

1 TITLE: Fecal contamination, parasite risk, and waterhole use by wild animals in a dry  
2 deciduous forest

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4 SHORT TITLE: Fecal contamination and waterhole use

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## 22 ABSTRACT

23 Waterholes are critically important to animal survival in dry habitats but are also a  
24 potential source of parasite exposure. Avoiding feces may effectively reduce parasite  
25 transmission risk, but may also impose costs, including greater travel distances to locate  
26 less contaminated resources. We studied factors influencing wild, water-dependent red-  
27 fronted lemurs' (*Eulemur rufifrons*) selection of waterholes, including factors related to  
28 trade-offs between energy expenditure and parasite avoidance. Research took place in a  
29 dry deciduous forest in western Madagascar characterized by water scarcity during a  
30 pronounced local dry season. We tested whether fecal contamination influenced lemurs'  
31 water selection with an experiment that gave lemurs a choice between clean and fecally  
32 contaminated water disinfected by boiling. We also monitored lemurs' use of natural  
33 waterholes to determine how conspecific fecal contamination and travel distance  
34 influenced lemurs' use of waterholes. Red-fronted lemurs displayed a strong preference  
35 for clean water in the experiment. At natural waterholes, we found a significant negative  
36 interaction between frequency of previous lemur visits and fecal contamination, and a  
37 longer return time to waterholes with increasing fecal contamination, revealing that  
38 lemurs returned to less contaminated waterholes more frequently and sooner. We also  
39 found that lemurs prioritized shorter travel distances over feces avoidance. Together,  
40 these results suggest that red-fronted lemurs exercised their preferences for avoiding  
41 parasite risk in their natural waterhole choices by avoiding highly contaminated  
42 waterholes, especially when waterholes were equidistant. Thus, fecal contamination and

travel distance influence water selection in water-scarce habitats, with potential impacts on habitat use and ecological interactions.

KEYWORDS: drinking, feces avoidance, field experiment, lemur, parasite avoidance, water

#### SIGNIFICANCE STATEMENT

Animals can take many measures to avoid becoming infected with parasites. One strategy involves avoiding reliable indicators of parasite presence, such as feces. Although avoiding feces may have many benefits, it may also have costs, such as when essential resources, like waterholes in a dry forest, inevitably become contaminated by the animals that use them. Using a choice experiment, we demonstrated that wild red-fronted lemurs preferred to avoid fecal contamination of water sources. From observations of lemurs' waterhole choices, we determined that lemurs exercised this preference most when choosing among nearby waterholes, thus prioritizing energy conservation, and secondarily reducing the costs of parasite exposure risk. Avoidance of feces may thus have effects on lemurs' patterns of habitat use and ecological interactions.

## 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 (Moore 2002; Hart 2011; Curtis 2014; Poirotte et al. 2017). In this coevolutionary dynamic, many parasites 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 and 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 the context of resource scarcity, costs of avoiding parasites might be especially high if uncontaminated resources are limited. For example, in a theoretical model, selectivity for mates that are uninfected with a sterilizing sexually transmitted pathogen would be favored even if costs of avoidance are high, but unfavorable if the pathogen is highly prevalent in the population, leaving few uninfected alternative 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.

Avoiding parasite transmission might also be costly if doing so requires forgoing resources or expending additional energy. For example, foraging Eurasian oystercatchers (*Haematopus ostralegus*) most frequently consumed cockles (*Cerastoderma edule*) that were 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 forewent more food in areas with higher tick abundance (Fritzsche and Allan 2012). In both cases, avoidance of parasites had 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, 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 and Hawley 2010). In other cases, parasite avoidance is performed flexibly. For example, 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 and MacIntosh 2015). 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 fecal contamination influences mammals' choice of drinking water. In a series of experiments, domestic cattle (*Bos taurus*) preferred clean water to feces-contaminated water, and individuals with access to clean water gained more weight than those with access to pond water (Willms et al. 2002). Elephants (*Loxodonta africana*) in Kruger National Park used artificial water sources more often than had lower levels of *Escherichia coli* contamination, implying that

the elephants avoided fecal contamination in water (Ndlovu et al. 2018). Such avoidance should be beneficial, given that fecal-orally transmitted parasites can often be transmitted through contaminated water (Palumbo et al. 2002) and the moist microhabitat around water promotes the survival of infectious stages of parasites (Perry 1999). Increased bodily contact with water and wet surfaces around water sources can increase parasite prevalence across individuals, as observed with *Schistosoma* in olive baboons (*Papio anubis*) (Müller-Graf et al. 1997), or across seasons, as found with *Strongyloides* in white-faced capuchins (*Cebus capucinus*) (Parr et al. 2013).

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 the dry habitat-adapted lemur (*Lemur catta*) and the rainforest-adapted lemur species (*Varecia* spp.), suggesting that despite the potential costs of selectivity for clean water in a water-scarce habitat, avoiding fecal contamination in water was still favored. Alternatively, the preference for clean water observed in this study could be an artifact of captivity. 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 studied the water selections of red-fronted lemurs (*Eulemur rufifrons*) 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 available water

sources and surrounding areas show evidence of contamination. We investigated the hypothesis that wild red-fronted lemurs prefer to drink water with lower risk of parasite transmission. 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 natural 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 lemur utilization. We investigated each of these factors alongside our main hypothesis about parasite avoidance.

## MATERIALS AND METHODS

### 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 decades (Kappeler and 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), rainfall is extremely rare, and the small river in the study site eventually dries up, leaving only several small, standing waterholes (hereafter referred to as a natural waterhole, or “NWH”) in the riverbed by the

end of the dry season (Fig. 1a). The NWHs typically form at depressions in the riverbed where water collects, often with steep banks on one or two sides. In most cases, lemurs drink near one or the other open sides, so that they can reach the water from less steep ground, and they typically remain close to the bank where 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 and Kappeler 2004).

--- FIG. 1 HERE ---

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. It was not possible to record data blind because this study involved focal animals in the field.

#### Experimental study

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 (CRA et al. unpubl. data). For each AWH, we placed two 10-l black rubber buckets (livestock feed buckets) into two shallow holes dug into the ground in an area of the habitat that was well-hidden from forest trails and covered from aerial predators. The gap around the edge of the bucket was filled with soil to make the top of the bucket level with the surrounding earth, and the buckets were filled with water (Figs

1b, c). We added additional water every two to three days (as needed). Water buckets were emptied, cleaned with dish soap, and refilled with water once per week. 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 respectively. At each AWH, we filled one bucket with 10 l of clean, filtered water from the supply also used for drinking by the researchers and support staff at the Kirindy Forest Research Station. We filled the other bucket with 10 l of feces-contaminated water 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 min 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.

Before commencing the water quality experiment, we found that one group (F) had a strong preference for one side, even though the two water sources were identical. To control for such cases of side bias, we switched the side of the buckets containing the

fecally contaminated and clean water choices between the first four days and the last four days of the water quality experiment.

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-sec videos with a five-second delay in the dark using an infrared flash, which was undetectable by the lemurs. These settings reflected the maximum recording time that could be set on the cameras in light and dark conditions to minimize battery loss to the flash. 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 for two to three 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 touching and tongue lapping the water and could contain pauses while the individual remained at the waterhole edge. 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).

#### 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 from the cameras at the NWHs and recorded the dates, times, and group identities (if collared) of red-fronted lemur visits to the NWHs. We discarded any videos that did not include red-fronted lemurs.

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

237 be distinguished. To deal with this issue, we applied a conservative heuristic based on the  
238 typical duration of camera activations we recorded by marked groups: we considered  
239 repeated camera activations for less than one hour by uncollared individuals to comprise  
240 a visit by one group, and counted repeated camera activations by uncollared individuals  
241 extending for each additional hour as an additional visit by another group. This rule may  
242 have slightly over- or under-estimated the number of visits by unmarked groups on any  
243 single day, but it was applied consistently across waterholes.

244 We also assumed that activation of the cameras by red-fronted lemurs around a  
245 NWH indicated that lemurs drank from the NWH, even in the very few instances that  
246 drinking was not directly observed. Based on personal observations in the field, if lemurs  
247 were close enough to the NWH to activate the camera, they most likely drank from the  
248 NWH. This might occur if they were within the range of the camera but did not re-  
249 activate the camera while drinking (when they often stayed still except for small mouth  
250 movements not detectable by the camera), or if they moved outside of the camera's range  
251 or frame to drink, which may have prevented the camera from recording the drinking  
252 event.

253 In addition to monitoring the use of waterholes by all lemur groups in the  
254 population, we monitored the movements of several groups more closely during July-  
255 September 2017 using GPS collars (Collar 1AA, 55g, 1600mAh, e-obs GmbH,  
256 Gruenwald, Germany). During routine tranquilizer darting, a GPS collar was affixed to  
257 one adult male from each of five habituated study groups (A, B, F, J, and Q). We  
258 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. 2011, 2013).

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). Each week, we also obtained descriptive data for each camera-monitored NWH, including the longest diameter 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. Defecation into the water directly was observed rarely, involving only a the few cases in which tree branches extended over the waterhole, and 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 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 measuring fecal contamination at mid-week, and across the two days after measuring 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 the 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. This analysis ensured that our heuristic for counting uncollared groups did not interfere with this analysis and controlled 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. 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. We calculated the distance between this resting site and all NWHs that were monitored during the week that the visit occurred. 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). The NWH that was selected in the lemur group's visit received a 1, and unselected NWHs, i.e. the monitored available NWHs during the same week, received a 0. The conditional logistic regression thus compared the selected waterholes to a representative sample of unselected

alternatives available at the time of the choice to determine how travel distance, fecal contamination, and waterhole length influenced lemurs' choices.

## RESULTS

### 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  $5.4 \pm 1.9$  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). (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 37 instances of lemurs drinking clean water, and 3 instances of feces-contaminated water ( $p < 0.0001$ , binomial test).

### Observational study

We collected observational data over 16 weeks across two field seasons for a total of 174 observations, where each observation corresponded to one waterhole in a given week. Each week we monitored a subset of between 6 and 17 NWHs with 20 cameras. This

subset varied from week to week as waterholes dried, and depended on the number of cameras required to completely monitor the edges of waterholes of different sizes. 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 in most weeks were not visited at all by lemur groups (n=72 observations of 0 visits in one week).

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. 2a; Table 1). 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. 2b). The model that included this interaction (Table 1) 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.

--- TABLE 1 HERE ---

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,  $z=-0.5$ ,  $p>0.05$ ).

--- FIG. 2 HERE ---

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. 3; 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,  $z=3.05$ ,  $p<0.05$ ).

--- FIG. 3 HERE ---

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,  $z=1.63$ ,  $p>0.05$ ).

## DISCUSSION

Broadly, our experimental results suggest that avoidance of fecal contamination is a factor in lemurs' choice of water sources. Our experiment provides 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).

The 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 in the surroundings was lower, and returned to them after longer delays in time when fecal contamination in the surroundings was higher. In other words, the results of our observational study indicate that lemurs may have chosen alternative NWHs when the NWH that they used previously

became increasingly fecally contaminated. Together, these findings suggest that despite generally high levels of fecal contamination in the areas surrounding commonly used NWHs, lemurs mediated their exposure to fecal parasites by changing their choice of NWH.

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 had less fecally contaminated surroundings, 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 is a secondary priority behind minimizing energy expenditure in lemurs' choice of water source. When we directly compared NWHs that lemurs chose to unselected alternatives, distance from the previous resting site was 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 into how fecal contamination influenced lemurs' selections of one 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 after longer delays 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 (Pereira and Kappeler 1997; Fichtel and Hammerschmidt 2002; Fichtel and Kappeler 2002). Predators such as fossa (*Cryptoprocta ferox*) and Madagascar harrier hawks (*Polyboroides radiatus*) were observed to use the waterholes regularly; evidence suggests that lemurs and predators commonly use the same waterholes, but at different times of day (CRA et al. unpubl. data). 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 few 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 one bucket despite the fecal contamination is perplexing, but is consistent with side bias observed in other experiments (Jackson et al. 1998; Tebbich et al. 2007; Buttelmann and Tomasello 2013). 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 shared resources similar to these waterholes, the microhabitat surrounding the waterholes may represent an area of increased parasite transmission risk (Nunn et al. 2014), and contact rates, even among sick, lethargic animals, are likely to be higher near waterholes (Franz et al. 2018). Empirically, the increased density that results from animals congregating around a water source increases parasite transmission risk (Vicente et al. 2007). In dry areas, water sources may serve as a hub of parasite transmission between individuals, groups, and even species (Vicente et al. 2007; Barasona et al. 2017). In general, in water-scarce environments, waterholes are likely to 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 parasitological 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 and 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 1989, 1999), and 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 away until the rainy season begins, the probability of parasite transmission at waterholes is predicted to be high. The heightened risk may make strategies to avoid highly contaminated waterholes especially necessary in this context.

In summary, the experimental component of this study found that wild red-fronted lemurs preferred to drink clean rather than feces-contaminated water. 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 and Kappeler 2016). In addition, travel distance represented a substantial cost to lemurs in their water acquisition and likely limited the set of waterholes among which they could choose. Despite apparent constraints that 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 delaying returning to highly contaminated waterholes.

We conclude that parasite avoidance is an important, but not the primary, factor driving water selection in our study species and likely in other similar water-scarce systems. Given the potential for water to influence animal movements (Purdon and van Aarde 2017; Ndlovu et al. 2018) and interspecific interactions (Sirot et al. 2016), avoidance of fecally contaminated waterholes could have diverse, cascading ecosystem impacts. These findings should motivate future work on how animal behavior is impacted by parasite risk in water, and by water more generally.

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521 Compliance with ethical standards

522 The protocol for this research was approved by Duke University's Institutional Animal

523 Care and Use Committee (Protocol A008-17-01) and the Malagasy Ministry of the

524 Environment, Water, and Forests. All applicable international, national, and/or

525 institutional guidelines for the use of animals were followed.

526 Conflict of interest

527 The authors declare that they have no conflicts of interest.

528

529 Data availability

530 The datasets generated and analyzed during the current study are available from the

531 corresponding author on reasonable request.

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FIGURE CAPTIONS

**FIG. 1** Photos of water sources in this study. a. A red-fronted lemur drinks from a natural waterhole (NWH) in the drying Kirindy River bed. b. Artificial waterhole (AWH) setup. Clean water is on the left, and feces-contaminated water on the right. c. Two red-fronted lemurs drink from an AWH, and a motion-activated camera trap records their choice of water source

**FIG. 2** Interaction plots showing that the relationship between visits after measurement and visits before measurement is mediated by fecal contamination. a: Visits to natural waterholes by all groups; b: Visits to natural waterholes by collared groups only. Shade of data points corresponds to the level of fecal contamination, with lighter points representing lower levels of fecal contamination. A “jitter” function was applied to the count data to make points more visible. Waterholes that were commonly visited before we measured them were less likely to be visited afterward if they had higher levels of contamination

**FIG. 3** Lemurs returned to more fecally contaminated waterholes after longer time intervals. Each point represents the time delay between subsequent visits of a lemur group to the same waterhole, and the fecal contamination present at the waterhole during the week of the group’s first visit. Line represents the fitted linear mixed model, which predicts the time to return to a waterhole based on fecal contamination and waterhole length as fixed effects, and waterhole identity, group identity, and week as random effects. Variables scaled by z-score

674 TABLES

675 **TABLE 1** Results from generalized linear mixed models testing the effect of visits before  
676 measurement of fecal contamination and fecal contamination on visits after measurement. Based  
677 on their AIC scores, the interaction model was better supported by the data than the model  
678 without an interaction. All fixed effects were standardized by z-score. The shaded rows are  
679 statistically significant ( $p < 0.05$ )

| Model                       | Fixed effects                        | Estimate | Std. err. | z     | p     | Random effects | Variance | Std. dev. |
|-----------------------------|--------------------------------------|----------|-----------|-------|-------|----------------|----------|-----------|
| Interaction (all groups)    | Intercept                            | -0.75    | 0.21      | -3.54 | <0.05 | Waterhole ID   | 0.35     | 0.59      |
|                             | Visits before measure                | 0.77     | 0.11      | 6.98  | <0.05 |                |          |           |
|                             | Feces measured within 5m of NWH edge | 0.46     | 0.11      | 4.39  | <0.05 |                |          |           |
|                             | Waterhole length                     | -0.14    | 0.15      | -0.99 | 0.32  | Week           | 0.16     | 0.40      |
|                             | Visits before * feces measured       | -0.30    | 0.07      | -4.10 | <0.05 |                |          |           |
| No interaction (all groups) | Intercept                            | -0.91    | 0.23      | -3.79 | <0.05 | Waterhole ID   | 0.49     | 0.70      |
|                             | Visits before measure                | 0.47     | 0.09      | 5.24  | <0.05 |                |          |           |
|                             | Feces measured within 5m of NWH edge | 0.27     | 0.10      | 2.50  | <0.05 | Week           | 0.25     | 0.50      |
|                             | Waterhole length                     | -0.05    | 0.15      | -0.36 | 0.72  |                |          |           |
| Interaction (collared only) | Intercept                            | -1.83    | 0.32      | -5.78 | <0.05 | Waterhole ID   | 0.81     | 0.90      |
|                             | Visits before measure                | 0.46     | 0.15      | 3.05  | <0.05 |                |          |           |
|                             | Feces measured within 5m of NWH edge | 0.16     | 0.17      | 0.98  | 0.33  |                |          |           |
|                             | Waterhole length                     | 0.12     | 0.20      | 0.60  | 0.55  |                |          |           |
|                             | Visits before * feces measured       | -0.21    | 0.11      | -2.01 | <0.05 |                |          |           |

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