently below 15%. Average C_4 plant proportions in *Macrotermes* diet range from 5 to 15%, with the lowest C_4 proportions in Kayan, Senegal, the highest proportions at Bakoun, Guinea (Fig 2.2). The $\delta^{13}C$ values of all termites measured in this study are indistinguishable from C_3 plants and can thus not be considered a C_4 food resource. Termite $\delta^{13}C$ values across sites averaged at -24.3±1.3‰ (1σ), with the highest average $\delta^{13}C$ values found at the site of Bakoun in Guniea with a mean of -23.8±1.2‰ (1σ) (Fig 2.1) that is far from a typical C_4 plant $\delta^{13}C$ value of -12.5±1‰ [80]. Termite $\delta^{15}N$ values varied between sites, ranging from -2.3‰ to 8.0‰, demonstrating considerable differences in plant baselines between sites [3]. Comoé GEPRENAF revealed the highest mean $\delta^{15}N$ termite values (4.0±2.2‰ 1σ), whereas Gashaka in Nigeria showed comparatively low values (-1.5 ±0.8‰ 1σ).

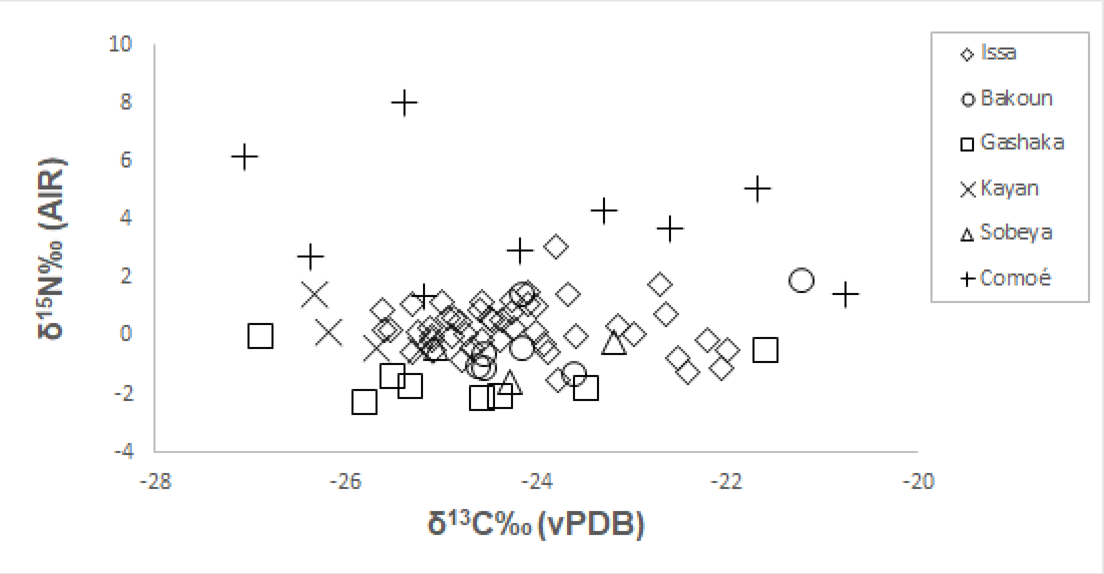

Fig 2.1 Scatter plot showing the variation in δ^{13}C and δ^{15}N values of *Macrotermes* samples across the six savanna woodland sites in this study.

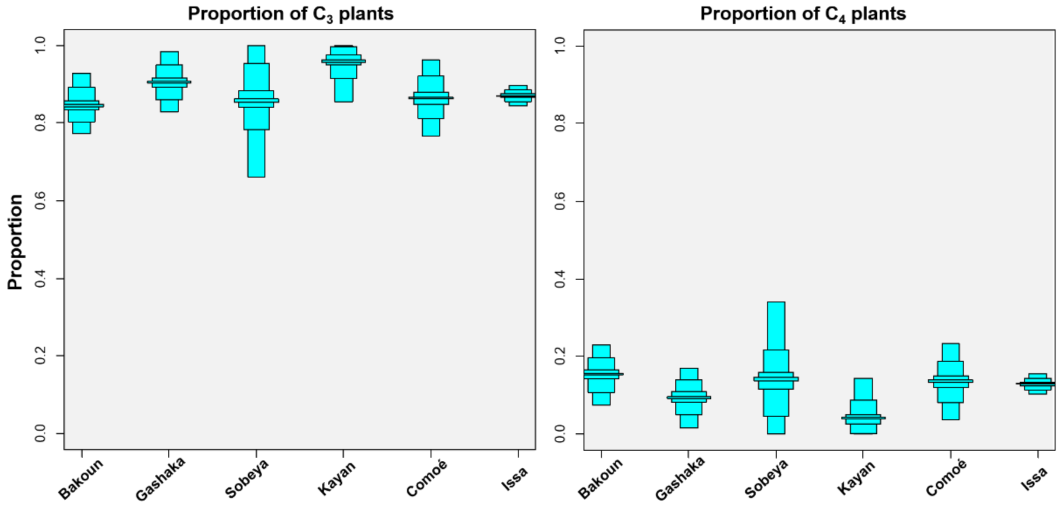

Fig 2.2. Boxplot illustrating the proportions of C_3 and C_4 plants in *Macrotermes* diets across sites as estimated by a Stable Isotope Mixed Model with credibility intervals set to 95, 75, and 25% (proportion of 1.0 = 100%).

The raw data from the Issa termites are presented separately in Table 2.3. The results of our LMMs suggest a strong influence of both fixed effects habitat and caste on the $\delta^{13}C$ values of *M. subhyalinus* specimens from 12 different mounds at Issa (χ^2= 10.4, df = 4, p < 0.001), but no effect on the $\delta^{15}N$ values (χ^2= 2.4, df = 4, p = 0.649). The effect of caste on the $\delta^{13}C$ values was highly significant (χ^2= 31.0, df = 2, p < 0.001). Estimates indicate that major soldiers are on average 0.6‰ lower in $\delta^{13}C$ than workers and on average 0.7‰ lower than minor soldiers (Fig 2.3). In the $\delta^{13}C$ model, the effect of habitat was significant (χ^2= 9.1, df = 2, p = 0.010) with estimates suggesting 1.7‰ lower $\delta^{13}C$ values in savanna woodland (miombo) areas and 0.4‰ lower $\delta^{13}C$ values in gallery forest compared to newly colonizing forest areas (Fig 2.4). We present the estimates of the $\delta^{13}C$ and $\delta^{15}N$ models in Appendix 2.1 and Appendix 2.2 Tables.

Table 2.3 *Macrotermes subhyalinus* $\delta^{13}C$ and $\delta^{15}N$ values by caste from the Issa Valley. Habitat abbreviations are as follows: CU= forest-mixed, closed understory, F= fallow, FB= forest on bamboo, FC= forest-colonizing, FR= forest on rock, GF= gallery forest, OU= forest-mixed, open understory, SH= savanna-herbs, SW= savanna-wooded.

Habitat	**Caste**	**$\delta^{13}C$**	**$\delta^{13}C$ Mean**	**SD (1σ)**	**Min**	**Max**	**$\delta^{15}N$**	**$\delta^{15}N$ Mean**	**SD (1σ)**	**Min**	**Max**
All habitats	**All castes**		**-24.2**	**1**	**-25.6**	**-22**		**0.3**	**0.9**	**-1.5**	**3.1**
FC	major soldier	-24.7					0.1				
FC	major soldier	-25.1					0				
GF	major soldier	-25.1					-0.1				
GF	major soldier	-24.2					0.8				
GF	major soldier	-25.6					0.2				
GF	major soldier	-25					1.2				
GF	major soldier	-24.9					0.6				
GF	major soldier	-25.5					0.2				
GF	major soldier	-24.8					0.5				
GF	major soldier	-25.6					0.9				
GF	major soldier	-24.1					1.1				
GF	major soldier	-25.3					1.1				
SW	major soldier	-22.4					-1.2				
SW	major soldier	-24.6					1.2				
SW	major soldier	-23.9					-0.5				
SW	major soldier	-23.8					-1.5				
All habitats	**major soldier**		**-24.7**	**0.8**	**-25.6**	**-22.4**		**0.3**	**0.8**	**-1.5**	**1.2**
FC	minor soldier	-24.6					-0.1				
FC	minor soldier	-24.4					-0.2				
GF	minor soldier	-23.9					-0.3				
GF	minor soldier	-24					1				
GF	minor soldier	-24.9					0.6				
GF	minor soldier	-24.6					0.9				
GF	minor soldier	-24.5					0.6				
GF	minor soldier	-25.1					0.3				
GF	minor soldier	-24.4					0.5				
GF	minor soldier	-22.6					0.8				
GF	minor soldier	-24					0.1				
SW	minor soldier	-22.1					-1.1				
SW	minor soldier	-23.7					1.5				
SW	minor soldier	-23.1					0.3				
SW	minor soldier	-22.5					-0.8				
All habitats	**minor soldier**		**-23.9**	**0.9**	**-25.1**	**-22.1**		**0.3**	**0.7**	**-1.1**	**1.5**

Habitat	**Caste**	**$\delta^{13}C$**	**$\delta^{13}C$ Mean**	**SD (1σ)**	**Min**	**Max**	**$\delta^{15}N$**	**$\delta^{15}N$ Mean**	**SD (1σ)**	**Min**	**Max**
FC	worker	-25.3					-0.6				
FC	worker	-24.8					-0.8				
GF	worker	-23.6					0				
GF	worker	-24.1					1.5				
GF	worker	-25.1					-0.5				
GF	worker	-24.3					1.2				
GF	worker	-24.5					0.6				
GF	worker	-25.2					0.2				
GF	worker	-24.7					-0.4				
GF	worker	-24.9					0				
GF	worker	-22.7					1.8				
GF	worker	-24.2					0.3				
SW	worker	-22					-0.5				
SW	worker	-23.8					3.1				
SW	worker	-23					0.1				
SW	worker	-22.2					-0.1				
All habitats	**worker**		**-24**	**1.1**	**-25.3**	**-22**		**0.4**	**1.1**	**-0.8**	**3.1**

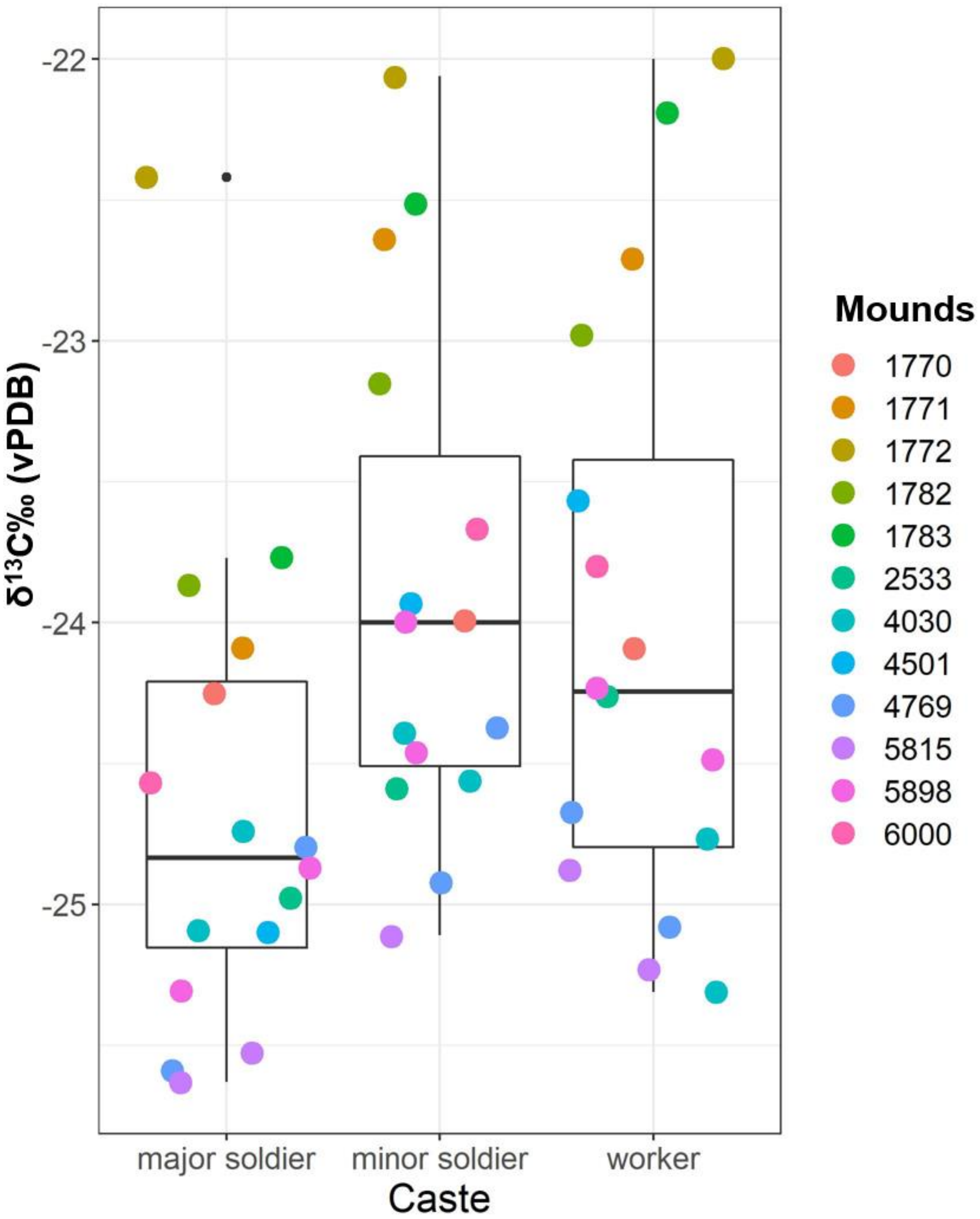

Fig 2.3 Whisker boxplot illustrating the effect of caste on the $\delta^{13}C$ values of Issa Valley *Macrotermes subhyalinus*.

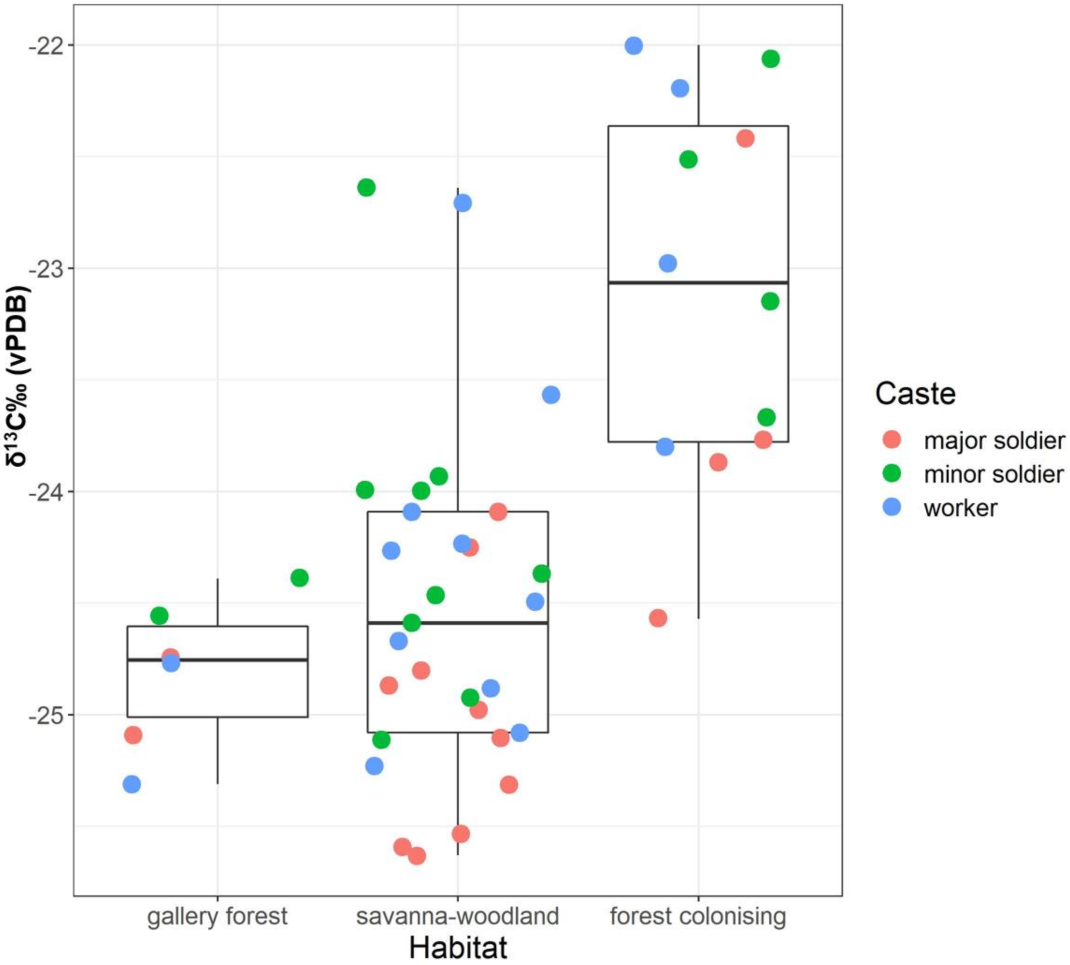

Fig 2.4 Whisker boxplot illustrating the effect of habitat type on the δ^{13}C values of Issa valley *Macrotermes subhyalinus*.

2.4 Discussion

We report here the δ^{13}C and δ^{15}N values of termites belonging to the genus *Macrotermes* from six savanna woodland chimpanzee field sites in equatorial Africa. All sites except for Gashaka in Nigeria bear evidence that chimpanzees utilize these termites as a feeding resource [67]. We interpret the considerable range δ^{15}N values reported here to be primarily a result of differences in the plant baseline values between the six sites [3]. Although these $\delta^{15}N$ values add to the published database on *Macrotermes* isotope values, our research interests are primarily concerned with $\delta^{13}C$ values which will be the focus of our discussion. Across sites we quantified the potential C_4 plant consumption in these termites and found that C_4 plants are a marginal and insignificant part of *Macrotermes* diets (5-15%, Fig 2.2). The range of *Macrotermes* $\delta^{13}C$ values (mean -24.3±1.3‰) are indistinguishable from C_3 plants and thus these termites cannot be considered a C_4 food resource (C_4 mean = -12.5 ± 1.0‰). The *Macrotermes* spp. isotope values that we report here are important in the context of subtle ^{13}C-enrichments observed in isotopic values from savanna dwelling chimpanzee populations. Relatively high $\Delta^{13}C$ values were detected in chimpanzees from Senegal, which suggests potential input of C_4 resources in chimpanzee diets [4]. Here we provide mixing models results for *Macrotermes sp.* samples collected at Kayan which suggest minimal (5%) input from C_4 vegetation (Fig 2.2), therefore positioning C_4 vegetation as an unlikely contributor to the comparatively high $\delta^{13}C$ values of -23.0‰ [3] measured in the Kayan chimpanzees. Wessling and colleagues [4] report even higher $\delta^{13}C$ values of -21.7‰ within a population of chimpanzees further to the north of Senegal, but *Macrotermes* samples from that site were not analyzed here. While it is parsimonious to assume that other chimpanzee communities in Senegal rely on *Macrotermes* consumption to similar degrees as the nearby Fongoli chimpanzees [43], our termite isotope data do not suggest that this feeding behavior will considerably affect their $\delta^{13}C$ values. The highest average $\delta^{13}C$

value of *Macrotermes* in this study come from Bakoun (-23.8‰) which is at the low end on the range of $\delta^{13}C$ values found in chimpanzees. Instead, our results support the assumption that these Senegalese chimpanzees may engage in wild C_4 plant consumption or even crop-raiding on domestic C_4 plants [4]. However, we cannot exclude the possibility that termites at other chimpanzee sites in Senegal rely more heavily on C_4 resources as they were not sampled in this study.

Our study also aimed to test the effect of caste on *Macrotermes* $\delta^{13}C$ and $\delta^{15}N$ values. *Macrotermes* worker castes feed other colony members. Workers forage for food that they may eat themselves or store in reserves. Soldiers by contrast, depend on the workers to directly feed them pieces fungal comb material [37]Additionally, the worker castes support the growth of the *Termitomyces* fungal comb with their feces which helps to promote the production of fungal nodules that are additionally consumed by some termites within the colony [37,40] . Carbon fractionation occurs in the plant matter, fungal combs, and *Termitomyces* nodules within a *Macrotermes* colony [40,62]. The consumption of these food sources varies between colony members based on caste and age-class [37,39], but the exact contributions of *Termitomyces* nodules in food processing within a mound remains uncertain [40]. We incorporated a trophic enrichment factor into our mixing model according to comparable Δ-data available at the time of analysis (but see [40]). However, our trophic enrichment factor was not altered based on caste and we did not record the age-class of termites. These uncertainties, in addition to probable differences in carbon fractionation between *Macrotermes* species, impose a possible limitation that may be considered in the context of the results from our mixing model. The data reported here from the Issa Valley, in which we collected individuals from each caste from 12 mounds, suggests that minor soldiers and workers are significantly higher in $\delta^{13}C$ than major soldiers (Fig 2.3) and that there is no detectable effect of caste on the $\delta^{15}N$ values. Young workers may subsist partly on ^{13}C-enriched *Termitomyces* nodules [37] which may account for the difference in $\delta^{13}C$ values observed between workers and major soldiers here and in previous research [40]. Differences in $\delta^{13}C$ values between major and minor soldiers has also been reported for *Macrotermes* in Kenya [40] but the underlying mechanism remains unclear as both similarly depend on being fed fungal comb material. The depletion of $\delta^{13}C$ values in major soldiers compared to other castes adds important context to the data reported for the Issa Valley.

We found that in the Issa Valley, habitat type also had a significant effect on $\delta^{13}C$, but not on $\delta^{15}N$ values. According to the habitat description protocol [71,72],"forest-colonizing" describes habitats in which a mature forest expands into a non-forest area (i.e. savanna woodland in this case), whereas "gallery forest" describes forests in direct proximity to a river. And "savanna-wooded" describes areas that are dominated by grasses or ferns but also contain significant interspersed wooded vegetation. Termites foraging within savanna wooded sites may reasonably be expected to have the highest $\delta^{13}C$ values due to the relative abundance of C_4

grasses. However, our mixing model demonstrates that Issa Valley termites within forest-colonizing habitats were more enriched in $\delta^{13}C$ than either gallery forests or savanna-wooded habitats (Fig 2.4). These results may be attributable to the “canopy effect” in which dense forest canopies produce depleted $\delta^{13}C$ values in understory vegetation less exposed to sunlight and atmospheric carbon [3,85,86]. The young and small trees from a colonizing forest segment may not cause a canopy effect as much as the mature trees in the gallery forest or even the sparse, yet larger, trees within a savanna-wooded habitat. Another possibility is that the forest-colonizing habitat provides less suitable food and the termites compensated by foraging on comparatively more C_4 sources. However, we did not conduct vegetation plots at the termite-mounds for this study and thus any causal explanations are necessarily limited.

We collected termites at various wet and dry seasons at the six sites in our study. While Issa and Gashaka samples were collected in both wet and dry seasons, the samples from the other four sites were collected during either one or the other (Table 2.1). Some chimpanzee communities preferentially feed on *Macrotermes* during the rainy season while other communities termite-fish throughout the year [66]. It is worth noting that the samples from Kayan were collected during the rainy season and that the nearby chimpanzee community at Fongoli are known to feed on *Macrotermes* throughout the year [43]. It is conceivable that these termites may have higher $\delta^{13}C$ values in the dry season if they are more dependent on C_4 grasses at that time. However, our findings suggest that *Macrotermes* at savanna woodland chimpanzee sites do not provide δ^{13}C values comparable to C_4 resources in either rainy or dry seasons.

Several scholars have proposed that termites could have been exploited by hominins with the use of tools [87,88] comparable or even more derived than what we see in chimpanzees across Africa today (summarized in [27]). Bone tool replicas used to dig into *Trinervitermes* mounds developed striation marks significantly similar to bone tool fossils discovered in Swartkrans [87]. Lesnik [88] replicated Backwell and d'Errico's 2001 method of experimental bone tool use on both *Trinervitermes* and *Macrotermes* mounds for comparison. Although not able to fully distinguish between *Trinervitermes* and *Macrotermes* wear patterns, Lesnik's analysis introduced *Macrotermes* as an appealing alternative hypothesis to *Trinervitermes* as a genus targeted by hominins. Although we cannot refute this hypothesis, our data suggests that *Macrotermes* at the six savanna woodland sites in our study are isotopically distinct from C_4 resources and thus unlikely to have contributed to the enriched δ^{13}C values found in some chimpanzees and early hominins.

Further, one should note that previous studies [58,60] which found substantially higher δ^{13}C values in *Macrotermes*, were conducted in environments outside of the range of extant chimpanzees, which suggest that these locations lack the climate and vegetation structure chimpanzees need to survive. Direct comparisons between the *Macrotermes* isotope values presented here and in previous studies are further complicated due to varying sampling methodologies. Schyra and team [60] report δ^{13}C values approximately 4-5‰ higher relative to the mean values reported here. However, only the workers were sampled in the former study which hinders comparisons given the inconsistencies in δ^{13}C between castes of the same colony reported here and elsewhere[40,62]. Still, it is unlikely that worker termites would be that dissimilar from soldiers and thus, these results are worth careful consideration to the interpretations made here. More notably divergent, however, are the values reported in Boutton, Arshad, and Tieszen's flagship study on *Macrotermes* isotope values within two Kenyan grassland habitats [58]. Among the nonreproductive castes, the δ^{13}C values reported were roughly -15‰ at Kaijado and -19‰ at Ruiru. The researchers exclusively sampled termite head tissues in that study so as to minimize isotopic variation due to sampling various body parts [58]. Again, incomparable methodologies obfuscates direct comparison to the present study in which we sampled the complete termite as various body parts differentially affect isotopic signatures.[89,90]. Additionally, as the objective of this study is to assess the isotopic value of *Macrotermes* as a food source, the samples here also include termite gut content which may have an effect on the resulting isotope values. Nevertheless, these dissimilar results from the Kenyan grassland sites add important context to the present study and further highlight the influence of habitat on *Macrotermes* diets. Still, the data presented here on whole termite bodies from extant chimpanzee habitats are likely to be more relevant to isotopic ecology of chimpanzees as well as hominins which are hypothesized to live in similar savanna woodlands environments

[91,92]. Our overall results indicate that *Macrotermes* inhabiting savanna woodland habitats in Africa can reveal C_3 plant-based diets and do not seem to uphold as a reliable source of high $\delta^{13}C$ values in chimpanzees. Our data further illustrate the value of cross-site comparisons and the importance of corresponding habitat data when considering the isotopic signatures of potential food resources in primate isotope ecology and paleodietary analyses.

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Chapter 3

A chimpanzee’s time to feast: Seasonality of *Macrotermes* flight hole activity and alate dispersal flights detected by termite-fishing experiments and camera traps in the Issa Valley, Tanzania

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Abstract

Investigation into chimpanzee termite-fishing behavior has provided invaluable insights into the evolution of animal tool use and culture. However, research to date often overlooks how the ecology of termites may shape the nature of this predator-prey interaction. Though anecdotal links between meteorological factors, termite dispersal flights and the ability of chimpanzees to termite-fish have been made, these relationships were never empirically tested. In this study, we examined if and how the reproductive ecology of mound building termites (*Macrotermes subhyalinus*) affects the availability of this resource to termite-fishing chimpanzees in the Issa Valley, Tanzania. We utilized in-person termite-fishing experiments, remote camera trap footage, as well as meteorological data to model both the availability of termite prey across seasons for chimpanzees as well as the timing of dispersal flights of the termite reproductive caste (alates). Additionally, we describe the predator diversity that characterizes alate swarming events during their seasonal dispersal flights. We identify strong relationships between meteorological factors, *Macrotermes* alate dispersal flights and vulnerability of the colony to predation. As rainfall accumulates, *Macrotermes* become more abundant in their flight holes and are thus more vulnerable to researcher termite-fishing experiments. This increased accessibility to *Macrotermes* continues until alate dispersal flights which we also find are linked to cumulative rainfall as well as negatively correlated to wind speed. This type of baseline data on *Macrotermes* ecology is fundamental when forming hypotheses and designing studies into topics such as chimpanzee culture, tool-use, and foraging cognition.

Keywords: termite-fishing, tool-use, swarming behavior, opportunistic predation, experimental primatology, *Pan troglodytes schweinfurthii*

3.1 Introduction

In 1960, Jane Goodall observed chimpanzees (*Pan troglodytes)* at Gombe stream in western Tanzania construct tools out of vegetation to use them to “fish” for termites (van Lawick-Goodall, 1968). As termite-fishing was the first evidence of non-human animals making and using tools in the wild, much research has since been devoted towards studying this behavior, particularly cultural variations in technique and learning, across chimpanzee populations (Boesch et al., 2020). Researchers assign much significance to the study of chimpanzee termite-fishing with its implications for primate cognition, culture, tool-use, and even hominin evolution. Additionally, paleoanthropologists have used chimpanzee models of termite-fishing (Lesnik, 2014) along with evidence from stable-isotope analysis (Sponheimer et al., 2005) and experimental tool use-wear analysis (Backwell and d’Errico, 2001; Gardner, 2019) to hypothesize that termite-fishing was a foraging strategy employed by hominins (*Paranthropus robustus*). However, the influence of termite prey behavior and ecology within this predator-prey interaction is often overlooked.

In fact, the reproductive life cycle and ecology of termites may represent a significant obstacle for chimpanzees to utilize this nutritious food resource. Despite the diversity of termite genera present in chimpanzee habitats, chimpanzees primarily feed on the large-bodied genus of *Macrotermes* (Lesnik, 2014). *Macrotermes* (*Macrotermitinae*) is a genus of fungus-cultivating termites distributed across Africa and South-East Asia. *Macrotermes* are considered ecosystem engineers in part for their ability to concentrate nutrient-rich soils during the construction of their large, epigeal mound structures that can rise several meters out of the earth (Abe et al., 2009; Dangerfield et al., 1998; Jouquet et al., 2006). In mature colonies, the winged-reproductive caste, known as alates, annually disperse *en mass* from the epigeal surfaces of these mounds to establish new colonies. *Macrotermes* colonies spend much of their time deep underground to avoid desiccation and predation. In preparation for seasonal dispersal flights, however, the worker castes construct passageways between the nest interior and the surface of the mound and open flight holes for the alate caste to disperse (Darlington, 1986; Mitchell, 2007). These passageways are sealed off by freshly worked soil patches constructed by the worker caste in order to minimize invasions into the colony before and after flights.

In her pioneering work with chimpanzees at Gombe, van Lawick-Goodall (1968) wrote about the connection between *Macrotermes* seasonal dispersal flights at the onset of the rainy season and the ability of chimpanzees to effectively termite-fish. However, this initial field observation was never empirically tested. McGrew and colleagues (1979) cited these early observations and found a correlation between monthly rainfall patterns and the quantity of chimpanzee tools found on termite mounds at Mt. Assirik in Senegal and Gombe (McGrew et al., 1979). The relationship between the onset of the rainy season and the onset of the presence of termite-fishing tools suggested a relationship either with termite accessibility or *Macrotermes* annual reproductive cycle, or both. However, some chimpanzee populations continue to termite-fish for *Macrotermes* despite seasonal variations in rainfall patterns (Sanz and Morgan 2013 for review). To date, though, we lack data on termite presence and accessibility throughout the annual cycle (Collins and McGrew, 1987, 1985; Koops et al., 2013; McBeath and McGrew, 1982; McGrew and Collins, 1985). Termite dispersal flights are still occasionally referenced as a potential contributing factor to seasonal patterns of termite-fishing (Sanz et al., 2004; Sommer et al., 2017). Yet the relationship between the seasonal reproductive cycle of *Macrotermes* and *Macrotermes*-fishing by chimpanzees remains poorly understood. Such data are useful for primatologists seeking to understand the availability of insect food resources similar to how phenology data are collected on important fruiting trees to provide metrics of fruit availability (Marshall and Wich, 2013). *Macrotermes* represent key sources of protein, fat, and minerals to chimpanzees that feed on them (Deblauwe and Janssens, 2008; O'Malley and Power, 2014). Thus, tracking fluctuations in the availability of this resource across seasons is a key and yet overlooked measure of resource availability in chimpanzee habitats. Additionally, identifying the environmental conditions that lead to alate dispersal flights and the corresponding influx of *Macrotermes* soldiers near mound surfaces may clarify mechanisms that contribute to observed seasonality of chimpanzee termite-fishing in some communities (Koops et al., 2013; Sanz and Morgan, 2013).

By documenting changes in availability of *Macrotermes* to chimpanzee exploitation, we can more accurately test hypotheses on tool-use variation between chimpanzee communities. The particular driving factors of the emergence, maintenance, and intraspecific variation of tool use behaviors between great ape populations are uncertain (Fox et al., 1999; Sanz and Morgan, 2013). Fox and colleagues (1999) have previously described three competing, but non-mutually exclusive hypotheses: the “necessity” hypothesis, the “opportunity” hypothesis, and the “limited-invention” hypothesis. That termite-fishing is practiced year-round by Fongoli chimpanzees is inferred to be an adaption to a lack of alternative protein sources (Bogart and Pruetz, 2011). This interpretation of the seasonality and intensity of this tool-use behavior within the Fongoli population supports the “necessity” hypothesis (Sanz and Morgan, 2013). However, to test which hypotheses best explain seasonality in termite-fishing in any chimpanzee community, we must first assess the extent to which, if any, *Macrotermes* is an ephemeral resource for a particular community of termite-fishing chimpanzees.

Termite alate dispersal flights are significant ecosystem events not only due to the alates themselves serving as a critical nutrient pulse for many animal species (Silva et al., 2021), but also due to the increased activity of the other castes close to the epigeal mound surface before and after dispersal flights, which leave them vulnerable to predation from termite-fishing chimpanzees. The ability to reliably track and observe alate dispersal patterns can therefore reveal valuable insight not only to entomologists but to ecologists, and primatologists in particular, who are interested in the availability of this food resource to higher trophic levels. Identifying which, if any, meteorological factors predict alate dispersal flights as well as the associated opening of flight holes atop mounds, is one avenue for understanding the availability of this resource. A few studies have examined the meteorological factors influencing *Macrotermes spp.* dispersal flights. Mitchell (2008) observed *M. natalensis* dispersal flights from 11 swarming seasons in Pretoria, South Africa. Rainfall was emphasized as the most important factor as a minimum 5mm threshold had to be met after a dry season for initial flights to begin. Additionally, flights occurred between 17-19°C, relative humidity range between 39%- 90%, and also occurred primarily during windless conditions (Mitchell, 2008). Neoh and Lee (2009) later reported that meteorological conditions also affected the dispersal flight timing of two sympatric *Macrotermes* species (*M. gilvus* and *M. carbonarius*) in Penang Island, Malaysia. Although both species dispersed during windless conditions, the authors emphasized interspecific differences in the timing of dispersal flights. For instance, *M. gilvus* flights generally occurred during the warmer and more humid months (March-September), before dawn, and lasted for hours. By contrast, *M. carbonarius* flights tended to occur between November-January, more often at dusk, and lasting for only a few minutes. The authors suggest these temporal niches reduce competition between these sympatric species as foraging intensity is highest during the period of alate production (Neoh and Lee, 2009; Schuurman, 2006). These studies of *Macrotermes* dispersal flights suggest the ecological shifts that trigger flights vary by region and species, and may commonly require windless conditions.

Dispersal flights are remarkable ecological phenomenon for entomologists, as well for those interested in the broader implications to the wider food web. This influx of nutrients may contribute to the increased relative abundance of some mammal species around *Macrotermes* mounds (D'Ammando et al., 2022; Fleming and Loveridge, 2003). Predator abundance may also play a role in the timing of alate dispersal flights (Neoh and Lee, 2009; Nutting, 1969). Thus, accounting for the diversity and abundance of predators during alate dispersal flights may positively contribute to an understanding of how *Macrotermes* contribute to the food web as well as the biotic pressures that may affect flight timing.

Reports on the diversity of predators at *Macrotermes* dispersal flights have been constrained by the difficulties associated with reliably observing these ephemeral events as well as possible bias introduced by human observer presence. Observations in person can take several months, and the synchrony of dispersal flights within a location makes it difficult to observe multiple dispersal flights in a season without a large team of researchers. Whilst trapping alates may be ideal for studies focused solely on recording the density and intensity of alate dispersion (Silva et al., 2021), insect trapping methods are limited in their ability to precisely chronicle dispersal flights by hour of day or observe surrounding ecological phenomena such as predation events. In the most detailed published report on predation events observed in person during *Macrotermes* flights, researchers recorded in person *M. subhyalinus* dispersal flights across two years in Kenya. The authors were able to identify multiple species including 26 bird, 3 reptiles, and 1 non-human mammal as predators during these events (Dial and Vaughan, 1987). The overwhelming bias of avian predators and relative paucity of terrestrial ones may be an artifact of researcher presence during these events, as larger bodied, terrestrial species may be more wary of approaching a termite mound with humans in the area (Lasky and Bombaci, 2023). Additionally, daytime observations of dispersal flights are overwhelmingly biased towards noting diurnal predators whereas nocturnal predators may also feed on these swarming insects. New approaches are required to observe the diversity of vertebrate predators that exploit alate dispersal flights. Remote observations made via camera-trap footage offer a unique opportunity to overcome the many limitations of human observers.

Remote camera-trapping (CT) is an important methodology for non-invasively and cost-effectively collecting ecological data. In a 2015 review of CT studies, most studies focused on mammals (94.7%), and on carnivores (64.7%) in particular (Burton et al., 2015). Recently, however, researchers have demonstrated the use of CTs to observe insect-plant interactions (McElveen and Meyer, 2020; Naqvi et al., 2022; Pegoraro et al., 2020; Steen, 2017). Naqvi and colleagues showed that both automatic and scheduled recordings significantly outperformed human observers in collecting data on insect pollinator interactions (Naqvi et al., 2022). Additionally, CT in agricultural contexts may help the monitoring of pests (Lima et al., 2020; Preti et al., 2021). Despite these examples, CT is mostly constrained in its ability to monitor insect ecology due to the size of study organisms unable to trigger camera recordings. However, CT might still prove useful to ecologists interested in observing and chronicling termite alate dispersal flights due to the large abundance of swarming insects as well as the presence of vertebrate predators able to trigger the cameras. CT could offer a chronologically precise and resource-efficient alternative to human observers for monitoring dispersal flights events simultaneously across multiple termite mounds. Additionally, CT may be able to record predator interactions during alate dispersal flights that would otherwise remain unobserved.

In this study, we utilized multi-year CT footage, meteorological data, and termite-fishing experiments at 13 *M. subhyalinus* mounds in a miombo woodland mosaic ecosystem in western Tanzania. First, we employed observational and termite-fishing experiment data (emulating chimpanzee termite-fishing behavior) on termite activity at flight holes to monitor the seasonal availability of this food resource for the termite-fishing chimpanzee population in the area (Almeida-Warren et al., 2017; Stewart and Piel, 2014). Second, we sought to identify the meteorological predictors of *M. subhyalinus* alate dispersal flights by comparing the onset of dispersal flights to meteorological data recorded on site. Finally, we documented the diversity of fauna that predate on this highly-seasonal but abundant food resource. In order to understand the role of alate dispersal events on chimpanzee termite-fishing behavior, we first need to understand the relationship between ecological conditions and both the ability for researchers to extract termites and alate dispersal events. More specifically, we sought to test the following hypotheses and predictions:

- The abundance of *Macrotermes* occupying flight holes and available to termite-fishing chimpanzees is highly seasonal given chimpanzee termite-fishing is highly seasonal at the Issa Valley. We predicted that termites within flight holes would increase during the onset of heavy rains after the dry season and decrease during the late wet season after which alates no longer disperse.

- The occurrence of *Macrotermes* dispersal flights in the Issa Valley are significantly correlated to meteorological conditions. We predicted that dispersal flights would occur more after the first 100mm of rainfall and during both windless and rainless conditions and that flights would precipitously decrease as rain continues to accumulate into the late wet season.

- Multiple species of Issa vertebrates will feed on and compete for *Macrotermes* alates during their annual dispersal flights, as had been described in previous studies of *Macrotermes* alate dispersal events.

3.2 Methods

3.2.1 Study site

The Issa Valley is a ca. 85km^2 mosaic landscape located in the Tongwe West Forest Reserve in western Tanzania. The landscape consists of steep valleys and plateaus ranging from 900-1800 meters above sea level. The mosaic of habitat types in the Issa Valley include miombo woodlands, swamps, grasslands, rocky outcrops and patches of riverine forests (Drummond-Clarke et al., 2022). The site is continuously monitored by a team of researchers. Issa Valley has marked seasonality with a distinct six month dry season (~May-October) in which rainfall is less than 100mm/month and a wet season (~November-April) that accounts for most of the annual rainfall averaging 1,245 mm per year (Piel et al., 2017). *Macrotermes* mounds are abundant in both miombo and riverine forests throughout the site. Thirteen of the fourteen colonies of the species M. *subhyalinus* in this study were taxonomically identified by SP with support by Rudolf Scheffrahn at Fort Lauderdale Research & Education Center (Phillips et al., 2021).

3.2.2 Experimental termite-fishing

We collected observational data of *Macrotermes* activity at the surface of all study mounds in order to monitor termite surface activity across multiple seasons and years (2018-2022). Fourteen termite mounds were visited twice a month between October 2018 and January 2022 to collect data on the flight hole activity (FHA) of *M. subhyalinus* colonies. We define FHA by our ability to collect termites from alate flight holes at the epigeal surface of a mound by copying chimpanzee termite-fishing techniques in a controlled experimental setting. The term FHA is distinct to the term surface activity, which has been described in the literature as any *Macrotermes* activity (e.g. foraging) by the colony above the surface but not necessarily at the epigeal mound. For our systematic termite-fishing experiments, we constructed vegetative tools in the style of Issa chimpanzees (Almeida-Warren et al., 2017; Stewart and Piel, 2014) and then attempted to emulate Issa chimpanzee termite-fishing technique and insert these tools at 30cm deep for exactly 10 seconds into 5 passageways atop of the mound for 5 trials during each mound visit. This often required first gently removing freshly laid soil patches to expose flight holes. We then noted if termites bit into the tool and were retrieved. FHA was defined as the ability of experimenters to retrieve termite prey in a trial. As a previous study suggested savanna termite activity generally increases with the rainy season (Davies et al., 2015), we increased our FHA sampling effort during the periods of October 2018-January 2019 as well as between August 2018 and January 2022 during which we visited each mound twice a week to conduct our termite-fishing experiment. For subsequent statistical analyses we included FHA as the overall proportion of successful experimental fishing trials, binned by week, as a response variable.

3.2.3 Camera traps monitoring swarming and predation events

Between 2016 and 2019, we deployed motion-triggered cameras (Bushnell Trophy Cam HD Aggressor, Bushnell CORE) in front of 13 *Macrotermes* mounds in an area of ca. 5km^2 in the Issa Valley. Seven of these cameras were in place and recorded continuously since 2016. In October 2018, six more cameras were placed in front of additional termite mounds based on direct observations of recent *Macrotermes* activity near the mound surface. All cameras were placed ca. 1m high on nearby trees and set to record in 60s intervals 24 hours a day with 1-s time intervals between videos every time motion is detected. SD cards and batteries were replaced twice monthly. All resulting camera trap videos (n= 42,384) were assessed by the authors and/or volunteer undergraduate students in the PEMA lab at University of California Santa Cruz (see Acknowledgements). We recorded all instances of alate dispersal flights detectable by numerous large (up to 45 mm) (Scheffrahn, 2008) winged insects departing in a vertical trajectory from the mound surface. In parallel to flight departure, we could often observe numerous alates moving around on the mound surface itself. These dispersal flight detections were often, but not always, associated with the presence of vertebrate predators who triggered the cameras. Any video in which one or more alate could be observed emerging from a flight hole a top the mound was considered as an alate dispersal flight for analysis. We also identified the predator type investigating and feeding on dispersing termites and differentiated diurnal (7:00h to 18:00h) from nocturnal (18:00h to 7:00h) predation events. For subsequent statistical analyses we included the presence or absence of the start of an alate swarm at a given hour as a binomial response variable.

3.2.4 Meteorological data

We collected continuous weather data to observe whether meteorological factors significantly predicted the onset of *Macrotermes* dispersal flights. An environmental weather logger (HOBO RX3000 Remote Monitoring Station) was deployed at the central field camp at Issa Valley to continuously collect meteorological data. The station tracked daily precipitation, barometric pressure, temperature, wind speed, gust speed, and relative humidity in five-minute intervals year-round since 2017. For the year 2016, only precipitation data were gathered via the environmental weather logger.

3.2.5 Data analysis

We performed all statistical data analyses and data handling in R, version 3.4.4 (R Core Team, 2018). We first explored how precipitation predicts the presence of termites at flight holes (FHA) in our sample of *Macrotermes* mounds. We considered the variable of precipitation in two ways. First, we calculated the rolling weekly average of daily total precipitation (hereafter weekly rainfall) to consider how our response FHA is influenced by ongoing precipitation. Second, we calculated the total accumulated precipitation since the onset of the previous seasonal dry spell (hereafter cumulative rainfall) to address whether a rainfall threshold triggers termite activity. We included a quadratic term for cumulative rainfall in our model because we predicted that flight hole activity would increase as rainfall accumulates initially but then decline as rain continues to accumulate into the second half of the wet season as most mounds disperse their annual brood of alates. Annual seasonal dry spells were identified as the first period of 30 or more days without daily rainfall ≥ 0.3 mm. We tested for the Pearson’s correlation coefficient to observe to what degree these two rainfall variables correlated and whether one had to be dismissed for this analysis.

To model the influence of precipitation predictors on FHA, we then built a generalized linear model (GLM) with binomial distribution and logit link function by using the glmer function in R (lme4 package, Bates et al., 2014). The response variable FHA consisted of a two-column matrix with weekly counts of successes and failures during fishing-experiments attempting to collect termites from flight holes. As we did not observe a significant correlation between our predictors of weekly rainfall and cumulative rainfall, both terms were included as covariates into the model. The covariates were log transformed to reduce the skewness in their distributions. All data used in this model can be found in Table S1. We tested for collinearity in our model by finding the variance inflation factors (VIF) in our model and we found values around one.

In a second model, we investigated whether some of the environmental predictors described by Mitchell (2008) were associated with alate dispersal flights (ADF). In particular we considered wind speed, atmospheric pressure, temperature, relative humidity, and precipitation on the outcome variable ADF similar to Mitchell 2008. Additionally, we included cumulative rainfall since the onset of latest dry spell (see above) as another predictor variable. Again, we included a quadratic term for cumulative rainfall to account for the predicted quadratic nature of our model response. Each of these meteorological variables were included as a mean value of the hourly data preceding our variable ADF. For our analysis of alate dispersal flights, we constructed a data frame including all instances when a dispersal flight was documented and the corresponding meteorological data from the preceding hour of the start time of each event. Additionally, we randomly selected one hour from each day in which no dispersal event was observed along with the corresponding meteorological variables for that hour. Since alate dispersal flights are rare events and thus can lead to non-converging models, we ran a modified GLM with a binomial distribution and logit link function using the logistf function in the logistf package ("logistf: Firth's Bias-Reduced Logistic Regression," 2022) with the argument "flic=TRUE". This function and argument reduce bias in logistic regression of rare events by a post-hoc adjustment of the intercept (Puhr et al., 2017). We systematically checked for correlations between all predictor variables. We found that relative humidity correlated with temperature and precipitation. We chose to exclude relative humidity from our model as we judged that *Macrotermes* colonies are more likely to respond to changes in temperature or direct rainfall rather than changes in relative humidity alone. The final covariates in this model were all zero-transformed and included the following: wind speed, temperature, precipitation, barometric pressure, cumulative rainfall, as well as a quadratic term for the cumulative rainfall. Our response variable was a binomial (0=no, 1=yes) whether a dispersal flight began at the hour in our data frame. All data used in this model can be found in Table S2. We tested the VIF in our model and found all values around one.

3.3 Results

3.3.1 Experimental termite-fishing

We conducted 1,924 termite-fishing trials from 2018-2022 to determine when *Macrotermes* occupied termite mound flight holes. Of these experiments, 363 were successful in extracting termites occupying flight holes. There was only one mound in which experimenters were never able to extract termites during the study period. The results of our logistic regression analysis on FHA suggested that both weekly rainfall and cumulative rainfall had significant effects on the weekly proportion of FHA (Table 3.1). FHA significantly increased as weekly rainfall increased (Log-odds= 0.12, p = .006) (see Fig 3.1). FHA was also significantly affected by cumulative rainfall (Log-odds= -0.85, p < .001). In this case, the proportion of successful experimental trials increased as rainfall accumulated until 200 mm, but decreased sharply thereafter (see Fig 3.2).

Table 3.1 Model summary of logistic regression of flight hole activity. Cumulative rainfall is the quadratic term.

	Log-odds	**Standard Error**	**p-value**
Intercept	-20.28	1.71	
Weekly Rain	**0.12**	**0.04**	**0.006**
Cumulative rainfall	**-0.85**	**0.07**	**<0.000**

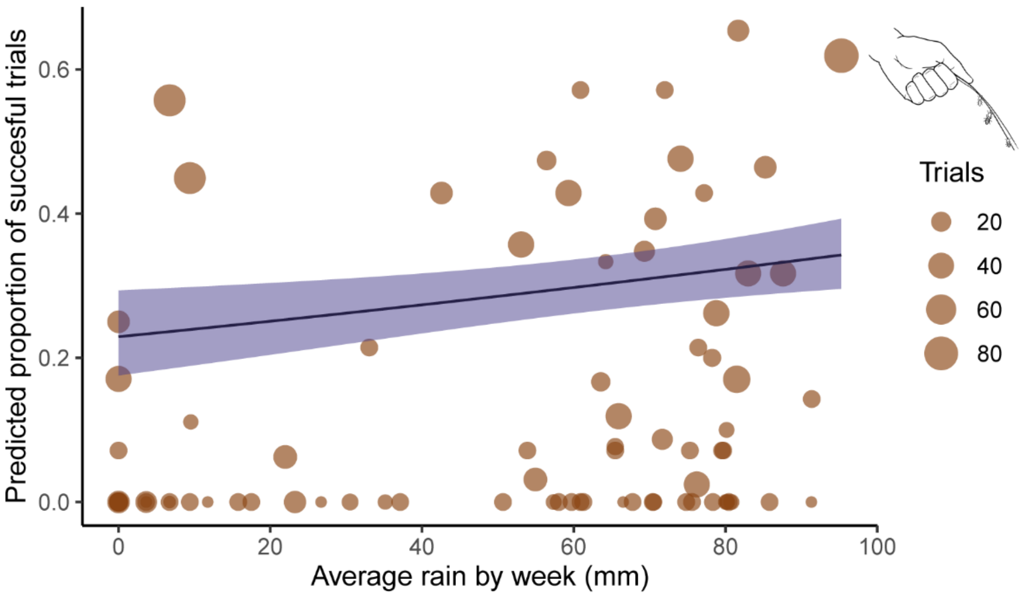

Fig 3.1 Simulation of mean weekly precipitation against proportion of termite-fishing success at flight holes. Each brown dot represents the termite fishing trials binned per week and their size represents the number of trials. The black line represents the predicted proportion of success based on 1000 randomly simulated data points when the other covariate is held at 0. Blue ribbon represents the 5 and 95 confidence intervals.

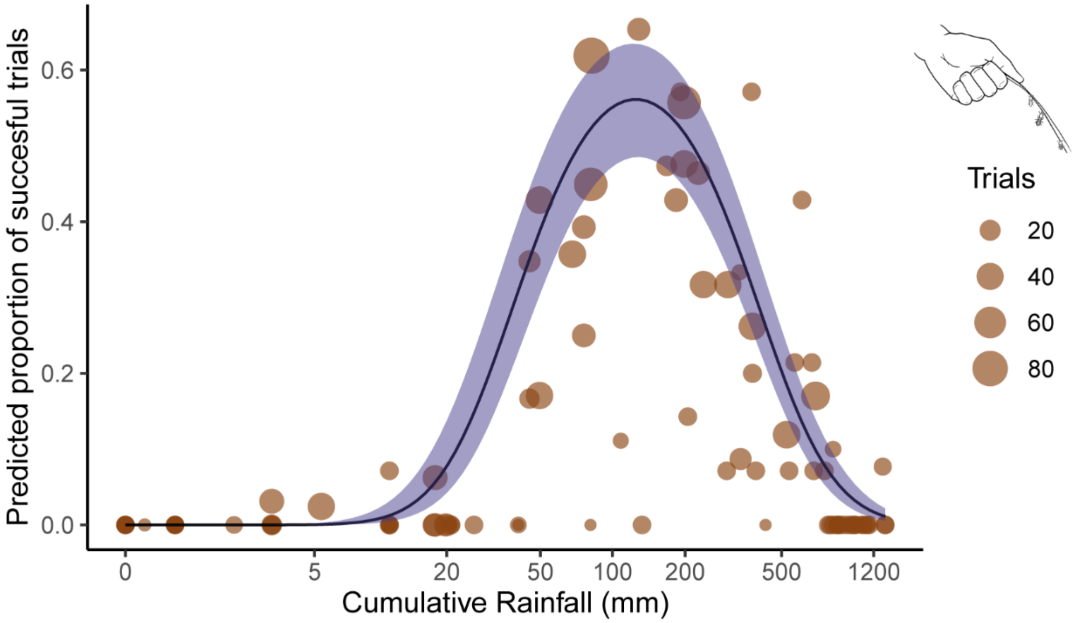

Fig 3.2 Simulation of total rain accumulation since the latest onset of a dry season against the proportion of termite-fishing success at flight holes. Each brown dot represents the termite fishing trials binned per week and their size represents the number of trials. The black line represents predicted proportion of success based on 1000 randomly simulated data when the other covariate is held at 0. Blue ribbon represents the 5 and 95 confidence intervals. Cumulative rainfall is presented on the logarithmic scale in order to retain detail of trial success in early rain accumulation while also visualizing the entire breadth of cumulative rainfall on site.

3.3.2 Alate dispersal flights

From the analysis of the camera trap footage, we observed 59 dispersal events across 44 different days. All dispersal flights occurred during the wet season. Two of the mounds had no evidence of dispersal flights during the entire study period. When a flight was captured for a mound during a given season, 76% of the time we recorded at least one subsequent flight (range 1-5) at that mound over the season. We found that wind speed and cumulative rainfall had significant effects on the timing of dispersal flights (Table 3.2). The likelihood of a dispersal flight occurring decreased significantly as wind speed increased (Log-odds= -0.88, p < .001). The likelihood of a dispersal flight occurring significantly increased initially as cumulative rainfall increased but then began to decrease after approximately 400 mm of rainfall (Log-odds= -1.06, p < .001).

Table 3.2 Model summary of meteorological effects on the likelihood of alate dispersal flights. Cumulative rainfall is included as the quadratic term.

	Log-odds	**Standard Error**	**p-value**
(intercept)	-2.09	0.28	
Wind speed	**-0.88**	**0.32**	**<0.001**
Rain	0.47	0.31	0.078
Barometric pressure	0.01	0.17	0.953
Temperature	0.28	0.19	0.130
(Cumulative rainfall)^2	**-1.15**	**0.43**	**0.001**

3.3.3 Predator diversity

We observed a total of 104 predation events with at least 18 predator species across nine orders. All observations of predator abundance and diversity are listed in Table 3.3 and specific examples are shown in Fig 3.3.

Table 3.3: Taxonomy of alate predators and number of predation events observed for *Macrotermes* dispersal flights between 2016 and 2019.

Order	*Genus*	*species*	Common	Events
Accipitriformes	*unidentified*	*unidentified*	Raptor	17
Accipitriformes	*Terathopius*	*ecaudatus*	Bateleur	1
Artiodactyla	*Philantomba*	*monticola*	Blue duiker	3
Artiodactyla	*Tragelaphus*	*scriptus*	Bushbuck	1
Bucerotiformes	*Lophoceros*	*alboterminatus*	Crowned hornbill	2
Carnivora	*Genetta*	*tigrini*	Large spotted genet	10
Carnivora	*Helogale*	*parvula*	Dwarf mongoose	1
Carnivora	*Bdeogale*	*crassicauda*	Bushy-tailed mongoose	19
Carnivora	*Genetta*	*tigrini*	Large spotted genet	10
Carnivora	*Viverridae*	*civetticus*	African Civet	4
Chiroptera	*unidentified*	*unidentified*	Bat	10
Musophagidae	*Turaco*	*rossae*	Ross's turaco	5
Primate	*Cercopithecus*	*ascanius*	Red-tail monkey	3
Primate	*Pan*	*troglodytes*	Chimpanzee	10
Primate	*Otolemur*	*monteiri*	Greater galago	1
Rodentia	*Cricetomys*	*ansorgei*	Giant pouched rat	6
Squamata	*Varanus*	*albigularis*	Rock Monitor lizard	3
Unidentified	*Turaco*	*unidentified*	Unidentified turaco	1
Unidentified	*unidentified*	*unidentified*	Unidentified bird	5

Fig 3.3 Predation events at *Macrotermes* mounds observed via camera trap footage. (A) Red tailed monkeys alongside Bushy tailed mongoose, (B) chimpanzee eating an alate with termite-fishing tool in hand, (C) rock monitor lizard, (D) African civet.

3.4 Discussion

Our findings suggest that there is a strong link between meteorological factors, particularly cumulative rainfall, and *Macrotermes* reproductive ecology. The termites' preparation for annual dispersal flights during the early onset of the wet season appears to offer Issa chimpanzees an opportunity to exploit *Macrotermes* colonies using their specialized fishing tools. We show that the dispersal flights themselves offer additional opportunities for chimpanzees, as well as a wide variety of vertebrates, to opportunistically exploit this nutrient dense resource. This study presents an empirical analysis linking seasonal *Macrotermes* ecology with the ability to extract termites in the style of one chimpanzee tool-use tradition. Similar studies at other termite-fishing chimpanzee sites are warranted to further clarify reported differences in the seasonality of this tool-use behavior.

3.4.1 Experimental termite-fishing

The first objective of this study was to identify if there is a relationship between precipitation patterns and the availability of *Macrotermes* prey for termite-fishing chimpanzees in the Issa Valley. We found that precipitation was associated with flight hole activity of *Macrotermes* both as a weekly average and as a cumulative value (Table 3.1). Given the large dispersion of data observable in Fig 3.1, it does not appear that heavy precipitation during one week is necessarily a better predictor for termite extraction compared to a week with less, but still some, precipitation. Rather, that there is at least some recent precipitation is the more reliable predictor for termite extraction (i.e. after the onset of the wet season). This indicates that FHA is much higher once the wet season has started compared to dry season experiments, which aligns with reported seasonality of termite-fishing from many (Sanz and Morgan, 2013), but not all (Bogart and Pruetz, 2011; Sanz et al., 2014) chimpanzee communities.

While the dry versus wet season distinction on FHA is important to demonstrate empirically, we gain even higher resolution on FHA seasonality by analyzing cumulative rainfall as a predictor of FHA. When comparing Fig 3.1 and Fig 3.2, it is clear that cumulative rainfall is a better predictor of FHA than weekly average rainfall. We did observe that some colonies open their flight holes and occupy them with soldiers after the initial onset of rains aligning with previous reports of *Macrotermes* pre-flight behavior (Mitchell, 2008). However, the proportion of mounds with active flight holes was highest between roughly 50-200 mm of cumulative rainfall (Fig 3.2). During this period, colony members are closer to the surface in anticipation of dispersal flights and are especially vulnerable to intrusion from predators, including termite-fishing chimpanzees. FHA then decreases during the late wet season after most alates have dispersed so as to reduce risk to the colony. By gathering data on the termite availability, we can begin to address the dynamic role termites play as part of a seasonal multi-species interaction rather than as a consistent resource for exploitation by chimpanzees.

Our results add to the discussion on observed chimpanzee tool use variation specifically by examining how ecological factors play a role in seasonality in one community. Researchers have posited that Fongoli chimpanzees will termite-fish in response to lack of other available protein sources (Bogart and Pruetz, 2011) and this explanation aligns with the "necessity" hypothesis of tool-use variation between great ape sites (Sanz and Morgan, 2013). However, in the Issa Valley, Giuliano (2022) found that both general food availability and hunting of mammalian prey by chimpanzees was highest during the transitionary period between the late dry season and early wet season or August-December (Giuliano, 2022), coinciding with peak FHA reported in this study. These results do not support a "necessity" hypothesis that termite-fishing by chimpanzees in the Issa Valley is a response to nutritional scarcity. Instead, we find that the ability for researchers to experimentally extract termites is strongly linked to *Macrotermes* behavior, itself driven by meteorological variables during early wet season. Therefore "opportunity" rather than "necessity" appears to best explain the seasonality of termite-fishing by chimpanzees in the Issa Valley. *Macrotermes* are a rewarding source of nutrition that are sought by Issa chimpanzees when they are available even in times of relative food abundance.

However, some chimpanzee communities do exploit *Macrotermes* year-round, which indicates that the temporally limiting opportunity to reliably termite-fish described in this study is not a uniform phenomenon across chimpanzee communities. Even chimpanzees at Gombe Stream, which is ~ 140 km away from the Issa Valley, are documented to successfully termite-fish year-round. Still, termite-fishing at Gombe Stream peaks in the early wet season suggesting the intensity of this behavior is also influenced by the flight preparation behavior of *Macrotermes* colonies (Goodall, 1986; McGrew, 1992). Additionally, there are communities of central chimpanzees (*P. t. troglodytes*) that utilize termite-fishing “tool-sets”, which are more sophisticated than the termite-fishing tools reported in eastern chimpanzees (*P. t. schweinfurthii*) (Sanz et al., 2014). Sanz and Morgan (2013) concluded that termite-fishing in these communities does not increase in response to preferred food scarcity. Instead, termites are considered a high-quality resource that would be energetically rewarding year-round and consistent access is suggested to be feasible due to the particular tools used by Goualougo chimpanzees in the Republic of Congo (Sanz and Morgan, 2013). As discussed above, western chimpanzees (*P. t. verus*) from Fongoli, Senegal are also known to forage for termites throughout the year but their tool-sets are not as sophisticated and consist mainly of fishing probes and rarely perforation sticks (Bogart, 2005). Our results suggest that Issa chimpanzees are unable to reliably extract *Macrotermes* year-round with their termite-fishing tools which consist only of the fishing probe (Almeida-Warren et al., 2017). Whether a more complex tool-set with perforating and/or puncturing sticks would enable year-round access is currently unclear, although preliminary experimental attempts to use robust puncturing and perforating sticks at the study mounds at Issa suggest that they would not reliably help to increase access to *Macrotermes* due to the hard ground (S. Phillips, unpublished). A “limited invention” hypothesis of tool use variation posits that tool use is simply rare and unlikely to be invented and maintained (Sanz and Morgan, 2013). Our interpretation from preliminary experiments, however, is that soil is likely to be much tougher and less penetrable in the miombo woodland ecosystem, even during the wet season, than what is found in the rainforests of the Republic of Congo. Future avenues of research could include comparative termite-fishing experiments with various tool-set traditions at sites where chimpanzees are known to predate on *Macrotermes* year-round perhaps by following a protocol inspired by Lesnik and Thackery’s experiments with bone tools at *Trinevitermes* mounds (Lesnik and Thackeray, 2007). Such experiments could further clarify documented distinctions in *Macrotermes* seasonality and the extent of its effect on chimpanzee cultural variability.

Macrotermes in the Issa Valley are only available for a brief period, but chimpanzees may improve their access with effective foraging skills. Though evidence of termite consumption at the Issa Valley is a seasonal behavior (Giuliano, 2022; Stewart and Piel, 2014), whether or not attempts to termite-fish by this community are similarly biased towards the onset of the wet season has yet to be documented. Dr. Karline Janmaat argues for the importance of measuring a primate’s inspection events as well as their *nonevents* at a resource as one way to measure a primate’s expectations for resource availability and cognitive abilities (Janmaat, 2019). Chimpanzees may use weather cues, particularly rainfall, similarly to effectively forage for termites in the Issa Valley. For instance, we can hypothesize that Issa chimpanzee termite-fishers taking cues from environmental conditions would attempt to fish after the initial onset of rains following a long dry spell and generally not during the late wet season or dry season. This use of indirect weather cues would save time and energy constructing tools, visiting mounds, and attempting to extract termites from inaccessible termite colonies. Data collection efforts to test this hypothesis in the Issa Valley are underway.

3.4.2 Alate dispersal flights

Our second objective was to test whether meteorological patterns predict alate dispersal flights. By doing so, we further clarify how seasonal meteorological patterns influence *Macrotermes* ecology and, in turn, chimpanzee exploitation of these colonies. We tested the effects of multiple meteorological predictors on *Macrotermes* ADF. We found that flights most often occurred during the first few hundred millimeters of seasonal rainfall and noticeably decline in frequency after ~ 400 mm (Fig 3.4). There are several, non-mutually exclusive, explanatory mechanisms by which cumulative rainfall predicts the dispersal of *Macrotermes* alates. The saturation of soils likely aids the ability for alates to establish subterranean colonies. Moisture hastens the development of alates in some species, and the timing of flights based on rain accumulation may synchronize with the most productive time for workers in young colonies to forage for food (Nutting, 1969; Sands, 1965). Perhaps ~200 mm of rainfall in the Issa Valley is the best time for nuptial pairing of alates to connect, reproduce, and build colonies in time for workers to forage when vegetative resources that *Macrotermes* prefer at Issa are most abundant (Phillips et al., 2021). That dispersal flights peak in a similar range of cumulative rainfall to the ability of researchers to successfully extract termites from flight holes further links chimpanzee termite-fishing ability to *Macrotermes* reproductive ecology. Researchers could consider tracking the alate dispersal flights of *Macrotermes* at other chimpanzee termite-fishing sites to test whether their reproductive cycle is correlated to peak termite-fishing intensity.

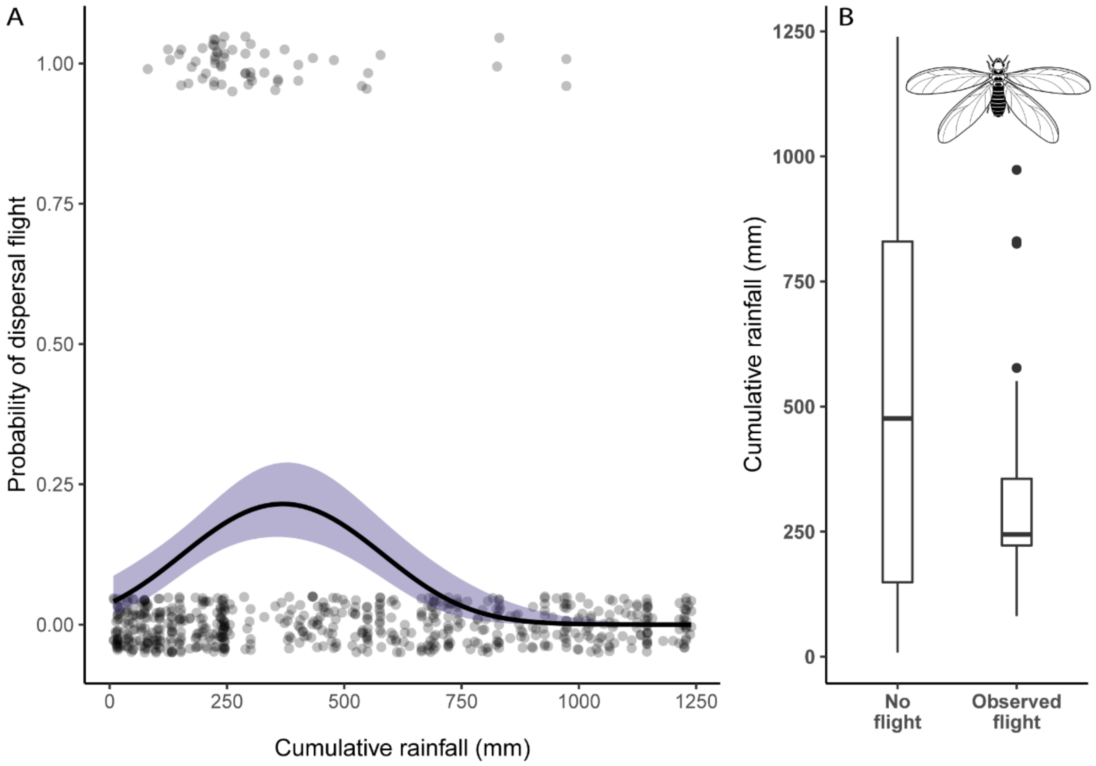

Fig 3.4 A) Scatterplot of dispersal flight binomial data with superimposed logistic regression fit and confidence intervals for the effect of cumulative rainfall. Each dot scattered around the value 0 represents no observation and each dot scattered around value 1 represents an observation of an onset of a dispersal flight. Points are jittered to visualize the number of

observations for this binary dataset. **B)** Box and whisker diagram for cumulative rainfall values. Horizontal lines represent lower 25% quartile, median, and upper 25% quartile.

Wind speed is another factor clearly relevant to the dispersal of the alate caste. Previous studies noted that *Macrotermes* flights tend to occur during windless conditions (Darlington, 1986; Mitchell, 2008; Neoh and Lee, 2009). However, this is the first study to empirically report the influence of wind speed on *Macrotermes* dispersal flight timing. Decreased wind speeds are a factor influencing termite dispersal flight timing but the phenomenon shows variability. For example, *Odontotermes formosanus* in Southern China actually dispersed during high winds, travelling as far as 743 m with the aid of prevailing wind (Hu et al., 2007). Messenger and Mullings found that *Copototermes formosanus* in New Orleans (USA) traveled as far as a kilometer with wind (Messenger and Mullins, 2005). In these cases, wind apparently aids the dispersal of termite alates by increasing the distance they can travel. However, such species appear to be the exception rather than the rule for termites. It has been previously suggested less wind may increase the likelihood of post flight pairings whereas alates that disperse during high wind conditions would likely be too scattered to reliably connect (Neoh and Lee, 2009; Nutting, 1969). In the present study, we find that *Macrotermes subhyalinus* most commonly disperse at wind speeds less than 0.5 mph and always less than 1 mph (Fig 3.5). It strikes us as unlikely that chimpanzees, or other predators, respond to a decrease in wind speed by visiting *Macrotermes* mounds in hopes of capturing alate prey given the rarity of these events.

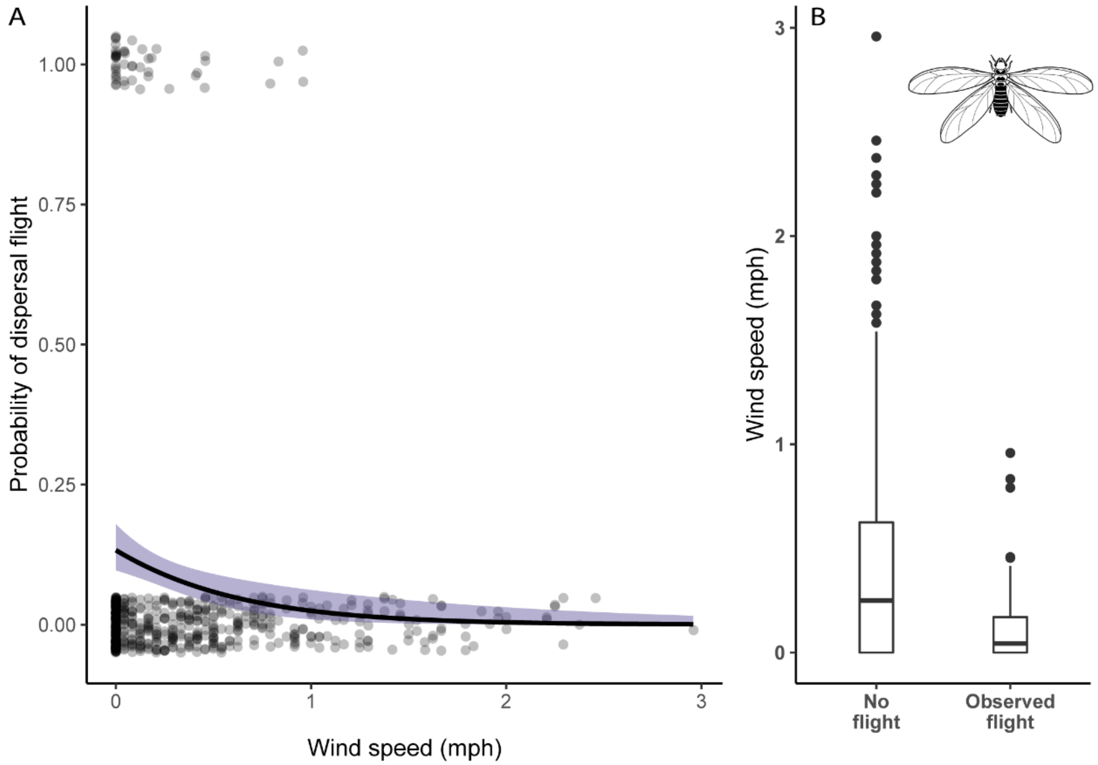

Fig 3.5 A) Scatterplot of dispersal flight binomial data with superimposed logistic regression fit and confidence intervals for the effect of wind speed. Each dot on 0 represents failure to observe onset of a dispersal flight and each dot at 1 represents a success (total n=59). Points are jittered around 0 and 1 to better visualize the number of observations for this binary dataset. **B)** Box and whisker diagram for wind speed values. Horizontal lines represent lower 25% quartile, median, and upper 25% quartile.

While our results illuminate some of the mechanisms driving dispersal flights, they surely do not account for the full picture as flights are still quite rare even under ideal circumstances (Fig 3.4 & 3.5). Notably, light and time of day is frequently considered a major factor for flight timing of many species (Mitchell, 2008; Nutting, 1969) though we did not consider those factors in this study. In addition to the abiotic factors that we considered, there are likely several biotic factors affecting ADF. Predation pressure, for instance, may influence termite flight timing (Neoh and Lee, 2009; Nutting, 1969). We can see that flights began in both nocturnal (n=27) and diurnal (n=32) conditions and thus were similarly vulnerable to both types of predators. However, we only recorded the presence of predators during these flights and did not analyze feeding duration or estimate number of alates consumed. Future studies may consider analyzing these factors from camera trap footage to gain insight on whether predator pressure may play an important role in alate dispersal flight timing.

3.4.3 Predator diversity

Our third objective in this study was to observe and document the diversity of predators of *Macrotermes* alates during dispersal flights. We observed a minimum of 19 distinct species predating on alates (Table 3.3). Among the most common predators to feed on dispersing alates was the chimpanzee (n=10). By examining alate predation events, we discovered another way in which *Macrotermes* colonies positively contribute to the diet of chimpanzees. The alate caste of *Macrotermes* may be particularly valuable to chimpanzees due to their relatively high dietary fat content compared to other insect prey options (O’Malley and Power, 2014). Generally, we observed that chimpanzees would arrive to the mounds already with a tool, indicating their intention to termite-fish, and then opportunistically feed on alates. Additionally, many other miombo woodland mammals exploit *Macrotermes* mounds during dispersal flights (Table 3.3). The high abundance of an accessible and nutrient-rich prey likely influences the diversity and frequency of broad mammalian visits to termite mounds (D’Ammando et al., 2022; Fleming and Loveridge, 2003).

Camera trap evidence of *Macrotermes* dispersal flights offer ecologists a unique opportunity to observe rare inter-specific interactions (see Fig 3.3). We observed twenty-two incidences in which two or more species of predators occurred simultaneously at a mound during a dispersal flight. Half of these species-interactions events involved bats as well as a terrestrial predator (e.g., African civet, bushy tailed mongoose, large spotted genet) concurrently feeding. However, there were eleven dispersal events in which one predator supplanted another predator on the mound. Four of these events involved a bushy-tailed mongoose that displaced a giant pouched rat and two more when the same species of mongoose displaced a large spotted genet. In another case, a group of red-tailed monkeys displaced a bushy-tailed mongoose. We documented only one incident in which a chimpanzee directly overlapped with another predator and, in this case, it supplanted a red-tailed monkey. Our camera trap evidence indicates that alate dispersal flights are an ecological phenomenon that attract many vertebrates at once creating an ecological hotspot of activity.

Our results are markedly different from those of Dial and Vaughn’s (1987), who reported on predator diversity during *Macrotermes* flights in Kenya. Most notably, they report little evidence of mammalian predators during flights observed in person while simultaneously reporting much higher bird diversity than what we found. We attribute most of this difference to the different methodologies utilized between these two studies. In-person observations would be heavily biased towards diurnal predators as well as biased against elusive species. Additionally, larger bodied wildlife are more likely to flee from humans (Lasky and Bombaci, 2023). However, our method introduces its own bias by limiting the scope of observation to animal occurrences within the view of the camera. Still, camera trap monitoring adds considerable value by providing an opportunity to assess temporal niche partitioning of both diurnal and nocturnal predators that are unlikely to occur simultaneously when human observers are present. Though our method may not be ideal for entomologists primarily concerned in the abundance of alates dispersed, ecologists benefit from broad environmental monitoring that CTs allows, and primatologists gain access to data that reveals how the seasonality of *Macrotermes* dispersal flights influence chimpanzee termite-fishing behavior.

3.4.5 Conclusion

In summary, the results of this study indicate that the seasonality of termite-fishing in the Issa Valley is likely driven in large part by the seasonal reproductive ecology of *Macrotermes,* which is in turn driven by meteorological factors, in particular cumulative rainfall. This explanation aligns with the “opportunity” hypothesis of cultural variation in chimpanzee tool use behavior (Fox et al., 1999;

Sanz and Morgan, 2013) in this case on seasonality. Similar research on *Macrotermes* reproductive life cycle is warranted at other chimpanzee termite-fishing sites in order to clarify differences in seasonal exploitation of this resource. Researchers can then further examine the appropriateness of hypotheses on tool use variation between cultures by testing whether alternative tool sets work equally as well across chimpanzee habitats in which the seasonal availability of *Macrotermes* has been described. Additionally, establishment of this resource availability enables us to closely examine chimpanzee foraging cognition by analyzing how inspection rates and strategies change across seasons. We suggest a dynamical interplay between seasonal meteorological factors, *Macrotermes* reproductive ecology, chimpanzee tool-use, and opportunistic predation events.

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Chapter 4

Wild Chimpanzees use rainfall as a cue to inspect termite mounds for fishing

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Abstract

How and when to inspect for hidden and ephemeral food sources presents a cognitive challenge for wild animals. Observational data suggests a high degree of seasonality in the chimpanzees preferred termite prey at the site of Issa, Tanzania. This allows us to consider hypotheses on chimpanzees’ ability to predict termite activity and to most efficiently termite-fish based on corresponding rainfall cues. Further, sex differences in foraging cognition, as has been observed in some measures of tool use proficiency, may be identifiable between male and female inspection efforts and efficiency at extracting hidden termite prey. We analyzed 6,806 minutes (113 hours) of camera trap videos recorded over 48 months (from 2016-2019) from termite mounds and documented chimpanzee inspection behavior in the Issa valley, western Tanzania. Our results suggest that Issa chimpanzees used rainfall cues to initiate foraging for termites and adjusted the time period in which they prepare tools in advance and inspect termite mounds. Additionally, females spent more time at termite mounds than males, but did not significantly differ from males in time spent inspecting mounds or in efficiency at extracting termite prey. Chimpanzee planning and foraging behaviors have implications for hominin decision making, forethought, and seasonal landscape utilization.

Keywords: Tanzania; Great ape; Cognition; investigation, tool-use, weather, insectivory, camera-trapping

4.1 Introduction

Many chimpanzee (*Pan troglodytes*) communities across Africa are known to make organic tools, insert them into the flight holes of mound building termite colonies, and then extract and consume the termites that latch onto the tool[1,2]. This behavior was first described by Jane Goodall in free-ranging chimpanzees (*P.t.schweinfurthii)* in Tanzania and revolutionized our perspective on tool-use traditions in non-human animals[1]. The specific techniques of this termite-fishing behavior can vary considerably between chimpanzee communities that practice it, and as such termite-fishing is considered a socially learned, cultural behavior[3,4]. Studies on the mental processes necessary to produce and use various tool sets such as those used for termite-fishing have uncovered cognitive abilities of wild chimpanzees[5,6] in addition to enriching our understanding of great ape culture[7]. For instance, some central African chimpanzees (*P. t. troglodytes*) demonstrate hierarchical organization[8], or the utilization of a logical behavioral sequence with various steps and subroutines, in their use of 2-3 distinct tools to procure underground termite prey[5,9].Complex perishable tool use such as this also has behavioral implications for early hominin behaviors and foraging opportunities[10,11].

Another important aspect to termite-fishing has received far less attention and may offer additional insight into wild chimpanzee foraging cognition: when and how do chimpanzees decide the appropriate times to termite-fish? In the Issa Valley of Tanzania, human experimenters have demonstrated that *Macrotermes* are inaccessible to chimpanzee style termite-fishing techniques throughout most of the year[12]. Termites are vulnerable to termite-fishing almost exclusively during the early onset of the wet season. During this time, the colony's worker caste construct passageways connecting chambers in the nest interior to the mound's surface for the reproductive alate caste to disperse[13,14].Following these mound dispersal events, which typically occur before 500mm of rainfall [12], successful extraction of termites using chimpanzee termite-fishing techniques was rare for human experimenters. *Macrotermes* are considered a high-value[2,15,16], ephemeral resource rather than a fallback food for this community[12,17]. Issa chimpanzees would greatly benefit from an ability to predict the availability of *Macrotermes* and adjust their time invested in exploring this valuable resource.

Field-based observational studies allow researchers to test primate cognitive abilities within a relevant naturalistic context[18,19]. Janmaat[18] asks three questions that help to establish guidelines for observational studies of wild cognition: "What do chimpanzees fail to find?", "What do chimpanzees not do?" and "Under what particular conditions do chimpanzees plan?"[18]. In her review, Janmaat outlined examples from her own work in Taï National Park (Ivory Coast) in which her and her team recorded instances of chimpanzees inspecting feeding trees that did not contain ripe fruit[20], chimpanzees approaching but not inspecting trees[21], and chimpanzees departing earlier from nests to travel to and feed on ephemeral fruits that may be subject to high competition for feeding[22]. She identified how intuitive statistics, interseason memory, and planning are necessary skills for chimpanzees to effectively forage on highly ephemeral fruit in their natural habitat[18]. With this framework, much can be learned from the non-events (i.e. bypassing a mound) of termite-fishing, the unsuccessful or “negative-bouts”[23], as well as from the conditions in which chimpanzees do prepare tools in advance, attempt, and succeed in extracting termites. Studying wild chimpanzee termite-fishing and inspection behavior offers a chance to contextualize observed cognitive skills within a relevant ecological context. Implications of such helps us identify potential adaptive pressures, such as temporality of embedded high-value foods, that may help drive the evolution of intelligence in primates[18,24].

To termite-fish at a *Macrotermes* mound, one must invest time into both tool production and inspection behavior. Teleki[25] wondered how Gombe chimpanzees in Tanzania were able to even locate flight hole passageways on the mound’s surface. In the Issa Valley, the outlines of *Macrotermes* alate flight holes, which are often covered by a thin layer of freshly worked soil, are commonly visible to human observers. Chimpanzees have been reported to employ a suite of sensory skills to locate productive termite mounds including visually scanning the mound surface[25], scratching away at freshly worked soil to expose passageways[1], and sniffing tools to detect termite pheromones after inserting it into the mound[6]. Additionally, constructing tools to termite-fish is a time-costly activity and would not be expected to occur in high frequency unless a reward is anticipated. For example, Musgrave and colleagues[26] found that chimpanzee mothers that relinquished their tools to offspring spent 33% less time termite-fishing in the immediate 30 second interval afterward. The authors interpreted this reduction in termite-fishing time as a cost to the tool-donor[26]. Tool preparation requires a degree of forethought that is regarded as more challenging, and more rare in the animal kingdom, than just using tools[6]. The majority of plants used to construct tools by chimpanzees in the Issa Valley are located more than 10 meters away, and out of sight, from the nearest termite mound[27]. Temporal differences in chimpanzee inspection effort at mounds and tool preparation can help us to elucidate when chimpanzees expect to find termite prey.

Several studies have documented a female-bias in chimpanzee tool-use efforts[23,28–30], skill acquisition[31], and even tool acquisition from their mothers[32]. Lonsdorf[31] demonstrated that young female chimpanzees at Gombe both learned how to termite-fish and became more efficient in their termite-fishing abilities at a younger age compared to males. Goodall documented that female chimpanzees at Gombe spent more time termite-fishing throughout the year as well as more time inspecting termite mounds before peak availability[23]. Goodall's findings support a hypothesis that termite-mound inspection behavior is a skill that females rely more heavily upon than males. Perhaps female chimpanzees' willingness to inspect mounds more often leads overall greater access to termites, whereas males prefer only to termite-fish when termites are most abundant[23]. Musgrave and colleagues suggest that increased seasonality of termite-fishing behavior at Gombe may accentuate differences in learning ability between the sexes[33]. Understanding the possible sex biases in inspecting and planning for termite-fishing may provide insight into the degree to which female-biased foraging skills characterizes observable chimpanzee cognition in addition to hypotheses on the role female primates may have played in the evolution of tool use[34,35]. Table 4.1 lists the null hypotheses that were tested in this study.

In the current study we sought to determine:

1. Whether rainfall predicts a chimpanzee presence and inspection behaviors on termite mounds
2. Any effect of rainfall on the success of termite-fishing for Issa chimpanzees
3. If sex influences Issa chimpanzee foraging behavior and termite-fishing efficiency at mounds

4.2 Methods

The Issa Valley study area covers ca. 85 km^2 and is located in within the Tongwe West Forest Reserve in western Tanzania. It is a savanna mosaic habitat with varied vegetation types dominated by miombo woodlands and including grasslands, and thin strips of riparian forests. There are two distinct seasons: the wet season, which typically occurs between November and April when nearly all annual rain falls, and a dry season (< 100 mm/month) occurring between May and October. The Issa chimpanzee community is known to termite-fish at the large, epigeal

Macrotermes mounds that are found throughout the different vegetation types[36]. So far, *Macrotermes subhyalinus* is the only species of *Macrotermes* that has been taxonomically identified [37], including several colonies described in this study.

At 13 *Macrotermes* mounds, we deployed motion-triggered cameras (Bushnell Trophy Cam HD Aggressor, Bushnell CORE) to monitor termite and chimpanzee behavior (for termite behavior see Phillips at al. 2023). Seven of these 13 cameras have been deployed since 2016. We deployed an additional six cameras in October 2018. We placed cameras on nearby trees approximately one meter from the ground, facing the mounds, and set them to record for 60 second intervals when triggered by motion, 24 hours a day[12,38]. Every two weeks, we replaced camera batteries and SD cards and downloaded the video data. Since 2016, we recorded local precipitation data every five minutes using an environmental weather logger (HOBO RX3000 Remote Monitoring Station) located at the research station.

For this study, we first filtered all resulting videos (n = 42,384) to examine only those that include chimpanzees (n = 9,955), which resulted in 6,806 minutes (113 hours) of video footage for analysis. Note that sometimes camera’s settings would reset and have incorrect settings for weeks in which they did not record full 60 second videos but instead recorded 5 or 15 second videos. We organized sequential videos of chimpanzee parties into distinct bouts, based on date, time, and location in which they occurred. Bouts occurring at the same location and day would be separated and classified into new bouts if there was 15 or more minutes in between videos of a chimpanzee at the same mound to try and account for the possibility that one chimpanzee party may leave before another party arrives and begins a separate inspection process within the same hour. Observations (n= 2,006) include all behaviors of an individual during a single bout or instances of an individual bypassing a mound.

We used the free open-source Behavioral Observation Research Interactive Software (BORIS) to construct our ethogram (Table 4.2 shows the full ethogram with a description of all behaviors recorded), review footage of chimpanzees at termite mounds, and carefully log events of chimpanzee termite mound inspection behavior and successful termite-fishing for data analysis. Chimpanzee termite mound inspection behavior includes anytime a chimpanzee utilizes their visual, olfactory, or tactile senses to attempt to locate a productive flight hole at a termite mound. Each behavior recorded was associated with a focal subject. We used behaviors of adult and independent subadult individuals (moving about and termite-fishing fully independently) for this study. We did not include data from dependent (i.e., unweaned) subadult chimpanzees. The models that test for possible effects of sex were carried out on a subset of the observations in which sex could be identified with certainty (n = 1,388). This resulted in 131.2 hours of focal observation data for which sex could be identified. In many instances, an individual’s behavior could not be positively confirmed during portions of the observation because only a portion of their body was visible. We removed all instances in which chimpanzees were at a mound but their exact behavior could not be determined for models testing specific termite-fishing or inspection behaviors. However, these instances were included in model 4A (described below), which tested sex bias in overall time spent at mound, if the individual's sex could be positively identified.

Table 4.1 List of null hypotheses to be tested against mixed-effect models

Model #	**Null hypotheses**
1	Chimpanzees arrive with tools regardless of rainfall
2A	Chimpanzees inspect mounds regardless of rainfall
2B	Duration of inspections does not change with rainfall
3A	Termite-fishing success is not influenced by rainfall
3B	Duration of termite-fishing bouts does not change with rainfall
3C	Termite-fishing efficiency does not change with rainfall
4A	Sex does not influence duration at mounds
4B	Sex does not influence duration inspecting mounds
4C	Sex does not influence termite-fishing efficiency

Table 4.2 Ethogram of chimpanzee behaviors at *Macrotermes* mounds analyzed in BORIS. State events are behaviors that record the duration of a behavior while point events do not record duration. Inspection behaviors include olfactory inspection, tactile inspection, and visual inspection.

Behavior	**Type**	**Description**
Fishing duration	State event	Begins after first confirmed successful dip. Ends when an individual leaves a mound or stops termite-fishing.
Inspection duration	State event	Begins when an individual employs first inspection technique. Ends after first successful dip or individual leaves.
Presence at mound duration	State event	Begins when individual arrives within approximately one meter of mound. Ends when individual leaves frame for last time.
Resting at mound duration	State event	When an individual is at a mound but is not fishing or using inspection techniques for a minute or more.
Arrival on mound with tool	Point event	Individual arrives at mound for the first time for that bout with tool already in mouth or hand.

Changing insertion in mound	Point event	When an individual changes the flight hole that they are inserting the tool into.
New tool	Point event	Individual creates or replaces a new tool (different than arriving on mound with tool)
Olfactory inspection	Point event	When an individual sniffs a tool after inserting it into the mound, sniffs their finger after touching flight hole, or sniffs flight hole directly.
Passing mound	Point event	When an individual is seen in frame but walks by the mound without stopping to employ any inspection technique or termite-fish.
Successful dip	Point event	When an individual dips a tool into the mound and successfully extracts and consumes termites confirmed by seeing them chew but not for tool modification.
Tactile inspection	Point event	When an individual intentionally removes a portion of the mound's surface with their hand or foot. Or when they scratch at one spot on the mound with their finger.
Unsuccessful dip	Point event	Each time a tool is inserted into the mound but the individual does not subsequently insert the tool into their mouth and chew.
Visual inspection	Point event	When the individual stops traveling and their gaze is either staring directly at one portion of the mound or scanning the surface.

We analyzed data in R version 3.4.4[39]. Previous research on termite ecology at Issa showed that cumulative rainfall and weekly rainfall had an effect on seasonal termite activity patterns[12]. The rainfall associated variable "weekly rainfall" is defined by a rolling average value of the daily precipitation from the seven days preceding a given observation, whereas "cumulative rainfall" is the total accumulated precipitation since the onset of the most recent dry season and the day of the observation. We checked for Pearson's correlation coefficient between these two variables to ensure that they did not correlate. We identified the onset of annual dry seasons as the first period of 30 or more days without daily aggregate rainfall ≥ 0.3 mm[12].

We ran nine regression models (see below) to test our hypotheses and better understand the relationship between precipitation and chimpanzee arrival with prepared tools, inspection behavior, and termite-fishing as well as the influence of sex on behaviors at termite mounds. All models were mixed-effect models including a random nested parameter of an individual subject within an individual mound. Three of our models were logistic regressions prompting us to use generalized linear mixed-effect models (GLMM). We utilized linear mixed-effect models (LMM) for the six non-logistic models.

Models 1 through 3C include the same independent fixed effects of weekly average rainfall, cumulative rainfall, and the random fixed effect of individual within a mound. We additionally included a quadratic term for cumulative rainfall, predicting that chimpanzee behaviors would similarly increase in frequency or duration as rain initially began to accumulate but decline as rainfall became heavy and more consistent in the late wet season. All of the rainfall covariates were zero-transformed. We considered the possibility that chimpanzees may incorporate both very recent rainfall trends (weekly average rainfall) as well as broader seasonal rainfall (cumulative rainfall) at the same time when making decisions about visiting termite mounds. Thus, for each of our models that included rainfall covariates (models 1-3C), we used ANOVA to compare the fit of model in which the rainfall parameters interact versus a model in which the rainfall parameters do not interact to determine whether the interactive model significantly outperformed the non-interactive model.

The covariates in Models 4A, 4B, and 4C differ from our preceding models in that they do not include rainfall terms. Instead, we included sex as a predictor variable along with the same random fixed-effect as before of subject at a given mound. This resulted in a smaller dataset of observations to work from than in the previous models (n = 1,388). We calculated rate of successful dips by dividing the total number of successful dips of one individual during their bout by the duration of time they spent at the mound. All dependent variables in these models were log-transformed to reduce the skewness in their distributions.

We examined the variance inflation factor (VIF) values for models with two or more fixed effects and always found values under 1.5 (Table 4.3) suggesting collinearity was not an issue. For each model with a gaussian distribution, we examined their histogram of residuals, qq plots, as well as the residual values against fitted values. No resulting plots violated normality assumptions. Additionally, the response variables for all models with gaussian distributions were log transformed to reduce skewness. For every model (1-4C), we ran an analysis of variance test (ANOVA) to compare our explanatory model with a respective null model which excluded all fixed effects and included only the random slope term of variation by chimpanzee at a specific mound.

Table 4.3: List of mixed-effect model formulas. The abbreviation avg. rain = average weekly rainfall and cml. rain = Cumulative rainfall. ANOVA tests used to compare the fit of interactive versus non-interactive models with rainfall covariates.

Model	**model formulation**	**Distribution**	**Interactive terms**	**VIFs**
1	Arrive with tool ~ avg rain * (cml rain + I(cml. rain^2)) + (1|mound / subject)	Binomial	Yes ($p = 0.012$)	< 1.3
2A	Inspect binomial ~ avg rain * (cml rain + I(cml rain^2)) + (1|mound / subject)	Binomial	Yes ($p < 0.001$)	< 1.3
2B	Inspect duration ~ avg rain + (cml rain +I(cml rain^2)) + (1|mound / subject)	Gaussian	No ($p = 0.28$)	< 1.2
3A	Fishing binomial ~ avg rain * (cml rain + I(cml rain^2)) + (1|mound / subject)	Binomial	Yes ($p < 0.001$)	< 1.5
3B	Fishing duration ~ avg rain + (cml rain +I(cml rain^2)) + (1|mound / subject)	Gaussian	No ($p = 0.808$)	< 1.2
3C	Fishing efficiency ~ avg rain + (cml rain +I(cml rain^2)) + (1|mound / subject)	Gaussian	No ($p = 0.181$)	< 1.2
4A	Duration at mound ~ sex +(1|mound / subject)	Gaussian	N/A	N/A
4B	Inspection duration ~ sex +(1|mound / subject)	Gaussian	N/A	N/A
4C	Fishing efficiency ~ sex +(1|mound / subject)	Gaussian	N/A	N/A

4.3 Results

We analyzed 2,006 observations of adult or independent immature chimpanzees at termite mounds over the four-year period 2016-2019. From these observations, we recorded a total of 165.6 hours of chimpanzee behavior at termite mounds. Four out of the six mixed-effect models with rainfall covariates suggest either weekly rainfall, cumulative rainfall, or a combination of both is associated with the probability that chimpanzees inspect a mound or succeed in extracting termite prey. Only one out of the three remaining models suggested a sex bias in how chimpanzees spend time at termite mounds.

4.3.1 Chimpanzee arrival at mound with a tool

Model 1 was significant compared to the null model ($p < 0.001$), driven by the effects of cumulative rainfall ($p < 0.001$) and the interaction between cumulative rainfall and weekly average rainfall ($p = 0.003$) (see Appendix 4.1 for details). There were 592 instances in which a chimpanzee arrived at a termite mound with a termite-fishing tool. In 323 of these instances, the chimpanzee arrived at a mound with a tool, but was not able to successfully termite-fish.

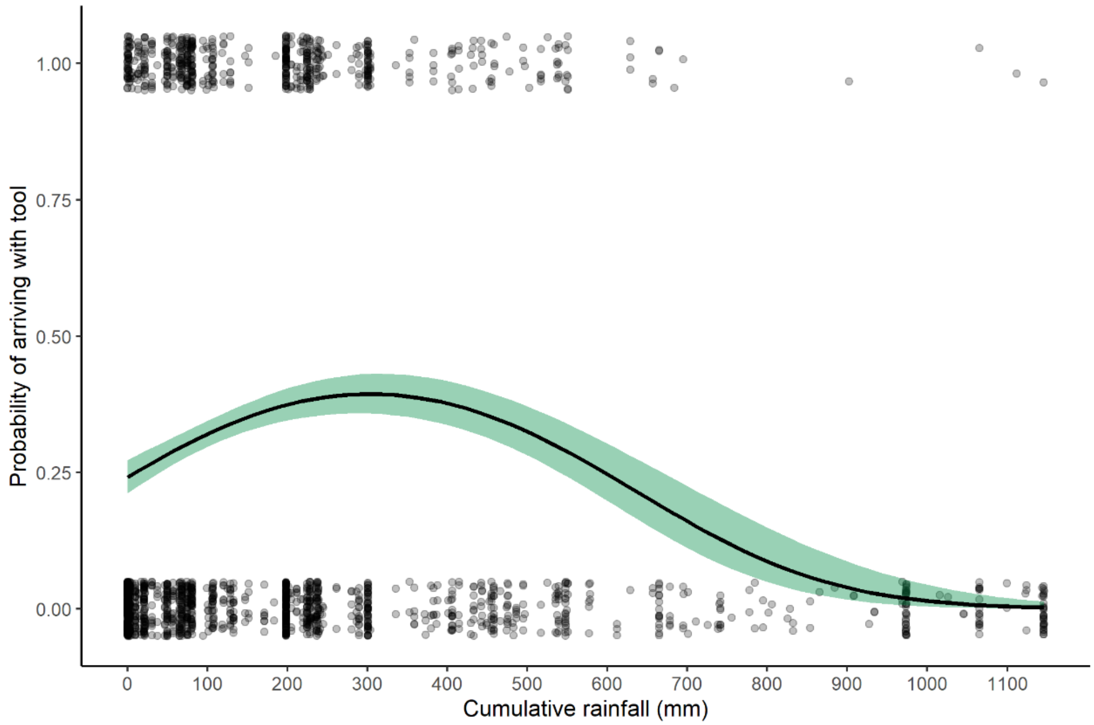

Fig 4.1 The effect of cumulative rainfall on chimpanzees arriving to termite mounds with a termite-fishing tool. Shaded band represents 95% confidence interval. Points are jittered in order to view number of observations. The probability that a chimpanzee arrived to a mound with a prepared tool rises immediately after the first seasonal rains and declines dramatically in the late wet season.

4.3.2 Chimpanzee inspection behavior

Model 2A performed significantly better than the null model ($p < 0.001$). Weekly rainfall ($p = 0.002$), cumulative rainfall ($p < 0.001$) as well as the interaction between weekly rainfall and cumulative rainfall values ($p < 0.001$) were significant predictors for the occurrence of a chimpanzee inspection behavior (See Appendix 4.2 for details). There were 789 instances in which we identified an individual to use a specific inspection technique (olfactory, tactile, or visual). In 328 of these instances successful extraction of termites occurred in 461 instances the individual left the mound before they were observed to successfully termite-fish. In some cases, a bout would begin with chimpanzees already successfully extracting termites before inspection behavior could be observed.

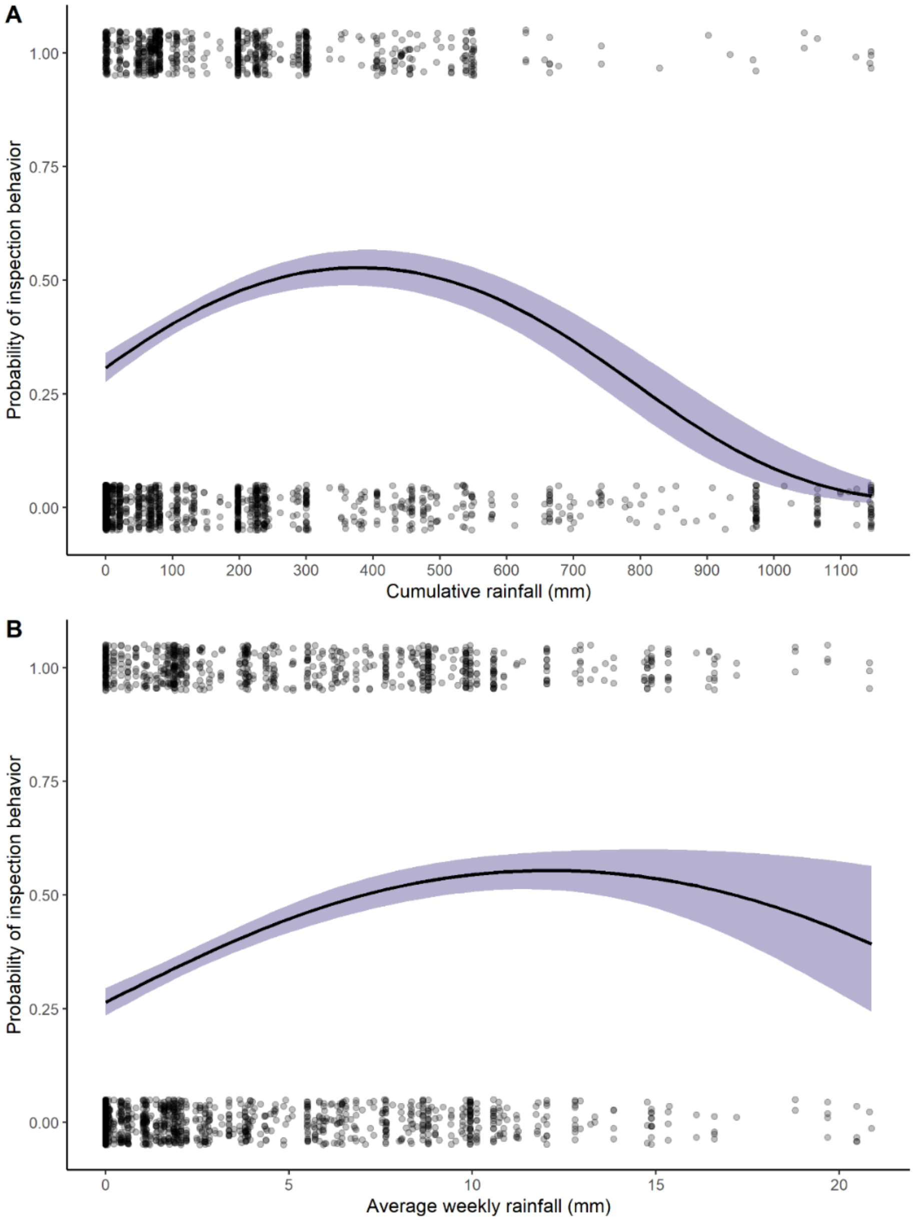

Fig 4.2 A The effect of cumulative rainfall on the binary outcome of whether a chimpanzee inspected a termite mound for termite fishing viability. **B** The effect of average weekly rainfall on whether a chimpanzee inspected a termite mound for termite fishing viability. Shaded bands represent 95% confidence intervals. Points are jittered in order to view number of observations. Interaction between rainfall terms has a significant effect on probability of inspection behavior. Probability of inspection increases with initial seasonal rains and increased average weekly rainfall. Inspection probability decreases into the late wet season and there is less precision in probability estimates at the highest range of average weekly rainfall.

Model 2B performed better than the null model (p = 0.015), driven by the effect of cumulative rainfall (p = 0.044) on the duration of inspection behaviors (See Appendix 4.3 for details). The average length of time that a chimpanzee would inspect a termite mound was 62 seconds with a standard deviation of 78 seconds. The maximum amount of time a chimpanzee spent inspecting a termite mound was 11.9 minutes.

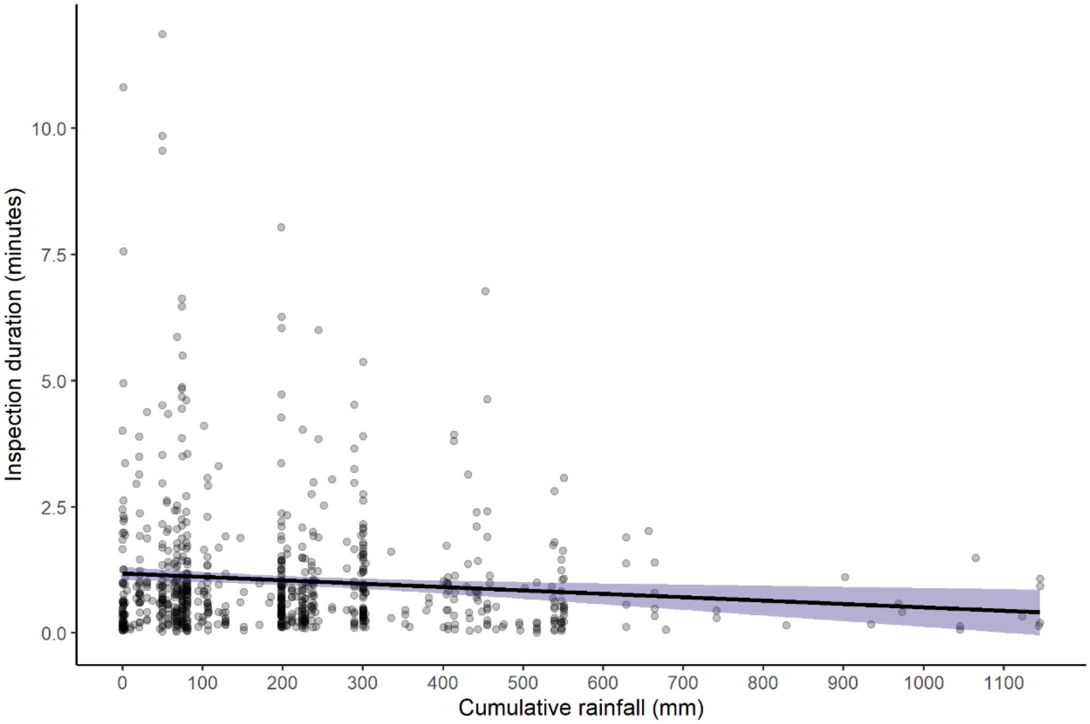

Fig 4.3 The effect of cumulative rainfall on the duration of inspection behavior at termite mounds. The shaded band represents 95% confidence interval. The duration of inspections steadily decline as rainfall accumulates into the late wet season.

Chimpanzee termite-fishing behavior

Model 3A performed significantly better than the null model ($p < 0.001$), which was driven by the significant effects of cumulative rainfall ($p < 0.001$) and the interaction between cumulative rainfall and weekly average rainfall ($p = 0.002$) (see Appendix 4.4 for details). There were 522 instances in which an individual chimpanzee had a successful termite-fishing bout.

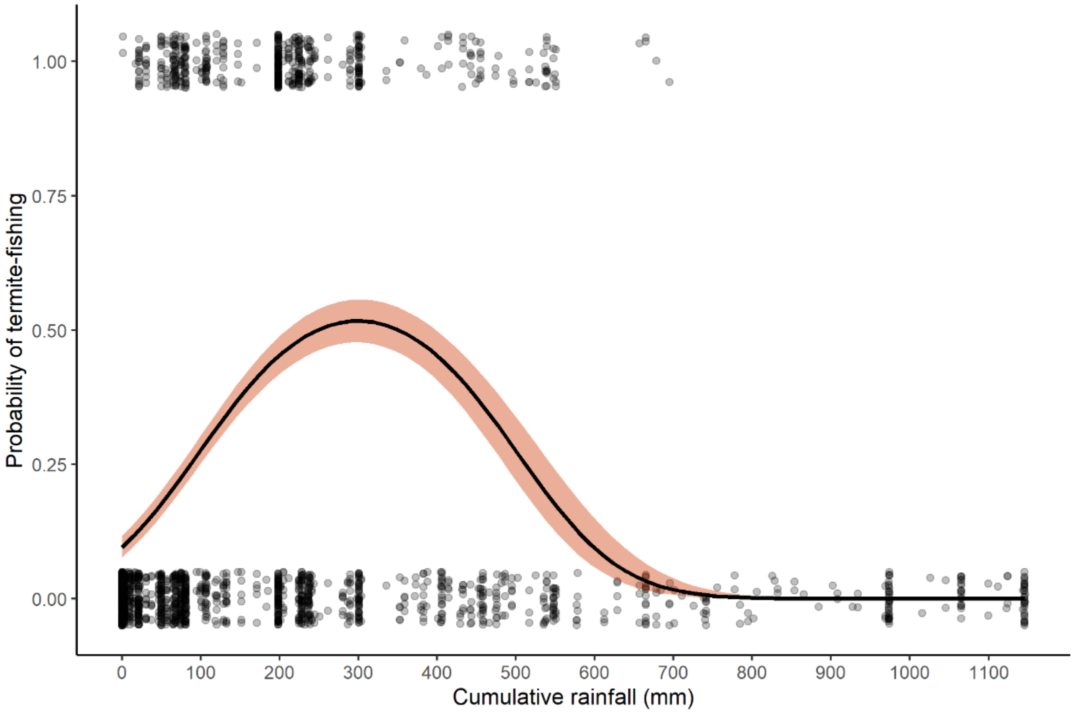

Fig 4.4 The effect of cumulative rainfall on the binary outcome of whether a chimpanzee successfully extracted termites from a termite mound. The shaded band represents 95% confidence interval. Points are jittered in order to observe number of observations. The probability for a successful termite-fishing bout to occur begins to rise after first rains of the wet season and peaks at around 300mm of cumulative rainfall.

Model 3B did not perform significantly better than the null model suggesting no effect of rainfall on the duration of termite-fishing bouts. Model 3C did not perform significantly better than the null model suggesting no effect of rainfall on the amount of successful extraction of termites within a termite-fishing bout.

Sex-bias in chimpanzee behavior at mounds

Model 4A performed significantly better than the null model ($p < 0.001$), suggesting that on average females spend more time at mounds than males, with 7.7 min versus 4.6 min respectively. Model 4B did not perform significantly better than the null model suggesting no sex bias in the duration of termite mound investigation behavior. Model 4C did not perform significantly better than the null model suggesting no sex bias in the rate at which males and females dipped successfully for termites.

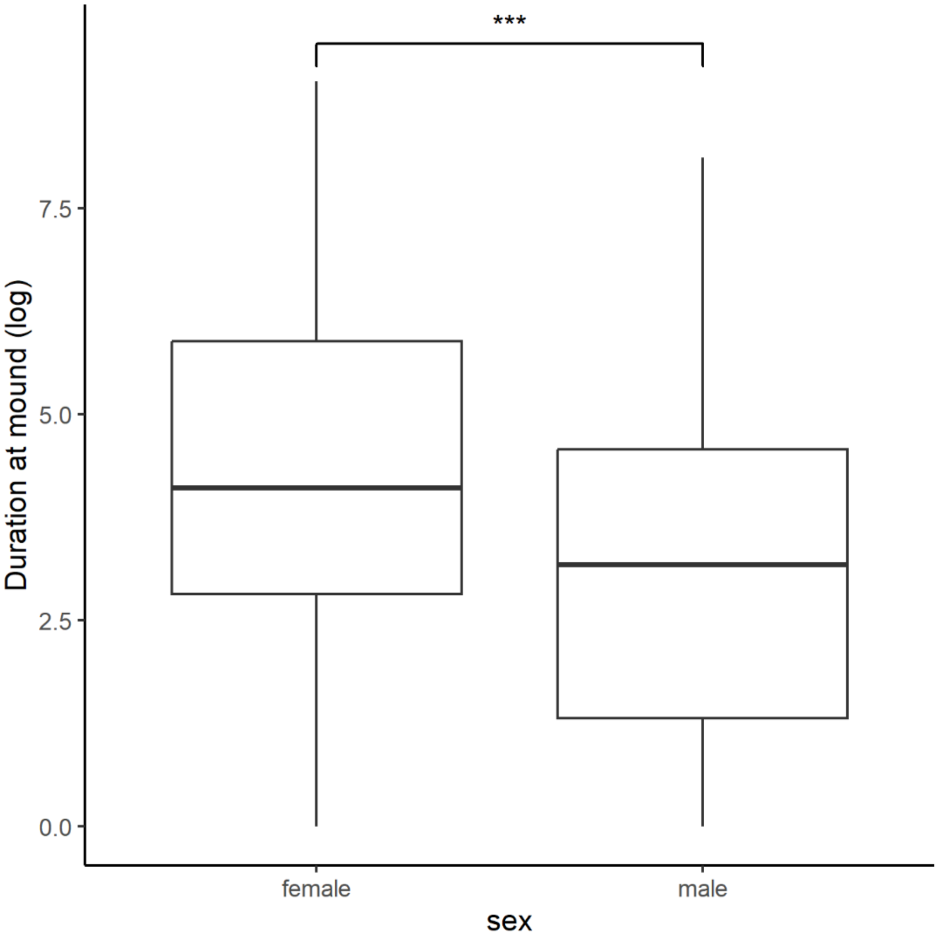

Fig 4.5 The effect of sex on the overall duration chimpanzees spend at mounds (y axis is log transformed), showing that females spend significantly more time at termite mounds than males.

4.4 Discussion

The combined results of the present study and its precursor on Issa *Macrotermes* seasonality[12] demonstrate how seasonal variation in *Macrotermes* surface activity is an obstacle that chimpanzees navigate by utilizing rainfall cues. During the late wet season and throughout the dry season, *Macrotermes* are rarely accessible at the surface of their mounds[12]. During that time, Issa chimpanzees rarely prepare tools or inspect termite mounds (Fig 4.1 & 4.2). During that time, we regularly observe chimpanzees bypassing mounds without attempting to inspect them. These non-events[18] suggest that Issa chimpanzees understand that *Macrotermes* prey are not accessible during late wet season or dry season conditions and do not invest time and energy into exploring this food resource.

Only once the rains begin, Issa chimpanzees begin to make tools, inspect termite mounds increasingly frequently, and have preliminary success in extracting termites. That there is a steep rise in inspection rates and arrivals with tools at the first onset of rains (Fig 4.1 & 4.2), but a less steep rise in successful termite-fishing bouts (Fig 4.4) suggests that chimpanzees anticipate their ability to find termites based on rainfall cues even if they are not quite yet accessible at a given mound. This can be considered an instance of "informative failing"[18] as the inspection behavior yet inability to find embedded foods provides information on the chimpanzees' expectations. These failures to termite-fish are different from the non-events prevalent in the late wet season and dry season when chimpanzees bypass mounds without stopping to inspect or attempt to termite-fish.

As rainfall further accumulates in the 50-100mm range, *Macrotermes* become more accessible at the surface of their mounds[12] and we observed an increase in the proportion of successful termite-fishing bouts (Fig 4.4). Direct observations suggest that termite-fishing behavior begins at almost the same time as the onset of

Macrotermes accessibility, rather than being related to a reduction in preferred plant food availability or hunting of mammalian protein[17]. These findings show clearly that Issa chimpanzee tool use is driven by the opportunity to gather a high value, ephemeral resource (termites) rather than the necessity to rely on fallback foods during a period of resource scarcity[4,40].

Converging analyses on the evolution of primate intelligence make the case that reliance on high-value, but time-costly resources better explain, and are more correlated to, increased cognitive capacities in primates rather than intricate social systems[24,41]. The utilization of specific weather cues to predict and forage for high value resources is a cognitive ability that has been previously identified in wild primate populations. Janmaat and colleagues[42] found that mangabeys *(Lophocebus albigena johnstonii*) in the Kibale Forest of Uganda are more likely to revisit fig trees (*Ficus* sp.) on days with higher average maximum temperatures and solar radiation. These conditions are known to influence the maturation of figs and thus the researchers concluded mangabeys are able to efficiently predict fig availability based on weather cues. Our study shows that Issa chimpanzees appear to similarly benefit from the ability to adjust their tool construction and inspection effort at termite mounds depending on rainfall conditions. This conclusion identifies the temporality of not only plant foods but also eusocial insects as a possible selective pressure contributing to cognitive adaptation as well as tool use in primates[43].

Besides these cognitive skills, the analysis of hierarchical organization and sequential behavior necessary for effective tool use has helped to illuminate cognitive abilities in previous studies[44]. Corrective guidance is a cognitive skill that refers to the ability of an individual to adjust a behavioral sequence and correct their anticipatory schema in response to cues by pausing, skipping, abandoning, proceeding, and correcting errors in sequential steps [6,8]. Close analysis of inspection behavior routines at termite mounds were outside the scope of this study. Yet this may be a fruitful avenue for future study based on the results of model 2B in which we observe a difference in the duration of inspection sequences (Fig 4.3). Chimpanzees are likely adjusting inspection sequences as a form of corrective guidance depending on various mound conditions and the outcome of previous inspections tests (e.g. sniffing tools or scratching soil, see supplementary video). Future research may illuminate the structure of these routines and whether behavioral sequences vary significantly between individuals, sexes, or communities.

Based on our findings, it is not apparent that female chimpanzees are more skilled at extracting termites in the Issa Valley or that they rely on inspection behavior more than males. Females may have a stronger preference for termites[35] compared to males and thus invest more time overall in pursuing termite prey (Fig 4.5). This is consistent with the broad conclusions of Goodall[23] who found that female chimpanzees at Gombe are more willing to forage for termites outside of times of peak availability. Yet, when overall time at mound is controlled for at Issa, females and males spent similar amounts of time inspecting mounds and successfully extracting termites. Similarly, no difference was found in mean dipping latencies, a measure of termite-fishing efficiency, between male and female chimpanzees in the Goualougo triangle[45]. We may have expected, based on Goodall's findings, that females would spend more time inspecting mounds because they could be more willing to inspect mounds during times of low termite availability. This does not appear to be the case unless higher inspection durations before peak availability are balanced by females requiring less time to locate productive flight holes during periods of high termite availability. Still, we do observe a sex-bias at Issa in overall presence at termites mounds that is consistent with a broader pattern of female chimpanzees generally investing more time into tool use behaviors[28–30,35,46].

4.4.1 *Conclusions*

Issa chimpanzees adjust their tool preparation and mound inspection efforts in response to the onset of rains after the extended dry season. By utilizing the rainfall cues to predict termite accessibility, Issa chimpanzees are rewarded with a higher probability to successfully termite-fish. Our study demonstrates that primate foraging cognition is not only useful for feeding on ephemeral and nutrient dense plant foods, but also plays and important role in predation on eusocial insects. We suggest that this ability may likely extend to other forms of foraging and species-interactions which could be explored in the future, such as hunting immature monkeys[47,48] or foraging for macrofungi[49]. In addition to tool-use[43], the utilization of weather cues may be a cognitive adaptation allowing for the exploitation of seasonally embedded, high-value food resources. Identifying these resources limited in time and/or space as well as the patterns of cognition among the primates that forage for them are important aspects for evaluating hypotheses on the evolution of intelligence in primates and other non-human animals[18,24].

Termite fishing behaviors are also of notable interest to hominin foraging patterns since their first potential linkage to *Australopithecus robustus*[50]. Stable carbon isotopic signatures among South African hominins have led to speculation that termites may have played an important dietary role for hominin populations [51,52], though these C_4 signatures are not necessarily derived from termites [2,37,53,54]. Early human populations certainly had access to termite mounds and may have utilized various tools, like chimpanzees, to access them [55]. Our results suggest that hominins would have had rainfall as a predictive cue for successful termite foraging. This nutrient-dense and low-risk resource would have been seasonally predictable and readily available as a dietary supplement, potentially for multiple species of Plio-Pleistocene hominins. And though wood tool use is a perishable technology, the multi-step and planned technological use reported here for Issa chimpanzees fits well within what Pascual-Garrido and colleagues[56] call a “perishable-to-lithic behavioral continuum” (pg.3). Documenting plant technological use and quantifying the circumstances in which these technologies are used is a primary and necessary step toward both understanding the nuanced foraging behaviors of extant chimpanzees and toward developing testable hypotheses for past hominin behaviors.

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Chapter 5

Conclusion and Future Directions

This dissertation explored the topics of *Macrotermes* dietary ecology, surface activity, and chimpanzee foraging behavior at termite mounds. Each of the preceding chapters highlight ways in which devoting research effort to the behavior and ecology of termite species have major implications to our interpretations of chimpanzee behavior as well as hypothesized foraging behavior of extinct hominins. In this chapter, I will consider some of the important conclusions from these studies and consider future directions researchers may take to further investigate open research questions.

Chapter 2 reported the carbon and nitrogen stable isotope values from six savanna-woodland chimpanzee sites across the entire range of chimpanzees in equatorial Africa. We did not find evidence that *Macrotermes* termites are contributing to comparatively high carbon isotope ratios observed in some chimpanzee communities nor are they likely to have contributed to the strong C_4-signal seen in some hominins. Though this research effort did not provide additional support for hypotheses of hominin *Macrotermes* consumption, it nevertheless contributes significant context to the field of paleodietary ecology by narrowing likely sources deriving from C_4-plants to the diets of some hominins. We can say now that evidence of *Macrotermes* consumption by hominins is not likely to be found through carbon isotope analysis.

Of course, this absence of evidence is no reason to predict hominins would have been unable or unwilling to extract this nutrient rich resource that were likely distributed in their environments. The use-wear analysis of experimental bone tool replicas have supported theories that Swartkrans material were inserted into termite mounds (d'Errico & Backwell, 2009; Gardner, 2019) and, more, could have specifically been used for perforating passageways into *Macrotermes* mounds (Lesnik, 2011, 2014). Chapters 3 and 4 provided context to paledietary hypotheses to suggest that if hominins exploited *Macrotermes* they would have been likely to face seasonal constraints on the availability of this resource and, additionally, that they could have potentially navigated these constraints by adjusting their foraging efforts in relation to rainfall cues as is seen in the Issa Valley.

It is also possible that hominins utilized more sophisticated tool sets for extraction at least than what is observed in Eastern chimpanzees (*P. t. schweinfurthii*). Whether more complex tool-sets would increase access to *Macrotermes* in a landscape such as the Issa Valley remains an open question. Chimpanzees (*P. t. troglodytes*) at the Goualougo Triangle utilize wooden tools to both puncture and perforate into termite nests allowing them access to inner chambers of nests independent of any seasonal variation in termite surface activity (Sanz et al., 2004). Would such puncture and / or perforation tools work similarly in the Issa Valley? If Goualougo style tool-sets do increase accessibility at *Macrotermes* mounds in the Issa Valley that would lend support to the limited-invention hypothesis which suggests that tool-use variation occurs because traditions are rare and thus unlikely to be either invented or maintained (Fox et al., 1999). Although use-wear experiments thus far support the theory that Swartkrans bone tools were used at termite mounds (d'Errico & Backwell, 2009), including at *Macrotermes* mounds (Lesnik, 2011) it has not been established that they serve a function beyond what can be accomplished with tool sets made of plant material used by chimpanzee communities to extract termites. Experimental comparisons between the utility of different tool technologies in increasing access to *Macrotermes* prey in the Issa Valley, or other sites in which access is restricted, could help us to further interrogate models of hominin *Macrotermes* foraging as well as debates on the diversification of technologies in the great ape clade (Fox et al., 1999; Sanz & Morgan, 2013).

During my field season in the Issa Valley from August 2021 until January 2022, I conducted a pilot study to explore the feasibility of experiments testing the utility of three different technologies for accessing *Macrotermes*. I was inspired by the approach of Lesnik and Thackery (2007) that found using bone tools to dig at *Trinervitermes* mounds was more efficient than breaking into the hard crusts of the mound with stone tools (Lesnik & Thackeray, 2007). I did my best to procure tool replicas according to available literature. I cleaned the soft tissue and grease off bone tools before using stone tools to fracturing them to size (9-19cm) (Lesnik, 2011) and located nearby sticks for perforation (min 47cm, Ø 5mm) and puncturing (41cm, Ø 10mm) according to Goualougo chimpanzee tool set specifications (Sanz et al., 2004). Twice a month, I conducted termite-fishing experiments at *Macrotermes* mounds by attempting to use each technology (fishing probe / finger, vegetative chimpanzee tool-set, and bone tool replicas) to break into the hard-outer crust of mounds. The goal was to test whether chimpanzee tool sets or bone tool replicas could increase access to termites when fingers and fishing probe techniques were unsuccessful at extracting termites.

I found it quite difficult to control for physical effort and technique. Perforation motions, that I understood to be the method Lesnik used to reproduce Swartkrans striations, turned out to be a futile effort at the surface of *Macrotermes* mounds in the Issa Valley. Instead, I tended to use the bone tools to dig into mounds in a fashion similar to how a trowel would be used. Unfortunately, the exact methods used were not rigorously controlled nor documented as I explored various ways to try to make any progress into the hard soil. There were three incidences out of over a hundred experiments in which either a bone digging tool or wooden perforation tool increased my ability to access *Macrotermes* that were otherwise inaccessible using the Issa chimpanzee technique. Neither bone tools nor perforation / puncturing tool-sets offered reliable consistent help in accessing otherwise inaccessible *Macrotermes* colonies and, furthermore, that it would be unlikely chimpanzee or hominin communities would be willing to maintain either technological tradition in this type of environment. However, this preliminary impression from a pilot study is far from conclusive. I still believe a more structured study of this nature is warranted in order to test to what extent the limited-invention hypothesis might explain variation in termite-fishing technologies observed in chimpanzee communities. I also believe such a study could help to clarify the utility, or lack thereof, of bone tools at *Macrotermes* mounds. I would encourage future researchers to invest more time and preparation into such a study as well as documenting experimental methods with video camera footage for proper data analysis.

This dissertation reports how *Macrotermes subhyalinus* at Issa Valley spend the majority of the year far underneath epigeal soil and inaccessible to experimental termite-fishing. After the onset of rains following the dry season, termites would open up flight hole passageways connecting the nest interior to the surface of the mound. During this time, usually lasting 2-3 months, termites are accessible to human researchers. After the first few hundred millimeters of rainfall and during low wind conditions, the reproductive caste of termites would disperse for their annual flights and the colony would rarely be accessible to termite-fishing until the onset of the next wet season. It is my hope that primatologists and conservationists invest into similar studies at sites across Africa. Besides the immediate benefit of tracking seasonal resource availability for an endangered primate, this type of data will be useful for researchers interested in the mechanisms underlying observed variation in tool use intensity and seasonality between communities of termite-fishing chimpanzees. Even at sites without chimpanzees, targeted placement of cameras at *Macrotermes* mounds may help conservationists track the presence and density of elusive, human-shy vertebrates that opportunistically feed on dispersing alates.

As mentioned in chapter 4, there are additional studies on chimpanzee cognition possible through close examination of the inspection techniques employed by chimpanzees at *Macrotermes* mounds. For instance, some manual skills of great apes require a cognitive ability known as “hierarchical organization” that refers to the ability to organize a behavioral sequence according to which steps logically precede each other (Boesch, 2013; Byrne, 2004). Hierarchical organization in termite mound inspections may resemble an ordered sequence such as: observing that termites are likely to be accessible given recent rainfall conditions, traveling to a mound with a tool, visually scanning the mound’s surface for termite flight holes, removing recently constructed soil patches, attempting to termite-fish, and smelling the inserted fishing probe or flight hole if unsuccessful. Inspection sequences at termite mounds is an overlooked category of behavior in chimpanzee termite-fishing studies. The identification of this behavior opens the door to future descriptive analyses and cross-cultural comparisons of this behavior in chimpanzee communities across Africa.

I began my dissertation research with the observation that extracting termites like a chimpanzee was not as straight-forward as I expected. From there I noticed a need to consider the behavior and ecology in order to properly contextualize chimpanzee termite-fishing behavior (but see also Sanz et al., 2014). In order to investigate the cognitive abilities underlying primate tool use, and discuss what these abilities can tell us about ancient hominins, we must understand the extent of the ecological obstacle being solved through tool-use. *Macrotermes* are nutrient rich (O’Malley & Power, 2014) but elusive organisms that require intelligence to successfully forage upon. It is my hope that primatologists and anthropologists will elaborate upon the research findings presented in this dissertation by considering the ways in which the particular ecology and behavior of eusocial insects present unique challenges and opportunity for apes.

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Appendices

Appendix 2.1 Model estimates, standard error (SE), and t-values for each fixed effect in the model testing for habitat and caste differences in Issa termite $\delta^{13}C$ values.

fixed effect	**estimate**	**SE**	**t**
(Intercept)	-25.262	0.606	-41.662
Habitat - gallery forest	0.408	0.646	0.632
Habitat - savanna-woodland	1.728	0.677	2.551
Caste - minor soldier	0.708	0.112	6.300
Caste - worker	0.647	0.110	5.864

Appendix 2.2 Model estimates, standard error (SE), and t-values for each fixed effect in the model testing for habitat and caste differences in Issa termite $\delta^{15}N$ values.

fixed effect	**estimate**	**SE**	**t**
(Intercept)	-0.310	0.731	-0.423
Habitat - gallery forest	0.883	0.777	1.137
Habitat - savanna-woodland	0.310	0.816	0.38
Caste - minor soldier	-0.002	0.168	-0.01
Caste - worker	0.086	0.165	0.52

Appendix 2.3 Plant stable isotope data for the site of Comoé GEPRENAF used in the stable isotope mixing models.

country	**location**	**year**	**sample**	**genus**	**species**	**habitat**	**$\delta^{15}N$‰**	**%N**	**$\delta^{13}C$‰**	**%C**
Côte d'Ivoire	Comoé GEPRENAF	2014	leaves	*Nauclea*	*latifolia*	Forest – mixed, open understorey	2.5	1.6	-29.5	30.9
Côte d'Ivoire	Comoé GEPRENAF	2014	leaves	*Dialium*	*guineensis*	Forest – mixed, open understorey	3.1	2.2	-28.8	29.6
Côte d'Ivoire	Comoé GEPRENAF	2014	leaves	*Diospyros*	*mespiliformis*	Forest – mixed, open understorey	0.7	1.3	-30.9	32.2
Côte d'Ivoire	Comoé GEPRENAF	2014	leaves	*Nauclea*	*latifolia*	Savannah – herbs	1.7	1.4	-29.5	29.7
Côte d'Ivoire	Comoé GEPRENAF	2014	leaves	*Ceiba*	*penthandra*	Gallery forest	1.6	1.9	-28.2	28.6

Appendix 4.1 Model estimates, standard error, z values, and p-values for Model 1 described in chapter 4. The abbreviations avg. rain = average weekly rainfall and cml. rain = Cumulative rainfall. “z” indicates fixed effects were zero-transformed

Fixed effects:	*Estimate*	*Std. Error*	*z value*	*Pr (>|z|)*
(Intercept)	-0.54045	0.244888	-2.20693	2.73E-02
z.avg.rain	0.228196	0.072229	3.159366	1.58E-03
z.cml.rain	0.169929	0.117935	1.440876	1.50E-01
I(z.cml.rain^2)	-0.22532	0.061197	-3.68185	2.32E-04
z.avg.rain:z.cml.rain	-0.45373	0.114538	-3.96134	7.45E-05
z.avg.rain:I(z.cml.rain)	0.168552	0.07025	2.399325	1.64E-02

Appendix 4.2 Model estimates, standard error, z values, and p-values for Model 2A described in chapter 4. The abbreviations avg. rain = average weekly rainfall and cml. rain = Cumulative rainfall. “z” indicates fixed effects were zero-transformed.

Fixed effects:	*Estimate*	*Std. Error*	*z value*	*Pr (>|z|)*
(Intercept)	-0.54045	0.244888	-2.20693	2.73E-02
z.avg.rain	0.228196	0.072229	3.159366	1.58E-03
z.cml.rain	0.169929	0.117935	1.440876	1.50E-01
I(z.cml.rain^2)	-0.22532	0.061197	-3.68185	2.32E-04
z.avg.rain:z.cml.rain	-0.45373	0.114538	-3.96134	7.45E-05
z.avg.rain:I(z.cml.rain)	0.168552	0.07025	2.399325	1.64E-02

Appendix 4.3 Model estimates, standard error, and t values for Model 2B described in chapter 4. The abbreviations avg. rain = average weekly rainfall and cml. rain = Cumulative rainfall. “z” indicates fixed effects were zero-transformed.

Fixed effects:	*Estimate*	*Std. Error*	*t value*
(Intercept)	3.58043	0.08175	43.795
z.avg.rain	0.06194	0.04256	1.455
z.cml.rain	-0.1613	0.07959	-2.027
I(z.cml.rain^2)	-0.00689	0.0407	-0.169

Appendix 4.4 Model estimates, standard error, z values, and p-values for Model 3A described in chapter 4. The abbreviations avg. rain = average weekly rainfall and cml. rain = Cumulative rainfall. “z” indicates fixed effects were zero-transformed.

Fixed effects:	***Estimate***	***Std. Error***	***z value***	***Pr (>|z|)***
(Intercept)	-0.7814	0.4087	-1.9119	5.59E-02
z.avg.rain	0.053682	0.08289	0.647623	5.17E-01
z.cml.rain	1.069739	0.143554	7.45181	9.21E-14
I(z.cml.rain^2)	-1.55012	0.183629	-8.4416	3.13E-17
z.avg.rain:z.cml.rain	-0.58222	0.14917	-3.90309	9.50E-05
z.avg.rain:I(z.cml.rain)	0.43323	0.140688	3.079368	2.07E-03

List of supplementary files

Table S1 can be found at https://doi.org/10.3389/fevo.2023.1289433

Table S2 can be found at https://doi.org/10.3389/fevo.2023.1289433

Supplementary video can be found at https://doi.org/10.5281/zenodo.11582806

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