Water Scarcity, Distribution, and Quality as Drivers of Lemur Behavior

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Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Evolutionary Anthropology in the Graduate School of Duke University

2019
ABSTRACT

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Abstract

Because water is essential for life, when it is scarce, it may be one of the most important drivers of animal behavior. Despite its clear importance, water is relatively poorly studied in terms of its impact on primate behavior, and previous research has been limited to observational studies. This dissertation takes a combined experimental and observational approach to study behavior related to water acquisition in captive and wild lemurs. Specifically, I investigated how several dimensions of water sources influence lemur behavior, including their parasite transmission risk, spatial distribution, and predation risk. In experiments, I manipulated the fecal contamination of water with several species of lemurs in captivity at the Duke Lemur Center, and found that lemurs strongly preferred clean to feces-contaminated water in captivity (Chapter 2). I expanded this initial study to a more comprehensive examination of the impact of water scarcity on the behavior of wild red-fronted lemurs (Eulemur rufifrons) in Kirindy Forest, Madagascar. This wild study population requires drinking water to survive, but water is extremely limited during the dry season. I experimentally manipulated water availability in the habitat by introducing artificial water sources, and I tracked how changes to the distribution of water influenced the ranging patterns of the lemurs recorded by GPS collars. Lemur groups shifted the intensity of their habitat use relative to natural and experimental water availability (Chapter 3). Using a similar experimental
approach to the study in captivity, I determined that wild, water-limited lemurs also preferred to drink clean water. Based on lemur groups’ selection of natural water sources as measured with camera traps, wild lemurs also selected water sources with lower fecal contamination more frequently, but with some constraints. Lemurs were more likely to return to waterholes and returned to them after shorter time intervals when they had lower levels of fecal contamination in the areas around them. However, lemurs’ natural waterhole choices reflected that fecal contamination was a secondary factor determining water source selection, behind travel distance (Chapter 4). Finally, I examined how predation risk, i.e. the presence of fossa (Cryptoprocta ferox) and Madagascar harrier hawks (Polyboroides radiatus), influenced red-fronted lemurs’ spatiotemporal patterns of waterhole use. Red-fronted lemurs used waterholes at times of day when predators were least likely to be present (Chapter 5). This study, with its combined experimental and observational approach, identifies water as an important factor that shapes wild primate behavior. I found that lemurs were flexible in their responses to changes in water distribution, parasite risk, and predation risk. I suggest that water should be the focus of future behavioral research in primates, especially given the relevance of water for human evolution and the potential for climate change and human land use to further alter water availability.
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1. Introduction

1.1 Adaptations for water acquisition

Water is among the most essential nutrients for animal survival and reproductive success. Yet, remarkably, animals manage to inhabit even the most extremely water-scarce habitats (Schmidt-Nielsen & Schmidt-Nielsen, 1952). Although physiological adaptations support the survival of some animals in arid habitats, many adaptations that facilitate water acquisition in dry habitats are behavioral. Water is acquired from several possible sources: “metabolic” water is produced via oxidation of organic compounds, “preformed” water is ingested as a component of food, and “free” water is ingested independently (Chew, 1965; Frank, 1988; Jéquier & Constant, 2010). How much water is acquired through each of these routes may be influenced by physiological water requirements, environmental variables, and behavioral traits.

Some mammals can survive without drinking free water, acquiring all their water from within foods and via metabolism, and they prefer foods that will provide the greatest quantity of preformed water or have the greatest potential to produce metabolic water (Frank, 1988; Knut Schmidt-Nielsen, 1964). For example, desert-adapted bannertail kangaroo rats (Dipodomys spectabilis) adjust their food preferences with changes in humidity to maximize metabolic water production (Frank, 1988). Merriam’s kangaroo rats (Dipodomys merriami) increase the preformed water content of the seeds that comprise their diet by caching them in burrows with higher humidity prior to
Ingestion (Nagy & Gruchacz, 1994). Ringtailed lemurs (*Lemur catta*) in spiny desert habitats exploit invasive prickly pear cacti (*Opuntia spp*.), a food item with high preformed water content (Kelley, 2011; Kelley, 2013).

Animals that have water requirements exceeding water acquired metabolically and via food must drink free water. Free water can take either decentralized forms that may be more commonly available in rainy habitats, such as wet leaves, wet bark, or collection in treeholes (Sharma et al., 2016), or more centralized forms, such as streams, rivers, or waterholes (Barton, Whiten, Strum, Byrne, & Simpson, 1992; Rose & Fedigan, 1995; Scholz & Kappeler, 2004). Which of these forms of water is most commonly used may be based on a number of factors, including substrate use patterns, predator risk, and availability of water in these various forms in the habitat (Sharma et al., 2016). In this dissertation and this introductory chapter, I focus on centralized free water sources, such as waterholes. Here, I review the evidence that water distribution and quality influence primate behavior, consider how waterholes might offer a unique opportunity to investigate several ecological risks, and argue that empirical investigations of the role of water in human and primate evolution should represent a high priority in evolutionary anthropology.

### 1.2 Impact of water on primate behavior

Early descriptive studies of wild primates living in dry habitats commonly detailed behaviors related to water as an integral part of a species’ ecology. For example,
Kummer described that hamadryas baboons (*Papio hamadryas*) frequently dug holes in the river bed with their hands, creating pools of water that they could drink from (Kummer, 1968). In his observations, Kummer also detailed what he perceived to be an increased predator risk at waterholes, especially those with nearby vegetation that could easily hide a predator (Kummer, 1968). Altmann & Altmann included a chapter in their account of *Papio cynocephalus* ecology titled “Water and Food,” in which they described social interactions at waterholes, predator vigilance behaviors, frequency of drinking, and temporal patterns of drinking throughout the day and across seasons (S. Altmann & Altmann, 1970). In their preliminary descriptions of patas monkey (*Erythrocebus patas*) behavior, Struhsaker and Gartlan noted that during the peak of the dry season, the few available waterholes served as hubs of intergroup encounters, opportunities for changes in male harem leadership, and interspecific interactions, including with predators (Struhsaker & Gartlan, 1970). Indeed, some primatologists have even argued that focusing on primates’ visits to waterholes may be more effective for studying primate behavior than following groups under certain conditions, particularly for the study of interspecific interactions and when primate groups are not well habituated (Beck & Tuttle 1972). Hence, primatologists have long acknowledged that waterholes have an important ecological influence on behavior for some primate species in dry habitats or during dry periods.
From these initial accounts, hypotheses about how water influences primate behavior in dry habitats were generated. Several more recent studies subsequently investigated behaviors related to water, including ranging behavior. For example, waterholes have been shown in observational studies to influence patterns of habitat use and movement in olive baboons (*Papio anubis*) (Barton et al., 1992), red-fronted lemurs (*Eulemur rufifrons*) (Scholz & Kappeler, 2004), white-faced capuchins (*Cebus capucinus*) (Campos & Fedigan, 2009), and chacma baboons (*Papio ursinus*) (Noser & Byrne, 2007, 2014). A study of black howler monkeys (*Alouatta pigra*) investigated the factors influencing drinking behavior, including heat stress and water availability in food (Dias, Rangel-Negrín, Coyohua-Fuentes, & Canales-Espinosa, 2014). These findings represent important strides in our preliminary understanding of how water influences primate behavior.

Acquiring water has also been suggested to play an important role in the survival and evolution of early human populations (Binford, 1980; Conkey, 1980; Speth, 1987). The expansion of populations of early *Homo* into arid or seasonally arid savannah regions (Stanley, 1992) must have depended upon behavioral adaptations for locating and discerning the quality of water sources. Finlayson hypothesized that water was a primary selective pressure in early human evolution, proposing that the demands of traveling across arid savannah habitats between scattered water sources selected for the specific cognitive, morphological, and cultural adaptations of modern humans.
While Finlayson’s hypothesis draws support from the fossil record, a comparative approach (i.e. wild primate studies) would facilitate investigation of the specific ecological conditions under which water drives the evolution of adaptive traits.

In modern humans, Taylor proposed the “tethered nomadism hypothesis” to explain human settlement location and subsequent movement patterns. He hypothesized that to balance the needs for water and food in a dry habitat, humans would adopt a pattern of wandering in search of food within boundaries that were determined by a safe radius of travel from a central water source within their territory (Taylor, 1964). Other researchers have noted the importance of permanent water sources in the ecologies of some hunter-gatherer groups, such as the !Kung, for whom permanent waterholes serve as hubs for inter-group trading of commodities or mates (Binford, 1980; Lee, 1976; Tomaselli & Homiak, 1999).

Recently, scholars have recognized that traditional approaches to studying water use and adaptations to water scarcity in humans are insufficient in a world with changing institutional, climatic and demographic challenges (Wutich and Brewis 2014). These concerns are mirrored in primatology; climate change is projected to cause disruptions to patterns of water availability (Trenberth et al. 2014), making human and non-human primate populations throughout the world vulnerable to dehydration, increased waterborne parasite exposure, and other potential health risks. Across the
field of evolutionary anthropology, scholars have recognized that water is an important component of ecology, but the application of modern experimental and methodological techniques to questions related to water has been slower than to other resources like food, which has been the focus of intensive primatological research for decades (Clutton-Brock, 1977; Janson & Chapman, 1999; Sterck, Watts, & van Schaik, 1997; van Schaik, 1989; Wrangham, 1980).

1.3 Waterholes represent peaks in the landscape of risk

That relatively little attention has been paid to water resources as drivers of primate behavior in recent years is surprising because water is especially essential to the survival, health, and reproductive success of species that live in seasonally arid or drought conditions – as many primate species do. In addition, locating, navigating to, and making decisions about water resources may involve complex perceptive, cognitive, and social processes that have long been foci of research in primatology and evolutionary anthropology. In dry habitats, centralized water sources, such as waterholes, may represent hubs of inter-individual, inter-group, or inter-specific encounters – a possibility raised by the earliest studies of waterholes in primates (Altmann & Altmann, 1970; Kummer, 1968; Struhsaker & Gartlan, 1970). As such, waterholes may represent a unique opportunity to study how many ecological factors influence primate behavior, including their relative importance in determining behavioral outcomes. In this dissertation, I focus on several of these factors: water
distribution, parasite risk, and predation risk. I also consider the effects of intergroup competition. I introduce each of these factors briefly here.

The distribution of water may be among the most important factors driving water-dependent primate movements in dry habitats. For example, olive baboons were more likely to spend time in quadrats of their home ranges that contained water than those without water during the dry season (Barton et al., 1992). Red-fronted lemurs traveled several kilometers outside of their home ranges to waterholes when their home ranges did not include a water source, and groups that traveled the farthest distances established secondary core areas near waterholes during the dry season (Scholz & Kappeler, 2004). White-faced capuchins’ home ranges changed relative to the distribution of water during the dry season (Campos & Fedigan, 2009). Chacma baboons made direct long-distance trips to waterholes during the dry season (Noser & Byrne, 2007, 2014).

Though obtaining water is essential to primate survival and health, water can also pose health risks (Nunn & Altizer, 2006). Among the most infamous waterborne parasites of humans are Cryptosporidium, Giardia, and Vibrio cholerae, all of which cause diarrheal disease, which can be life threatening. Beyond these examples, almost any fecal-orally transmitted parasite of primates has the potential to be transmitted through contaminated water, including helminths, protozoa, bacteria, and viruses (Palumbo, Ziglio, & Van der Beken, 2002). Natural water sources in arid environments also provide
a moist microhabitat that promotes the survival of infectious stages of parasites in the soil (Perry, 1999), and increased bodily contact with water and wet surfaces can increase the prevalence of parasites, as reported for *Schistosoma* in olive baboons (Müller-Graf et al., 1997).

In dry areas, water sources are likely to serve as hubs of parasite transmission between individuals, groups, and even species (Krecek et al., 1990). Across primate species, higher parasite richness is associated with more intense range use (Nunn & Dokey, 2006), which may result from the presence of an important resource within a group’s range. Increased overlap of multiple groups’ ranges around critical resources, such as waterholes during dry seasons, has been shown to increase parasite transmission theoretically and empirically (Bastos et al., 2000; Bengis, Kock, & Fischer, 2002; Nunn, Thrall, & Kappeler, 2014; Vicente et al., 2007).

Given this risk, primates living in dry habitats are expected to be sensitive to parasite-relevant qualities of water to minimize their exposure to parasites. Non-human primates possess behavioral strategies for preventing parasite exposure in several other contexts (Poirotte et al., 2016; Poirotte, Massol, et al., 2017; Sarabian & MacIntosh, 2015; Sarabian, Ngoubangoye, & Macintosh, 2017). Humans are highly sensitive to water quality in some cultures: American subjects report in surveys that they would refuse to drink treated, recycled waste water even if it is cleaner than bottled water (Rozin et al., 2015). Thus, primates are expected to avoid parasite risk when possible at waterholes.
Waterholes in dry habitats also present a high degree of predation risk for animals that use them, both because predators may be drawn to water for their own physiological needs, and because predators might exploit prey’s reliance upon water. Given an increased risk of predation at waterholes (de Boer et al., 2010), prey may avoid predators by selecting water sources that are better hidden (Sánchez-García et al., 2012), selecting water sources with fewer predators nearby (Valeix et al., 2009), or increasing vigilance behaviors and/or group size to reduce predator risk around the edge of waterholes (Burger, 2001; Freese, 1978; Périquet et al., 2010; Valeix et al., 2009).

In addition to the risks posed by predators, as water becomes increasingly scarce during the dry season, primate groups are more likely to encounter one another at waterholes, accompanied by the risk of agonistic interactions and injury (Freese, 1978; Rose & Fedigan, 1995; Struhsaker & Gartlan, 1970). At waterholes, male white-faced capuchins were primarily vigilant of males in other groups, rather than toward predators or other group members (Rose & Fedigan, 1995). Patas monkeys anecdotaly increased their rates of aggression between groups as water became increasingly scarce during the dry season (Struhsaker & Gartlan, 1970). However, the overlap of multiple groups at waterholes could also improve vigilance for predators and dilute the risk of predation (Burger, 2001).

The “landscape of fear” is a concept that was introduced to explain how predation risk shapes landscape-level habitat use by prey (Laundre, Hernandez, &
Ripple, 2010). This so-called landscape represents the relative levels of predation risk in a habitat as peaks and valleys, which correspond to the fear of predation a prey experiences in different parts of its habitat. Peaks in the landscape of fear, where perceived predation risk is highest, would be avoided by prey (Laundre et al., 2010). In other words, prey’s perception of predation risk can shape its habitat use on a landscape scale. This phenomenon was famously documented when wolves (Canis lupus) were reintroduced to Yellowstone National Park, resulting in changes to elks’ (Cervus elaphus) habitat use that were greater than would be expected through direct predation; instead, elks avoided particular habitat types in which perceived predation risk was higher (Creel et al., 2005). Recently, this concept of the landscape of fear was applied to parasite infection risk and termed the “landscape of disgust,” an emotion hypothesized to motivate parasite avoidance (as fear motivates predator avoidance) (Buck, Weinstein, & Young, 2018; Weinstein, Buck, & Young, 2018). The landscape of disgust suggests that avoidance of perceived parasite risk could shape host habitat selection in similar ways to predation risk. Multiple forms of risk may be thus plotted across a landscape and considered to have an influence on the patterns of habitat use of a species (Fig. 1).
Figure 1: Waterholes may represent peaks in multiple landscapes of risk.

In dry habitats, for species that must drink free water, waterholes might represent peaks in multiple landscapes of risk. Each of the dimensions I have discussed above could represent a distinct landscape of risk: risk of parasite transmission, risk of predation, risk of intergroup aggression. If these layers of risk are overlaid, and there is a peak in each dimension around waterholes, then waterholes would represent intersecting locations in which multiple dimensions of risk are elevated. The concept of the landscape of fear predicts that animals would avoid areas with high perceived risk, but if drinking water is essential, waterholes may be unavoidable. This conglomerata
of circumstances offers a unique opportunity to examine the impacts of these various factors on animal behavior.

1.4 Overview of dissertation chapters

The present dissertation investigates the role that waterholes play in the ecology of lemurs that require free drinking water and live in water-scarce habitats. I examine major axes along which waterholes can vary, in terms of the risks and costs that are associated with drinking from them. These axes of variation include the energetic costs of travel to water (as a function of the spatial distribution of waterholes relative to other resources or habitat), predation risk at waterholes, risk of intergroup competition and aggression, and parasite transmission risk.

In Chapter 2, I present a study of parasite avoidance behaviors in captive lemurs’ water choices at the Duke Lemur Center. I test how lemurs balance the risks of fecal pathogen transmission with potential nutritional or other benefits of feces ingestion in their selection of water sources. In an experiment, lemurs were given a choice between clean water and water with disinfected fecal material added, which contained cues associated with fecally transmitted parasites, but minimal risk. This chapter establishes the foundation for further investigation of lemurs’ water choices related to parasite risk in the wild.

The remaining chapters result from my field seasons studying wild red-fronted lemurs in Kirindy Forest CNFEREF (Kappeler & Fichtel, 2012), western Madagascar

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during the mid-late dry season in 2016 and 2017. This population comprises several collared and habituated groups of lemurs that regularly drink from standing waterholes during the dry season (Scholz & Kappeler, 2004).

Chapter 3 investigates how the distribution of water sources influences ranging patterns in wild red-fronted lemurs. Although water is generally considered an important driver of animal movements in dry habitats, its independent impact on primate habitat use has not been investigated (Barton et al., 1992; Campos & Fedigan, 2009; Kelley, 2011; Kelley, 2013; Noser & Byrne, 2014). Thus, the possibility remains that other resources such as food or sleeping sites co-occur with water and are instead responsible for the apparent influence of water on habitat use. In this chapter, I track changes in the spatial distributions of lemur groups’ habitat use in response to experimentally adding and removing “artificial waterholes” in the habitat. I also monitor how the lemurs’ habitat use changes as natural waterholes dry up throughout the dry season. Using dynamic Brownian bridge movement models (Horne et al., 2007; Kranstauber et al., 2012), I estimate utilization distributions for lemur groups before, during, and after the addition of the artificial waterholes and compare them to one another.

In Chapter 4, I test whether red-fronted lemurs choose water sources with lower perceived parasite risk, and evaluate how avoidance of parasites trades off with travel distance. In an experiment similar to that presented in Chapter 2, I offered red-fronted
lemurs a choice between clean, filtered water, and water with disinfected conspecific fecal material added, and I recorded their selection. I also examine whether lemurs mitigate parasite risk, measured as fecal contamination in the surrounding area, in their decision to return to natural waterholes that they previously visited, or in the duration of time between visits. I also examine whether travel distance and avoiding overlap with other groups might explain lemurs’ waterhole choices. I investigate these hypotheses using data collected by GPS collars and camera traps positioned around the edge of waterholes.

Because red-fronted lemurs’ two main predators, fossa (*Cryptoprocta ferox*) and harrier hawks (*Polyboroides radiatus*) must also drink water, the risk of encountering predators may be higher than average at natural waterholes. In Chapter 5, using camera trap data, I examine whether lemurs avoid waterholes that are frequently visited by predators. I also use circular statistics, which are appropriate for time datasets, to determine whether the timing of red-fronted lemurs’ waterhole use, as measured by camera trap activations, differs from the timing of camera activations by predators at the waterholes (Ridout & Linkie, 2009).

### 1.5 Conclusion

This dissertation represents, to my knowledge, the most thorough investigation of the role of water in primate ecology to date. Using multiple technologies, including GPS collars and video camera traps, I assess the influence of water on multiple aspects
of primate behavior, including ranging and habitat use, parasite avoidance, and predator avoidance. I also use cutting-edge statistical techniques, including Brownian bridge movement models, methods for quantifying camera trap data, and mixed conditional logistic regressions, to test my hypotheses. If waterholes indeed represent peaks in the landscape of risk, then my study design offers the opportunity to integrate and evaluate the relative importance of multiple factors on the water-related behaviors of red-fronted lemurs, with implications for their behavior and ecology more broadly. This project takes advantage of numerous characteristics of the study system in Kirindy Forest in Madagascar, including red-fronted lemurs’ dependence on water and the availability of multiple waterholes that vary in their characteristics, to investigate how these characteristics influence the behavior of red-fronted lemurs.
2. Water choice as a counterstrategy to fecally transmitted disease: an experimental study in captive lemurs

2.1 Introduction

Parasites and pathogens have shaped host evolution through the fitness costs that infections exert on their hosts, with effects on host genomes, immune defenses, and behavior (Moore et al., 2002; Nunn & Altizer, 2006). Parasite infection requires two sequential steps of host-parasite interaction: first, the host must come into contact with an infectious stage of a parasite; second, the host must be susceptible to infection, or incapable of mounting an effective immune response (Combes, 2001). Host strategies to prevent parasite infection must interfere with at least one of these steps, and may involve multiple behavioral, immunological, and other physiological adaptations (Combes, 2001). Host behavior is arguably the most important factor mediating exposure; hence, behavioral counterstrategies to disease have received much attention (Hart, 1992; Moore, 2002; Nunn & Altizer, 2006; Curtis, 2014). An effective behavioral counterstrategy acts as a first line of defense against parasites and precludes the need for costly immune responses, and a variety of behaviors are known to reduce the risk of parasite infection in animals (Hart, 1990; Hart, 1994).

Although avoiding infectious stages of parasites may provide fitness advantages, doing so is often a difficult task for hosts. To increase the probability of transmission, fecal-orally transmitted endoparasites are expected to benefit from being undetectable
by hosts (Hart, 2011). Although animals can sometimes distinguish parasite infection status in conspecifics or environmental contamination (Garner et al., 2007), many parasites, especially those that require ingestion by the host, are often impossible to detect at low abundance (Hart, 2011). Difficulty of detection likely prevents hosts from avoiding some endoparasites directly; selection may instead act upon animals’ ability to avoid more easily detectable stimuli associated with parasite presence in the environment, such as feces, rather than cues of the parasites themselves, to reduce the probability of exposure (Hart, 1990; Cooper et al., 2000). Because endoparasites are frequently transmitted via the fecal-oral route, and because in natural environments most conspecifics are probably infected with parasites, avoidance of feces may be an effective strategy for reducing parasite exposure (Cooper et al., 2000; Sarabian & MacIntosh, 2015).

Because food and water are common transmission pathways for parasites (Slifko et al., 2000), hosts may be able to reduce the risk of ingesting infectious stages of parasites by choosing to consume food or water with lower levels of fecal or other contamination (Hutchings et al., 2003). However, animals may also experience a cost when rejecting essential resources through choosy behavior (Hutchings et al., 2000). For example, a recent study of hygienic behavior in free-ranging Japanese macaques (Macaca fuscata) demonstrated that the macaques’ willingness to consume feces-contaminated food depended on the quality of the food item and that food washing behaviors differ
between males and females (Sarabian & MacIntosh, 2015). In that study, the authors tested whether the macaques consumed food items from different substrates, which included fresh conspecific feces and a plastic notebook cover. The macaques consumed 100% of peanuts (preferred food item) but only 37% of wheat grains (less preferred) from the fresh feces, while consuming 100% of both food items from the plastic notebook cover. Thus, the macaques reduced their potential caloric intake for the benefit of avoiding parasite exposure. These results suggest that both the costs of forgoing a fitness-relevant resource and the risk of parasite infection contribute to decisions about which resources to ingest.

Remarkably little research has focused on how parasite risk in water sources shapes behavioral tradeoffs, despite the diversity of potentially costly parasites and pathogens that are routinely transmitted via drinking or contacting water, such as *Cryptosporidium, Giardia, Blastocystis, Paragonimus*, and *Schistosoma* (Slifko, Smith, & Rose, 2000). Dairy cattle, one of the few species in which water preferences have been studied, prefer clean water to fecally contaminated water (Willms et al., 2002). Additionally, cattle given clean water spend more time feeding, less time resting, and have heavier calves than cattle given pond water to drink (Willms et al., 2002). These findings suggest that water preferences, or learning processes associated with those preferences, could be shaped by natural selection.
Despite the benefits of avoiding fecal contamination for the sake of reducing exposure to parasites, animals do not exclusively avoid feces; in fact, a wide range of taxa intentionally consume feces (coprophagy), including insects, rodents, lagomorphs, pigs, horses, dogs, and non-human primates (Soave & Brand, 1991; Hirakawa, 2001). In these taxa, coprophagy may have an adaptive function, such as enhancing nutritional extraction or providing specific micronutrients or microbes (Soave & Brand, 1991). Contrary to assumptions about the parasite exposure risks of this behavior, in some species, coprophagy may actually serve an anti-parasite function. For example, the gut microbiota of bumblebees that consume conspecific feces are more robust to infection by a virulent trypanosome parasite than the microbiota of individuals prevented from consuming feces (Koch & Schmid-Hempel, 2011). This example illustrates that more research is needed to understand the costs and benefits of coprophagy, and the contexts in which these costs and benefits apply.

In primates, coprophagy has historically been considered an aberrant behavior associated with captivity (e.g. Hook et al., 2002), though recent work in captive chimpanzees suggests this may not be the case (Hopper et al., 2016). A number of authors have suggested adaptive explanations for the consumption of feces in wild primates (Hladik et al. 1971; Harcourt & Stewart, 1978; Krief et al., 2004; Sakamaki, 2010; Bertolani & Pruetz, 2011). Wild ring-tailed lemurs have been observed to consume heterospecific (human, domestic animal) fecal matter during the dry season, and authors
have proposed coprophagy as an adaptation for nutrient acquisition in times of scarcity, or for older individuals who lack the dentition to process preferred foods (Fish, Sauther, Loudon, & Cuozzo, 2007; Gemmill & Gould, 2008). Other than accounts in this one population, whether coprophagy occurs more broadly in wild lemurs is unknown.

Given that some lemurs engage in coprophagy, lemurs present an opportunity to investigate the tradeoffs between parasite avoidance and nutrient acquisition involved in water selection. Lemurs also inhabit diverse habitats, in which rainfall, and thus water availability, ranges from high and consistent in rainforests to low and often seasonal in arid areas, with variable seasonality of water sources in many other regions, such as the deciduous forests of Madagascar’s west coast (Goodman & Benstead, 2007; Mittermeier et al., 2010). In rainforest habitats where water is not limiting, species that drink water may be able to afford a higher level of selectivity for clean water than they would in habitats that are dry or seasonally dry, so being choosy could increase the risk of dehydration. Rainfall patterns are likely important for parasite transmission because they affect parasite desiccation and survival (Perry, 1999) and the buildup of fecal material (and fecally-transmitted parasites) in and around standing water during dry seasons (Patz et al., 2000; Nunn & Altizer, 2006; Nunn et al., 2014).

In the current study, we investigated behavioral responses to cues of fecal contamination (as a proxy for the presence of water-borne parasites) by studying the water preferences of captive lemurs at the Duke Lemur Center (DLC). We investigated
water quality preferences using a choice test paradigm in five species of lemurs in captivity: *Eulemur coronatus* (crowned lemur), *Eulemur mongoz* (mongoose lemur), *Lemur catta* (ring-tailed lemur), *Varecia rubra* (red ruffed lemur), and *Varecia variegata* (black and white ruffed lemur). These five species were chosen because they were observed to drink water regularly in captivity (pers. obs. and pers. comm. with DLC staff), and because they were available for testing in sufficient numbers. In these experiments, lemurs were allowed to choose between paired water sources that differed in their degree of fecal contamination. For ethical reasons, fecal material was disinfected to minimize the risk of pathogen transmission.

We tested three hypotheses. Under the avoidance hypothesis, lemurs exhibit behavioral strategies that reduce their probability of consuming water that poses a risk of parasite transmission. Although we did not present lemurs with water sources that posed a true infection risk, the cues of fecal contamination may be a reliable enough indicator of parasite risk to elicit avoidance (Hart, 1990). Under this hypothesis, we predicted that lemurs would prefer to drink un-manipulated (i.e. clean) water more often than water with feces added to it.

Under the nutritional benefits hypothesis, we predicted that lemurs would actually prefer water that contained fecal material, which may be expected for animals that are coprophagic. To the best of our knowledge, the species in this study vary in the regularity with which they are observed to ingest feces in captivity. Anecdotally, *Varecia*
spp. engage in this behavior especially frequently, but all of these species may perform conspecific coprophagy at least occasionally in captivity (pers. obs. at the DLC and pers. comm. with DLC staff).

Finally, under the environmental constraints hypothesis, lemurs adapted to dry or seasonally dry habitats would not show a preference in either direction, because being overly selective presents a high risk of dehydration in these habitats. We predicted that *V. rubra* and *V. variegata*, species adapted to the rainforests of Madagascar’s east coast where water is abundant and regular, would have stronger preferences for clean water than *L. catta*, a species adapted to the spiny desert habitat in the south of Madagascar where water choice is likely to be more limited.

In addition to investigating the lemurs’ drinking preferences overall, we also determined the sensitivity of lemurs to fecal contamination by adjusting the concentration of feces in water. We also conducted an additional test to control for habituation in which we investigated whether visual novelty drives preferences for one water source over another, rather than the fecal cues themselves.

### 2.2 Methods

#### 2.2.1 Study subjects

We conducted behavioral experiments with 60 captive lemurs at the DLC in Durham, NC. We conducted 69 trials: 23 trials in distinct groups for Experiment 1, 25 trials for Experiment 2, and 21 trials for Experiment 3. The tested individuals were
members of the five species listed above. The lemurs used in this study were captive-born and have lived at the Duke Lemur Center for most or all of their lives. At the DLC, the lemurs are checked regularly for gastrointestinal parasites and de-wormed if they have active infections. The lemurs are used for many different research studies, all of which are non-invasive. The choice paradigm involving fecally contaminated water that we used has not been used at the DLC previously, and thus the subjects had no prior experience with the experiments that we conducted. The lemurs are housed in conspecific pairs or groups in indoor enclosures with outdoor access, and groups ranged in size from 1 to 7 individuals (mean group size = 2.9). During warm weather, some groups are allowed to free-range in outdoor forest enclosures. Trials were conducted in both indoor housed and free-ranging groups.

2.2.2 Experiments

We conducted three behavioral experiments, which are described in detail below: (1) a choice test with a concentrated fecal stimulus, (2) a choice test with a diluted and filtered fecal stimulus, and (3) a habituation test, which involved making both choices appear visually novel by adding color dye to them. Experiments took place between April 2014 and July 2015. Members of each group were tested together; i.e. individuals were not separated from their cohabitants. Individuals were identifiable by collars, shaved markings, or naturally occurring characteristics. Each group was used in each experiment one time. If the group was housed indoors, each experiment lasted 60
minutes and the subjects were restricted from accessing the outdoor portion of their enclosures during the trial. If the group was free-ranging in the forest enclosure, the trial lasted approximately 20 minutes (21.1±1.8 min). This shorter and more flexible time period was used for outdoor experiments because the subjects could move away from the experiment area freely.

In all experiments, the subjects were presented with a simple choice between two bowls of water. The metal bowls were identical to one another and similar in appearance to the bowls in which the lemurs normally receive water and food. One bowl always contained the control water condition: 500 mL of un-manipulated tap water, which is the same water that is normally provided to the lemurs daily ad libidum. The other bowl held the experimental, parasite-relevant (i.e. fecally contaminated) water condition, which varied by trial type as explained below (Fig. 2).

2.2.2.1 Experiment 1: Choice test with concentrated fecal stimulus

In this first experiment, we aimed to determine whether the subjects exhibited preferences for clean water using a strong stimulus. The experimental condition was tap water with feces added to it. A 10-15 g sample of fresh fecal material was collected opportunistically from the test group and was disinfected by boiling it in 100-200 mL of water for at least one minute. This small volume of adulterated water was refrigerated overnight. Immediately prior to the trial, tap water was added to increase the volume of liquid to 500 mL. Faecal material was visible floating in the water, and the water smelled
of faeces to human observers, despite the process of boiling and refrigeration. This level of contamination was chosen because there was a very apparent difference between the two water sources to human observers, and we expected that results from this test would provide clear evidence of a preference in one direction or the other. In other words, the goal of this experiment was to conduct a strong test of the hypotheses, with either preferences for or aversions to potentially contaminated water expected to be easily provoked.

Based on the requirements of the Institutional Animal Care and Use Committee, to completely avoid the minute risk of transmitting parasites between lemur groups or species, the fecal sample added to the water in a given trial was collected only from the group being tested. Every effort was made to standardize the experimental stimulus across trials, but because each experimental stimulus was created independently for each test group, some variability in the characteristics (e.g. color) of the stimulus was unavoidable. In addition to mitigating any ethical or health concerns with the methods, creating a unique experimental stimulus for each trial minimized the chance that an uncontrolled factor unique to a single experimental stimulus influenced all trials, and ensured that all subjects were presented with conspecific fecal contamination of the experimental stimulus.
2.2.2.2 Experiment 2: Choice test with dilute and filtered fecal stimulus

The goal of this experiment was to more closely replicate realistic conditions in nature, as compared to the marked difference in visual and olfactory characteristics between the control and experimental water sources in Experiment 1. The experimental water condition was prepared as in Experiment 1, except with a smaller amount of feces (5 g). Additionally, prior to refrigeration, the adulterated water was strained through a coffee filter to remove solids. The water sources were more difficult to distinguish from one another for human observers, and both visual and olfactory cues from the experimental water were less apparent in this experimental condition, although the control and experimental conditions were discernible by appearance and smell to human observers. In this experiment, we sought to explore the lemurs’ sensitivity to cues of contamination, because no information is available about the level at which lemurs (or any primate) can detect fecal contamination in water.

2.2.2.3 Experiment 3: Habituation test

Neither the control nor the experimental water source in our first two experiments was particularly unusual-looking or smelling compared to the typical surroundings in the subjects’ housing conditions. However, we wanted to rule out the possibility that the subjects were simply habituated to drinking clean water, since fecal contamination in water may be relatively rare in their captive environments due to regular cleaning. To control for the possibility that habituation might influence the
subjects’ drinking preferences, we prepared the water as in Experiment 1 and added several drops of blue food dye to both the control and experimental sources. Unlike the monochromatic nocturnal lemurs, the species tested in this experiment can perceive the color blue because they are either dichromatic (like most non-primate mammals) or trichromatic (like many other primates) (Jacobs, 2008; Leonhardt et al., 2009); thus, adding blue color dye made both sources of water appear novel. Some differences in color between control and experimental conditions were apparent to human observers, and floating feces remained visible. The color dye did not alter the smell of the water to human observers.
2.2.2.4 Experimental protocol

At the start of the trial, the two bowls of water were placed approximately 25 cm apart from one another inside the enclosure. In the indoor enclosures, the bowls were placed in wire stands that secured them to the metal mesh of the enclosure wall just

Figure 2: Experimental setup. a: Experiment 1, concentrated fecal contamination; b: Experiment 2, dilute fecal contamination; c: Experiment 3, habituation.
above the ground. These wire bowl stands prevented the water bowls from being knocked over, and are used to hold water bowls in the enclosures at the DLC regularly. The water bowls were introduced into an area of the enclosure from which the subjects were excluded, and the location through which the subjects were allowed to enter was approximately equidistant from both bowls. In the outdoor enclosures, the bowls were placed on the ground, as the fences were typically too far from the location of the lemurs to be reasonably used to hold the stands. The water sources were placed in an area more than 10 m from the location where the lemurs were observed, and the two bowls were placed approximately equidistant from the group.

Each experiment was video-recorded. At the end of the test period, the video recording was stopped and the water bowls and stands were removed from the enclosure. Two independent observers coded the videos. We recorded the occurrence of each drinking event, which individual performed it, and which water condition the individual chose. A subset of 12% of the trials was randomly selected and coded by both observers to assess inter-observer reliability.

The experiments were all presented to subjects in the same order (Experiment 1, followed by Experiment 2, followed by Experiment 3) with a period of at least four weeks between experiments. While the groups and individuals used in each experiment overlapped substantially, there was some variation in the individuals and compositions of groups used across experiments due to captive management decisions unrelated to
our project (e.g., when a juvenile lemur reached maturity and was moved out of her natal group between experiments, or a group was used for another research project for an extended period of time, which prevented its use for some parts of our study).

2.2.3 Analysis

To test the avoidance hypothesis and the alternative nutritional benefits hypothesis for all three experiments, we conducted a two-sided binomial test to determine whether the count of lemurs’ selections of clean and dirty water sources differed significantly from chance (i.e., with a probability of 0.5). We first analyzed all drinking events. However, one condition for the binomial test is independence of data points. This condition is not met for repeated drinking events by an individual in the same trial, and may not be met for drinking events by different individuals within a single trial if social factors (such as copying or avoidance of more dominant individuals) play a role in decision-making. Although we have no evidence for these effects, to account for the possible non-independence of data points, we re-analyzed two subsets of the data: (1) only the first drink performed by each individual that participated, and (2) only the first drinking event of each trial.

To compare *L. catta* to *Varecia spp.* in tests of the environmental constraints hypothesis, we used Fisher’s exact test for count data. To maximize sample size for the comparison, we combined *V. variegata* and *V. rubra* and considered all drinking events rather than a subset. We elected not to use a mixed model approach to analyze these
data because we simply wanted to compare counts of ‘successes’ (clean drinks) and ‘failures’ (experimental drinks), rather than investigating the effect of a particular predictive variable on the outcome of the trial.

**2.3 Results**

In total, we observed 164 drinking events: 43 instances of drinking in Experiment 1, 78 in Experiment 2, and 43 in Experiment 3 (Fig. 3). Of the 69 total trials, 50 were conducted indoors and 19 were on free-ranging animals. Twenty-four female individuals and 27 male individuals participated in the experiments by drinking from at least one of the water sources on one occasion. The vast majority of individuals who participated were adults, though several juveniles at different stages of maturity were included. Inter-observer reliability for the occurrence of drinking events was 96% (all drinking events except one were agreed upon between observers; a camera adjustment interfered with observing the drinking event on which coders disagreed).
Figure 3: All drinking events recorded in the three experiments. Significant difference from chance (0.5) in binomial test is denoted by * (p<0.0005) and ** (p<0.0001).

When all drinking events were included, we found clear statistical support in favor of the avoidance hypothesis, rather than the nutritional benefits hypothesis, in Experiment 1 (40 clean drinking events out of 43 total, $p<0.0001$, binomial test). In Experiment 2, the lemurs drank from the clean water in 56 instances and from the experimental water in 22 instances, again supporting the avoidance hypothesis (56 clean out of 78 total, $p=0.0001$, binomial test) (see Fig. 4). In Experiment 3, the lemurs chose the clean water in all 43 instances, which also supports the avoidance hypothesis and
suggests that lemurs did not simply drink the water that was most familiar (43 clean out of 43 total, \(p<0.0001\), binomial test).

When only the first drink by each individual was included in the analysis from Experiment 1, we observed 26 instances of drinking from the clean water, and 0 from the experimental water (\(p<0.0001\), binomial test). In Experiment 2, we observed 23 drinking events from the clean water and 9 from the experimental water (\(p=0.02\), binomial test). In Experiment 3, we observed 20 instances of drinking from the clean water and 0 from the experimental (\(p<0.0001\), binomial test). Thus, when we subsetted the data to account for non-independence within individuals, evidence continued to favor the avoidance hypothesis.

In Experiments 1 and 3, the same pattern held (\(p<0.0001\), binomial test). In Experiment 2, however, involving a more dilute experimental condition and smaller sample size, effects were no longer statistically significant: we observed 10 instances of drinking from the clean water source, and 5 from the experimental (\(p=0.30\), binomial test).

We did not have any a priori expectations about sex differences, but we conducted a comparison of water preferences across sexes post-hoc. We expected Experiment 2 to be the strongest test of a sex difference because so few drinking events of the experimental water occurred in Experiments 1 and 3. We conducted a Fisher’s exact test and found no difference between males and females in their preference in
Experiment 2 (males: 36 clean drinking events vs. 13 experimental; females: 20 clean vs. 9 experimental drinking events; \( p=0.80 \)).

We found no statistically significant differences between the dry-habitat species (\( L. \ catta \)) and the wet-habitat species (\( Varecia \ spp. \)) (\( p>0.05 \) in all three experiments, Fisher’s exact tests). However, in Experiment 2, \( L. \ catta \) differed significantly from \( Varecia \ spp. \) in the count of trials in which they participated (participation in 2 out of 9 trials for \( L. \ catta \) versus 8 out of 8 in \( Varecia \ spp. \), \( p=0.002 \), Fisher’s exact test). In the other experiments we found no difference between \( L. \ catta \) and \( Varecia \ spp. \) in terms of participation (\( p>0.05 \), Fisher’s exact tests). We did not expect trial location to have influenced these results because participation was roughly equivalent between trials conducted indoors (66% of trials) and outdoors (63% of trials).
2.4 Discussion

Our results support the hypothesis that lemurs use behavioral strategies to reduce the potential risk of parasite transmission via drinking water. Moreover, this hypothesis was supported when fecal concentrations were higher (more easily detectable to human observers) and lower (less detectable to human observers), and when both water sources were altered to appear novel (opposing the importance of habituation as a factor in the choice of clean water). The only result that did not provide support for the avoidance hypothesis involved the subset of data that included only the first drinking event of the trial for Experiment 2. It is possible that small sample sizes, or
some other effect of sampling only the first individual to participate in a trial, are responsible for this non-significant result. Another possibility is that the preference for clean water is no longer evident when we account for non-independence of selections by individuals of the same group, or when we statistically ‘remove’ social factors by analyzing only the first drinking event in the trial. We did not observe this same change in significance when we removed social factors in Experiments 1 or 3. Hence, these findings may suggest that social factors are more important for decision-making when the options exhibit more subtle differences, as in Experiment 2. Though we attempted to ‘remove’ social factors in this analysis, all of these trials were conducted in a social setting; to truly remove social effects on drinking decisions, we would have to conduct trials with isolated individuals, which was not done in this study.

Our findings in Experiment 3 suggest that lemurs did not avoid drinking water that appears an unusual color (i.e., the lemurs did ingest the clean, blue-colored water). In the experiments presented here, we did not assess habituation to all characteristics of the water, including whether novel smells or novel floating material may have played a role in the lemurs’ decisions. Based on observations of lemurs at the DLC (inadvertently) defecating directly into their water dishes, we consider it unlikely that neophobia or neophilia to the experimental water is responsible for our findings. However, we did not directly assess habituation to characteristics of water other than color in the experiments presented here.
Although we attempted to account for factors that may have influenced water selection besides the level of fecal contamination, variables beyond our control may have influenced water choice, such as some element of favorability in the placement of the water sources not apparent to us, or some activity by other (non-testing) animals or animal technicians in nearby enclosures. It is also possible that social factors influenced water choice in some manner not accounted for by our analysis, such as competition for access to one water source, or conformity to the choices of others in the group. The slight inconsistencies in the individuals and group composition across the different experiments, while beyond our control, may also have had some impact on the trials, as these social dynamics would possibly have been in flux from one time point to the next. While such variables may have influenced individual trials, we have no reason to expect that they biased the results in a consistent direction across trials.

We found no difference between dry habitat- and wet habitat-adapted species in terms of preferences for clean water in all three experiments. We did, however, find that dry-habitat *L. catta* participated significantly less than wet-habitat *Varecia spp.* in Experiment 2. While we had no *a priori* expectations about rates of participation across species, the exceptionally low rate of participation in *L. catta* (only 22% of trials) may suggest that this species had more difficulty distinguishing clean from dirty water, and may be better suited than *Varecia spp.* to forgoing water when its quality is uncertain. If individuals were able to detect some potential contaminant in the experimental water,
but could not reliably distinguish it from the control water, perhaps the safest decision, particularly for a species that is adapted to live in dry habitats, would be to forego drinking from either option. This might be considered modest support for the environmental constraints hypothesis, although no difference was observed in the frequency of clean water choices across the species.

Previous studies have found sex differences in some behaviors around water relevant to parasite transmission (e.g. Müller-Graf, et al., 1997; Sarabian & MacIntosh, 2015), with males tending to perform some behaviors that put them at risk for parasite transmission at higher rates than females. Ingestion of fecally contaminated items was not previously found to differ significantly between males and females, however (Sarabian & MacIntosh, 2015). Consistent with the latter finding, we did not detect any evidence of a sex difference in terms of drinking fecally contaminated water.

The feces in this experiment were disinfected by boiling; hence, the water sources posed little true risk to the animals. As a result, subjects selected water sources based on cues associated with fecal contamination, rather than some cue specific to parasites themselves. It remains unknown whether lemurs are capable of detecting the presence of actual parasites in water in the absence of other cues about water quality. Some species have been reported to detect and avoid specific cues of parasites (e.g. Poirotte et al., 2017), while others avoid feces regardless of parasite infection status (e.g. Cooper et
al., 2000). Future experiments could investigate whether the presence of parasites themselves influences the avoidance of water.

By removing constraints associated with water scarcity and other factors, use of captive animals for these experiments made it possible to isolate the effects of water quality on behavioral preferences for clean water. The subjects had continuous access to clean water until immediately before the experiments, and could thus choose their preferred water without the influence of extreme thirst or risk of dehydration. While we cannot make strong claims about the behavior or preferences of wild lemurs, our results suggest that, in the absence of other factors, clean water would also be preferred in wild lemurs.

We were surprised to observe so few instances of lemurs drinking the fecally contaminated water, considering that some species in this captive population have been observed to practice coprophagy regularly. Our results raise some interesting questions about the little-studied behavior of coprophagy in non-human primates, including under what circumstances the benefits to be gained from this behavior outweigh the associated risks, and how animals might distinguish between those different scenarios.

Consuming one’s own feces or the feces of a parent or familiar group-mate may present few risks in terms of exposure to new parasites, but many potential benefits from a nutritional or commensal microbial standpoint. However, fecal contamination from an unknown source or in the absence of appropriate social-environmental context (as in our
experiment) could present a very large risk in terms of parasite exposure. Along similar lines, the microbial community and parasite developmental stages of fresh feces may differ substantially from that of fecally contaminated water, such that engaging in coprophagy would not necessarily present precisely the same benefits or risks as ingesting fecally contaminated water. In addition, benefits of coprophagy for microbiome maintenance may be effectively gained with only occasional consumption of feces (which is consistent with the frequency of some observations in the wild, e.g. Harcourt & Stewart, 1978). If so, a useful strategy might be to avoid fecal contamination the majority of the time, but to engage in ingestion of feces, or in this case, fecally contaminated water, in some small proportion of decisions. Under this framework, the relatively infrequent choices of fecally contaminated water that we witnessed may function to maintain the gut microbiome. Many questions about the function, phylogeny, ontogeny, and mechanism of coprophagy remain, and will require additional research.

Observational and anecdotal evidence suggests that animals do, at least sometimes, avoid endoparasites, but some of these accounts have been contradicted or inconsistent over time (see e.g., Hausfater & Meade, 1982; Markham et al., 2016). Experimental studies, such as ours, have provided more traction on the factors that influence and trade off with parasite avoidance behaviors, including tests on lobsters (Behringer et al., 2006), house finches (Bouwman & Hawley, 2010), killifish (Krause &
Godin, 1996), mice (Kavaliers, Choleris, & Pfaff, 2005), and tadpoles (Kiesecker et al., 1999). As might be expected, the results depend on the particular host-parasite system; thus, avoidance is not always observed in experimental tests. In a recent experimental study of primates (Macaca fuscata), for example, rates of avoidance were context dependent, with avoidance of parasite-relevant cues likely trading off with other factors, such as the value of food (Sarabian & McIntosh, 2015). Future experimental investigations will continue to clarify how natural selection and learning have shaped parasite and pathogen avoidance behaviors.

In conclusion, our finding that lemurs show strong preferences for clean over fecally contaminated water supports the avoidance hypothesis and suggests that water-borne infectious disease risk has acted as a selective force on lemur drinking preferences. We also found that preferences were more extreme when the level of contamination was higher; this suggests that these animals are able to judge the level of fecal contamination in water sources, or that individuals vary in the threshold of contamination that triggers an avoidance response. More generally, we argue that work on water preferences – and other types of behavioral counterstrategies to infectious disease – should consider the costs and benefits of avoiding resources that have implications for fitness, such as food and water. We expect that in wild lemurs experiencing drought, predator pressure, and competition for food, parasite avoidance may trade off with these other selective pressures.
3. Water availability impacts ranging behavior: an experimental and observational study of wild red-fronted lemurs in a dry deciduous forest

3.1 Introduction

In arid or seasonally dry habitats, water may be among the most important determinants of movement and habitat use for some species. Foraging ecologists have dedicated considerable effort to understand how food resources and foraging decisions determine animals’ habitat use (Davies et al., 2013; Kelley, 2013; Kittle et al., 2015; Owen-Smith, Fryxell, & Merrill, 2010; Seidel & Boyce, 2015; van Gils et al., 2015). Despite its critical importance, water is less commonly considered as a driver of ranging behavior. Given the increasing threat of extreme weather events predicted with future climate change (Schlaepfer et al., 2017; Trenberth et al., 2014; Wanders & Wada, 2015), understanding the flexibility of animals to respond to changes in water availability will be important for future conservation and management decisions of wild populations in dry environments. Many animal populations have suffered high mortality during droughts (Alberts, 2018; J. Altmann, Hausfater, & Altmann, 1985; Foley, Pettorelli, & Foley, 2008; Gordon, Brown, & Pulsford, 1988; Hamilton, 1985; Knight, 1995; Sapolsky, 1986), highlighting the potential strength of selection on adaptations for acquiring water and/or minimizing water intake requirements.

An extensive literature has examined the influence of food distribution on animal ranging. For decades, numerous studies have demonstrated that provisioning food
reduces home range size in a large array of vertebrate taxa (e.g. Chhangani & Mohnot, 2006; Eifler & Eifler, 1999; reviewed in Boutin, 1990), indicating that the distribution of food influences animals’ habitat use and movement patterns. The location of water is also expected to drive habitat use for certain dry-habitat living species, but controlled experiments related to water use are less common than experiments on feeding behavior (Janson, 2014; Janson & Di Bitetti, 1997).

In wild animals, work investigating this topic has primarily focused on large herbivores, including elephants (Purdon & van Aarde, 2017), desert mule deer (Marshal, Bleich, Krausman, Reed, & Andrew, 2006), and mountain sheep (Bleich, Marshal, & Andrew, 2010). Several studies provided observational evidence that primate ranging responds to the distribution of available water (Barton et al., 1992; Campos & Fedigan, 2009; Noser & Byrne, 2007; Scholz & Kappeler, 2004). In addition, experimental and observational studies have found that ranging of domestic animals of economic importance, such as cattle, is driven by the location of water sources (Draganova, Yule, Stevenson, & Betteridge, 2016; Ganskopp, 2001; Hodder & Low, 1978; Hunt et al., 2007).

In wild animals, previous studies of the impacts of water on ranging were observational in design, leaving open the possibility that water may co-occur with other valuable resources rather than directly driving ranging patterns. Supporting this possibility, even desert-adapted small mammals that do not drink water increase their use of areas near introduced water sources because they are richer in foods like insects.
or succulent plants. Similarly, some bats forage above water pools in addition to drinking from them (Voigt & Kingston, 2015). These examples highlight that in the absence of field experiments, the apparent influence of water on habitat use could be misattributed to the overlapping distribution of other resources, such as food, sleeping sites, or territory boundaries, rather than water alone.

Here, we take a combined experimental and observational approach to test the hypothesis that the spatiotemporal availability of water resources determines patterns of habitat use in wild red-fronted lemurs (*Eulemur rufifrons*) in Madagascar. We expected that this species, although not physiologically adapted to survive without water, is behaviorally adapted to flexibly adjust its patterns of habitat use in response to changes in water distribution. We used GPS collars to quantify lemur movement patterns relative to the locations of naturally occurring water sources in a dry habitat. Following an observation period, we also introduced artificial water sources into the lemurs’ home ranges. We expected that if water, independent of other resources such as food or sleeping sites, influences habitat use, providing water in areas of the habitat previously lacking water would cause increased use of these areas, and reduced occupancy in areas near previously used water sources. Conversely, if water is used only incidentally or opportunistically when lemur groups were already nearby, or otherwise had no independent influence on lemurs’ ranges, the introduction of water would have no discernible influence on the ranging patterns of the lemurs.
Specifically, we tested three predictions of the hypothesis that the availability of water influences habitat use in red-fronted lemurs. First, compared to patterns of habitat use before the artificial waterhole experiment, we predicted that lemurs would spend more time in areas of the habitat close to introduced water sources during the experiment, and less time close to naturally occurring water sources. Second, after the experiment ended, we expected these patterns to reverse, with lemurs ranging more intensively near natural waterholes and less so near artificial waterholes after the experiment. Finally, we predicted that changes in natural water availability would be reflected in the lemurs’ habitat use. We focused specifically on changes near one of the remaining waterholes at the end of the dry season. We expected lemur groups to use the area surrounding this waterhole more as other sources of water dried over the course of the dry season.

3.2 Methods

3.2.1 Study system

We studied a wild, collared, and habituated population of red-fronted lemurs living in Kirindy Forest, a dry deciduous forest in western Madagascar. Kirindy Forest CNFEREF is a protected forestry reserve, and the red-fronted lemurs at this site have been studied for more than 20 years (Kappeler & Fichtel, 2012). Rainfall is extremely rare during the local dry season (May-October), when the small river that flows through the study site dries up until only several small standing waterholes remain in the riverbed
in the late dry season. Red-fronted lemur groups drink from these waterholes regularly, and previous observational work demonstrated that non-resident groups make long trips to reach water sources within the study site (Scholz & Kappeler, 2004). It is unknown whether this species can survive on water from its diet alone, but observations suggest that red-fronted lemurs require drinking water in this habitat based on the frequency with which they drink from waterholes in the riverbed (referred to as “natural waterholes” or NWHs for the remainder of this paper).

3.2.2 Experimental procedure

To monitor the lemurs’ habitat use, we affixed GPS collars (Collar 1AA, 55g, 1600mAh, e-obs GmbH, Gruenwald, Germany) to one adult male from each of four resident groups while he was incapacitated from routine tranquilizer darting. These groups’ ranges overlap substantially and are close to the river year-round (groups B, F, J, and Q). The devices were programmed to take one GPS fix every hour for a total of 24 fixes per day. Collars attempted to connect to satellites for two minutes, and if unsuccessful, would re-initiate the following hour. We collected data on the lemur groups’ geographic positions for 15 weeks during the dry season (July 5-October 18, 2017) and downloaded the data remotely from the collars using a basestation device (e-obs BaseStation II). One group (J) was collared two weeks later than the other groups due to logistical challenges of darting. Red-fronted lemur groups are cohesive, and the
movements of one individual are likely representative of the entire group (Pyritz et al., 2013; Pyritz, Kappeler, & Fichtel, 2011).

Each lemur group was observed 2-3 days per week between the hours of 0700-1100 and 1300-1700, and their use of any water source was recorded. For another study, the NWHs were also monitored with motion-activated video camera traps (Bushnell 119875C Trophy Cam). These records combined with direct observation were used to approximate the NWH that was used most habitually by each group, although the groups would occasionally use different NWHs.

Following a five-week baseline observation period (three weeks for Group J), we conducted a five-week experiment in which an artificial waterhole (AWH) was installed for each group at a location that the group was known to frequent, based on observations during the dry season of the previous year and suggestions from experienced Malagasy field assistants. Each AWH consisted of two 10-L black rubber buckets (livestock feed buckets) placed in shallow holes dug into the ground in an area of the habitat judged to be hidden from forest trails and covered from aerial predators (Fig. 5). Two motion activated video cameras (same as used at the NWHs, see above) were positioned in the area surrounding the AWH to monitor lemurs’ use of the AWHs. Soil was filled in around the edges of the bucket so that the top edge of the bucket was flush with the surrounding earth. Water (10 L) was added to each bucket until it was full, to make the water level even with the earth. For the first three weeks of the
experiment, the water added to all of the buckets was transferred from one NWH; in the final two weeks, the quality of the water was manipulated for another experiment not discussed here (similar to captive experiments published in Amoroso et al., 2017). Water of the same quality as or cleaner than the naturally available water was present in at least one of the buckets at all times for the duration of the five weeks. The water was topped off every 2-3 days and the buckets were dumped, scrubbed with dish soap, rinsed, and refilled with new water once per week.
Figure 5: Artificial waterhole (AWH) experimental setup.

Water was initially added to the AWHs on 23 July 2017. After one week, when review of the videos from the cameras revealed that lemurs had not located the AWHs
on their own, we took advantage of previous research in this population that trained the
lemurs to approach a clicker (e.g. as used in dog training) for experiments and routine
weighing. We used this clicker noise during the second week after the AWH installation
to opportunistically attract each group to the AWH when they were located nearby
(within 100 m of the AWH). We repeated this procedure a total of two times per group
during the second week after the AWH installation, and did not repeat the procedure
again. Upon observing the lemurs’ apparent neophobia toward the AWHs, we
attempted to make the AWHs appear more similar to NWHs by adding a handful of soil
and leaves from the surrounding area to the buckets; following this adjustment, the
lemurs drank from the AWHs. After the second use of the clicker, at least one individual
from every group drank from the AWH, which we judged to indicate that the lemurs
would be aware of the new water source without any further intervention. At
subsequent visits, all individuals of the focal groups were observed to drink from the
AWHs.

For the purposes of statistical analysis, we marked the beginning of the
experimental period after the end of the clicker use, once all lemur groups located and
drank from their AWHs independently. We expected that, given the short distance and
time period over which we affected the lemurs’ movement, the clicker did not have large
impacts on our measurements. But if using the clicker on two occasions per group
detectably influenced the range estimates of the lemur groups, this would have occurred
before the start of the experimental period. Thus, if the use of the clickers introduced any bias into the analysis, we expected it to bias the results in the opposite direction of our predictions, by increasing the time lemurs spent near the AWHs before we marked the start of the experiment.

During the experimental period we continued to follow the lemur groups and monitor their use of AWHs with the motion-activated video camera traps, specifically noting the dates and times when lemurs were observed drinking from the AWHs. The experimental period lasted for five weeks, and was marked as complete when the water remaining in the AWHs dried up after we stopped replenishing them. We continued to monitor the lemur groups via GPS collars for five more weeks after the experiment ended.

3.2.3 Analyses

We quantified the probabilities of lemur groups inhabiting particular areas of the forest using a dynamic Brownian bridge movement model (dBBMM) (function brownian.bridge.dyn in package “move” v.3.0.2 in R v.3.5.1) (Horne et al., 2007; Kranstauber et al., 2012; R Development Core Team, 2011). A dBBMM estimates the probability that a moving object is located in an area based on known points taken in sequence over relatively short time intervals, assuming a conditional random walk, or Brownian motion between the start and end points (Horne et al., 2007; Sawyer, Kauffman, Nielson, & Horne, 2009). The model estimates a probability density
connecting each pair of successive points that incorporates the location error and the
time elapsed between points. Thus, it estimates a larger area of probability around
points with higher error estimates or between points with a longer time interval, and
accounts for the temporal autocorrelation in the data. In this study, the location error of
each GPS fix was estimated by the GPS device itself. In addition, the dBBMM
dynamically estimates the Brownian motion variance, a measure of diffusiveness in the
animal’s path, based on a user-defined sliding “window” and “margin” (Kranstauber et
al., 2012). After plotting the raw movement paths and visually examining the data, we
judged that setting the “window” to 13 time steps (i.e. approximately two windows per
day) and “margin” to 3 was sufficient to identify relevant changes in movement
behavior. Using all successive points in each individual’s trajectory, we thus estimated
the probability of occupancy for each group in the habitat.

To determine how probability of occupancy changed across experimental
conditions, we created dBBMMs to compare habitat use before and during the
experiment, and during and after the experiment. The paired dBBMMs were determined
on the same geographic grid, so that probability estimates were directly comparable
across conditions. A 70 m grid was drawn for each group that included a 300-500 m
buffer around the group’s extent (depending on the size of the range, per requirements
of the brownian.bridge.dyn function). Then, for each analysis, a subset of those grid
squares within 600 m of the AWH of interest for the group was selected. This process
maximized the sample size of estimated probabilities without exceeding the minimum true sample size of GPS points collected for any group during a single period of the study, and eliminated the large number of grid cells with zero probability from the buffers around the extent of the range. We subtracted the probability distribution of the earlier time period from that of the later time period to create a distribution of the change in probability from one time period to the next. We calculated the distance from each location in this distribution (the center of each grid cell) to the AWH for that group. We repeated this analysis to compare habitat use before and during the AWH experiment and to compare during and after the experiment for each lemur group.

Our approach of subtracting the two probability distributions from one another allowed for straightforward interpretation. If the lemurs used a particular area consistently before, during, and after the experiment, the probability of occupying the relevant grid square would not change across these timepoints. Our method thus detected changes in intensity of use in either direction from one timepoint to the next. In this analysis, we assumed that increases in probability in the areas closest to the AWHs during the experiment were a consequence of the new water availability in this area. We also expected the lemurs to change their ranging intensity in other areas of the forest: not only reduced ranging in areas near NWHs in response to our experiment, but also in response to unrelated changes in their surroundings, such as in food availability and inter-group dynamics.
We used a model-fitting approach to determine how distance from water source influenced the change in intensity of ranging before, during, and after the experiment. We compared the AIC scores of an exponential model to a null (intercept-only) model to determine which model the data supported (using the gnls function of the “nlme” package in R; Pinheiro et al., 2018). To control for spatial autocorrelation, each null model was fitted with several spatial correlation structures, and the one that produced the lowest AIC score was applied to both the null and exponential models for each group-timepoint comparison (corStruct argument in the gnls function, see Appendices A and B for details). A model was considered more favorable than the alternative if it had a ΔAIC > 2. Thus, if an exponential model were better supported by the data than the null – i.e., if it had a lower AIC score by more than two units – we interpreted this finding to indicate that the presence or absence of a water source in a particular area of habitat increased or decreased the lemurs’ probability of using that area, respectively.

In addition to the analysis of the probability of occupancy within the whole ranging area relative to the distance from the artificial water source, we selected a subset of the grid cells that we predicted to be especially sensitive to changes in water availability: those cells within a 140m buffer (two grid cells’ distance) of a line drawn directly between a group’s AWH and an NWH that they were observed to use. We expected to see the strongest relationship (i.e. with less noise) between the distance from AWH and change in probability of occupancy along this line. We used generalized least
square regressions of these points from all groups combined (to increase sample size) to compare the fit of the maximum likelihood linear model that included distance to a null (intercept-only) model (using the lme function of the nlme package in R; Pinheiro et al., 2018). We controlled for group by including it as a random effect in both models and for spatial autocorrelation by adding a spatial correlation term to both models, following the procedure described above.

Finally, we investigated changes in natural waterhole use as the dry season progressed. At the end of the dry season, water only remained in one small area of the riverbed (in several NWHs, including NWH17), and the NWHs used previously by three groups (B, F, and J) had dried up. If water availability has a major impact on lemurs’ habitat use, we expected that probability of occupancy would be higher in locations closer to NWH17 in the late dry season, as compared to earlier in the dry season (i.e., before and after the experimental manipulation). For these three groups, we repeated the analysis of the change in probability along the line between the NWH used before the experiment and NWH17, following the same procedure described above for the three groups combined. Group Q was not included in this analysis because a NWH that they typically used before the experiment was still available after the experiment, so we expected little change in the probability of this group’s occupancy near this NWH.
3.3 Results

Across the 15-week period, the GPS devices collected a total of 9474 points, or an average of 2496 points per device (SD: +/- 241.7). The average location error estimated by the devices was 15.99m (SD: +/- 12.74m). Lemur groups took approximately two weeks (15.25 +/- 1.7 days) to begin using the AWHs on their own after the initial addition of water. After the start of the experimental period, lemur groups visited and drank from the waterholes on average once per day (23.19 +/- 19.8h), excluding activations of the camera or observations within an hour of each other, which we considered part of the same visit. All individual members of each group were observed to drink from the AWHs.

Plots of utilization densities for the groups before, during, and after the experiment revealed an apparent change in habitat use intensity during the experiment, although these changes are more marked in some groups than others (Fig. 6). Two types of changes were visible: changes to the boundaries of the utilization densities and to the intensity of use within the previous boundaries. The pre- and post-experiment plots also demonstrated how natural fluctuations in water availability influence ranging, causing increased intensity of use by groups in new areas of the forest where the only waterholes remained by the end of the study period.
Figure 6: Utilization densities for each group at the three experimental time periods.
When assessing change in ranging patterns as a function of distance from the AWH, we found that the exponential model offered a better fit to the data for all four groups in the comparison of ranging patterns before and during the experiment, and for three of four groups in the comparison of ranging patterns during and after the experiment (Table 1). These results are consistent with changes in habitat use toward the AWH, as predicted. When comparing models of the change in occupancy after compared to during the experiment for Group F, the null model was better supported by the data than the exponential model. For all groups, the grid squares closest to the AWH were used more during the experiment relative to periods before and after the experiment (Figs. 7 and 8). Areas at various distances from the AWH also underwent changes in the intensity of use. These changes may be stochastic, or due to ecological factors that were not this study’s focus, such as natural changes in food availability, interactions with other groups, or movements of predators.
Figure 7: Comparison of models of change in probability of occupancy.
Support for the exponential model over the null model indicates that the locations closest to the AWH underwent the most significant changes in the probability of occupancy, and in the predicted direction. Shaded cells indicate which model had the lower AIC score, or is more likely given the data. Absolute values of the difference in AIC scores between the two models are provided in the rightmost column, which had to be >2 to support one model over the other.
When we analyzed only the points along the line between a group’s AWH and its habitually used NWH before the experiment, we found that distance was a statistically significant negative predictor of change in probability of occupancy (distance coefficient $= -0.000036$, $P < 0.01$, $t = -3.48$, DF = 143; Fig. 9, Appendix A). A linear model that included distance was better supported by the data than the null (Table 2).
Support for the linear model in the first subset (AWH-previous NWH) indicates an increase in probability of occupancy in the areas where water was experimentally added, and a decrease in areas previously used for water. Support for the linear model in the second subset (NWH17-previous NWH) indicates a response to natural water drying: increased use of areas close to a remaining NWH (17), and decreased use of areas near the dried NWH.

Shaded cells indicate which model had the lower AIC score, or is more likely given the data. ∆AIC refers to the absolute value of the difference in AIC scores between the two models.
Analysis of the change in probability of occupancy after the experiment relative to before the experiment, focusing only on the points along the line between the NWH used habitually before the experiment and the closest remaining NWH after the experiment (NWH17), also revealed better support for a linear than null model (Table 2). Distance was a significant negative predictor of change in probability in this subset of the data, indicating that lemurs were more likely to spend time in the areas near NWH17 after the experiment than before the experiment (distance coefficient = -0.00044, 0 100 200 300 400 500 600 Distance from AWH

Change in Probability During-Before Group

B
F
J
Q

Figure 9: Change in probability along line between AWH and NWHs.
In general, the lemurs were also less likely to spend time near their previously used NWH after than before the experiment. In other words, as a NWH dried up, lemurs shifted their ranges away from this NWH and toward a different NWH that still contained water, suggesting that the presence of water was the primary reason for lemur groups to range here initially.

Figure 10: Change in probability along line between early NWH and closest remaining NWH (NWH17).
3.4 Discussion

The main finding of this study is that the spatiotemporal availability of water influences the habitat use patterns of wild red-fronted lemurs. Specifically, we demonstrated that experimental and natural changes in the distribution of water altered the boundaries of the area in which groups ranged and shifted the density of occupancy within the boundaries of the ranging area. Adding water to areas of the habitat resulted in increased intensity of ranging in these areas, and removal of the water caused a reduction of intensity. This pattern was observed in all groups except Group F, which did not reduce its intensity of ranging near the AWH after it had been removed. We assume this can be attributed to the AWH having been introduced into an area of the habitat that remained favorable for reasons independent of the presence of water. Given our experimental approach, we are able to isolate the effects of water, and can conclude that water is a direct and important determinant of movement and habitat use in this species. Overall, these findings imply a high degree of behavioral flexibility in the areas ranged by our study groups as a function of accessing water resources.

As suggested by the case of Group F, patterns of habitat use are complex and likely respond to water availability, but also to other ecological factors, such as food availability, social context, and habitat suitability (Alba-Mejia et al., 2013; Clapp & Beck, 2016; Crofoot, 2013; Marshal et al., 2006; Poirotte, Benhamou, et al., 2017; Rehnus, Marconi, Hackländer, & Filli, 2013; Viana et al., 2018). Although our experimental
approach gives us more confidence in attributing specific importance to water for
determining lemur ranging, experimental conditions cannot be perfectly controlled in
wild populations. Given the noise introduced by other environmental changes (e.g.
feeding trees) that likely influence lemur ranging, the strength with which our results
support the influence of water is notable and suggests that water availability is an
important driver of the ranging patterns of these lemur groups.

Previous observational evidence has supported that water can influence habitat
selection in large mammalian herbivores (Bleich et al., 2010; Marshal et al., 2006; Purdon
& van Aarde, 2017) and primates (Barton et al., 1992; Campos & Fedigan, 2009; Noser &
Byrne, 2014; Scholz & Kappeler, 2004) living in arid habitats. The experimental approach
taken in this study – introducing and removing water sources over a short time scale –
provides a great deal of inferential power in terms of the effects of water on the behavior
of dry-habitat living species that drink free water, and corroborates these previous
studies.

Our findings suggest that water distribution has a major impact on animal
behavior. While this is known for large, terrestrial animals and species of economic
importance, only a handful of studies have investigated the effects of water availability
in various forms and distributions, and typically with observational or indirect methods
rather than experimental approaches. For example, ring-tailed lemur’s (Lemur catta)
ranges in an arid, semidesert scrub environment mapped onto the distribution of a food
resource with high water content (Kelley, 2011, 2013). Similarly, provisioning of water made it possible for introduced, hybrid brown lemurs (*Eulemur spp.*) to live in Berenty Reserve, which would otherwise be too dry for them to survive (Jolly, 2013; Pinkus, Smith, & Jolly, 2005). Introduced water sources are also important drivers of large herbivore and small mammal density in arid and semi-arid habitats (Chamaillé-Jammes, Valeix, & Fritz, 2007; James, Landsberg, & Morton, 1999; Loarie, Aarde, & Pimm, 2009; Purdon & van Aarde, 2017; Switalski & Bateman, 2017). Given this evidence, distance from water sources is likely to be an important predictor of species ranges, which has some support in lemurs, and has already been put into practice for estimating population sizes (Herrera et al., 2018; Mercado Malabet & Colquhoun, 2018).

Ranging behaviors are a clear choice of behavior to consider relative to water availability and distribution. But this study also raises questions about how animals detect, locate, and keep track of the status of ephemeral water sources that vary across years. For example, in this study, the lemurs did not detect or use the AWHs on their own for an entire week after introduction. This suggests that an essential cue of the presence of water may have been missing in our artificial setup, or that memory may play an important role in determining the areas where search efforts are expended. Relatedly, research on elephants suggests that more experienced mothers may be more successful in supporting their calves through droughts in part because they remember the locations of critical water sources when others have dried up (Foley et al., 2008). In
addition to the tasks of finding and remembering the locations of water, the lemurs must also navigate and coordinate group movements to water sources. In a previous study of this population, red-fronted lemurs responded to spatially separated drinking platforms by reducing group cohesion in transit to these platforms, suggesting consensus costs of group coordination in this context (Pyritz et al., 2013).

Variation in water quality may also influence habitat use, yet we have little understanding of the characteristics of water that are important to animals. For example, captive lemurs prefer clean water to water that is contaminated with feces (Amoroso, Frink, & Nunn, 2017; Chapter 2, this dissertation). Evidence in wild elephants suggests that they also prefer to avoid feces in their selection of water (Ndlovu et al., 2018). Open questions include whether other wild animals exercise these same preferences for clean water, whether enough variation is present in naturally occurring water sources to exercise such preferences, and whether water scarcity might mediate these preferences. Studies on sensory ecology, group coordination, and parasite avoidance are logical next steps for investigating the impacts of water on animal behavior and evolution.

Our approach and findings provide a potential intervention for species facing unpredictability of water resources in the future, as global climate change brings with it more severe weather events, including possibly more droughts or longer periods of reduced rainfall (Desbureaux & Damania, 2018; Schlaepfer et al., 2017; Trenberth et al., 2014). Changes to the distribution and quality of water sources may also be among the
many complex impacts of more intensive human land use on ecosystems (Baker, 2003; DeFries & Eshleman, 2004; R. B. Jackson et al., 2001). In this study, lemurs proved to be highly flexible in their use of water resources in new areas of the habitat, suggesting that introducing of artificial water sources may support survival through challenging times. However, more research is needed to understand the impacts of provisioning water for individual species and for ecosystems more broadly (Simpson, Stewart, & Bleich, 2011).

In conclusion, we provide the first experimental evidence of the effects of water availability on wild primate ranging patterns. Undoubtedly, water ought to be an important factor in the ecologies of other wild animals in dry habitats. Future work should consider adapting this simple study design to determine whether these results are replicable in different species and habitats, and to understand the factors that influence variation in responses to water availability. Ultimately, water is expected to become an increasingly important factor for survival of many animals as a result of global climate change; thus, understanding the selective pressures exerted by water demands on animal behavior represents a high priority.
4. Experimental and observational evidence that wild red-fronted lemurs avoid fecal contamination and reduce travel costs in their selection of water sources

4.1 Introduction

In the arms race between hosts and parasites, selection is expected to favor parasites that are increasingly difficult for hosts to detect, and hosts that more effectively detect and avoid parasites (Curtis, 2014; Hart, 2011; Moore, 2002; Poirotte, et al., 2017). In this coevolutionary dynamic, many parasites may succeed in avoiding detection by hosts. Thus, hosts may also be selected to identify and avoid cues that are reliably associated with parasite risk, including environmental cues such as fecal contamination (Curtis, 2014; Sarabian & MacIntosh, 2015; Sarabian et al., 2017).

Avoidance of infection risk indicators, like feces, may have immediate health and ultimate fitness benefits, but also associated costs. In contexts of resource scarcity, costs of avoiding parasites might be especially high if uncontaminated resources are limited. For example, selectivity for mates that are uninfected with a sterilizing sexually transmitted pathogen would be favored even if the costs of avoidance are high, but this might change if the pathogen is highly prevalent in the population, leaving few uninfected options (Kokko, et al., 2002). Similarly, preferences for clean water resources may be most costly when the need for water is high, water availability is low, and most or all alternative options are fecally contaminated. With these examples in mind, we
expect that preferences for avoiding contamination should be flexible and depend on the interaction of multiple factors, including environmental context and individual need.

Avoiding parasite transmission might also be costly if doing so requires forgoing resources or expending additional energy. For example, foraging oystercatchers (*Haematopus ostralegus*) most frequently consume cockles (*Cerastoderma edule*) that are intermediate-sized, apparently resulting from a trade-off between maximizing energy intake (which would favor larger cockles) and minimizing parasite risk (which is lower in smaller cockles) (Norris, 1999). Similarly, several mammal species were observed to forego more food in areas with higher tick abundance (Fritzsche & Allan, 2012). In both of these cases, avoidance of parasites has costly repercussions in terms of energy intake.

Although existing accounts of parasite avoidance tend to highlight the remarkable efforts that hosts make to avoid parasite infection (e.g. Hart, 2011; Curtis, 2014), hosts do not always avoid parasites, perhaps because the costs of infection are outweighed by the energetic or other fitness-related benefits of consuming a resource (Hutchings et al., 2000; Hutchings, Kyriazakis, & Gordon, 2001). For example, house finches (*Carpodacus mexicanus*) risk infection with a pathogenic bacterium by feeding near sick, contagious conspecifics because they have a competitive advantage over these lethargic individuals, and can increase their food intake (Bouwman & Hawley, 2010). In other cases, parasite avoidance is performed flexibly. Japanese macaques (*Macaca fuscata*) adjust the frequency with which they consume food items that are in contact
with feces based on the quality of the food item (Sarabian & MacIntosh, 2015). Mandrills
(*Mandrillus sphinx*) avoid grooming the perianal area of partners harboring a large
richness of protozoan parasites, but groom the same areas of partners with fewer
protozoa (Poirotte et al., 2017). Together these examples highlight that in decisions
related to parasite exposure, nutritional or social benefits may outweigh the costs
associated with parasite infection.

A handful of previous studies have considered how feces avoidance influences
mammals’ choice of drinking water. In a series of experiments, domestic cattle preferred
clean water to feces-contaminated water, and individuals with access to clean water
gained more weight than those with only access to pond water (Willms et al., 2002).
Elephants (*Loxodonta africana*) in Kruger National Park used artificial water sources more
often when those water sources had lower levels of *Escherichia coli* contamination,
implying that the elephants avoided fecal contamination in water (Ndlovu et al., 2018).
The authors of this study proposed that the degree to which a water source influences
animal movements may depend in part on the parasite transmission risk of the water.
Taken together, these studies suggest that given its influence on growth, survival, and
reproductive success, water quality may impact animal behavior, with potentially broad
impacts for ranging, habitat choice, and local landscape use.

Waterholes may represent a hotspot for parasite transmission during dry
periods, given that almost any fecal- orally transmitted parasite can be transmitted
through contaminated water, including helminths, protozoa, bacteria, and viruses (Palumbo et al., 2002). The areas around water sources are also likely to provide a moist microhabitat that promotes the survival of infectious stages of parasites in the soil (R. Perry, 1999). Increased bodily contact with water and wet surfaces can increase the prevalence of parasites across individuals, as reported for Schistosoma in olive baboons (Papio anubis) (Müller-Graf et al., 1997), or across seasons, such as for Strongyloides in white-faced capuchins (Cebus capucinus) (Parr, Fedigan, & Kutz, 2013). Contact with the ground at waterholes might also contribute to host species differences in parasite richness, such as that between the sympatric Verreaux’s sifaka (Propithecus verreauxi) and red-fronted lemur (Eulemur rufifrons). Red-fronted lemurs are more commonly terrestrial, drink from standing waterholes during the dry season, and were found to harbor more than 10 species of gastrointestinal parasites; Verreaux’s sifakas, which are more arboreal and do not drink from waterholes, only harbor one species (Clough, 2010; Clough, Heistermann, & Kappeler, 2010; Springer & Kappeler, 2016). These examples illustrate the role that waterholes might play in parasite transmission for some host species.

A previous study in captive lemurs investigated how fecal contamination influenced lemurs’ water choices (Amoroso et al., 2017). In that study, five species of lemurs avoided drinking fecally contaminated water and preferred to drink clean water. This finding was consistent in both a dry habitat-adapted lemur species (Lemur catta)
and a rainforest-adapted lemur species (Varecia variegata), suggesting that despite the potential costs of selectivity for clean water in a water-scarce habitat, avoiding fecal contamination in water is still favored. Alternatively, the preference for clean water observed in this study could be an artifact of captivity, where dry habitat-adapted species have more access to clean water than they would in the wild. Wild animals in dry habitats may be less selective in their water choices because clean water sources are less abundant and could require greater travel distances.

To understand how wild lemurs navigate the tradeoffs between parasite avoidance and resource acquisition, we focused on red-fronted lemurs in a dry deciduous forest in western Madagascar. Red-fronted lemurs require water for survival, yet water is relatively scarce in their habitat, and most water sources and surrounding areas show evidence of contamination. We investigated the hypothesis that parasite transmission risk has exerted selective pressures on red-fronted lemurs’ water preferences, such that lemurs prefer to drink water with lower risk of parasite transmission. In this study, we operationalized the risk of parasite transmission as the amount of conspecific fecal material surrounding the water sources. In choice experiments at artificial water sources, we predicted that lemurs would choose to drink clean water more frequently than feces-contaminated water. Additionally, in an observational study, we predicted that lemurs would be more likely to drink from naturally-occurring waterholes with lower levels of conspecific fecal contamination in
the area surrounding them. We expected several other factors to modulate this predicted relationship, including the scarcity of water sources at different locations in the habitat, the energetic costs of traveling to water sources, and the subsequent buildup of fecal material at waterholes as a result of lemurs’ use of them. We investigated each of these factors alongside our main hypothesis about parasite avoidance.

4.2 Methods

4.2.1 Study system

Our study took place in Kirindy Forest, a dry deciduous forest in western Madagascar. Kirindy Forest CNFEREF is a protected core area of the Aire Protégé Menabe Antimena, where wild red-fronted lemurs have been studied for more than 20 years (Kappeler & Fichtel, 2012). This population of red-fronted lemurs includes several habituated groups comprised of collared, identifiable individuals, and an unknown number of unmarked groups. During the local dry season (May-October), rain falls extremely rarely. The small river in the study site dries up across this period, leaving only several small, standing waterholes (referred to as natural waterholes, or “NWH” for the remainder of this paper) in the riverbed by the end of the dry season (Fig. 11A). The NWHs typically form at depressions in the riverbed where water collects, often with steep banks on one or two sides. Thus, in most cases, lemurs drink near one or the other open sides, so that they can reach the water from less steep group, and they typically remain close to the bank such that they are protected on at least one side. Previous
observational work has documented that red-fronted lemurs drink from these NWHs regularly, sometimes making long treks to the water sources from outside of the study site (Scholz & Kappeler, 2004).

Figure 11: Photos of water sources in this study.

4.2.2 Experimental study

The current study comprised both an experimental and observational component. The observational component was conducted during the mid-late dry season, July-October 2016 and July-September 2017, and the experimental manipulation was conducted only in August-September 2017. For another study of the impacts of water distribution on lemur ranging behavior, we installed an artificial waterhole (AWH) within the home range of each of four marked groups for five weeks (Chapter 3, this dissertation). For each AWH, we dug two shallow holes into the ground and placed
a 10-l black rubber bucket (livestock feed bucket) in each, in an area of the habitat that was well-hidden from forest trails and covered from aerial predators (Figs. 11B & 11C). The gap around the edge of the bucket was filled with soil to make the top of the bucket even with the surrounding earth, and then 10 liters of water were added to each bucket until it was full. We added additional water every 2-3 days (as needed). We dumped and cleaned the buckets with dish soap once per week and refilled the water. For the first three weeks of the experiment, the water added to all of the buckets was transferred from one NWH.

For eight days in the remaining two weeks of the experiment, we manipulated the quality of the water for three marked groups (B, F, and J), comprising 7, 4, and 10 individuals at the time of the study, respectively. At each AWH, we filled one bucket with 10 l of clean, filtered water from the supply that is also used for drinking by the research and support staff at the Kirindy Forest Research Station. We filled the other bucket with 10 l of feces-contaminated water, which was prepared in the same manner as in the previous captive study (Amoroso et al., 2017). Specifically, we collected 150 g of fresh fecal material opportunistically from each lemur group on the day prior to the introduction of the water into the AWH. We added the feces to one liter of clean (filtered) water and boiled this mixture for at least 1 minute to disinfect it. The feces-contaminated water was allowed to cool overnight and was then added to one of the buckets at the AWH along with an additional 9 l of clean (filtered) water. This resulted
in water that had a fecal contamination level of 15 g/l, consistent with a previous study of lemurs’ water preferences in captivity (Amoroso et al., 2017). Fecal material was visible in the water, and the water smelled of feces to human observers.

Between the first four days and the last four days of the water quality experiment, we switched the bucket that contained the fecally contaminated and clean water choices from the bucket on one side to the bucket on the other side, to control for any side bias or preference that the lemurs might have had. In fact, before the start of the water quality experiment, while the two water sources were identical, we found that one group (F) had a strong preference for one side.

We installed two motion-activated video camera traps (Bushnell 119875C Trophy Cam) at each AWH to monitor the lemurs’ use of each water source. The cameras recorded one-minute videos with a one-second delay during daylight, and 15-second videos with a five-second delay in the dark using an infrared flash (undetectable by the lemurs). Thus, the cameras could be activated at any time throughout the 24-hour period, which was necessary because red-fronted lemurs are cathemeral (flexible in the distribution of their activity periods throughout the 24-h cycle; Kappeler and Erkert, 2003). We also followed the three focal groups 2-3 days per week between 08:00-11:00h and 13:00-17:00h. In these observations we recorded the drinking events performed by each individual from the AWHs. A drinking event was defined as an individual lemur’s mouth contacting and tongue lapping the water. Pauses in drinking while the individual
remained at the waterhole edge were considered part of the same event. A new event was counted if the individual moved out of reach of the AWH and returned to drink again. We combined our direct observations of drinking with the camera trap-recorded drinking for our final dataset.

For the analysis of the experimental data, to control for non-independence of repeated drinking events by the same individual in a short window of time, we limited our dataset to only the first water choice by an individual on a given day. We also imposed a more stringent filter by subsetting the data to include only the first water choice by an individual during each of the experimental periods, i.e. under each of the arrangements of the clean and feces-contaminated water options. We performed one-sided binomial tests on each of these datasets to discern whether the counts of drinking from the clean water source were significantly higher than an expected probability of 0.5 (i.e., chance).

4.2.3 Observational study

To determine whether fecal contamination influenced lemurs’ use of NWHs, we monitored the frequency of red-fronted lemur group visits to a subset of between 6 and 17 of all available NWHs each week, using the same motion-activated video cameras as in the experiment. We selected the subset of monitored NWHs to include as wide a variety of waterhole characteristics as possible, including NWHs that varied in size, location, and lemur use, based on our initial impressions and previous experience; thus,
some waterholes were not monitored during the study. We reviewed the videos recorded by the cameras at the NWHs and scored the dates, times, and group identities (if collared) of red-fronted lemur visits to the NWHs. Both collared and uncollared groups of red-fronted lemurs used the NWHs, and their visits often overlapped with one another. Collared groups were easily identified, so that overlapping camera activations by collared and uncollared groups could be counted as visits by more than one group to the waterhole. However, if multiple groups of uncollared lemurs overlapped in their activation of the waterhole cameras, they could not be distinguished. To deal with this issue, we applied a heuristic based on the typical duration of camera activations we recorded by marked groups: we considered repeated camera activations for less than one hour by uncollared individuals to comprise a visit by one group, and counted repeated camera activations by uncollared individuals extending for each additional hour as an additional visit by another group. This rule may have slightly over- or under-estimated the number of visits by unmarked groups on any single day, but it was applied consistently across waterholes.

We also assumed any activation of the cameras around a NWH indicated that lemurs drank from the NWH, even in the very few instances that drinking was not directly observed. Based on personal observations in the field, if lemurs were close enough to the NWH to activate the camera, they most likely drank from the NWH. This might occur if they were within the range of the camera but did not re-activate the
camera while drinking (when they often stayed still except for small mouth movements that may have been blocked from the camera’s motion detection), or if they moved outside of the camera’s range or frame to drink, both of which may have prevented the camera from recording the drinking event.

In addition to monitoring the use of waterholes by all lemur groups in the population, we monitored the movements of several groups more closely during July-September 2017 using GPS collars (Collar 1AA, 55g, 1600mAh, e-obs GmbH, Gruenwald, Germany). During routine tranquilizer darting, a GPS collar was affixed to one adult male from each of five habituated study groups (A, B, F, J, and Q). We programmed the GPS devices to take 24 fixes per day: i.e., one fix per hour. At the time of each fix, the collars would attempt to connect to satellites for two minutes, and if unsuccessful, would re-initiate the following hour. We downloaded the data from the GPS collars remotely using a basestation device (e-obs BaseStation II). Red-fronted lemur groups are cohesive, and the movements of one individual are likely representative of the entire group (Pyritz et al., 2013, 2011).

We surveyed the riverbed within the limits of the study site every two weeks to record the locations of all NWHs using handheld GPS (Garmin GPSmap 62). In the middle of each week, we also obtained descriptive data for each camera-monitored NWH, including the length of the waterhole and the number of red-fronted lemur fecal droppings within 5 m of the edge of the camera-monitored NWHs. Thus, when we refer
to “fecal contamination” of waterholes, we refer specifically to the count of red-fronted lemur fecal droppings in the area within 5 m of the waterhole edge rather than to fecal contamination of the water directly. Although we do not measure the fecal contamination in the water itself, a relationship would be predicted between the level of fecal contamination in the area surrounding the NWHs and the level of fecal contamination of the water. Defecation into the water directly was observed rarely, particularly in the few cases in which tree limbs extended over the waterhole, but was not systematically recorded in this study.

Because fecal contamination of waterholes was predicted to increase from one week to the next, we investigated changes in the number of lemur group visits within a week. We expected that fecal contamination would increase as a result of lemur visits. If lemurs avoided more fecally contaminated waterholes, we expected that waterholes with a higher measured level of fecal contamination would be visited less by lemurs in the subsequent days: i.e. that the groups would be less likely to return to the waterholes that were more fecally contaminated by earlier visits. To test this prediction, we summed the number of visits by lemur groups for each waterhole across the two days before the measurement of fecal contamination at mid-week, and across the two days after the measurement of fecal contamination. We then created a generalized linear mixed model that predicted the number of lemur visits after our measurement, using the number of visits before our measurement, feces contamination, and waterhole length as
fixed effects, and with waterhole identity and week included as random effects. In a second model, we added an interaction term between the number of visits before our measurement and fecal contamination. We compared the model fit of these two models as approximated by Akaike Information Criterion (AIC) scores. We considered models with a lower AIC score to fit the data better than higher scores, with a cutoff of 2 units to determine the best fitting model.

We repeated all steps of the analysis of visits before versus visits after measurement of fecal contamination on a subset of the dataset, in which we included only visits by collared, individually-identifiable groups, to ensure that our heuristic for counting uncollared groups did not interfere with this analysis. We also tested an additional model in the collared-only analysis to control for the possibility that agonistic encounters with other groups may have decreased the likelihood for groups to return to a NWH. This additional model thus included a binary term that indicated whether or not any of the visits in the two days before measurement of fecal contamination occurred within an hour of another group.

To determine whether fecal contamination influenced a lemur group’s time to return to a waterhole, we calculated the period of time between subsequent visits of a group to each waterhole that it visited more than once. We created a linear mixed model that predicted the return time, using feces contamination and waterhole length.
measured during the week of the first visit as fixed effects, with waterhole identity, group identity, and week included as random effects.

Finally, to examine the role of travel distance and feces avoidance in the lemurs’ selection of waterholes, we examined how a waterhole’s fecal contamination and distance from most recent resting site influenced lemur groups’ waterhole choices among the available options. First, for each visit by a GPS-collared group to a NWH, we used the GPS data to identify the most recent previous resting site, which we defined as a location at which the collared lemur moved less than 30 m in the preceding hour. This distance cutoff was judged to be sufficient to capture resting locations because hours with movements smaller than 30 m corresponded to habitual times of resting/sleeping, and resulted in a time interval of <6 h between all waterhole visits and the previous resting point; this interval is reasonable given lemurs’ typical pattern of activity, which is characterized by relatively frequent rest. We calculated the distance between this resting site and all NWHs that were monitored during the week that the visit occurred. This set of monitored NWHs served as a representative sample of the available choice set of NWHs. In addition to the distance from the most recent previous resting site, we compiled data on the fecal contamination and length of the monitored NWHs as measured during the week of the visit.

We used a mixed conditional logistic regression (R package “mclogit”; Elff, 2009) to determine how lemurs’ choice of NWH was influenced by distance from previous
resting site, fecal contamination, and waterhole length measured during the same week as fixed effects, and waterhole identity as a random effect. Conditional logistic regressions compare a selected element to an unselected set of alternative elements, based on the characteristics of each of the elements in the set (Elff, 2009). For this analysis, the monitored available NWHs during the week of the visit comprised the set of alternative options. The NWH that was selected in the lemur group’s visit received a 1, and all unselected NWHs in the set received a 0. The conditional logistic regression thus compared the selected waterholes to unselected alternatives available at the time of the choice to determine how travel distance, fecal contamination, and waterhole length influenced lemurs’ choices.

4.3 Results

4.3.1 Experimental study

Our experiment yielded 114 observations of an individual’s first choice of water on different days, including the choices of 21 different individuals from three groups, with an average of 5.4 +/- 1.9 choices per individual (with a maximum possible eight choices per individual, if they drank from the AWH at least once on each of eight days of the experiment). Of these choices, 107 were from the clean water and 7 were from the feces-contaminated water (p<0.0001, binomial test; Fig. 12). (When all instances of drinking were included, the same pattern was upheld.) Of these 7 choices of the feces-contaminated water, 6 were performed by members of a group with a strong side bias...
for the bucket that contained the fecally contaminated water. When we analyzed only the first choice by each individual under each arrangement of the AWH (i.e. a maximum of two choices per individual), our dataset included resulted in 37 instances of drinking from the clean water, and 3 instances from the feces-contaminated water ($p<0.0001$, binomial test).

Figure 12: Waterhole choices by lemurs in the AWH experiment.
4.3.2 Observational study

We collected observational data over 16 weeks across two field seasons for a total of 174 lines of data, where each line corresponded to one waterhole in a given week. We recorded a total of 559 visits by lemur groups to the focal waterholes during the times that they were monitored for this analysis. Each waterhole received a mean of 3.2 visits by lemur groups in a week, with the number of visits in a week ranging from 0-27. Most waterholes on most weeks were not visited at all by lemur groups.

In our analysis of the visits after measurement of fecal contamination compared to beforehand, we found that the number of visits before measurement and fecal contamination were both significant positive predictors of the number of visits after our measurement. The interaction between visits before and feces contamination was significant and the coefficient was negative, indicating that the relationship between visits before and visits after was negatively mediated by the level of fecal contamination. In other words, lemurs were less likely to visit more contaminated waterholes after than before our measurement of them, and were more likely to visit less contaminated waterholes after than before (Fig. 13A; Table 3). The model that included the interaction was substantially better supported by the data (AIC=397.1) than the model without the interaction (AIC=420.0).

When we restricted this analysis to only the collared groups, we found support for the same interaction between visits and fecal contamination (Fig. 13B). The model
that included this interaction (Table 3) was slightly better supported by the data (AIC=224.1) than the model without the interaction (AIC=226.2). We did not include week as a random effect because the model could not converge when week was included.

In this analysis of the collared groups, we also added a term to control for the possibility that groups were avoiding returning to waterholes because of previous aggressive encounters with other groups. Adding a binary term for the presence of another group did not improve the fit of the model (AIC=225.8), and the presence of another group was not a significant predictor of visits after measurement (estimate=-0.09, z=-0.5, p>0.05).
Figure 13: Relationship between visits after and visits before measurement is mediated by fecal contamination. A: all groups; B: collared groups only.
Table 3: Results from generalized linear mixed models testing the effect of visits before measurement and fecal contamination on visits after measurement.

<table>
<thead>
<tr>
<th>Model</th>
<th>Fixed effects</th>
<th>Estim</th>
<th>SE</th>
<th>z val</th>
<th>p val</th>
<th>Rand eff</th>
<th>Var</th>
<th>SD</th>
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<td>Interaction (all groups)</td>
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<td></td>
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<td>&lt;0.05</td>
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<tr>
<td></td>
<td>Feces measured within 5m of NWH edge</td>
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<td>0.11</td>
<td>4.39</td>
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<td></td>
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<td></td>
<td>Waterhole length</td>
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<td>-0.99</td>
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<td>Week</td>
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<td></td>
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<td></td>
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<tr>
<td>No interaction (all groups)</td>
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<td>0.23</td>
<td>-3.79</td>
<td>&lt;0.05</td>
<td>Waterhole ID</td>
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<td>0.70</td>
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<tr>
<td></td>
<td>Visits before measure</td>
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<td>0.09</td>
<td>5.24</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Feces measured within 5m of NWH edge</td>
<td>0.27</td>
<td>0.10</td>
<td>2.50</td>
<td>&lt;0.05</td>
<td>Week</td>
<td>0.25</td>
<td>0.50</td>
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<td></td>
<td>Waterhole length</td>
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<tr>
<td>Interaction (collared only)</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Feces measured within 5m of NWH edge</td>
<td>0.16</td>
<td>0.17</td>
<td>0.98</td>
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<tr>
<td></td>
<td>Visits before * feces measured</td>
<td>-0.21</td>
<td>0.11</td>
<td>-2.01</td>
<td>&lt;0.05</td>
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</table>

To determine how the significant interaction between visits before measurement and fecal contamination might explain visits after measurement, we examined whether groups’ return time to a NWH increased with its fecal contamination. Among the collared groups, we found that average return time to the same NWH was 3.69 days, with a range of 6 hours to 40.9 days. The linear mixed model, which included waterhole identity, group identity, and week as random effects, indicated that lemurs took slightly longer to return to NWHs that had higher levels of fecal contamination in the areas.
surrounding them (Fig. 14; intercept=0.32, feces estimate=0.22, z=2.09, p<0.05). Lemurs also took longer to return to larger waterholes (length estimate=0.49, z=3.05, p<0.05).

Figure 14: Lemurs returned to more fecally contaminated waterholes after longer time intervals.

Finally, we examined how lemurs’ choice of each NWH among the possible set of alternative NWHs was influenced by distance from previous resting site, fecal contamination, and waterhole length measured during the same week as fixed effects, and waterhole identity as a random effect. The mixed conditional logistic regression,
which included waterhole identity as a random effect, revealed that the distance to the NWH from the most recent resting site was the most important variable determining lemurs’ choice of waterhole; i.e. lemurs were most likely to select waterholes that were closest to their most recent resting site (intercept=1.05, distance estimate=-2.63, z=-5.83, p<0.05). Fecal contamination and waterhole length were not significant predictors of NWH choice in the conditional logistic regression (feces estimate=0.31, z=1.05, p>0.05; length estimate=0.52, z=1.63, p>0.05).

### 4.4 Discussion

Broadly, our results suggest that avoidance of fecal contamination is a factor in lemurs’ choice of water sources. Our experiment provides clear evidence that lemurs prefer to avoid fecally contaminated water when they have two otherwise equal options from which to choose. This finding is consistent with an equivalent experiment in captivity (Amoroso et al., 2017, Chapter 2, this dissertation).

This preference for clean water in the experiment is reflected to some degree in lemurs’ choices of NWHs. Within a week, we found that lemur groups visited NWHs more frequently than previously when fecal contamination was lower, and returned to them after longer delays in time when fecal contamination was higher. In other words, the results of our observational study indicate that lemurs may have chosen alternative NWHs when previously used NWH became increasingly fecally contaminated. Together, these findings suggest that despite generally high levels of fecal contamination...
at commonly used NWHs, lemurs mediated their exposure to fecal parasites by choosing less fecally contaminated NWHs when those they used previously became highly contaminated.

However, the strategy of reducing visits to previously used and highly contaminated NWHs did not lead to homogenization of NWH use over time, which would be predicted if feces avoidance were the primary driver of waterhole choice. Although the lemurs more frequently returned to NWHs that were less fecally contaminated, they rarely or never used some other NWHs, which accordingly had little or no fecal contamination in the areas around them. Thus, feces avoidance may be considered a secondary factor in waterhole selection.

Based on our analysis of NWH distance from previous resting site, avoiding fecal contamination appears to be a secondary priority behind minimizing energy expenditure in lemurs’ choice of water source. When we directly compared distance and fecal contamination of the waterholes that lemurs chose to the unselected alternatives, distance from the previous resting site was clearly the most important factor that emerged from the model, and fecal contamination was not a significant predictor. An important caveat to consider in this analysis is that fecal contamination was only measured once weekly, but the contamination certainly varied from each visit to the next within a week. More fine-scale temporal data on fecal contamination around the
NWHs might provide better insight how fecal contamination influenced lemurs’ selections of NWH among the alternatives.

Other ecological variables not examined in this study may have also determined the suitability of certain NWHs for use by lemur groups. For instance, highly frequented NWHs may have been perceived as having lower predation risk than other NWHs. One of our models indicated that lemurs returned less frequently to larger NWHs. More frequent visits to smaller waterholes may indicate a greater certainty of the absence of water-dwelling predators like crocodiles, which are locally present, although were not observed during the study period. In fact, at waterholes, lemurs commonly exhibited behaviors consistent with threat perception and arousal, such as “woof” vocalizations and “tail wagging,” which are typical of responses to terrestrial predators and during inter-group encounters (Fichtel & Hammerschmidt, 2002; Fichtel & Kappeler, 2002; Pereira & Kappeler, 1997). In accordance with this interpretation, lemurs were anecdotally observed to defecate at high rates while approaching and being vigilant around the edges of NWHs, and eliminative behavior is a common fear response documented among domesticated animals and mice (Calvo-Torrent, Brain, & Martinez, 1999; Forkman et al., 2007). Still other characteristics of waterholes may have influenced their frequency of use by lemur groups, including nearness to highly suitable habitat, cover by vegetation or rock formations, presence of other groups, or consistency from year to year.
Drinking site fidelity or side bias may have played a role in the instances of lemurs selecting the feces-contaminated water in the experiment. Six out of the seven instances of lemurs choosing the feces-contaminated water were performed by members of group F, which had a strong side bias for one bucket when the buckets contained identical water. When we introduced feces-contaminated water into the bucket on the preferred side, three out of four individuals persisted in drinking from that bucket at least once. Under the second arrangement, the clean water corresponded to the preferred side, and all individuals chose the clean water. That this group so strongly preferred this bucket despite the fecal contamination is perplexing, but is consistent with side bias observed in other experiments (Buttelmann & Tomasello, 2013; S. Jackson, Nicolson, & Lotz, 1998; Tebbich et al., 2007). Excluding these instances of side bias would result in only one occurrence of drinking feces-contaminated water in the experiment.

Based on previous theoretical work that modeled parasite transmission around a shared resource similar to these waterholes (Nunn et al., 2014), the microhabitat surrounding the waterholes may represent an area of increased parasite transmission risk. Empirically, the increased density that results from animals congregating around a water source in a dry habitat increases parasite risk (Vicente et al., 2007). In dry areas, water sources may serve as a hub of parasite transmission not only between individuals and groups, but even between species (Barasona et al., 2017; Vicente et al., 2007). In
general, in water-scarce environments, waterholes may represent hotspots of parasite transmission risk.

The particular conditions that characterize the NWHs in this study may even augment this general risk. Although we did not measure parasite transmission risk directly in this study, we documented a large quantity of feces that accumulated in the areas immediately surrounding waterholes commonly used by lemurs. Previous research on this population of red-fronted lemurs has identified 10 species of gastrointestinal parasites, most of which are directly transmitted via the fecal-oral route (Clough, 2010; Clough et al., 2010; Springer & Kappeler, 2016). The areas around the waterholes where feces accumulated were also characterized by moist soils from the presence of water, which could support the development or persistence of infectious stages of parasites in the environment (Perry, 1999; Perry, 1989). Red-fronted lemurs contact the soil in these areas when they approach the edge of the waterhole. Increased bodily contact with water and wet surfaces can increase the prevalence of parasites, as reported for *Schistosoma* in olive baboons (Müller-Graf et al., 1997). However, because water is highly important to this population of red-fronted lemurs (Scholz and Kappeler, 2004), and because fecal contamination is unlikely to wash or clear away from water sources until the rainy season begins, the probability of parasite transmission at waterholes is predicted to be high, which is why strategies to avoid highly contaminated waterholes may be necessary.
In summary, the experimental component of this study found that wild red-fronted lemurs preferred to drink clean rather than feces-contaminated water, even though high selectivity for clean water could have been accompanied by a risk of dehydration in this dry habitat. That lemurs had this preference implies that parasites transmitted via feces have exerted strong selective pressures on this host species, a finding supported by the research on this population documenting a high species richness of gastrointestinal parasites (Clough, 2010; Clough et al., 2010; Springer & Kappeler, 2016). In addition, travel distance represented a substantial cost to lemurs in their water acquisition and may limit the set of waterholes among which they can choose. Despite apparent constraints that appear to have limited lemurs to using only a subset of available natural waterholes, lemurs mitigated some of the risk of parasite transmission by returning to waterholes less frequently after they became highly contaminated by feces from previous visits, and delayed returning to waterholes that were highly contaminated. We conclude that parasite avoidance is an important, but not the primary component of water selection in our study species and likely in other similar water-scarce systems. These findings should motivate future work on how animal behavior is impacted by parasite risk in water, and by water more generally.
5. Temporal differentiation of waterhole use between wild red-fronted lemurs and their predators.

5.1 Introduction

In water-scarce environments, water sources present a dilemma for animals that drink from them (Sirot, Renaud, & Pays, 2016; Valeix et al., 2009). Due to physiological demands, some animals may have no choice but to use the few available sources of water, leading to higher population densities and more intense habitat use around lakes, rivers, waterholes, and other sources of water (Bleich et al., 2010; Boroski & Mossman, 1996; Valeix, 2011). Higher densities of prey are predicted to attract predators, increasing predation risk (Freese, 1978; Valeix et al., 2009). Predators may also need to drink water, and water sources may be concentrated in open areas of the habitat with greater visibility to predators. Water sources therefore may shape the landscape of predation risk for prey in these systems.

Semi-permanent waterholes in arid environments represent an especially acute example of how water impacts predation risk and prey counter-strategies to predation. In these settings of water scarcity, predators may be able to exploit prey’s dependence on water. In African savanna habitats, for example, lions (Panthera leo) frequently ambush prey from the vegetation surrounding waterholes (Makin, Chamaillé-Jammes, & Shrader, 2017). In fact, the locations of lion kills of water-dependent prey species like buffalo (Syncerus caffer) and wildebeest (Connochaetes taurinus) are best predicted by their distance from waterholes (de Boer et al., 2010). Previous research has demonstrated
that lions’ preferred prey species avoided using waterholes that were associated with higher lion encounter risk, increased their vigilance, and avoided waterholes at night (Valeix et al., 2009). Several species also displayed increased group sizes at waterholes with higher predator risk (Valeix et al., 2009). Moreover, ungulates visited waterholes at night more frequently in areas where human hunting occurred during the day than in non-hunting areas (Crosmary, Valeix, Fritz, Madzikanda, & Côté, 2012).

To understand the role of waterholes in shaping predator risk in a wide variety of systems, systematic study of predation risk is required. However, observing predation events directly can be challenging, which is why accounts of predation that are anecdotal or comprise small sample sizes are invaluable for understanding predator-prey dynamics (e.g. Fichtel, 2009; Goheen, Swihart, Goheen, & Swihart, 2018; Heymann, 1987; Matsuda, Tuuga, & Higashi, 2008; Wright, Heckscher, & Dunham, 1997). In addition to these challenges, ecological characteristics of some predator and prey species, such as non-diurnal activity periods, may make these dynamics even more challenging for human researchers to observe directly (Kappeler & Erkert, 2003; Müller, Velo, Raheliarisoa, Zaramody, & Curtis, 2000).

Recent studies have circumvented these challenges through the use of motion-activated camera traps to monitor how the activity patterns of prey respond to predator activity (Linkie & Ridout, 2011). Through analysis of camera trap activations, researchers have found that leopards (Panthera pardus) prioritize encounters with prey species over
avoiding competitors, lions, in their diel activity periods (Mugerwa, Du Preez, Tallents, Loveridge, & Macdonald, 2017). Similarly, other studies have found that mammalian species partitioned their use of artificial water sources in dry habitats, perhaps as a strategy to minimize competition at these small, highly valuable resources (Edwards, Gange, & Wiesel, 2015; Edwards, Al Awaji, Eid, & Attum, 2017). Temporal overlap between predators and prey as estimated by camera traps has been shown to concord with scat analyses of predators’ diets, implying that data from camera traps may accurately reflect predation risk (Torretta, Serafini, Imbert, Milanesi, & Meriggi, 2017).

In this study, we examined the patterns of waterhole use by a water-dependent species, the red-fronted lemur (*Eulemur rufifrons*), in a seasonally water-scarce environment. Red-fronted lemurs are cathemeral, i.e., they exhibit a habitual pattern of activity that is characterized by flexible periods of activity and rest throughout the 24-hour period (Kappeler & Erkert, 2003). As a result, their patterns of activity, both at waterholes and generally, might be more flexible and less constrained than those of a more strictly diurnal or nocturnal species. In our study system, red-fronted lemurs select among several available waterholes along a riverbed, which could vary in predation risk.

Using camera trap data, we assessed how lemurs navigated this presumably high-risk environment relative to the presence of their two main predators, the fossa (*Cryptoprocta ferox*) and the Madagascar harrier hawk (*Polyboroides radiatus*) (Goodman et
Previous scatological research on fossa in this study population estimated that red-fronted lemurs comprise around 5% of the fossa diet (Rasoloarison et al., 1995).

Fossa are also cathemeral (Dollar, 1999), and their cathemeral activity pattern has been hypothesized to drive the cathemerality of lemurs (Colquhoun, 2006), although a consensus on the ultimate explanations for this unusual activity pattern in lemurs has not yet been reached (Donati & Borgognini-Tarli, 2006; Eppley, Ganzhorn, & Donati, 2015; Kappeler & Erkert, 2003; van Schaik & Kappeler, 1996; Wright, 1999). Madagascar harrier hawks have been observed to predate red-fronted lemurs (Karpanty, 2006). Accordingly, in prior experiments in this study population, red-fronted lemurs performed referential alarm calls in response to harrier hawk vocalization playbacks (Fichtel & Kappeler, 2002). This call also elicited a response of scanning the sky and moving lower in the tree canopy from exposed positions, behaviors that functioned to reduce aerial predation risk (Fichtel & Kappeler, 2002). Madagascar harrier hawks are diurnal, and may also exert pressures on red-fronted lemur activity periods (Karpanty & Wright, 2007).

In this system, we aimed to understand how and whether lemurs balance their water demands with potential predation risk. Specifically, we investigated the hypothesis that lemurs avoid predators in their spatiotemporal use of waterholes. We tested two predictions. First, we predicted that lemurs would avoid waterholes that were frequently visited by predators. Second, we expected that lemurs would use
waterholes at times of the day when predator species were less commonly present at
waterholes. Given the multiple waterholes available for red-fronted lemurs and their
predators to use and the flexibility of red-fronted lemurs’ activity patterns, this system
provides a distinct opportunity to investigate this hypothesis.

5.2 Methods

5.2.1 Study system

This study took place in Kirindy Forest CNFEREF, a protected core area of the
Aire Protégé Menabe Antimena. Kirindy Forest is a dry deciduous forest occupied by a
population of red-fronted lemurs that has been studied for over two decades (Kappeler
& Fichtel, 2012). This population comprises several groups that are habituated and
collared and an unknown number of uncollared groups.

Red-fronted lemurs drink water from small standing waterholes that form as the
river stops flowing during the local dry season between April and November (Scholz &
Kappeler, 2004). Previous work in this population suggests that these waterholes are a
driver of red-fronted lemur habitat use during the dry season, when water is not
available elsewhere. In addition to red-fronted lemurs, a number of other species drink
from waterholes during the dry season, including the fossa and the Madagascar harrier
hawk.
5.2.2 Data collection

To quantify the use of waterholes by lemurs, fossa, and harrier hawks, we monitored a subset of between 6 and 12 waterholes using motion-activated video camera traps (Bushnell 119875C Trophy Cam) positioned around the edge of waterholes continuously from July-September 2017. These cameras were active 24 hours per day and recorded one-minute videos during daylight and 15-second videos in the dark using an infrared flash. From these videos, we scored the dates and times of camera activations by all three species. From videos of red-fronted lemurs, we also recorded the group ID if the lemur group was collared. If multiple groups (e.g. two collared, or one collared and one uncollared) activated the camera, that was also noted. If more than one unmarked group overlapped at the waterhole edge, we would not be able to distinguish them. To deal with this challenge, we developed a heuristic for counting the number of groups. We considered repeated camera activations for less than one hour by uncollared individuals to comprise one visit. Repeated camera activations by uncollared individuals extending for each additional hour were considered an additional visit. This decision was based on the typical amount of time that known, marked groups spent activating the cameras during each visit.

5.2.3 Analysis

To determine whether predator risk shaped lemurs’ waterhole choices, we tested whether the number of visits to waterholes by predators predicted lemur groups’ use of
waterholes each week. We used generalized linear mixed models ("glmer" function in the lme4 R package; R Development Core Team, 2011) to test for a relationship between predator visits and lemur group visits to each monitored waterhole in a given week. Specifically, to predict the counts of lemur visits, our first model included the count of fossa visits as a fixed effect. The fixed effect in the second model was the count of Madagascar harrier hawk visits. Waterhole identity was included as a random effect in both models. Because our dependent variable was measured as count data, we modeled the data using a Poisson distribution.

To examine whether lemurs avoided using waterholes at times when predator species were present at waterholes, we estimated the overlap, Δ, between camera activations by lemurs and by each of their predators at the waterholes (using the "overlapEst" function in the overlap R package). The value of Δ can range from 0, representing no overlap to 1, or complete overlap. We chose the Δ4 estimator of overlap because the minimum sample size for all of our species exceeded 75, per the recommendations of the package developers (Linkie & Ridout, 2011; Meredith & Ridout, 2016; Rowcliffe et al., 2014). We also bootstrapped this estimate by resampling one thousand times from a kernel density fitted to the original data (using the “bootEst” function in overlap). To test for differences between the distributions of camera activations between lemurs and two of their predator species across the 24-hour cycle,
we performed Watson’s two-sample test of homogeneity. This is a test for circular datasets, such as time, and thus appropriate for our temporal data.

5.3 Results

Overall, our camera traps recorded 3728 videos of red-fronted lemurs at waterholes, 142 of fossa, and 144 of Madagascar harrier hawks (Fig. 15). We found considerable co-occurrence of red-fronted lemur groups at waterholes, with 33.9% of the first camera activations by marked groups (which can be definitively identified) occurring within an hour of another group’s first camera activation. This result indicates that in a large proportion of visits, both marked and unmarked groups were likely to have overlapped in time and space around the waterhole.
We found no statistical support for the prediction that lemur groups avoided using waterholes that were more frequently visited by predators (Fig. 16). Neither the count of fossa nor Madagascar harrier hawk visits to waterholes was a significant predictor of lemur visits, although both had small negative coefficients (Table 4).
Predators exhibited considerable heterogeneity from week to week in their waterhole use; an extreme example of this was a waterhole with seven visits by Madagascar harrier hawks in one week, and zero visits the following week.

Figure 16: Count of lemur group visits to a single waterhole in one week against the number of visits to the same waterhole in the same week by each predator species.
Table 4: Model specifications of visits to waterholes by lemurs as a function of frequency of predator use. N=61 observations.

<table>
<thead>
<tr>
<th>Model</th>
<th>Fixed effects</th>
<th>Estimate</th>
<th>Std Error</th>
<th>z value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lemur visits ~ fossa visits</td>
<td>Intercept</td>
<td>0.3</td>
<td>0.34</td>
<td>0.99</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>Scaled fossa visits</td>
<td>-0.009</td>
<td>0.062</td>
<td>-0.14</td>
<td>0.89</td>
</tr>
<tr>
<td>Random effects</td>
<td>Variance</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Std Dev</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waterhole ID</td>
<td>2.55</td>
<td>1.60</td>
<td></td>
<td></td>
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<table>
<thead>
<tr>
<th>Model</th>
<th>Fixed effects</th>
<th>Estimate</th>
<th>Std Error</th>
<th>z value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lemur visits ~ hawk visits</td>
<td>Intercept</td>
<td>0.33</td>
<td>0.33</td>
<td>0.98</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>Scaled hawk visits</td>
<td>-0.016</td>
<td>0.97</td>
<td>-0.16</td>
<td>0.87</td>
</tr>
<tr>
<td>Random effects</td>
<td>Variance</td>
<td></td>
<td></td>
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<td></td>
<td>Std Dev</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Waterhole ID</td>
<td>1.87</td>
<td>1.37</td>
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</tr>
</tbody>
</table>

However, we found that lemurs were relatively unlikely to overlap with predators in the timing of their waterhole use (Fig. 17). The time of lemurs’ waterhole use had two peaks, one in the morning at around 9:30h, and another in the evening at 17:45h. These peaks fell on either side of the peak in camera activations by the Madagascar harrier hawk, which tended to occur at midday. Fossa activated the waterhole cameras more consistently throughout the 24-h period, with slightly higher frequencies at night than during the day, especially between the hours of 18:00h and 02:00h.
Figure 17: Kernel density of activity throughout the 24-hour cycle by each species at waterholes as estimated by camera trap activations.

The coefficient of overlap between red-fronted lemurs and Madagascar harrier hawks was calculated as 0.396 (bootstrap mean: 0.439; 95% CI: 0.38-0.50). The distribution of camera trap activations by lemurs was statistically significantly different from that of harrier hawks (test statistic: 5.92; p<0.001; Watson’s Two-Sample Test of Homogeneity). For red-fronted lemurs and fossa, the coefficient of overlap was calculated as 0.328 (bootstrap mean: 0.403; 95% CI: 0.34-0.47). The distributions of camera trap activations by lemurs and fossa were also found to be statistically
significantly different from one another (test statistic: 4.65; p<0.001; Watson’s Two-Sample Test of Homogeneity).

5.4 Discussion

In this study, we investigated predator-prey interactions at shared water resources in a dry habitat. Given the dependence of predators and prey on a common resource in this system, interspecific interactions were expected to be intensified. We found support for red-fronted lemurs avoiding waterholes at times that fossa and Madagascar harrier hawks, potential predators, were commonly present. This result may indicate that lemurs can reliably avoid predation by visiting waterholes at times when predators are less likely to be present. Red-fronted lemurs may be an especially relevant species for investigation of temporal adjustments to predation, given their cathemerality, an activity pattern that peaks flexible throughout the 24-hour cycle. More broadly, the distinctive pattern of non-overlap among red-fronted lemurs and their predators at waterholes aligns with previous evidence of anti-predator behavior by prey at waterholes in other communities (Crosmary et al., 2012; Edwards et al., 2015; Valeix, 2011; Valeix et al., 2009).

We did not find evidence of red-fronted lemurs avoiding waterholes that were used by predators within the same week, perhaps because predators did not reliably use particular waterholes. We also did not find a positive relationship between red-fronted lemurs’ waterhole use and the waterhole use of the two predator species, which may
have reflected predators’ attraction to waterholes that were commonly used by lemurs. The timescale that we used to quantify waterhole use in this study may have been too coarse to detect patterns of spatial use consistent with avoidance of predators at waterholes. This may be true especially because predators were highly flexible in their waterhole use from one week to the next. The unpredictability in predator use may indicate that fossa and harrier hawks do not habitually use particular waterholes, but instead drink from them opportunistically on their movements through the habitat. The lack of relationship in either direction between lemurs’ and their predators’ waterhole choices might also indicate that fossa and Madagascar harrier hawks are using the waterholes primarily for their own water and thermoregulatory needs, rather than stalking them or otherwise exploiting them for prey availability.

Indeed, a major question that remains in this study is why predators do not seem to use the regularity of red-fronted lemurs’ visits to the waterholes to their hunting advantage. One possibility is that predators may be constrained in the timing of their own waterhole use. For example, harrier hawks may use the waterholes for thermoregulation; they were commonly observed on the camera traps to wade in the shallow water and beat their wings, which may indicate that they do not only drink at the waterholes, but may seek them out especially during the peak heat of the day to cool themselves. Another explanation may be that when predator and prey are both flexible in the timing of their activity (i.e. both fossa and red-fronted lemurs are cathemeral),
perhaps no perfect, stable solution to the arms race between predator and prey exists. In addition, this analysis does not take into account the temporal patterns of waterhole use by other species, especially small mammals, that might also serve as prey for the two predators. Future research should investigate a wider breadth of species involved in predator-prey dynamics in this community.

Still another possibility is that heightened vigilance and other antipredator strategies by red-fronted lemurs at waterholes are effective, and that these behaviors make it preventatively difficult for predators to target red-fronted lemurs at waterholes. We report a high rate of overlap with other groups at waterholes (33.9%), which likely reflects the outcome of lemurs’ use of only a subset of waterholes during particular hours of the day. Higher rates of overlap among groups would increase the number of individuals at the waterholes, which may reduce the costs of vigilance and increase drinking time for individuals. Such an effect has been demonstrated for larger groups at waterholes in other species, including coatis (*Nasua narica*) and white-faced capuchins (*Cebus capucinus*) (Burger, 2001; Burger & Gochfeld, 1992; Valenzuela & Ceballos, 2000). However, overlap among groups may also have increased risk of intergroup aggression or competition. How animals might balance the potential aggressive costs and predation risk benefits of group encounters at waterholes is an open question for future research.

In conclusion, we found evidence of temporal avoidance of two predator species by red-fronted lemurs, corroborating previous evidence of temporal differentiation by
predators and prey at waterholes in other systems (Crosmary et al., 2012; Sirot et al., 2016; Valeix, 2011). However, we failed to find support for our prediction that red-fronted lemurs avoid waterholes with more predators; lemurs were no more or less likely to use waterholes that predators also used. This study highlights a unique characteristic of water resources: unlike food resources, waterholes attract species from a broad range of ecological guilds, including prey species and their predators. For this reason, the timing of prey’s waterhole use may be under particular pressure to avoid overlapping with predators’ visits to waterholes (Edwards et al., 2017). Species with flexible activity patterns, such as the red-fronted lemur, may be especially responsive to the temporal pressures of predation, and represent a useful system for exploration of these questions. Future work should expand the investigation of temporal differentiation between predators and prey at water sources into diverse dry ecosystems to better understand the effects of the unique characteristics of this essential resource on interspecific interactions.
6. Conclusion

6.1 Overview of findings

In this dissertation, I examined how lemurs’ spatiotemporal waterhole use and their ecology more broadly are influenced by water distribution, and the risks of predation and parasite transmission at waterholes. I found that five species of lemurs in captivity exhibited strong preferences for clean over feces-contaminated water, regardless of whether they were adapted to rainforest or dry habitats (Chapter 2). I demonstrated with experimental and observational evidence that the ranging patterns of wild red-fronted lemurs were influenced by the patterns of water distribution (Chapter 3). My analyses indicated that red-fronted lemurs’ natural waterhole choices depended on several factors. Red-fronted lemurs chose waterholes that minimized their travel distance from their previous resting site, and modulated their returns to previously used waterholes based on the level of fecal contamination in the surrounding area (Chapter 4). Predator avoidance in lemurs’ waterhole choices manifested in the temporal patterns of their waterhole use, but did not seem to be a factor in lemurs’ spatial decision-making about waterholes (Chapter 5). Taken together, these chapters represent the most comprehensive perspective to date on the impacts of water on the behavioral ecology of a wild primate.
6.2 Water scarcity as a selective pressure on primate behavior

This dissertation also highlights several key characteristics of water that make it a unique resource in terms of its influence on primate behavior. First, especially in dry or seasonally dry habitats such as the one in which this research took place, standing waterholes may represent the only opportunity for some animals to acquire an adequate volume of water to meet their survival needs. Food resources like fruits and leaves are unlikely to provide enough water for red-fronted lemurs to survive on them for extended periods of time without access to water, thus providing no “fallback” alternative water source when water is scarce. For species that depend on water, like the red-fronted lemur, water scarcity is expected to exert a particularly strong selective pressure on behavior.

Second, among the resources that red-fronted lemurs consume, water resources have a unique capacity to transmit parasites. Some parasites have waterborne stages in their life cycles and are uniquely adapted to transmit via water. In addition, fecal-orally transmitted parasites can also be carried by water (Palumbo et al., 2002). The moist microhabitats surrounding water are also ideal for parasite survival (Perry, 1999; Perry, 1989).

Finally, in terms of predation risk, waterholes are likely to be independently attractive to predators because of predators’ water needs. Water resources could attract predators because of increased prey density around them, but in addition, if predators
require water, they may be more likely to incidentally encounter prey at waterholes while pursuing their own water needs. These chance encounters could represent substantial risk from the perspective of the prey.

6.3 Implications for climate change

Studies of primate water use are especially important given the increased potential for extreme droughts in the future as a result of global climate change (Desbureaux & Damania, 2018; Mukherjee, Mishra, & Trenberth, 2018; Trenberth et al., 2014). In this dissertation, I demonstrated that ranging patterns of red-fronted lemurs shifted in response to changes in the availability of water, with downstream effects on predator risk, parasite transmission risk, and frequency of intergroup encounters. In other words, water scarcity in droughts could have extremely broad-reaching impacts on red-fronted lemur ecology, and other species that must drink water are likely to undergo many of these same cascading effects during droughts.

Understanding how water distribution affects habitat use on an individual or group level also has important ramifications for understanding and predicting patterns of habitat use on a population or species level. Presumably, the geographic extent of water-dependent species’ geographic ranges are constrained by the availability of water, so the drying-up of water sources during long droughts has the potential to lead to local extinctions of these species. Conversely, periods that are more wet than normal, where water sources persist for longer durations of time, could result in population expansions.
Water needs are an important factor to consider in predictions of water-dependent species’ responses to climate change (Adams & Hayes, 2008).

Finally, the experimental component of this study, in which I provisioned water within lemur groups’ home ranges, has produced several insights with relevance for interventions and management of this wild population and others. Red-fronted lemurs proved to be highly flexible in their use of the artificial waterholes. This result indicates that water provisioning interventions could be effective in extreme cases, such as long-term droughts that might threaten survival of large proportions of the population. However, in this study, the lemurs were apparently unable to locate the artificial waterholes independently within two weeks of their introduction. Based on this observation, the artificial waterholes may have lacked some sensory cues of the presence of water, or the lemurs may have had a fairly strictly defined search area for water, limited to areas they have previously known it to exist. Either way, provisioning water might be most successful when it is done in a location where animals are already expecting to find water. Although provisioning water in this manner is likely to be a successful strategy in terms of its effectiveness in reducing costs of dehydration, it may have unintended consequences, such as affecting rates of predator encounters, intergroup aggression, or parasite transmission. Thus, provisioning water is an intervention that should be considered cautiously, but ultimately may be necessary for species conservation in increasingly dry conditions as a result of global climate change.
6.4 Water scarcity in human evolution

Similar to the red-fronted lemurs in this study, early human populations are likely to have depended on the distribution of water (Binford, 1980; Conkey, 1980; Speth, 1987). Traveling across arid savannah habitats from one water source to the next may have selected for some of humans’ specific cognitive, morphological, and cultural adaptations (Finlayson, 2013, 2014). This study suggests that the foundations for many of these sensory, cognitive, and behavioral adaptations were already present in humans’ earliest primate ancestors. Cultural adaptations, such as the ability to transport water, were likely paramount for early humans to overcome the limitations of water distribution in determining their movements and patterns of habitat use, leading to the eventual worldwide range expansion characteristic of the species.

6.5 Future directions

The data collected in this study represent a unique dataset which includes many different factors that might influence red-fronted lemurs’ use of water. Although the study was not designed with this goal in mind, the possibility that waterholes represent peaks in red-fronted lemurs’ parasite transmission risk, predator risk, and risk of intergroup aggression offers the opportunity to pit these various factors against one another, to understand their relative importance in lemurs’ decision making about water (which could be generalized to their ecology more broadly). A future analysis would ideally include all of the variables investigated in this study in a single mixed
conditional logistic regression, to understand how travel distance, parasite risk, predator risk, and intergroup encounter risk relatively contribute to lemur groups’ decisions about which waterholes to visit. Using Bayesian interpolation methods, I would aim to replace missing data with estimates for relevant natural waterhole characteristics over shorter time intervals and for artificial waterholes, which would make this analysis possible.

If waterholes indeed represent hubs of parasite transmission risk between species, patterns of interspecific waterhole sharing could predict some of the most salient opportunities for spillover between species. Many species drink from waterholes in Kirindy Forest, and waterhole characteristics could influence the patterns of sharing, which may be non-random and based on each species’ preferences. Future work will build networks of waterhole sharing across species based on the patterns of waterhole use recorded by the video camera traps, and study how transmission dynamics vary as waterholes dry up across the dry season.

This dissertation considers the concept of the “landscape of fear” and the related concept of the “landscape of disgust.” While our understanding of fear in animals is relatively well-established, the emotion of disgust is not well defined or understood, even in humans. Theories of the ultimate function of disgust are highly diverse, and psychologists vary in the degree to which they believe disgust is uniquely human or shared among all animals. An additional direction for my future research includes
investigating disgust from a comparative perspective, both to understand its evolutionary history and to develop an evolution-based definition of disgust.
Appendix A

Model specifications for change in probability relative to AWH location for all points in each group’s range. Exponential model formula is \( y = a \times \exp(b \times x) + c \), where \( y \) is the change in probability and \( x \) is the distance from the AWH. Null model formula is \( y = c \).

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## Appendix B

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References


camera trap data. *Journal of Agricultural, Biological, and Environmental Statistics, 14*, 322–337.


Struhsaker, T. T., & Gartlan, J. S. (1970). Observations on the behavior and ecology of the


Biography

Caroline Rusk Amoroso graduated Cum Laude with distinction in research from the College of Agriculture and Life Sciences at Cornell University in May of 2012 with a Bachelor’s of Science in Animal Science. She published her undergraduate honors thesis. As a graduate student at Duke, Caroline has published several articles, including the second chapter of this dissertation. She has a paper in revision that resulted from a working group on disgust and psychiatric disorders funded by the Triangle Center for Evolutionary Medicine. Caroline is the recipient of the Integrative Bioinformatics for Investigating and Engineering Microbes Graduate Training Fellowship, the Triangle Center for Evolutionary Medicine Graduate Research Fellowship, and the James B. Duke Fellowship. She also obtained several research grants including the National Science Foundation Doctoral Dissertation Research Improvement Grant, Margot Marsh Biodiversity Foundation Research Grant, Primate Conservation, Inc. Research Grant, and two International Dissertation Research Travel Grants from the Duke Graduate School. Caroline served as President of Women in Science and Engineering (WiSE), a graduate student organization, for two years. She also served as the inaugural Administrative Intern for Duke Graduate School’s Sloan Scholars Program, which is dedicated to improving recruitment, retention, and graduation rates of underrepresented minority PhD students in STEM fields.