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Behavioral and Environmental Correlates of Parasite Burden in *Eulemur cinereiceps* from Southeastern Madagascar

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Abstract of the Dissertation

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The grey-headed lemur, *Eulemur cinereiceps*, is among the top 25 most endangered primates. This work investigated behavioral and environmental correlates of parasite exposure through the evaluation of parasite burden in two wild metapopulations of *E. cinereiceps* in southeastern Madagascar. Understanding the relationship between primate behavior, environment and primate disease risk is critical for the conservation of primates and their ecosystems. The presence and distribution of parasites among primate populations is the result of primate exposure to parasites through both behavioral and environmental variables. Primate exposure to parasites is often the result of either host social behaviors or habitat use behaviors.

Behavior data and non-invasive fecal sampling were collected during two field seasons in 2008 at the Agnalazaha and Manombo Forests. Both forests are comprised of low canopy coastal rainforest with mixtures of unforested areas, however the Agnalazaha forest is substantially more

disturbed when taking local community use and deforestation into account. Phenology and GPS data were also recorded at this time. This is one of the few studies to unite repetitive fecal sample collection and specific behavioral data from habituated and identified individuals. In addition, data collection at two field sites allowed for a comparison of environmental characteristics, primarily habitat disturbance.

This is the first study to identify parasites infecting *E. cinereiceps*. Four nematodes and one protozoan parasite were found in the fecal samples. Two pinworms, *Callistoura* and *Lemuricola*, were the most commonly found parasites during both field seasons. *Trichuris* was found in only one forest fragment during the second field season. Additionally, an *Entamoeba* species and Ascarididae species were identified. All of the parasites identified in this study are likely transferred through fecal-oral contamination and are expected to be relatively asymptomatic in *E. cinereiceps*, allowing host-parasite interactions to be studied without strong confounding parasite-avoidance behaviors. In addition, this study may serve as a model for more virulent parasites in other systems.

A field and laboratory diagnostic study validated fecal parasite recovery techniques. Preservation solution had significant impacts on parasite recovery and results indicate that 10% formalin is superior to 90% ethanol. Recovery technique also had an impact on parasite recovery. Fecal sedimentation was a more sensitive method than fecal flotation, although the difference was not significant. When using 90% ethanol as a preservation solution, parasite recovery approached those stored in 10% formalin when using fecal sedimentation rather than fecal flotation. Maximum fecal parasite species richness occurred when at least 2-3 grams of feces, or 67-75% of the fecal sample was utilized in repeated fecal flotation trials. Flotation solution and homogeneity of the feces did not affect parasite recovery yields.

The behavioral and environmental correlates of *Eulemur cinereiceps* parasite infection parameters varied by parasite species. Overall, neither behavior, nor environmental variables were a better predictor of parasite burden in the Agnalazaha and Manombo *E. cinereiceps* populations. Parasite infection frequency and prevalence increased during the fall/dry season. In some cases this may relate to behavioral differences between the two seasons, although it is unlikely to be the result of increased physical contact due to infant births.

Callistoura infection was best predicted by social behaviors including group size and physical contact between conspecifics. *Lemuricola* infection was best predicted by habitat use behaviors, travel time and time spent on the ground, which are both likely mediated by environment disturbance variables. *Lemuricola* frequency and prevalence was significantly greater in the more disturbed forest where travel time and time spent on the ground were also significantly greater. These results support previous research on *Lemuricola* infection in other lemur host species. *Trichuris* infection was found during only one field season in only one forest fragment and it's potential for cross-species contamination is currently unknown. *Entamoeba* infection was best predicted by environmental variables and the corresponding habitat use behaviors. Previous studies identifying ascarid parasites in other lemur species found that infection correlated negatively with habitat quality and age. The ascarid eggs found in the current study do not resemble those found in other primate studies. Ascarid infection in *E. cinereiceps* was best predicted by environmental variables; ascarid eggs were recovered only from study groups whose home range overlapped areas of water. Although height in the canopy was predicted to correlate negatively with parasite burden due to the mode of fecal contamination transmission, height did not vary significantly with parasite species richness or any parameters of parasite burden.

Each of the parasites identified in this study are likely transferred between host individuals by fecal-oral contamination. However, the host behavioral and environmental variables mediating host exposure and parasite burden varies for each parasite species suggesting that even parasites with similar modes of transmission may transfer between hosts and spread through populations using different mechanisms. This further suggests that clumping parasite families or those with general similarities may distract from more detailed patterns of host-parasite infection in wildlife communities.

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Chapter 1. Primate Host Exposure and Gastro-intestinal Parasite Infection

1.1 Introduction to Host-Parasite Interactions

1.1.1 Primate Parasite Ecology

The area of primate parasite ecology has applications in primate conservation, disease ecology and has become increasingly important to human populations. Interest in primate parasitology dates it origins to the late 19th century, although most traditional studies investigated case studies or used experimentation infection (Hegner, 1928; Cowen and Wolf, 1945). Many studies investigated the potential for mammal and in particular primate susceptibility in order to draw evolutionary connections between host species, to determine what constitutes parasite host species specificity, and to examine possible host-parasite symbiosis (Kellogg, 1914; Cleveland, 1926; Hegner, 1928; Hegner, 1937). Human parasite ecology was determined to be a combination of health care, individual sanitary behaviors, individual social behaviors, and complex group social behaviors (Cort, 1942).

Parasites play a role in community ecology and may influence host population size, fitness, and behavior (May and Anderson, 1978; Nunn et al., 2004). Primates represent an order of largely endangered or threatened mammals, and yet concerns for primate extinction have raised a comparatively lesser interest in parasite ecology than in the other processes affecting primate population decline (Altizer et al., 2003; Pedersen et al., 2007; Clough, 2010). Host-parasite interactions in wild populations are relevant to wildlife management projects and conservation efforts (Dobson and Lyles, 2000).

Parasites transmitted through contamination may pose unusually high risks to small or threatened populations (Woolhouse, 2001). Parasites may have a negative effect on host

individuals as well as entire host populations (Pedersen et al., 2007). Endangered primates, as well as those living in small fragmented communities, may be at greater risk from parasites due to a reduction in genetic diversity and a potential reduction in their ability to respond to pathogenic threats (Altizer, 2003). More information is currently needed about the spread of infections diseases and their impacts on endangered host communities (Pedersen et al., 2007). Indeed, as primate species become extinct, host-specific parasites of these primates will also become extinct resulting a loss of biodiversity with direct and indirect effects on various trophic levels (Grompper and Williams, 1998).

Parasite infections in wild host populations are the result of both host exposure to infective parasite stages and host susceptibility (Wilson et al., 2002). Host susceptibility depends on genetic and physiological host characteristics (Nunn and Altizer, 2006). While host exposure depends on environmental factors, parasite characteristics, host social behavior, and host spatial behavior (Altizer et al., 2003, Figure 1.1). Parasites may be transferred to a new host through direct contact, direct ingestion, direct inhalation, or indirect mechanisms such as the ingestion of an intermediate host, or biting vectors (May and Anderson, 1978; Loehle et al., 1995).

The mechanisms behind host exposure are often a response to host social and spatial behaviors, and environmental characteristics (Figure 1.1). In a broad study of emerging diseases, small changes in host behavior had the most significant affects on host exposure patterns (Schrag and Weiner, 1995).

In this dissertation, I attempt to link 1) host social behaviors, 2) habitat use behaviors, and 3) host environments to parasite burden in an effort to determine the best predictors of host exposure and parasite transmission patterns. This study focuses on the links between relatively asymptomatic parasites and their critically endangered host, and may serve as a model for the

links between more virulent parasites and their potential impact on host fitness and ultimately host extinction.

1.1.2 Demographic Correlates of Gastro-intestinal Parasite Burden with Emphasis on Primates

Immune system effectiveness and differences in behavior can be the result of demographic variables. Therefore demographic correlates of gastro-intestinal parasite burden should be evaluated as potential mediators of behavioral or physiological correlates of parasite infection (Freeland, 1976; Altizer et al., 2003; Chapman et al., 2009b). Many studies were unable to differentiate between these possibilities, and therefore, there is more literature on demographic correlates of parasite burden than research convincingly linking differences in behavior to variability in parasite burden (Table 1.1).

Studies associating age and parasite burden have reported mixed results across primate and other mammal studies and are likely both parasite and host species specific. Some studies found that parasite burden varied with age for one parasite and not others (Lilly et al., 2002; Clough, 2009). Juvenile gorillas had significantly higher rates of strongylate and threadworm parasites than adults, but not ascarid, whipworm or tapeworm parasites (Lilly et al., 2002). The results of a study of *Eulemur rufifrons* found a trend towards higher prevalence of whipworm (*Trichuris*) in older individuals, but found no age-based differences in pinworm (*Lemuricola*) infection rates (Clough, 2009). In a semi-captive population of *Mandrillus sphinx*, nematode prevalence increased significantly with age in females, but not males (Setchell et al., 2007). Additionally, research in ruminants indicates that juveniles are prone to higher parasite loads (Bowman and Lyne, 1995; Cote et al., 2005). However, a number of studies in wild primates have found no association between age and parasite burden (*Pan troglodytes*: Meuhlenbein,

2005; *Colobus angolensis palliates*: Okanga et al., 2006; *Alouatta pigra*: Vitazkova and Wade, 2007). Within a meta-analytic framework I compared host and parasite species where infection varies by age, and host and parasite species where infection does not vary by age. I found no patterns to explain the variability.

Gender, like age, is typically associated with habitat use, social behavior, and immune system efficiency, and is therefore expected to predict variability in parasite burden (Freeland, 1976; Altizer et al., 2003; Chapman et al., 2009a). A study on red squirrels, *Sciurus vulgaris*, attributed differences in male and female parasite load to different spatial use patterns between the sexes (Bertolino et al., 2003). However, results from primate-parasite studies have been inconsistent, in part because behavioral mediators were not always incorporated into the research. While several primate studies have found no difference in male and female parasite burden (*Alouatta palliata*: Stoner, 1996; *Colobus angolensis palliatus*: Okanga et al., 2006; *Chierogaleus medius*: Schwensow et al., 2007; *Mandrillus sphinx*: Setchell et al., 2007; *Alouatta pigra*: Vitazkova and Wade, 2007), others have found parasite-specific differences (*Eulemur rufifrons*: Clough, 2009) or host-specific difference (*Propithecus verreauxi* and *Lemur catta*: Loudon, 2009). Clough (2009) found that male *Eulemur rufifrons* had significantly higher prevalence of *Lemuricola* eggs during one field season, however during the second field season prevalence for all individuals was 100%. Clough (2009) also found the female *E. rufifrons* had a significantly higher prevalence of protozoan parasites than males, however, no differences were seen in parasite species richness (PSR) and *Trichuris* infections. In another study Loudon found significantly higher fecal endoparasite prevalence in male than female *Propithecus verreauxi* (2009), while finding no difference between male and female *Lemur catta* (2009). Gender differences in parasite load may be the result of behavior and parasite transmission, however,

Clough (2009) did not study *E. rufifrons* behavior and Loudon (2009) was largely unable to link behavior differences between male and female *Propithecus verreauxi* to parasite prevalence differences.

Associations between group size and parasite infections are likely to depend on transmission mode of the parasites and host behavior (Freeland, 1976; Arneberg et al., 1998; Altizer et al., 2003; Chapman et al., 2009a). Chapman et al. (2009a) predict that as group size increases, parasite burden from direct contamination should decrease, and that parasite species richness should increase. Host behaviors such as proximity and travel time are predicted to correlate with group size (Chapman et al., 2009a).

Conversely, several studies suggest that primate parasite prevalence and diversity are both positively correlated with group size (*Cercocebus albigena*: Freeland, 1979; *Cynomys* spp. ectoparasites: Hoogland, 1979; Primate cross species analysis: Nunn et al., 2004; Cross mammal species analysis: Vitone et al., 2004; *Eulemur rufifrons* PSR: Clough, 2009), while a negative relationship between group size and parasite prevalence was seen in wild ungulates (Ezenwa et al., 2006). A study of two groups of *Procolobus rufomitratus* found that the larger group had a lower prevalence of *Trichuris* infection (Chapman et al., 2009b). Chapman et al. (2009b) attributed this result to frequent fission in the larger group. Freeland (1979) attributed the positive correlation between group size and protozoan parasite species richness in *Cercocebus albigena* but not *Papio anubis* to behavioral differences between the host species. *C. albigena* groups are closed with less frequent transfers between groups than *P. anubis*. Thus, *C. albigena* groups may be better able to protect themselves from the introduction of novel parasites (Freeland, 1979). Similarly, Ezenwa (2003) found that parasite prevalence increased with group size in African bovids only in closed groups with infrequent transfers. And yet, in other studies,

no association between group size and parasite infections were found (*Lemuricola* and *Trichuris* in *Eulemur rufifrons*: Clough, 2009; *Oesophagostom* and PSR in *Procolobus rufomitratus*: Chapman et al., 2009a). The relationship between group size and parasite infection is likely a function of both parasite species characteristics and host group behavior.

Parasites infections may result in lower fecundity (Cheney et al., 1988) or harm individual fitness, and are thus expected to constrain the growth of host populations (Anderson and May, 1979). Population density is a combination of population growth rate, population size, habitat size and often habitat quality (i.e.: fragmentation, food availability). Host density in turn may affect dietary, ranging, and social behavior. Research on the association between population density and parasite infection has been widely conducted with inconsistent results. Ectoparasites, rather than endoparasites, are more likely to be affected by host population density (Nunn et al., 2003; Ezenwa et al., 2006).

However, host density is also predicted to correlate positively with infections from directly transmitted endoparasites as a result of increased contact between host individuals (Arnberg et al., 2001; Altizer et al., 2003), habitat reuse (Ezenwa et al., 2006), or both (Morland and Paulin, 1998; Chapman et al., 2009a). In support of this prediction, a number of studies have reported a positive correlation between host density and either endoparasite prevalence or parasite species richness (protozoa in *Cercocebus albigena*: Freeland, 1979; broad study mammals: Arneberg et al., 1998; red squirrel, *Tamiasciurus hudsonicus*: Bertolino et al., 2003; *Trichuris* in *Colobus guereza*: Chapman et al., 2005; *Canis familiaris*: Rubel and Wisnivesky, 2005; African bovids: Ezenwa et al., 2006; broad study of carnivores: Lindenfors et al., 2007; *Procolobus rufomitratus* and *Cercocebus galeritus galeritus*: Mbora and McPeek, 2009). However, other studies have reported a negative correlation between host density and parasite

burden (PSR in 12 Indian mammals: Watve and Sukumar, 1995; *Trichuris* in *Piliocolobus tephrosceles*: Chapman et al., 2005; pinworm in *Alouatta pigra*: Vitazkova and Wade, 2007). And still others have reported no relationship (*Alouatta fusca*: Stuart et al., 1993; deer mice, *Peromyscus maniculatus*: Meagher, 1999; PSR in a cross species analysis: Vitone et al., 2004; *Microcebus murinus*: Raharivololona and Ganzhorn, 2009). As with other demographic variables, the inconsistency of results across studies suggests that patterns between host density and parasite infection are both host and parasite species specific (Chapman et al., 2005; Lindenfors et al., 2007).

1.1.3 Behavioral Correlates of Gastro-Intestinal Parasite Burden with Emphasis on Primates

Social system is expected to play a role in disease ecology through the potential for transmission between conspecifics (Nunn et al., 2004). Social barriers such as selective immigration, territorial behaviors and intergroup encounters are expected to prevent contamination-associated parasite species (Loehle, 1995). Loudon (2009) observed that the primate *Lemur catta* had larger group sizes and higher rates of both endoparasites and ectoparasites than *Propithecus verreauxi*. Similarly, Ezenwa (2002) found that nematode infections in African bovids were more prevalent in gregarious hosts. Ezenwa also found a positive correlation between nematode prevalence and territoriality (2002). It may be that the bovid hosts with more parasite species adopted more territorial behaviors, or it may be that territorial hosts come into physical contact during encounters with other groups, leading to higher rates of parasite transmission.

Although social system and conspecific proximity are often linked, Frenton et al., (2002) predict that transmission events at the individual level rather than density or frequency dependent

transmission drive the spread of disease at the metapopulation level. Therefore, individual behavior, rather than population dynamics should play the most important role in an individual's risk of contracting a disease. Anderson and May (1979) predict that host contact rate is one of the most important epidemiological parameters influencing parasite spread. Association, closeness, or proximity between hosts should positively increase parasite species richness (Altizer et al., 2003) and parasite prevalence through increased transmission opportunities (Freeland, 1976; Loehle, 1995). Proximity and physical contact are typically expected to increase the transfer of these parasites through direct contact with hosts, however, proximity between host conspecifics may also play a role in infection rates with parasites transferred through ingestion or inhalation. In a study of raccoons (*Procyon lotar*), when contact between individuals increased after experimental provisioning in one population, the prevalence of individuals infected with parasites increased from 7% to 45%, while a separate control population remained at a steady low infection rate (Grompper and Wright, 2005). A study on *Alouatta pigra* found that the most important factor predicting individual parasite infection is whether his/her group members are infected (Vitazkova and Wade, 2007). The study of *A. pigra* strongly suggests that proximity plays a role in exposure and thus endoparasite prevalence.

In the host-parasite relationship, host defense against parasite infections should include habitat use behaviors such as avoiding recently used areas (Freeland, 1976; Loehle, 1995; Chapman et al., 2009a) or using deposition (latrine) sites (Chapman et al., 2009a). Chapman et al. (2009a) predict that the frequency of parasite infections will increase with repeated use of sleeping sites or deposition sites. Freeland (1980) found that *Cercocebus albigena* are more likely to remain in an area longer after it rained than if the weather was dry. He attributes this behavior to the advantages of rainwater cleaning fecal contamination from the environment.

Habitat use of arboreal hosts includes height in the canopy as well as any time spent on the ground. Time spent on the ground should increase the potential for obtaining contamination-transmitted parasites, particularly in hosts foraging on the ground. A study of two lemur species found that *Lemur catta*, the more terrestrial species, had a higher prevalence of ecto- and endoparasites than *Propithecus edwardsi*, the more arboreal lemur (Loudon, 2009). However, these results may be confounded by the fact that *Lemur catta* is also found with larger group sizes than *Propithecus edwardsi* (Loudon, 2009) and parasite infections are expected to increase with an increase in group size (Chapman et al., 2009a).

The effects of home range size on parasite infections have not been consistent, however this may be due to over-simplification. It is likely that parasites with different modes of transmission, different life cycles, and multiple host species, as well as hosts with different spatial use patterns, will yield dissimilar relationships between home range size and parasite burden. A study of 69 anthropoids found that parasite species richness increased significantly with home range size in some host species, but not in others (Nunn et al., 2004). A study of 12 mammal species in India did not yield a significant correlation between parasite species richness and home range size (Watve and Sukumar, 1995). Alternatively, a study on carnivorous mammals and their directly transmitted parasites found that parasite species richness was negatively correlated with home range size (Lindenfors et al., 2007). Parasite infection in primates is expected to increase with home range overlap due to increased potential for parasite transmission from contaminated areas and increased opportunities for intragroup encounters (Chapman et al., 2009a).

1.1.4 Environmental Correlates of Gastro-Intestinal Parasite Burden with Emphasis on Primates

Parasite burden in primates is expected to vary with environmental factors, such as habitat type, habitat quality, microhabitat, nutritional availability, temperature, rainfall and seasonality due to their effects on host exposure (Chapman et al., 2009a).

Seasonality is expected to play a role in primate parasite burden, in particular, parasite prevalence is expected to increase in wetter seasons (*Equus ferus*, wild horses: Rubenstein and Hohmann, 1989; *Alouatta fusca*: Stuart et al., 1993; *Alouatta palliata*: Stoner, 1996; *Rangifer tarandus*, reindeer, *Marshallagia* and *Ostertagia*: Cote et al., 2005; *Capra aegagrus hircus* and *Capra aegagrus* goats: Hoste et al., 2005; *Mandrillus sphinx*: Setchell et al., 2007). However, this pattern is likely to be parasite and host specific. A study on the presence of ectoparasites by season in *Propithecus edwardsi* found that tick and fly infection intensity was higher during the warm-wet season, however this pattern was not found in leeches or mites (Wright et al., 2009). Similarly, a study on *Pan troglodytes* found that one strongylid, *Oesophagostomum stephanostomum* varied by season but two other parasitic worms, *Trichuris trichiura* and *Strongyloides fuelleborni* did not (Huffman et al., 1997). A study on two sympatric lemur species found that *Entamoeba* sp. infection rates increased in *Lemur catta* during the wet season, but this variation was not found in *Propithecus verreauxi* (Loudon, 2009).

Habitat quality is expected to negatively correlate with parasite burden, however, studies have yielded mixed results. A study of *Propithecus verreauxi* found no difference between parasite prevalence in populations living in a disturbed forest versus those living in a more pristine forest (Loudon, 2009). The same study found that the disturbed forest population of *Lemur catta* had higher coccidian parasite prevalence, and lower *Lemuricola* prevalence than the population inhabiting more pristine forest and that prevalence in *Trichuris* and *Entamoeba* did

not vary (Loudon, 2009). A study on *Microcebus murinus* in five littoral forest fragments found that populations from poor quality forests yielded significantly higher prevalence of Ascaridae and Cestoda parasites, but there were no differences in the significance of Stongylida, *Trichuris*, Oxyuridae and Coccidia parasites (Raharivololona and Ganzhorn, 2009). The same study found that in smaller forests, *Microcebus murinus* had a significantly higher prevalence of Oxyuridae parasites, while no differences were found in other parasite taxa (Raharivololona and Ganzhorn, 2009). Similarly, a study on *Colobus angolensis palliates* in three forest fragments, found that the population inhabiting the poor quality fragment had a higher prevalence of *Trichuris* than populations living in either the intermediate or high quality forests (Okanga et al., 2006). However, a study on *Alouatta pigra* populations in continuous and fragmented forests found no differences in *Controrchis*, *Trypanoxyuris minutus*, or *Giardia* prevalence (Vitazkova and Wade, 2007).

Habitat and microhabitat variability is expected to affect host potential for parasite exposure. Some examples of these factors include exposure to sunlight, humidity, food availability, terrain and cross-host transmission opportunities. An island study on wild horses, *Equus ferus,* found that the most important environmental factor in endoparasite infection was the group's home range location (Rubenstein and Hohmann, 1989). Group home range location in this study may have affected parasite exposure through soil/sand condition or group home range overlap.

However, studies on primate host and parasite species have not supported these findings. A study on *Lemur catta* populations at three sites, spiny desert, gallery forest, high altitude montane forest, found no differences in nematode or protozoan prevalence between populations

(Villers et al., 2008). Similarly, a study on *Papio anubis* groups found no difference in endoparasite prevalence between wild-foraging and crop-raiding groups (Weyher et al., 2006).

Parasite infection taxes a host's immune system, and even asymptomatic parasites may increase the effects of poor nutrition, further compromising overall fitness (Anderson and May, 1979; Coop and Holmes, 1996; Coop and Kyriazakis, 1999; Altizer et al., 2003; Chapman et al., 2006). A study on *Procolobus rufromitratus* suggests there is an indirect effect between a decrease in food availability and nematode infection (Chapman et al., 2006). A study on goats, *Capra aegagrus hircus* and *Capra aegagrus*, suggests that supplementary feeding increases resilience and resistance to nematode infections (Hoste et al., 2005). Forest productivity, or food availability is likely to indirectly affect host parasite burden.

Environmental variation, whether seasonal, due to home range location, habitat quality, or other variables, is likely to play a role in community ecology and therefore in a host's disease ecology.

1.1.5 Summary

An individual's disease risk is the culmination of a variety of factors (Figure 1.1; Altizer, 2003) including parasite characteristics, host behaviors and the environment. Parasite characteristics such as life cycle, host specificity, and mode of transmission are expected to directly affect a host's risk of exposure. Host social behaviors such as proximity to conspecifics and group spread, and habitat use behaviors such as height in the forest and time spent in travel, may affect an individual's exposure to parasites through direct contact with infected conspecifics, direct contact with contaminated resources, or indirectly through increased encounters with intermediate hosts. Environmental variables such as habitat quality, food

availability, and disturbance, may affect both host behaviors and parasite characteristics. In turn, all three variables, the environment, host behavior and parasite characteristics, affect transmission opportunities and host exposure to parasites. Host exposure directly affects an individual's disease risk, and this disease risk is the combination of individual susceptibility, the distribution of the infection, and the pathogen's persistence in a host population. Disease risk can be evaluated indirectly through parasite burden.

Previous research investigating links between the environment, host behaviors, parasite characteristics and parasite burden have yielded inconsistent results (Table 1.1). This study further investigates these relationships within two wild metapopulations of lemurs in southeastern Madagascar.

1.2 Introduction to the Host Study Species: *Eulemur cinereiceps*

Lemurs represent a unique adaptive radiation among primates. Evolutionary distinctions include dental specialization, morphological specialization, reproductive activity, and social behaviors (Martin, 1972). The grey-headed lemur, *Eulemur cinereiceps* (1975 Rumpler), is a senior synonym of the white-collared lemur, *E. albocollaris*, formally *E. fulvus albocollaris* (Johnson et al., 2008). *E. cinereiceps* is found in the eastern rainforest mountain corridor between Andringitra National Park to the northwest and the Mananara River to the south (Irwin et al., 2005) where they hybridize with *Eulemur rufifrons* (Johnson and Wyner, 2000). Genetically pure *E. cinereiceps* can also be found in coastal fragments between the Manampatrana and Mananara Rivers (Irwin et al., 2005; Mittermeier et al., 2006). Five of these small forest fragments are found at Agnalazaha and Manombo, the study sites for this research (Figure1.2).

E. cinereiceps is critically endangered with a decreasing trend and current threats are both natural and anthropogenic (Andrainarivo et al., 2010). The coastal fragments are located within the cyclone region of southeastern Madagascar and sustain regular damage (Ratsimbazafy, 2002; Bollen and Donati, 2006). Deforestation for agriculture, commercial logging, and individual logging, along with hunting and capture for pets, and regular use of the forests by human populations is continuing to limit the remaining habitat and threaten the survival of *E. cinereiceps*.

E. cinereiceps is a cathemeral, sexually dichromatic and dimorphic species (Johnson et al., 2002). Although *E. cinereiceps* is reportedly a fission-fusion species, at Agnalazaha and Manombo, the groups appear fluid, with group composition changing over approximately 3-5 month durations (unpublished data 2006-2008 Andriamaharoa, Ingraldi, Martin, Ralainasolo). *E. cinereiceps* is predominantly frugivorous (Ralainasolo et al., 2008; Ingraldi, 2010). Five nocturnal follows in the current study were conducted during the full moon on both cloudy and clear nights in September, October, and November, 2008 from 18:00-24:30. A total of 16.87 hours of nocturnal behavior data were recorded for two groups, and 614.43 hours of diurnal behavior data was recorded during the same time periods on 5 groups. *E. cinereiceps* was inactive during 80.72% of the nocturnal follows, and only inactive during 44.99% of diurnal follows (Martin, unpublished data).

Similar to other lemurs, *E. cinereiceps* possesses a dental/tooth comb comprised of medially oriented and ventrally elongated lower incisors. Although the evolutionary function of this dental specialization has long been debated (Jones, 1918; Gregory, 1920; Stein, 1936; Martin, 1972); early research on wild populations noted the function of the tooth comb in occurrences allo- and autogrooming (Cuvier and St. Hilaire, 1829). Allogrooming with the tooth

comb typically occurs in hard to reach places such as the lower back, top of the head and upper back (Martin, 1972; Martin, unpublished data). In *E. cinereiceps* autogrooming with the tooth comb frequently takes place on the tail (Martin, unpublished data). Both auto- and allogrooming by use of the tooth comb should increase an individual's potential for ectoparasites and some endoparasite exposure. Further potential for the exposure to parasites from conspecifics occurs during sleep when individuals often position themselves "nose-to-hind", and wrap their tails around the anterior region of conspecifics (Martin, unpublished data).

In addition to the tooth comb, *E. cinereiceps* have specializations common to brown lemurs including fast gut passage rates and scent marking glands on the wrists, chest, and anus (Fleagle, 1999; Spehn and Ganzhorn, 2000; Martin, unpublished data). *E. rufus/rufifrons* gut passage rate range from 129-388 minutes with an average of 247 minutes, a rate slower than *Varecia* and faster than *Hapalemur* (Spehn and Ganzhorn, 2000). The impact of gut passage time on gastrointestinal parasite infection is currently unknown. Scent marking behaviors may lead to an increase in exposure to parasites when individuals rub against potentially contaminated branches in their environment. In addition, anal scent marking may dislodge infective parasite eggs from the perianal area, thus contaminating the environment and potentially infecting individuals that scent mark over the infected individual's mark.

Eulemur cinereiceps was selected as the study species for this research for several reasons. It is a critically endangered species (Andrainarivo et al., 2010) and therefore results from this research may have a significant impact on conservation management programs. The coastal *E. cinereiceps* populations belong to two metapopulations, which provided an opportunity for environmental comparisons. A preliminary study found at least two asymptomatic nematode parasites in these populations. This provided an opportunity to study

parasite exposure in a host that is unlikely to have adopted behaviors to avoid these parasites, which provides models for parasite exposure across other lemur and primate species. The variable social structure of *E. cinereiceps* ensured that the study was able to include relatively few individuals and still provide enough variability to identify possible host behavior patterns associated with parasite burden.

1.3 Introduction to the Study Sites

Agnalazaha (S 23° 11.175' E 47° 43.095') and Manombo (S 23° 01.697' E 47° 43.838') are located in the Fianarantsoa province in southeastern Madagascar (Figures 1.2 and 1.3). The sites are 12.0 kilometers apart and between 0.75-5.40 kilometers from the coast of the Indian Ocean. The Malagasy people living in this region subsist mainly from the farming of rice in flooded fields, using regulated slash and burn techniques, as well as fishing. Ocean fishing is less economically accessible with the loss of trees large enough to make canoes due to selective logging and cyclone damage. Although commercial logging is only legal in one of the forest fragments (Manombo Classified Forest) commercial logging still takes place during the early hours of the morning in many fragments. Logging for personal use is allowed in the Agnalazaha forest and it is common to see people leaving the forest with several small tree trunks over their shoulder. Hunting lemurs is illegal in all forests, although the hunting of wild boar and crocodile (when present) are allowed, therefore, the presence of people in the forest with spears and machetes is also a common occurrence. The hunting of lemurs for the use of subsistence as well as a small pet trade still occurs in nearby forests (Lehman et al., 2006) and in at least one fragment from each of the Agnalazaha and Manombo forests (Johnson & Overdorff, 1999; Martin, personal observation 2008).

The Agnalazaha and Manombo forests are high in biodiversity including the Madagascan flying fox (*Pteropus rufus*), lesser hedge hog tenrec (*Echinops telfairi*), lowland streaked tenrec (*Hemicentetes semispinosus*), fossa (*Cryptoprocta ferox*), broad striped Malagasy mongoose (*Galidictis fasciata*), at least three species of chameleon, Madagascar day gecko (*Phelsuma madagascariensis madagascariensis)*, at least two species of giant snail, as well as many other vertebrates and invertebrates. A study in 1995 named Manombo as the forest with the highest diversity of land snails in the world (Emberton, 1995). In addition to *E. cinereiceps*, the Agnalazaha and Manombo forests are the home of the black and white ruffed lemur (*Varecia variegata editorum* only found at Manombo), the eastern bamboo lemur (*Hapalemur griseus*), Ramantsoavana's southern woolly lemur (*Avahi ramanantsoavanai*), James' sportive lemur (*Lepilemur jamesorum* only found at Manombo), the Geoffroy's dwarf lemur (*Cheirogaleus major*), Jolly's mouse lemur (*Microcebus jollyae*), and the aye-aye (*Daubentonia madagascariensis*) (all lemur taxonomy reflects 2011 IUCN standards).

This region of southeastern Madagascar has a yearly average rainfall of between 2,400-2,600 mm (Ratsimbazafy, 2002) and a temperature range of 7.5-36˚C (Ratsimbazafy, 2002). Cyclone Gretelle struck in 1997 with devastating effects to the coastal forests (Ratsimbazafy, 2002). Recent estimates of *E. cinereiceps* populations suggest that densities are back to pre-cyclone estimates, although the floral species richness may not have rebounded as fast (Johnson et al., In Press).

Agnalazaha is approximately 1,250 square hectares and consists of two distinct fragments of littoral forest, the Agnalazaha coastal fragment (ACS) and the Agnalazaha inland fragment (AIN) (Figure 1.3). Both fragments are characterized by white sandy soils and swampy areas, a low canopy (< 10 m), and a low altitude (0-20 m)(Ingraldi, 2010). The Missouri Botanical

Garden (MBG) manages the sites and works with local community leaders for sustainable use of the remaining forest. Both fragments are highly degraded and discontinuous, although they are separated by less than 50 meters in some areas. The land between them is comprised of privately owned fallow rice patties. Reforestation projects around AIN were not successful, however both tree species selection and geographic location may have played a role in their failure.

Manombo consists of an approximately 15,730 square hectares of lowland rainforest, littoral forest, and fallow rice paddies (Ratsimbazafy, 2002, Figure 1.3). Less than half of the Manombo area remains forested, and that which is left is highly fragmented mainly as a result of agriculture and selective logging for precious woods (Ralainasolo et al., 2008). Manombo consists of three forest fragments. The smallest fragment is an extremely disturbed swampy forest broken into several parcels. One of them was briefly searched for lemurs during this study, and contrary to local rumors, a group of *E. cinereiceps* was found (n = ~7). The larger fragments were used more widely in this study: the Manombo Special Reserve (MSR), is managed by Madagascar National Parks (formally ANGAP) and the Manombo Classified Forest (MCF), is managed by the Ministry of Environment, Forests and Tourism, and locally by the Interregional Direction of Environment, Forests and Tourism (DREFT). Both are lowland rainforest, with elevation from sea level to approximately 137 meters, and characterized by a relatively high canopy of approximately 20 meters (Ingraldi, 2010).

The MCF is more threatened than the MSR because its borders are not monitored in any way. Slash and burn agriculture and selective logging have widened the gap between the forest fragments with perceptible differences between 2002-2010 (Ralainasolo, personal communication) and it has become increasingly more difficult for *E. cinereiceps* to travel between fragments. In addition, the practice of Tandroho threatens the large bodied lemurs in

MCF. Tandroho is a method of hunting where a strip of forest is cleared (approximately 50m x 20m) creating gaps in the canopy too large to be crossed by the lemurs. Logs with snares on them are placed over the gap to act as a bridge and lemurs are caught as they cross the gap (Bollen and Donati, 2006). Several traps were seen during the current study. Current research by Durrell Wildlife Conservation Trust focused on *V.variegata editorum* from 1997-2005 and since 2006 has focused on *E. cinereiceps*. This research continues to represent important contributions to the communities around the forest and to the science community.

1.4 Introduction to the Host Study Groups

An early preliminary study took place at both Agnalazaha and Manombo in June-July 2006. At this time, I assisted in the habituation of four study groups, one from each of the main forest fragments at both sites. I also collected fecal samples from 31 individuals. Although I was not present, in July and August 2007, two colleagues collected fecal samples from 18 individuals at Agnalazaha and Manombo. Two main study periods took place during the spring/wet season January – April 2008, and the fall/dry season September – December 2008. During the spring/wet 2008 season I studied three groups, two from the Agnalazaha Inland Forest fragment, and one from the Manombo Classified Forest (Table 1.2). All groups were habituated previous to my arrival. I collected behavior data and repetitive fecal samples from 20 habituated individuals, and single fecal samples from 11 unhabituated *E. cinereiceps*. During the fall/dry 2008 season five groups were studied, three from the Agnalazaha Inland Forest fragment (two previously studied, one new), one from the Manombo Classified Forest, and one new group from the Manombo Special Reserve (Table 1.2, Figure 1.3). All groups had been habituated previous to my arrival. I collected behavior data and repetitive fecal samples from 23 habituated individuals,

and single fecal samples from 23 unhabituated *E. cinereiceps*. All groups increased or decreased in size between the study periods (Table 1.2).

1.5 Research Questions and Predictions

1.5.1 Research Questions

1. What is the most efficient and effective way to evaluate fecal parasite burden for *Eulemur cinereiceps*?

2. What are the gastro-intestinal parasites infecting the coastal populations of *Eulemur cinereiceps*?

3. What are the demographic variables, social behaviors and habitat use behaviors associated with parasite burden in *Eulemur cinereiceps*?

4. What are the environmental characteristics associated with parasite burden in *Eulemur cinereiceps*?

5. How do social behaviors, habitat use behaviors, and environmental variables interact and relate to parasite burden in *Eulemur cinereiceps*? Are social behaviors, habitat use behaviors, or environmental variables a better predictor of parasite burden in *Eulemur cinereiceps*?

1.5.2 Research Predictions

1. What is the most efficient and effective way to evaluate fecal parasite burden for *Eulemur cinereiceps*?

Preservation solution, recovery technique, and quantity of feces are expected to affect the precision of fecal parasite evaluation. When using fecal flotation, flotation solution specific gravity and consistency of the sample (i.e.: homogeneity) are expected to affect parasite recovery (see chapter 2). Chapter two evaluates field and laboratory fecal parasite recovery techniques.

2. What are the gastro-intestinal parasites infecting the coastal populations of *Eulemur cinereiceps*?

This is the first study of *Eulemur cinereiceps* parasites. Based on a preliminary study and previous research on other lemur species, both nematode and protozoan parasites infections are expected from the Agnalazaha and Manombo populations. See chapter 3 and figures 3.1 and 3.2 for information on the fecal parasites identified from Lemuriformes hosts and in particular, other *Eulemur* hosts. Chapter 3 investigates the fecal parasites identified from the coastal populations of *Eulemur cinereiceps* in southeastern Madagascar.

3. What are the demographic variables, social behaviors and habitat use behaviors associated with parasite burden in *Eulemur cinereiceps*?

Predicting the link between host behavior and parasite infection is difficult because it is an ever-changing relationship. Demographic variables are not expected to vary with the parameters of parasite infection in the coastal populations of *E. cinereiceps*. Although in theory age and sex are expected to be a predictor of parasite infection based on diet, habitat use and immune system strength (Freeland, 1976; Altizer et al., 2003), there has been little consensus from previous studies (Table 1.1). Group size is not expected to correlate with parasite burden. A relationship between group size and parasite burden is typically seen in host species with a low frequency of transfers between groups (Ezenwa, 2006) and because *E. cinereiceps* groups frequently change composition (Table 1.2), it is unlikely that group size will act as either a barrier against the introduction of new pathogens or to increase susceptibility. Additionally group home ranges frequently overlap, suggesting that exposure to contamination based parasites will not be affected by the size of an individual's group.

Primate-parasite literature has predominantly supported a relationship between social behavior variables such as proximity and group spread and parasite burden due to their potential to affect exposure to directly transmitted parasites (Ezenwa, 2002; Grompper and Wright, 2005;

Vitazkova and Wade, 2007; Loudon, 2009). The parasites in this study are directly transmitted through fecal-oral contamination, therefore I expect social behavior variables to affect the potential for individual host exposure. I predict a positive relationship for two reasons: 1) *E. cinereiceps* does not use "latrine sites" for fecal deposition. Therefore individuals will regularly walk in the feces either on the ground or on branches below other individuals. Feces on the hands and feet will be easily transmitted to the mouth of themselves and their conspecifics during tooth comb grooming bouts. 2) Several female parasites, such as *Lemuricola*, a pinworm found in a preliminary study of *E. cinereiceps,* oviposit eggs outside the host anus. Dental grooming of the lower back and tail would readily lead to consumption of these infective eggs. Therefore, individuals spending more time in physical contact with conspecifics will be at a greater risk of parasite exposure.

Habitat use variables such as time spent in travel, home range size, height in the canopy and time spent on the ground are expected to correlate with parasite burden due to their potential to affect exposure to parasites transferred through fecal contamination (Chapmen et al., 2009a; Loudon, 2009; Table 1.1). Individuals that spend more time in travel are expected to increase their potential exposure to parasites transferred through fecal-contamination throughout their home range. This is likely to significantly increase in *E. cinereiceps* groups with high levels of home range overlap. Similarly, as home range size increases, individuals are expected to increase their potential for coming across both novel parasites as well as those transferred through fecal contamination. This should only hold true as long as travel time increases with home range size or home range overlap increases with home range size. Otherwise, as home range size increases, individuals are less likely to encounter areas of previous use with fecal contamination before the rain washes the ground and trees clean. Height in the canopy and time spent on the ground are

predicted to represent the strongest correlation with parasite burden in *E. cinereiceps*. Since the parasites identified in this study are all transferred through fecal-oral contamination, individuals traveling across the ground are expected to encounter contaminated feces significantly more often than those that do not descend to the ground. Furthermore, individuals remaining higher in the canopy are also expected to encounter fewer parasite than those traveling, feeding, and resting at lower heights. This is because branches, leaves, and fruit will become contaminated with parasites when individuals defecate, and those hosts maintaining lower heights are more likely to encounter and ingest their conspecifics contaminated feces.

The behavioral correlates of *E. cinereiceps* parasite burden are addressed in Chapter 5.

4. What are the environmental characteristics associated with parasite burden in *Eulemur cinereiceps*?

Environmental variables such as seasonality, habitat quality, and microhabitat variability are expected to correlate with parasite burden. Parasite prevalence is expected to increase during the spring/wet season and decrease during the fall/dry season (Stuart et al., 1993; Stoner, 1996; Huffman et al., 1997; Semple, 2002; Stuart et al., 2002; Setchell et al., 2007; Loudon, 2009). *Eulemur cinereiceps* in poorer quality fragments are expected to yield greater parasite prevalence and parasite species richness (Okanga et al., 2006; Loudon, 2009; Raharivololona and Ganzhorn, 2009). Parasite prevalence and species richness is also expected to vary with microhabitat (Ruebnstein and Hohmann, 1989; Stoner, 1996). Chapter 5 discusses the environmental and behavioral correlates of *Eulemur cinereiceps* parasite burden.

5. How do social behaviors, habitat use behaviors, and environmental variables interact and relate to parasite burden in *Eulemur cinereiceps*? Are social behaviors, habitat use behaviors, or environmental variables a better predictor of parasite burden in *Eulemur cinereiceps*?

The distribution of parasite infection in wild host populations is determined by variability in host exposure and host susceptibility (Wilson et al., 2002). These factors can be difficult to tease apart, however, this study approaches parasite burden in terms of host exposure patterns through environmental, behavioral and demographic variability. Host behaviors, both social and habitat use, are expected to better predict *Eulemur cinereiceps* parasite burden than environmental variables. I expect that environmental variables will act indirectly on host parasite burden through host behavior, and that host behavior will primarily predict parasite exposure and thus parasite burden. Chapter 4 evaluates the environmental and behavioral correlates of *Eulemur cinereiceps* parasite burden, and Chapter 5 draws conclusions from the above chapters.

1.6 Figures and Tables

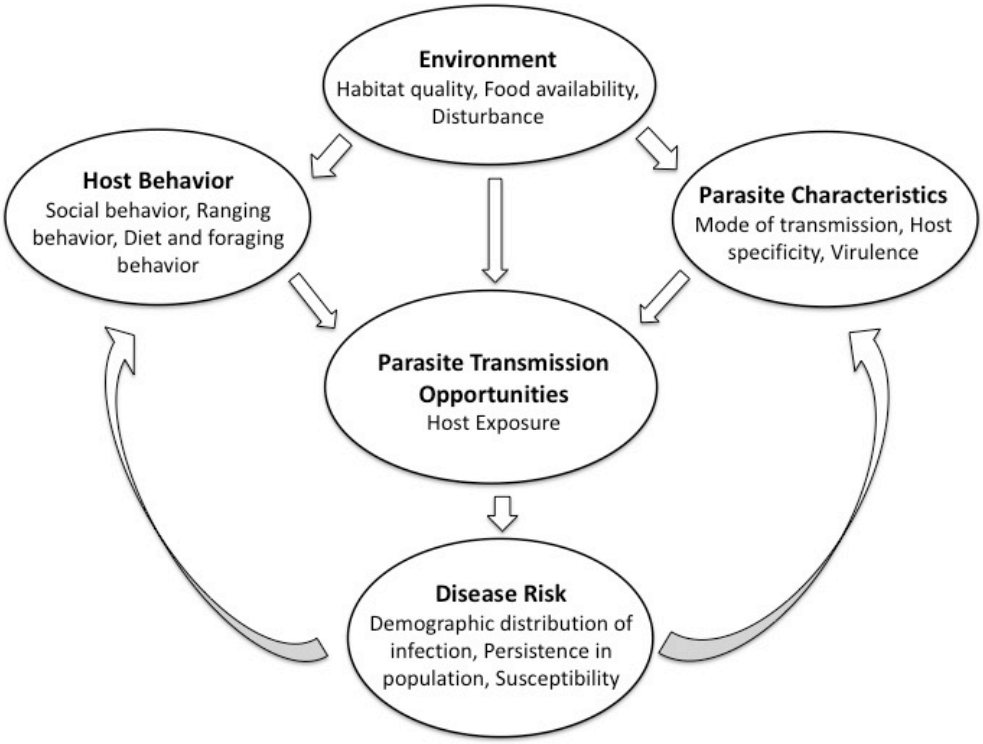

Figure 1.1 Parameters of primate parasite exposure and disease risk.
Adapted and altered from Altizer et al., 2003.

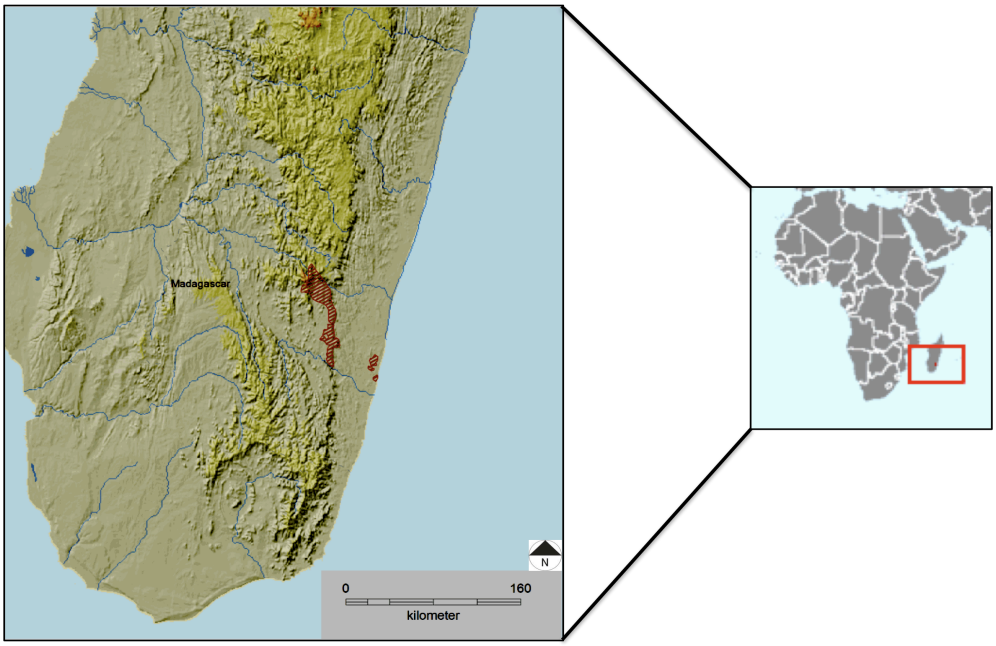

Figure 1.2 Range of wild *Eulemur cinereiceps*. Maps from IUCN Red List (2010).

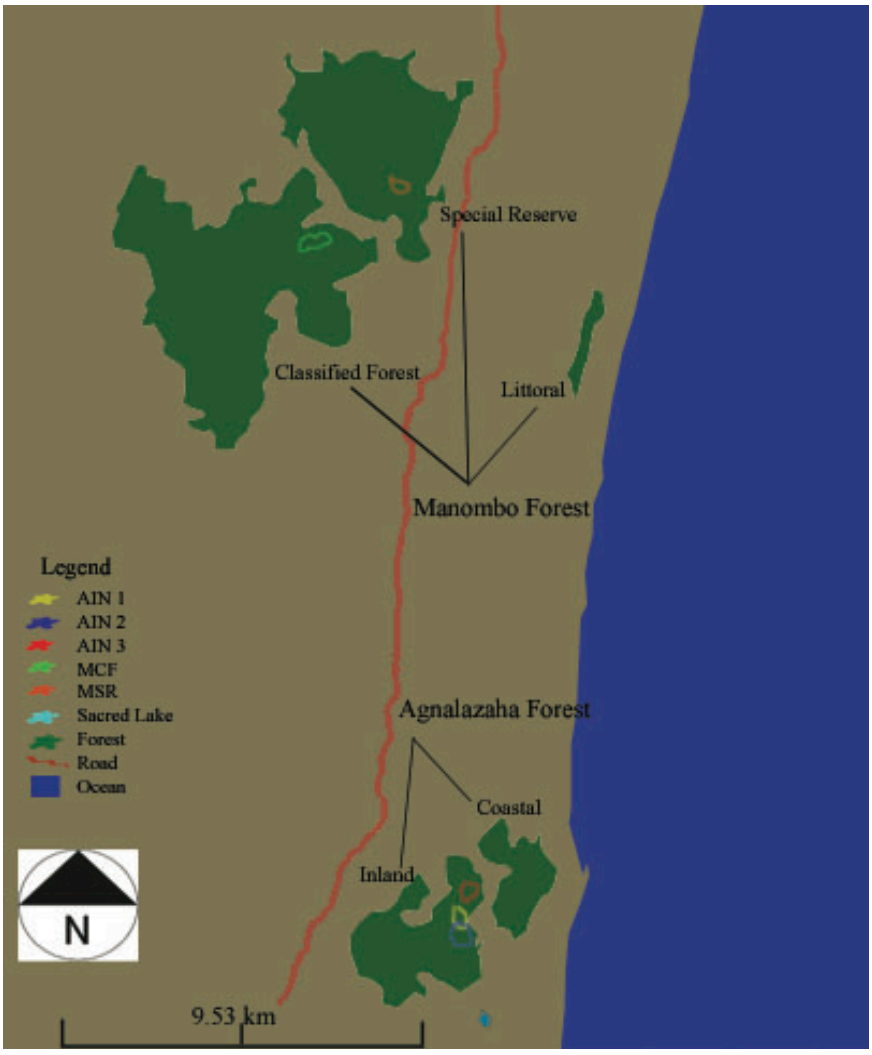

Figure 1.3 Map of study sites Manombo and Agnalazaha in southeastern Madagascar.

Table 1.1 Correlations and predictions in primate-parasite ecology.

Variable	Negative Correlation	Positive Correlation	No Correlation
Demographic Variables			
Age	Stongylate, threadworms in *Gorilla gorilla*: Lilly et al., 2002	*Trichuris* in *Eulemur rufifrons*: Clough, 2009; Nematodes in female *Mandrillus sphinx*: Setchell et al., 2007;	Ascarid, whipworms, tapeworms in *Gorilla gorilla*: Lilly et al., 2002; *Lemuricola* in *Eulemur rufifrons*: Clough 2009; Nematodes in male *Mandrillus sphinx*: Setchell et al., 2007; *Pan troglodytes*: Meuhlenbein, 2005; Nematodes in *Colobus angolensis palliates*: Okanga et al., 2006; Nematodes in *Alouatta pigra*: Vitazkova and Wade, 2007
Sex	Female *Eulemur rufifrons* higher protozoan parasite prevalence: Clough 2009	Male *Eulemur rufifrons* higher *Lemuricola* prevalence: Clough, 2009; Male *Propithecus verreauxi* higher nematode prevalence: Loudon, 2009	*Alouatta palliata*: Stoner, 1996; *Colobus angolensis palliates*: Okanga et al., 2006; *Cheirogaleus medius*: Schwensow et al., 2007; *Mandrillus sphinx*: Setchell et al., 2007; *Alouatta pigra*: Vitazkova and Wade, 2007, PSR and *Trichuris* in *Eulemur rufifrons*: Clough, 2009; nematodes in *Lemur catta*: Loudon, 2009
Group Size	Prediction: parasite burden from direct contamination: Chapman et al., 2009b; *Trichuris* in *Procolobus rufomitratus*: Chapman et al., 2009b	Prediction parasite species richness: Chapmen et al., 2009a; *Cercocebus albigena*: Freeland, 1979; Primate cross species analysis: Nunn et al., 2004; Cross mammal species analysis: Vitone et al., 2004; *Eulemur rufifrons* PSR: Clough, 2009; protozoan parasite species richness in *Cercocebus albigena*: Freeland, 1979	Protozoan parasite species richness in *Papio anubis*: Freeland, 1979; *Lemuricola* and *Trichuris* in *Eulemur rufifrons*: Clough, 2009; *Oesophagostom* and PSR in *Procolobus rufomitratus*: Chapman et al., 2009b
Social Behavior Variables			
Proximity		Predicted: Nunn et al., 2004; Predicted: Altizer et al., 2003; Predicted: Freeland, 1976; Predicted: Loehle, 1995	
Physical Contact		Predicted: Altizer et al., 2003; Predicted: Freeland, 1976; Predicted: Loehle, 1995	
Group Spread	Prediction: Chapman et al., 2009a; *Lemur catta* vs. *Propithecus verreauxi*: Loudon, 2009		
Habitat Use Variables			
Travel Time		Prediction parasite species richness: Chapman et al., 2009a	
Home Range Size		Some primate species: Nunn et al., 2004; Predicted parasite species richness: Chapman et al., 2009a	Some primate species: Nunn et al., 2004
Variable	Negative Correlation	Positive Correlation	No Correlation

Time on the Ground		*Lemur catta* vs. *Propithecus verreauxi*: Loudon, 2009	
Environmental Variables			
Micro-habitat/ Home Range Location		Microhabitat predicted to affect parasite burden: Chapman et al., 2009a; *Alouatta palliata* home ranges in wetter areas had increased parasite prevalence: Stoner, 1996	
Food Availability	Prediction: Altizer et al., 2003; *Procolobus rufromitratus*: Chapman et al., 2006		*Papio anubis*: Weyher et al., 2006
Forest Quality	Coccidian prevalence in *Lemur catta*: Loudon, 2009; Ascaridae and Cestoda in *Microcebus murinus*: Raharivololona and Ganzhorn, 2009; *Trichuris* in *Colobus angolensis palliates*: Okanga et al., 2006	*Lemuricola* prevalence in *Lemur catta*: Loudon, 2009	*Propithecus verreauxi*: Loudon, 2009; Stongylida, *Trichuris*, Oxyuridae and Coccidia in *Microcebus murinus*: Raharivololona and Ganzhorn, 2009; *Controrchis*, *Trypanoxyuris minutus*, *Giardia* prevalence in *Alouatta pigra*: Vitazkova and Wade, 2007
Season		Parasite burden increase with wetness: *Alouatta fusca*: Stuart et al., 1993; *Alouatta palliata*: Stoner, 1996; *Mandrillus sphinx*: Setchell et al., 2007; *Oesophagostomum* in *Pan troglodytes*: Huffman et al., 1997	*Trichuris trichiura* and *Strongyloides fuelleborni* in *Pan troglodytes*: Huffman et al., 1997

Table 1.2 Study groups for the Agnalazaha and Manombo forest fragments.

Group	S1 Adult Male	S1 Adult Female	S1 Juv. Male	S1 Juv. Female	S2 Adult Male	S2 Adult Female	S2 Juv. Male	S2 Juv. Female	S2 Infant Male	S2 Infant Female	S1 Total	S2 Total
AIN 1	2	2	4	0	2	2	3	0	1	1	8	9
AIN 2	2	1	2	1	1	1	2	1	0	1	6	6
AIN 3					0	1	2	1	1	0	N/A	5
MCF	2	2	3	1	1	1	0	1	0	0	8	3
MSR	1	1	1	0	2	2	2	0	1	1	3	8

Abbreviations: S1 = Season 1, Spring/Wet Season, S2 = Season 2, Fall/Dry Season, AIN = Agnalazaha inland forest groups 1, 2, and 3, MCF = Manombo Classified Forest, MSR = Manombo Special Reserve. Juveniles were born assumed born in 2006 or 2007; infants were born in 2008 during the study.

Chapter 2. Field and Laboratory Diagnostic Evaluation

2.1 Abstract

The interaction between non-human primates and their parasites is an area of increasing research interest in primate behavioral ecology. Opportunistic fecal collection can be a relatively simple, inexpensive and non-invasive way to gather parasitological data during existing field studies; however, preservation and analysis of the parasite stages found in fecal samples can be fraught with challenges and biases. This research evaluated the constancy of fecal preservation and parasite analysis for assessing endoparasite infection in the grey-headed lemur, *Eulemur cinereiceps* (= *E. albocollaris*). A total of 55 fecal samples from *E. cinereiceps* were used in 359 trials to compare preservation solutions (90% ethanol and 10% buffered formalin) and recovery methods. Results indicate that fecal sedimentation is a more sensitive method of recovery than fecal flotation, although using repeated floats on a sample significantly increased the sensitivity of fecal flotation. Samples stored in 10% buffered formalin yielded higher counts than their counterparts stored in 90% ethanol. Fecal flotation solution and homogeneity of the sample did not affect results. The necessary quantity of fecal sample for precise laboratory evaluation was addressed with consideration to research applications.

2.2 Introduction

2.2.1 Current Uses of Fecal Parasite Recovery

The application of parasitology to primate behavioral ecology research is an increasingly prevalent area of interest (Huffman and Chapman, 2009). Understanding a host's potential to harbor parasites promotes insight into individual and population fitness (Coop and Holmes,

1996), phylogeny and co-speciation (Hugot, 1999), conservation (Gillespie and Chapman, 2004) and possible zoonosis with human and non-human hosts (Ashford et al., 1990).

Although there have been several summaries of available and suggested methods for non-human primate parasitology (Gillespie, 2006; Greiner and McIntosh, 2009), few critical evaluations of fecal preservation and analysis techniques have been published. Thus it is difficult to interpret or compare results between studies. Method validation is critical to ensure the highest degree of efficiency, precision and accuracy. A study of wild chimpanzees found that the parasite species richness for an individual host increased with each consecutive fecal sample evaluated, up to 3 or 4 fecal samples (Muehlenbein, 2005). A study on human parasites found that 72% of parasite species were identified based on a single fecal sample if the parasite was found in more than 20% of the population (Branda et al., 2006). A study on two lemur hosts found that parasite loads varied widely from the same individuals in a population over a two-year study period (Clough, 2010). These studies highlight the necessity for diagnostic validation in field and laboratory research.

Various parasitological indices were evaluated in this study using 55 fecal samples from a wild population of grey-headed lemur, *Eulemur cinereiceps*, to validate field collection and laboratory recovery techniques. Preservation solution, recovery technique, aspects of fecal flotation and quantity of the feces were evaluated for precision.

2.2.2 Standard Procedures for Fecal Parasite Recovery

The following is based on procedures from Gillespie (2006), Greiner and McIntosh (2009), and Kutz (personal communication). Fecal samples collected in the field can be studied live, or preserved and studied later in a lab. Fresh fecal samples provide the most accurate results,

however samples need to be either frozen or preserved if they will not be evaluated shortly after collection. Many field sites do not have access to a laboratory, thus preservation has become a common step in non-human primate fecal parasite studies. Most samples are stored directly in a vial with preservation solution and later transported to a laboratory. Common preservatives include 70%, 90% or 95% ethanol (C_2H_6O), 10% formalin ($H_2C(OH)_2$), and 4% Potassium dichromate (KCh_2). Formalin is a better tissue preservative and is expected to better preserve parasites, while ethanol is used for molecular analysis. However, both 90% ethanol and 10% formalin are frequently used in primate fieldwork. Potassium dichromate is used most often in the study of coccidian and other protozoan parasites.

In the laboratory, two prevalent methods for egg recovery are fecal sedimentation and fecal flotation. The process of sedimentation is more time consuming and thus less frequently used. In fecal sedimentation and fecal flotation a portion of the fecal sample or a mixture of the fecal sample and preservative is separated, strained through cheese cloth and rinsed with tap water in a vial. The suspended fecal matter is spun in a centrifuge to speed precipitation. During fecal sedimentation, the supernatant is poured off and the resulting pellet is scanned in 40μl aliquots under a microscope.

During fecal flotation the resulting pellet is added to one of several solutions of a given specific gravity (SG) separating the fecal matter from the parasite eggs/cysts. The flotation solution allows the fecal matter to sink and the eggs to rise to the top during centrifugation. Time in the centrifuge varies between two and 10 minutes with five minutes as the most popular measure. As the eggs/cysts rise to the top of the vial, they adhere to a coverslip and the single coverslip is removed, placed on a microscope slide, and scanned for the presence of eggs/cysts/larvae. Popular fecal flotation solutions include: Magnesium Sulfate (MgSO4; SG

1.20), Zinc Sulfate (ZnSO4; SG 1.18–1.20), Sodium Nitrate Solution (NaNO3; SG 1.18–1.20), Saturated Salt (NaCl; SG 1.18–1.20), and Modified Sheather's Solution (SG 1.27). Eggs, cysts and larvae on the coverslip are then identified by morphology and counted.

The quantity of fecal sample needed for a reliable estimate of the parasite species richness and egg counts is currently not known. Too large a portion of the sample during fecal flotation will cloud a microscope slide making egg identification difficult. During fecal sedimentation, the amount of time it takes to run each sample in the laboratory is directly related to the amount of sample being evaluated.

2.3 Research Questions

1. Does a 10% formalin preservation solution affect egg and cyst recovery differently than a 90% ethanol solution?
2. Is fecal sedimentation a more sensitive recovery technique than fecal flotation for egg counts and parasite species richness (PSR)?
3. Is there any interaction between preservation solution and egg recovery technique?
4. Do the following aspects of fecal flotation affect egg recovery?
    a. Flotation solution: The specific gravity (SG) of the flotation solution may affect the sensitivity of fecal flotation. Flotation solutions are mixed at a specific gravity meant to separate the mixture during the centrifugation. The SG should be heavier than the eggs/cysts, but lighter than the majority of the fecal matter to properly separate them in the tube. If the SG is too heavy, the coverslip will become too clouded with fecal matter to identify parasite eggs/cysts. If the SG is too light, the eggs/cysts will sink to the bottom with the fecal matter. Is there a difference in egg

recovery between a normal modified Sheather's solution (SG 1.27) and a heavier modified Sheather's solution (SG 1.32)?

b. Consistency of the fecal sample: Another aspect of fecal flotation is the consistency of the sample. Many fecal samples are not homogeneous when collected, samples from many primate species are commonly comprised of partially digested leafy matter, fruit pulp, and seeds. Is there a difference in egg recovery when the liquid portion of the fecal-preservative mixture is used rather than the solid portion of the fecal-preservative mixture?

5. What is the quantity of the fecal sample needed for a reliable estimate of parasite species richness and egg counts for fecal flotation?

2.4 Method

2.4.1 Statistical Analysis and Terminology

Five field and laboratory techniques were tested for precision and efficiency. Because there is no way to confirm that samples collected in the wild are accurate, precision is the best measure available for method confirmation. In order to assess precision three measures were used: *parasite species richness* (PSR), the total number of parasite species found in a sample (Bush et al., 1996), *presence* of a parasite species, and *counts*, the number of eggs/cysts/larvae shed per gram, from each parasite species infecting a host (Bush et al., 1996). The term *samples* refer to feces collected from one lemur during a single instance of defecation and stored in preservative. Matched pairs represent the samples divided in half, or subsamples, with 2N sample size for analysis. The term *trial* refers to the number of tests run on each sample.

E. cinereiceps harbors at least six parasite species. The most commonly found eggs belong to two easily distinguished pinworm species *Callistoura* sp. and *Lemuricola* sp. and were used to measure egg counts. The rest of the parasite species were used only in assessing PSR.

Nematode egg counts showed non-normality and best fit a Poisson distribution. Egg counts were analyzed using a Poisson log linear regression analysis. Significance was determined using the Wald chi-square statistic (Quinn and Kerough, 2002). Presence and PSR were analyzed using Pearson's chi-square statistic. Research question five, regarding the quantity of feces needed for a reliable analysis was analyzed using a cumulative species richness curve for PSR, graphs plotting the cumulative variation in egg counts by gram, and the mean absolute percentage error (MAPE).

2.4.2 Fecal Samples Used to Address Each Research Question

All samples were collected from *Eulemur cinereiceps* at Agnalazaha and Manombo forests in the Fianaranasoa province of southeastern Madagascar. Feces were collected immediately after defecation, labeled, weighed, and preserved within 12 hours of collection. Preserved feces were transported to Susan Kutz's parasitology laboratory in the Faculty of Veterinary Medicine at the University of Calgary where they were evaluated over the following year.

For the first four questions, all tests run in the lab each used two trials of one gram of feces. Counts/gram results were averaged for the two trials. Except where indicated, evaluations used samples preserved in 10% formalin, with a Sheather's flotation solution (SG 1.27), and a mixture of solid and liquid fecal-preservation solution. Some samples were used in multiple tests. See Figure 2.1 for a flowchart of how each set of samples were used for the following tests.

1. Preservation solution:

Twenty-four samples were divided in half and stored in either 90% ethanol or 10% formalin. Ten of the 48 tests were evaluated using fecal sedimentation, and the other 38 were evaluated using fecal flotation.

2. Recovery technique:

Eleven samples were halved; one portion was evaluated using fecal sedimentation and the other was evaluated using fecal flotation for a total sample size of 22 matched pairs. Five of the original samples were preserved in 90% ethanol and six in 10% formalin.

3. Interaction between flotation solution and recovery technique:

Four samples were large enough to be divided in half and preserved in either 90% ethanol or 10% formalin. Each of these eight halves was then divided in half again and evaluated using either fecal flotation or fecal sedimentation. In this way, 16 portions of four fecal samples were evaluated using all combinations of recovery technique and preservation solution. In addition, all analyses of recovery technique and preservation solution are plotted in a visual display. Many samples were too small to divide more then once and therefore most samples were evaluated by comparing either recovery technique or preservation solution. However, to compensate for small sample size, a broad comparison of all analyses is available in Figure 2.3.

4a. Flotation Solution:

Out of the available solutions and their specific gravities (see above) sugar solutions were used because they are cost efficient and easy to manipulate for SG variation. A heavy modified Sheather's solution (SG 1.32) was compared with the regular modified Sheather's solution (SG 1.27) using 23 samples (46 matched pairs). Fourteen of the samples were preserved in 10%

formalin, and nine preserved in 90% ethanol. All samples were run in two single gram trials and comparisons were evaluated using fecal flotation.

4b. Consistency of the fecal sample:

Nine fecal samples were divided approximately in half based on consistency for a total of 18 matched pairs. Of the matched pairs, nine were comprised of a "liquid" mixture of feces and preservative solution, and nine were a "solid" mixture of feces and preservation solution consisting of mainly partially digested plant matter. All samples were preserved in 10% formalin. A 10^{th} sample was not evaluated due to laboratory contamination.

5. Quantity of fecal sample needed:

Samples preserved in 10% formalin were evaluated using fecal flotation with normal Sheather's solution SG 1.27, five minute flotation spin time, and using a mixture of solid and liquid portions from the samples. Between 10 and 24 trials were run for each sample.

Feces were measured before they were stored in preservative, and the required amount of feces was extracted from the sample as a mixture of feces and preservative using the formula:

$$\frac{(F_x + \text{preservative})}{F_{xof}} \times F_r = \text{Required amount of total fecal mixture}$$

Where F_x is the amount of feces in the sample and F_r is the required amount of feces needed for the study.

Samples were divided into a number of 0.25 or 0.33 fecal gram portions and each portion was evaluated separately. In order to assess how many grams of feces are needed for a precise fecal flotation analysis, three evaluation techniques were used.

The mean absolute percentage error (MAPE) was used to measure to accuracy of cumulative fecal sample trials using the following calculation:

$$M=(1/n)\Sigma\left|(A_t-F_t)/A_t\right|$$
$$t=1$$
$$n$$

Where *At* is the actual value, *Ft* is the forecast value and M is the mean absolute percentage error, n is the number of trials.

MAPE is typically used to measure forecast error, and in this case it was used to measure the error from average egg counts in small cumulative portions (0.25 or 0.33 grams) of seven samples. The MAPE values were plotted against size of the fecal sample to assess the quantity of the fecal sample against error. In other words, each sample was evaluated in its entirety in small 0.25 gram or 0.33 gram portions. The deviation percentage for egg counts in these portions was measured cumulatively for all possible combinations. For example, if a given sample has a mass of 3.5 grams, then it was evaluated in 14 cumulative portions of 0.25 grams. The *Callistoura* egg counts for each of these portions were used to evaluate error from the mean at each 0.25 gram increment (0.25, 0.50, 0.75, etc.). These values (using the above formula) give a measure of the error in egg counts for each increment of the sample.

Cumulative variation plots were used to determine the minimum weight needed from each fecal sample for precise *Callistoura* and *Lemuricola* egg counts per gram. Using small portions of the sample (0.25 or 0.33 grams), variation in egg counts from the final egg counts by cumulative fecal weight (i.e.: 0.25, 0.50, 0.75 grams etc.) was plotted for each sample. Estimates for reliable sample size can be determined as variation approaches zero. For this portion of the study, eggs from two species of pinworms were counted.

2.5 Results

2.5.1 Preservation Solution 90% Ethanol vs. 10% Formalin

Samples stored in 10% Formalin yielded significantly higher PSR than their counterparts stored in 90% Ethanol ($\chi^2 = 15.84$, $p \leq 0.001$, Table 2.1). A Chi-Square test of PSR comparing preservation solution using only samples evaluated by fecal flotation also found that the PSR is significantly higher in samples preserved in 10% formalin ($\chi^2 = 18.40$, $p \leq 0.001$, Table 2.1). A Chi-Square test could not be run using only samples evaluated by fecal sedimentation because all expected cells had counts less than five.

Feces preserved in 10% formalin had a significantly higher presence of *Callistoura* eggs than the portion of samples stored in 90% Ethanol ($\chi^2 = 14.722$, $p \leq 0.001$, Table 2.1). A Chi-Square test on presence of *Callistoura* eggs in samples only analyzed using fecal flotation also found significantly more samples with *Callistoura* preserved in 10% formalin than those preserved in 90% ethanol ($\chi^2 = 17.79$, $p \leq 0.001$, Table 2.1). A statistical test for samples evaluated using fecal sedimentation could not be run because all samples in both preservatives yielded *Callistoura* eggs when evaluated using fecal sedimentation.

Callistoura egg counts were compared by preservation solution while taking recovery technique (sedimentation vs. flotation) into consideration using a Poisson Regression Generalized Linear Model and found that samples stored in 10% formalin yielded significantly higher egg counts than samples stored in 90% ethanol, taking recovery technique into account (Wald $\chi^2 = 549.12$, $p \leq 0.001$, Table 2.1).

In a Chi-Square test *Lemuricola* eggs were found in significantly more samples preserved in 10% formalin than in their matched-pairs stored in 90% ethanol ($\chi^2 = 7.11$, $p = 0.008$, Table 2.1). A comparison of the *Lemuricola* egg counts for the samples stored in 90% ethanol and 10%

formalin was assessed using a Poisson Regression GLM taking recovery technique into account and found that samples preserved in 10% formalin yielded significantly higher *Lemuricola* egg counts (Wald $\chi^2 = 621.87$, $p \leq 0.001$, Table 2.1).

2.5.2 Recovery Technique

A Chi-Square test indicated no difference in the PSR found between samples using fecal flotation and fecal sedimentation (Table 2.2). Difference in the presence of *Callistoura* or *Lemuricola* eggs in the 11 samples could not be analyzed with a Chi-Square test. However, out of the 11samples, *Callistoura* was found in all of them when using fecal sedimentation, and in eight of them when using fecal flotation. The presence of at least one *Lemuricola* egg was found in three of the 11 samples when using fecal sedimentation and in six of the 11 samples when using fecal flotation.

Callistoura egg counts were compared using a Poisson Regression GLM (Table 2.2). The Poisson Regression indicates that fecal sedimentation results in significantly higher *Callistoura* egg counts than fecal flotation both with and without taking preservation solution into account (Wald $\chi^2 = 2916.43$, $p < 0.001$). A Poisson Regression GLM indicates that fecal sedimentation produces significantly higher *Lemuricola* egg counts than fecal flotation (Wald $\chi^2 = 5.12$, $p = 0.024$, Table 2.2). The same results were seen with a Poisson Regression GLM taking preservation solution into account (Table 2.2).

2.5.3 Interaction Between Fecal Preservation Solution and Egg Recovery Technique

Figure 2.2 plots *Callistoura* and *Lemuricola* egg counts by sample for five sets of matched-pairs for both recovery technique and preservation solution. All samples were divided in

half in the field and preserved in either 10% formalin or 90% ethanol. In the lab samples were further divided and evaluated using either fecal sedimentation or fecal flotation. Results appear similar between preservation solutions when using fecal sedimentation. The most inconsistent results occurred in samples preserved in 90% ethanol and evaluated using fecal flotation.

Figure 2.3 plots the cumulative PSR of samples preserved in 90% Ethanol and 10% Formalin by recovery technique. These samples are in matched pairs by preservation solution but not by recovery technique. Although sample size is small for fecal sedimentation (N=5), sedimentation appeared to yield fairly consistent results regardless of preservation solution. Samples evaluated using fecal flotation had higher PSR when preserved in 10% Formalin rather than when preserved in 90% Ethanol.

2.5.4 Fecal Flotation Solution

Two fecal flotation solutions, a normal modified Sheather's solution (SG 1.27) and a heavy modified Sheather's solution (SG 1.32) were compared. A Chi-Square test found no difference in the PSR for samples evaluated using a Sheather's solution with a heavier SG than the regular SG (Table 2.3). A Chi-Square test found no difference in the presence of *Callistoura* eggs from samples using the Sheather's solution with regular SG or the heavy SG (Table 2.3). A comparison of the presence of *Lemuricola* eggs in samples evaluated using normal and heavy Sheather's solution found no difference (Table 2.3).

Two Poisson Regression GLMs found no difference between the *Callistoura* egg counts from samples evaluated using normal and heavy Sheather's solution both when and when not taking preservation solution into account (Table 2.3). A Poisson Regression GLM comparing counts of *Lemuricola* eggs for each Sheather's solution while taking preservation solution into

account found that Sheather's solution SG 1.27 yielded significantly higher egg counts (Wald $\chi^2 = 48.20$, $p < 0.001$, Table 2.3).

2.5.5 Consistency of the Fecal Sample

A Chi-Square test indicated no difference in PSR between liquid and solid mixtures (Table 2.4). The presence of *Callistoura* eggs was not statistically analyzed since presence was found in all samples of both liquid and solid mixtures.

A Poisson Regression GLM indicated significantly higher *Callistoura* egg counts in samples comprised of the feces solid mixture than the liquid mixture ($\chi^2 = 9.40$, $p = 0.002$, Table 2.4). There is a disparity of over 200 in the egg counts in the matched pair of one sample and rerunning the Poisson Regression GLM without the outlier sample results in a reversal of significance with higher egg counts from the liquid mixture ($\chi^2=12.92$, $p < 0.001$, Table 2.4). The inconsistency of these results suggests they are unreliable.

No difference was found in the presence of *Lemuricola* eggs between the two mixtures (Table 2.4) and in only one sample-pair was *Lemuricola* found in one mixture and not the other. A Poisson Regression GLM for *Lemuricola* egg counts by homogeneity of the sample indicates no difference (Table 2.4).

2.5.6 Quantity of Fecal Sample

MAPE values for each of the seven samples and their averages have been visually depicted in Figures 2.4, 2.5, 2.6 and 2.7. Figure 2.4 plots the MAPE values and standard deviations for all eight samples at each cumulative fecal mass. The trend fits an exponential curve that levels out in all cases between two and three grams. Figure 2.5 plots the MAPE value of each

sample at each cumulative fecal mass. The goal MAPE value for this study is set at 10%. All the samples follow a general trend approaching a value of 10% between two and five grams. Figure 2.6 plots the MAPE values by a cumulative percentage of the fecal mass of each sample, rather than the actual mass of the sample. MAPE values approach 10% when between 60-80% of the fecal sample has been analyzed. Figure 2.7 plots the average MAPE values for all samples using cumulative percentages of fecal mass. The samples approached 10% MAPE when approximately 75% of the sample have been evaluated.

Variance in egg counts was assessed by plotting the variance for cumulative egg counts as a function of the cumulative fecal mass. Figure 2.8 plots the variance of *Callistoura* egg counts. The variance approaches zero between two and three grams for all samples. Figure 2.9 plots the variance of *Lemuricola* egg counts, which approach zero between two and four grams in all four samples that contained *Lemuricola* eggs.

Parasite species richness was assessed using a cumulative curve for seven fecal samples (Figure 2.10). In three of the samples, PSR remains the same despite an increase in cumulative fecal mass. In the other four samples, PSR reaches its peak between 0.75 and three grams.

2.6 Discussion

2.6.1 Preservative Solution

For many wildlife studies, immediate access to a laboratory is not available. In this case, fecal samples need to be frozen or preserved in the field. For proper fixation feces should be in at least a 1:10 feces:preservative ratio (Kutz, personal communication).

All statistical tests confirm that 10% formalin is a more sensitive preservation solution than 90% ethanol. When using fecal sedimentation as a recovery technique, 90% ethanol more

closely approaches the results of 10% formalin than when using fecal flotation. However, when assessing egg counts for *Callistoura* and *Lemuricola*, as well as the presence of *Lemuricola*, 10% formalin yields significantly higher results.

2.6.2 Recovery Technique

Overall fecal sedimentation appears to be a more sensitive technique than fecal flotation. Egg counts are significantly higher with both *Callistoura* and *Lemuricola* eggs when using fecal sedimentation rather than fecal flotation, even when taking preservation solution into account. The differences in recovery technique on parasite species richness and parasite presence are less substantial. Overall, the results from this study suggest that fecal sedimentation is more sensitive to egg counts, especially when using a 90% ethanol preservative, and that fecal sedimentation and flotation can both be used effectively for PSR and parasite presence.

2.6.3 Interaction Between Preservation Solution and Recovery Technique

The interaction between preservation solution and recovery technique suggests that samples preserved in 90% ethanol and 10% formalin may yield comparable results when using different recovery techniques. Fecal samples collected in 90% ethanol yielded lower PSR and egg counts, but when evaluated using fecal sedimentation, the results are more reliable. It may be possible to compare samples preserved in 90% ethanol to those preserved in 10% formalin, if fecal sedimentation is used for the analysis of those stored in 90% ethanol. Samples stored in 90% ethanol and evaluated using fecal flotation yielded the lowest and most inconsistent results.

2.6.4 Flotation Solution

Overall there was little difference between a heavy and normal modified Sheather's solution when using fecal flotation. The only difference was found in *Lemuricola* egg counts, which were significantly higher when using a normal Sheather's solution with a specific gravity of 1.27.

2.6.5 Consistency of the Fecal Sample

It was expected that the liquid sections would yield significantly higher egg prevalence and counts. The eggs are expected to attach themselves to the circumference of the fecal matter as it passes through the intestinal wall and then fall off with the softer fecal matter in the storage vial. Liquid portions of the sample were expected to yield higher egg counts and parasite prevalence. However, due to the conflicting results of *Callistoura* egg counts and because there was no difference in *Lemuricola* egg counts, I conclude that was no significant difference in the results from solid and liquid portions of the fecal sample.

2.6.6 Quantity of the Fecal Sample

MAPE values, variance in egg counts and a parasite species richness cumulative curve were used to assess the quantity of a fecal sample required for fecal flotation. It was determined that depending on the question being asked, it may be a better option to base the amount of fecal sample being used on a percentage of the fecal sample rather than number of grams. I used a goal MAPE score of 10%, however some studies may accept much higher scores.

If PSR is more important than egg counts, then approximately 2-3 grams of a fecal sample should maximize sensitivity when using fecal flotation. This equates to just under 60% of the

fecal sample. Although this portion of the study only evaluated eight samples, results were consistent. Approximately 2.5 grams, or 72% of the fecal sample, was needed for PSR to reach its maximum value in all samples except one. This suggests robustness for *E. cinereiceps* fecal evaluation. Fecal egg counts are more sensitive than PSR and parasite presence, therefore 3 grams (~77%) are recommended as a minimum for studies using egg counts. However, these measures may change with different host and parasite species. In the case of rare parasites, it may be advisable to study at least 3 grams or more than 75% of the fecal sample. This study did not evaluate the amount of feces needed to make an accurate assessment of the parasite infections of a given individual using fecal sedimentation. This question should be addressed in future research.

2.7 Conclusion

Although this study is not comprehensive of all aspects of field and laboratory work with gastro-intestinal parasites, the following important conclusions are drawn.

Ten percent formalin is a more reliable preservative than 90% ethanol, although it is also a carcinogen and mildly corrosive, making it less attractive for field work. When arrangements for the proper disposal of chemical storage, and handling of the chemical can be made, then 10% formalin is a better preservative for morphological fecal parasite analysis.

This study also suggests that fecal sedimentation is a more sensitive method for identifying parasite infections than fecal flotation. This may be subject to the consistency of the fecal matter within the sample and the parasites being studied and should be considered separately for each research question. Fecal flotation can be improved with repeated floats, which

also reduces the chance of error. Overall fecal flotation with repeated floats is an efficient option for fecal parasite recovery.

While homogeneity of the sample and fecal flotation solution did not impact the results of this study, they should still be considered in future work with different host and parasite species. In addition, some flotation solutions such as zinc sulfate are better applied to studies of protozoan cysts that may deteriorate in other materials.

Finally, the quantity of the fecal sample being studied remains an important factor in all analyses. At least 2 grams, and at least 60% of the fecal sample should be evaluated to maximize PSR. When using fecal flotation, 3 grams or 77% of the fecal sample is recommended to provide the most consistent nematode egg counts.

2.8 Figures and Tables

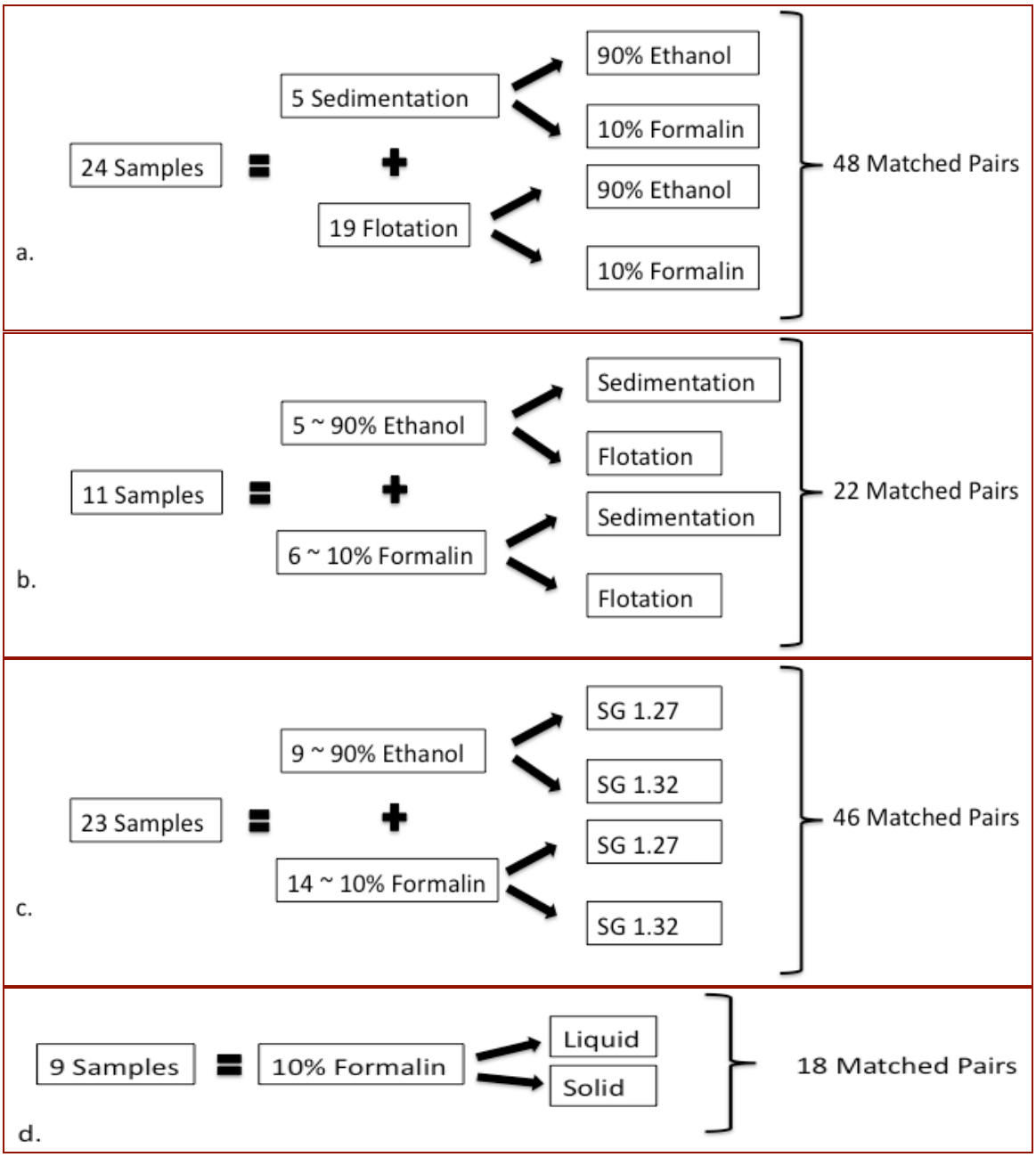

Figure 2.1 Fecal samples used to assess each research question.
a. Preservation Solution, b. Recovery Technique, c. Flotation Solution, d. Consistency of the fecal sample.
Note that some samples are used in multiple questions.

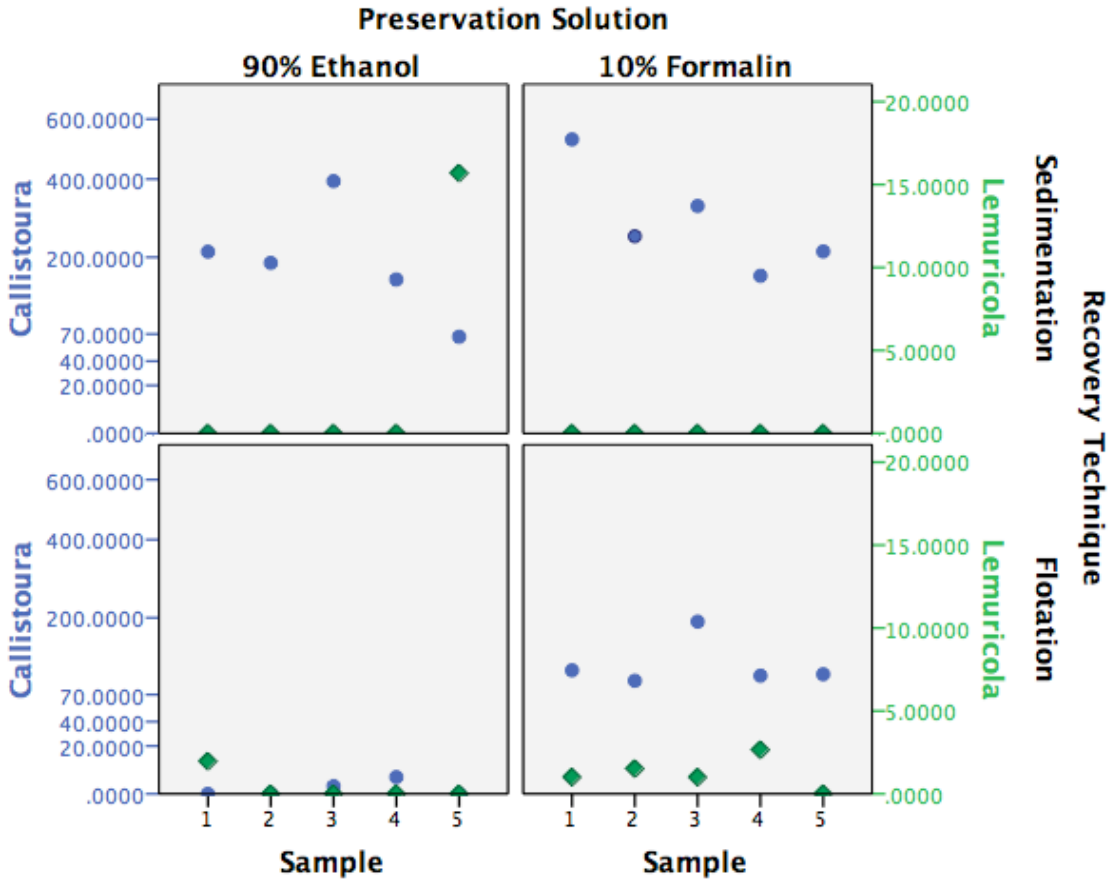

Figure 2.2 *Callistoura* and *Lemuricola* egg counts by sample, preservation solution and recovery technique for the same five samples.
Note diamond dots represent *Lemuricola* and circle dots represent *Callistoura*. Y-axis not on a linear scale.

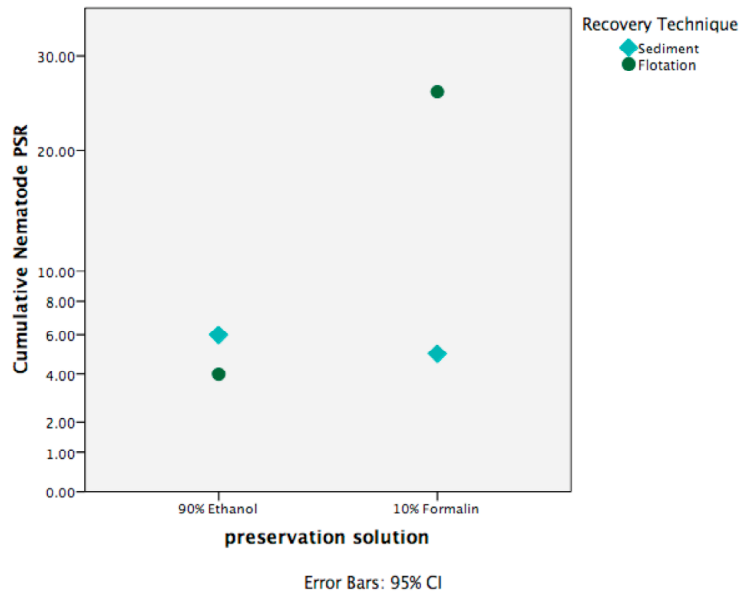

Figure 2.3 Cumulative nematode PSR by preservation solution for both egg recovery techniques. Note samples are matched by preservation solution and not recovery technique.

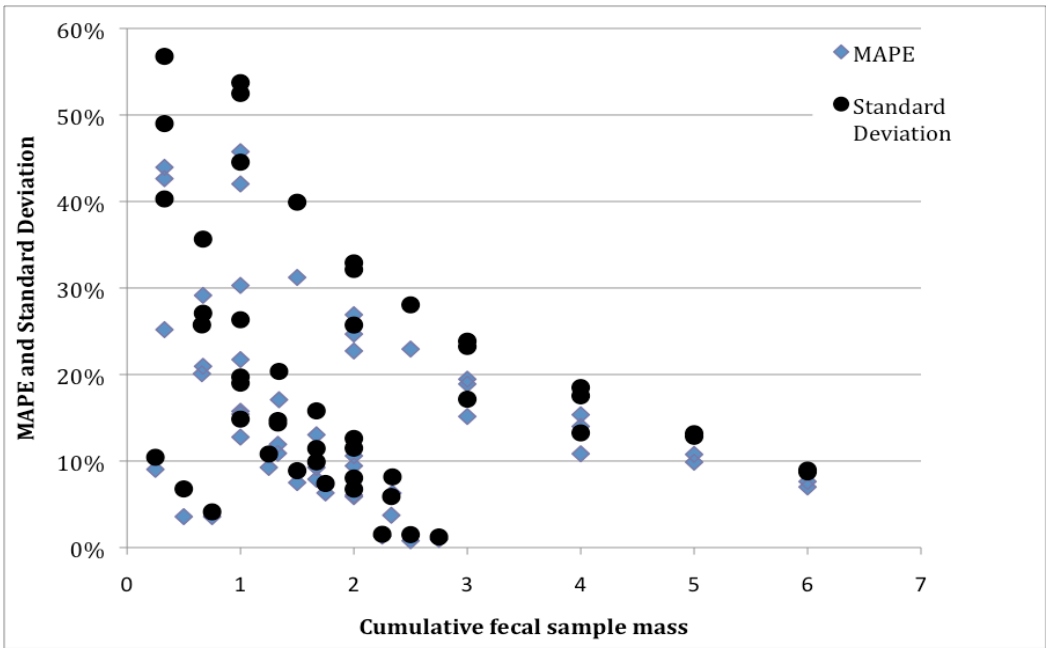

Figure 2.4 All MAPE percentages and standard deviations by cumulative fecal sample mass for each of seven samples.

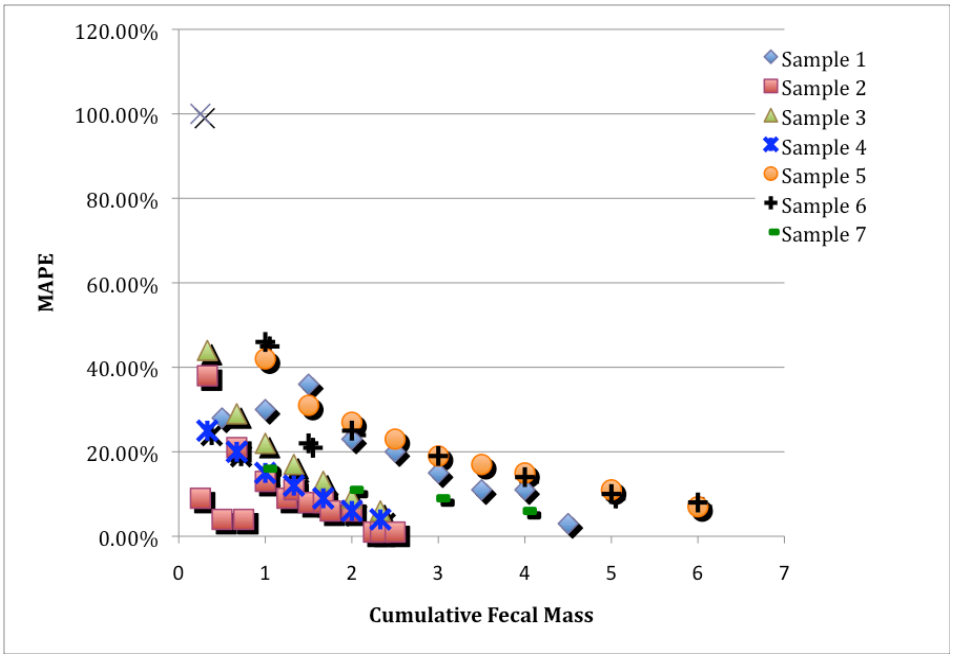

Figure 2.5 MAPE values by cumulative fecal mass for seven samples.

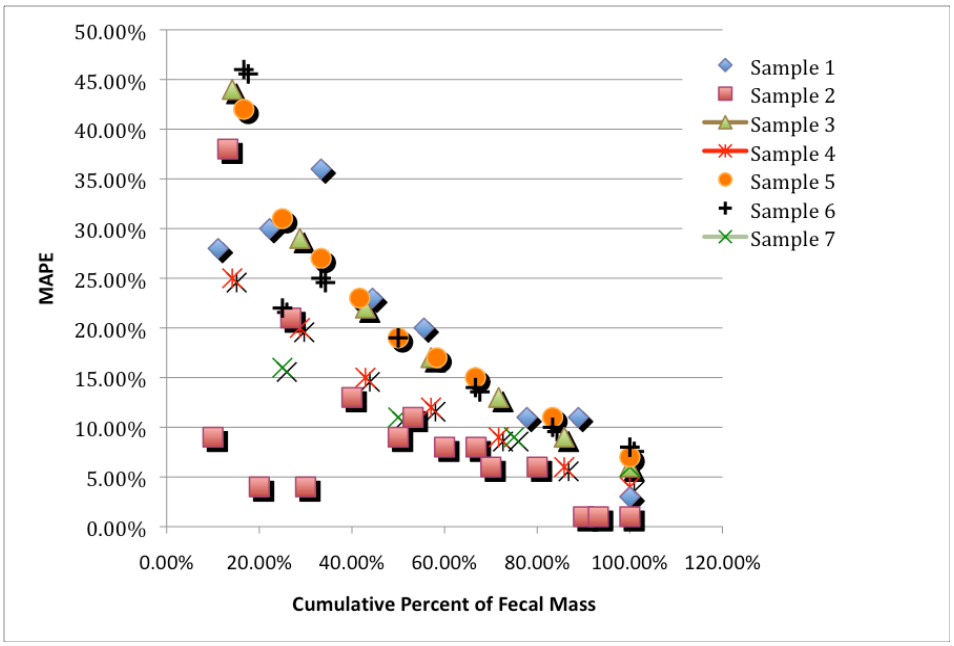

Figure 2.6 MAPE values by cumulative percentage of fecal mass for seven samples.

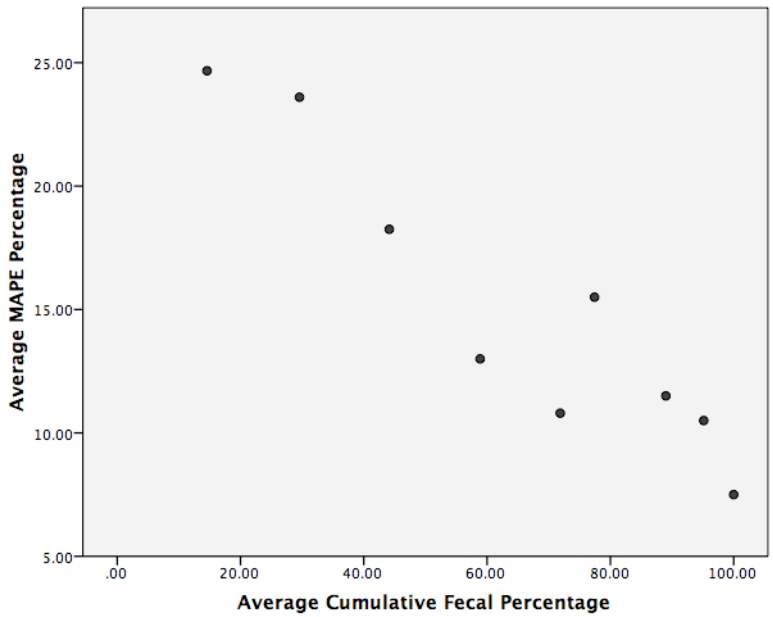

Figure 2.7 Average MAPE values for all seven samples by cumulative percentage of fecal mass.

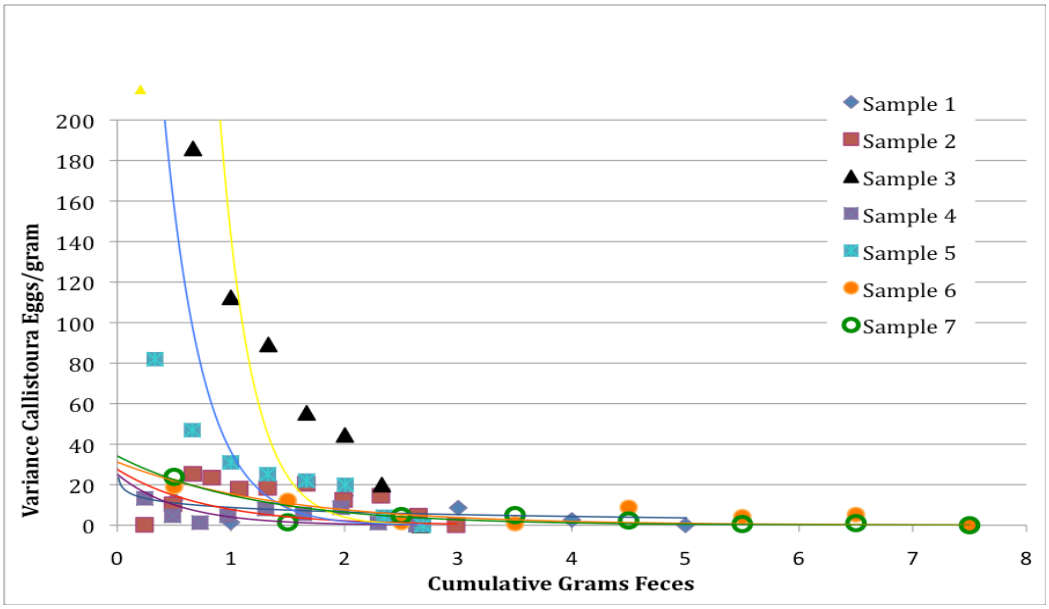

Figure 2.8 Variance in *Callistoura* egg counts by cumulative fecal mass for seven samples.

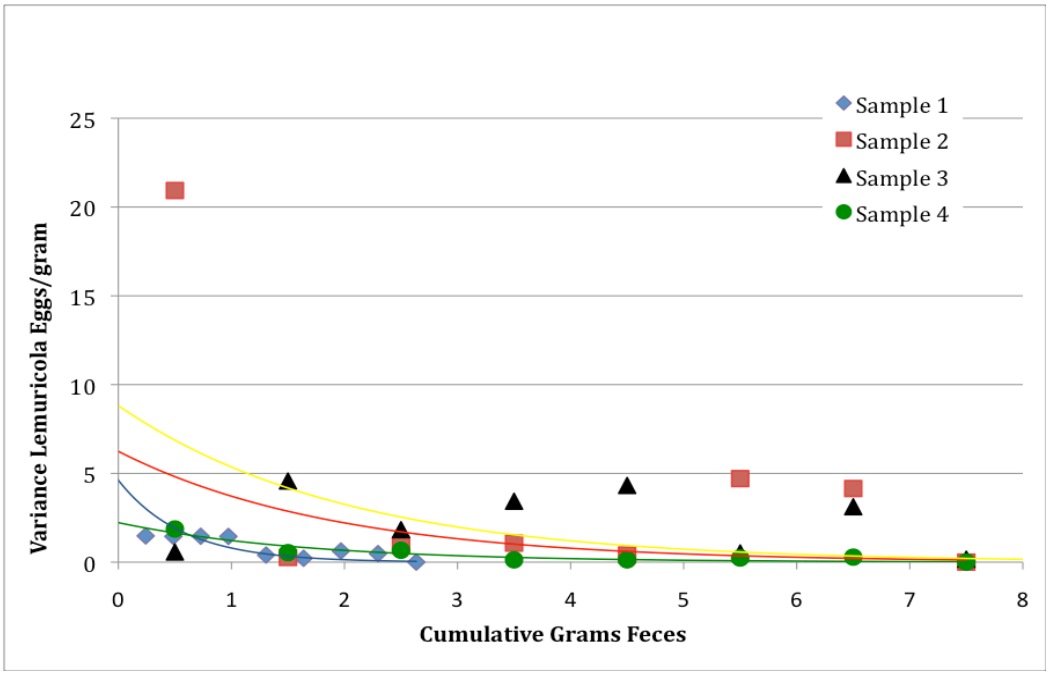

Figure 2.9 Variance in *Lemuricola* egg counts by cumulative fecal mass for four samples.

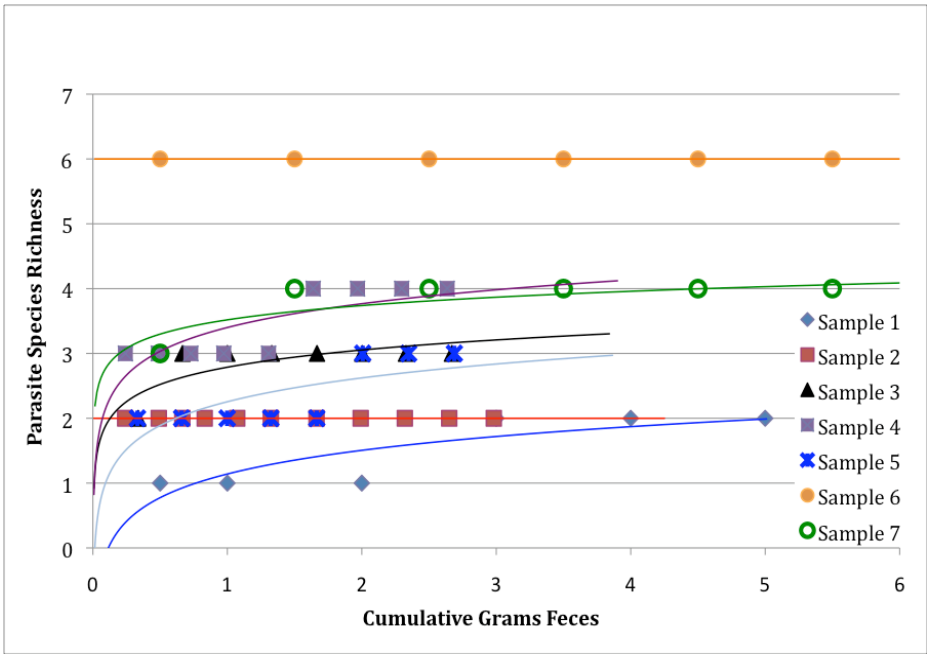

Figure 2.10 Cumulative parasite species richness by fecal mass for seven samples.

Table 2.1 Statistical analyses of preservation solution.

Predictor Variable	Response Variable	Offset Variable	Analysis	Sample Size	Sample Means	Test Statistic	Significance	More Successful Predictor
Preservation Solution	PSR		Chi-Square Test	48 Matched pairs		15.84	0.000*	10% Formalin
Preservation Solution	PSR	Only samples using fecal flotation	Chi-Square Test	38 Matched pairs		18.40	0.000*	10% Formalin
Preservation Solution	*Callistoura* Eggs Presence		Chi-Square Test	48 Matched pairs		14.72	0.000*	10% Formalin
Preservation Solution	*Callistoura* Eggs Presence	Only for samples using fecal flotation	Chi-Square Test	38 Matched pairs		17.79	0.000*	10% Formalin
Preservation Solution	*Lemuricola* Eggs Presence		Chi-Square Test	48 Matched pairs		7.11	0.008*	10% Formalin
Preservation Solution	*Lemuricola* Egg Counts	Recovery Technique	Poisson Regression	48 Matched pairs	Ethanol= 0.12, Formalin= 4.38	621.87	0.000*	10% Formalin
Preservation Solution	*Callistoura* Egg Counts	Recovery Technique	Poisson Regression	48 Matched pairs	Ethanol= 6.65, Formalin= 15.55	549.12	0.000*	10% Formalin

Note *indicates statistial significance where $p \leq 0.01$.

Table 2.2 Statistical analyses for recovery technique.

Predictor Variable	Response Variable	Offset Variable	Analysis	Sample Size	Sample Means	Test Statistic	Significance	More Successful Predictor
Recovery Technique	PSR		Chi-Square Test	22 Matched pairs		3.83	0.147	None
Recovery Technique	*Callistoura* Egg Counts	Preservation Solution	Poisson Regression	22 Matched pairs	Sediment= 28.06, Flotation= 3.78	2916.43	0.000**	Sedimentation
Recovery Technique	*Lemuricola* Egg Counts		Poisson Regression	22 Matched pairs	Sediment= 3.00, Flotation= 1.55	5.12	0.024*	Sedimentation
Recovery Technique	*Lemuricola* Egg Counts	Preservation Solution	Poisson Regression	22 Matched pairs	Sediment= 0.21, Flotation= 0.11	5.12	0.024*	Sedimentation

Note *Indicates significance where $p \leq 0.05$, **indicates significance where $p \leq 0.01$.

Table 2.3 Statistical analyses of flotation solution.

Predictor Variable	Response Variable	Offset Variable	Analysis	Sample Size	Sample Means	Test Statistic	Significance	More Successful Predictor
Flotation Solution	PSR		Chi-Square Test	46 Matched pairs		0.35	0.840	None
Flotation Solution	*Callistoura* Eggs Presence		Chi-Square Test	46 Matched pairs		0.34	0.555	None
Flotation Solution	*Lemuricola* Eggs Presence		Chi-Square Test	46 Matched pairs		0.11	0.743	None
Flotation Solution	*Callistoura* Egg Counts	Preservation Solution	Poisson Regression	46 Matched pairs	Normal Sheather's= 0.78, Heavy Sheather's= 0.83	1.54	0.215	None
Flotation Solution	*Lemuricola* Egg Counts	Preservation Solution	Poisson Regression	46 Matched pairs	Normal Sheather's= 1.97, Heavy Sheather's= 1.30	48.20	0.000*	Normal Sheather's solution
Flotation Solution	*Lemuricola* Egg Counts without outliers		Poisson Regression	44 Matched pairs	Normal Sheather's= 9.48, Heavy Sheather's= 5.04	31.48	0.000*	Normal Sheather's solution

Note *indicates significance where $p \leq 0.001$.

Table 2.4 Statistical analyses for sample consistency.

Predictor Variable	Response Variable	Analysis	Comment	Sample Size	Sample Means	Test Statistic	Significance	More successful variable predictor
Consistency	PSR	Chi-Square Test		18 Matched pairs		0.23	0.629	None
Consistency	*Callistoura* Egg Counts	Poisson Regression		18 Matched pairs	Liquid= 79.78, Solid= 93.22	9.40	0.002*	Solid
Consistency	*Callistoura* Egg Counts	Poisson Regression	Without outlier	18 Matched pairs	Liquid= 61.00, Solid= 47.75	12.92	0.000*	Liquid
Consistency	*Lemuricola* Egg Counts	Chi-Square Test		18 Matched pairs		0.23	0.629	None
Consistency	*Lemuricola* Egg Counts	Poisson Regression		18 Matched pairs	Liquid= 47.33, Solid= 52.78	2.67	0.103	None

Note *indicates significance where $p \leq 0.05$.

Chapter 3. Fecal Parasites Recovered from a Critically Endangered Lemur, *Eulemur cinereiceps*, in Southeastern Madagascar

3.1 Abstract

Primate parasitology is an area of increasing interest. Understanding the role of parasites in wild populations is a critical aspect of conservation programs and wildlife studies. A crucial part of understanding an animal's disease risk is identifying parasites in wild populations. *Eulemur cinereiceps* is a critically endangered primate from southeastern Madagascar. Only five small fragments of the forested coastal range remain, and these fragments are cut off from the continuous mountain population. This study identified four nematodes and one protozoan parasite through noninvasive fecal sampling of *Eulemur cinereiceps* in five forest fragments at Manombo and Agnalazaha. Little is currently known about the parasite species identified in this study. They are all expected to transfer between hosts through direct fecal-oral contamination, suggesting that cross-species transmission is a possibility.

3.2 Introduction

Identifying the parasite taxa found in any host species is critical to understanding the collective role of each organism in its environment (Altizer et al., 2003). It is also important for spotting potential zoonotic disease risk for an entire community and understanding the symptoms and preventive mechanisms for parasite species (Mathews, 2009). Parasite infections are the result of various factors including host immunity, host exposure and indices of the parasites themselves (Altizer et al., 2003; Sorci et al., 2003). Parasites may be transferred to a new host directly or indirectly. Direction transmission includes either direct contact (through the skin or mucus) or direct ingestion (in water, soil, or food sources). Indirect mechanisms include the

ingestion of an intermediate host such as insects or mollusks, or transfer through contact with indirect hosts such as mosquito bites (Loehle, 1995).

Although 94 of the world's 412 primate species are Lemuriformes, parasitism in lemurs has been understudied when compared to other primate clades (Irwin and Raharison, 2009). This becomes particularly important as an increasing number of lemur species become endangered due to habitat loss (Irwin et al., 2005). The *Eulemur* complex represents a group of closely-related brown lemurs found throughout Madagascar (Johnson, 2006). Although the endoparasites of many *Eulemur* species have been reported, to date, no studies have identified the endoparasites of the grey-headed lemur, *Eulemur cinereiceps*.

Figures 3.1 and 3.2 represent a selective phylogeny of the endoparasites found in *Eulemur* species. The diagram is not an exclusive taxonomy of these phyla, instead only superfamilies with taxa that parasitize lemurs are represented in the diagram. The listed genera have parasite taxa found in *Eulemur* hosts. Few protozoan parasites have been identified due to their small size and fairly indistinguishable morphology. The genera noted in Figure 3.1 should be considered cautiously and are likely representatives of some protozoan parasites infecting lemurs. Among *Eulemurs* as well as all lemurs, nematodes are the most commonly found parasites (Irwin and Raharison, 2009).

3.3 Method

3.3.1 Host Study Species and Sites

Eulemur cinereiceps is a cathemeral, primarily frugivorous primate weighing approximately 2.0kg and can be found in montane and coastal forests in southeastern Madagascar (Irwin et al., 2005; Ralainasolo et al., 2008; Ingraldi, 2010). This study took place at

the Manombo and Agnalazaha coastal forests in the Fianaranasoa province of southeastern Madagascar (Figure 3.3). The Agnalazaha forest is managed by the Missouri Botanical Gardens and is comprised of two fragments, approximately one kilometer apart and each approximately 10.63 km^2 and 3.36 km^2 in size (Ingraldi, 2010; Table 3.1). The Manombo forest is comprised of three fragments, two approximately 15.85 km^2 and 1.70 km^2 managed by Madagascar National Parks (Ingraldi, 2010; Table 3.1). The third fragment is approximately 29.10 km^2 and is managed by the Ministry of Environment, Forests and Tourism, and locally by the Interregional Direction of Environment, Forests and Tourism (Ingraldi, 2010; Table 3.1).

3.3.2 Fecal Sample Collection and Evaluation

A total of 311 samples were collected during 2006, 2007, and 2008 and evaluated for parasite egg and cyst recovery (Table 3.2). Fecal samples were collected directly after defecation, labeled, weighed and preserved with either 90% ethanol or 10% formalin. Samples were kept out of the sun and transported to parasitology laboratories following each field season. In the lab, samples preserved in 10% formalin were evaluated using fecal flotation in two or three single-gram repetitive trials (see chapter 2). A total of 209 samples were evaluated in 627 fecal flotation trials for egg and cyst recovery. In addition, 57 samples preserved in 90% ethanol were evaluated using two gram fecal sedimentation. This represents samples from a total of 31 individuals during 2006, 18 individuals during 2007, 31 individuals during the spring/wet 2008 season, and 45 individuals during the fall/dry season. This sample size is adequate for population assessment based on a study by Paulin and Morand (2000). The Baerman's nematode culture method was utilized in the field for an additional 36 samples for larvae analysis without success.

Additional samples stored in potassium dichromate were assessed for coccidian parasites and giardia without success. These samples are not included in any analyses or estimates.

3.4 Results

Fecal sample collection from 2006-2008 yielded four nematode parasites identified by morphology: *Callistoura* sp., *Lemuricola* sp., *Trichuris* sp., and an ascarid sp. Several larvae were found but have not been identified. Several protozoan species were found, although only one taxon, *Entamoeba* sp., was identified. Several ectoparasites were also found in fecal samples.

3.4.1 Nematoda

The most commonly found nematode egg was from the pinworm *Callistoura* sp. (Table 3.3). Based on egg morphology this is likely *C. blanci* (Chabaud et al., 1965; Figure 3.4) and was found in frequencies varying from 36.67% to 95.65% of individuals seasonally (Table 3.2). *Callistoura* belongs to the Pharyngodonidae family, which typically infects reptiles and amphibians (Faulkner et al., 2004). It is unknown whether *Callistoura* parasitizes any non-lemur hosts.

Lemuricola sp., another pinworm, was found in 9.68% to 54.72% of individuals seasonally (Figure 3.4; Table 3.2). *Lemuricola* belongs to the Oxyuridae family, a group of pinworms infecting human and nonhuman hosts (Table 3.3). This genus has eight species, all of which infect lemurs. Based on egg morphology and two potential adult specimen found in feces, the eggs found in *E. cinereiceps* likely belong to *L. vauceli* (Chabaud et al., 1965).

Eggs belonging to the whipworm *Trichuris* were found from samples in one forest fragment at Agnalazaha only after September 2008 (Figure 3.4; Table 3.3). *Trichuris* may have

been introduced to the population after earlier field seasons, or its absence may have been due to fluctuating prevalence in the population. Although they were only present in 17.78% of the fall 2008 individuals, they were present in 72.72% of the individuals sampled from the Agnalazaha coastal forest fragment where they were found (Table 3.2). The only *Trichuris* species identified in lemurs is *Trichuris lemuris* (Rudolphi, 1819 =*Trichocephalus*), however, egg morphology within this genus is too indistinct to confirm that the eggs from this study belong to *T. lemuris*.

Several ascarid eggs were also found (Figure 3.4; Table 3.3). Unfortunately, it was only identified after the analysis of the 2006 and 2007 samples and so its presence or absence cannot be confirmed before 2008. Ascarid eggs were found in 3.23% to 17.78% of the 2008 individuals sampled (Table 3.2). The only known ascarid to infect lemurs is *Acaris petiti* (Chabaud et al., 1964) from the family Ascarididae. Chabaud (1964) didn't describe eggs and thus the morphology cannot be matched from a fecal diagnosis. Egg appearance is different from those of other ascarids reported in lemurs (Anderson, 2000; Irwin and Raharison, 2009), as well as those known to infect humans and domesticated animals.

Two frequently found larvae were unidentifiable and may belong to the genera *Parahabdonema* or *Lemurostrongylus*, however, this cannot be confirmed without molecular analysis. The morphology of the larva is most consistent with the orders Strongylida and Rhabditida (Figure 3.2). Larvae from taxon 1 were an average of 302.50 µm x 11.50 µm (n=6), with a visible spicule-like appendage on the posterior end and no other well-defined internal structures (Figure 3.4). Larvae from taxon 2 were an average of 288.79 µm long and 10.90 µm wide (n = 13) with an extended posterior end (Figure 3.4).

3.4.2 Protozoa

An *Entamoeba* species was identified based on cyst morphology similar to those found in *Eulemur rufifrons* and classified through molecular analysis (Clough, 2010). *Entamoeba* is an Amoebida of which there are several species known to infect human and nonhuman primates (Figure 3.4; Table 3.3). *Entamoeba* were not recorded during analysis of the 2007 and spring/wet season 2008 samples due to an error in identification. In 2006 and 2008 the *Entamoeba* species were found in 12.9% to 26.67% of the individuals sampled (Table 3.2).

Based on morphology it is possible that *Balantidium* sp. were found in a number of samples. However, this could not be confirmed (S. Kutz, S. Upton, J. Kvičerová, M. Kvac, personal communication).

3.4.3 Ectoparasites

In addition to endoparasites, a number of ectoparasites were also found in the fecal samples. The most common ectoparasite is a louse taxa also found in *Varecia varecia editorum*, *Eulemur rufifrons* and *E. cinereiceps x E. rufifrons* hybrids (Martin and Baden, unpublished data; Martin and Delmar; unpublished data). The lice, shown in figure 3.3, appear morphologically similar to *Phthirpediculus brygooi*, which was recorded in *Eulemur mongoz* (SID, 2002).

3.5 Discussion and Conclusion

Callistoura and *Lemuricola* were the most commonly occurring infections among the coastal population of *Eulemur cinereiceps*, while the ascarid species, *Trichuris* and *Entamoeba* were rarely found. All of the parasites are transmitted directly through fecal contamination,

however, it is also possible that *Lemuricola* may additionally be transferred through direct physical contact between individuals (Irwin and Raharison, 2009).

The potential for cross-species transmission is increased with contamination based pathogens (Ezenwa, 2003). The nematode parasites found in this study have not been reported in any human populations and are not likely to represent a threat to local communities around the forest. Within the forest there exists the potential for cross-species contamination between lemur species, as well as other mammal species including domesticated animals such as zebu (*Bos indicus*) and pigs (*Sus scrofa domesticus*). Four of the five forest fragments are highly fragmented and frequently used by human and domesticated animals. The fragments at Agnalazaha have significantly higher rates of anthropogenic disturbance than those at Manombo (Ingraldi, 2010). It is unknown whether *Callistoura* may infect other mammals, reptiles or amphibians. *Lemuricola* has been found only in lemurs, however, the eggs are difficult to distinguish from *Enterobius*, another genus in the Oxyuridae family that infects humans. Different *Trichuris* species are known to parasitize humans, dogs, cats, pigs and mice, and their eggs are difficult to distinguish. *E. cinereiceps* have not been observed consuming fecal matter or soil, however, and in light of the absence of *Trichuris* eggs from 2006 and 2007 samples, it is possible that the *Trichuris* species infecting *E. cinereiceps* at Agnalazaha were introduced from another mammalian host. The ascarid species found in *E. cinereiceps* does not resemble eggs described from domesticated animals or lemurs and therefore the potential for zoonoses cannot be determined.

The *Entamoeba* species could not be identified, however, *Entamoeba coli* is the *Entamoeba* species most often reported in lemurs (Irwin and Raharison, 2009). *Entamoeba hystolica* is a human parasite found near the field sites of Manombo and Agnalazaha (C. Ingraldi,

personal communication), however potential cross over between human and lemur hosts has not been studied. Protozoan parasites infecting primates have been studied less frequently due to their small size and subsequent difficulty in identification. It is likely that there are many protozoan parasite species infecting lemurs yet to be discovered in Madagascar.

There were two taxa of larvae found in feces from *E. cinereiceps* (Figures 3.9 and 3.10). It is unusual for parasitic larva to be passed through the feces rather than eggs (Bowman and Lynn, 1995) and there are three possibilities for larva found in fresh feces. 1) Non-parasitic larvae living in the environment may be ingested with food and passed through the feces. However, the abundance of larvae found in each *E. cinereiceps* sample, the high prevalence across *E. cinereiceps* individuals, and the dispersal of individuals between two sites suggest these larvae are not being randomly swallowed during feeding. 2) Free-living larvae may be picked up off the ground with fecal samples after defecation. Again the prevalence and abundance of these larvae in *E. cinereiceps* feces suggests this is unlikely. In addition, similar larvae were found in mouse lemur, *Microcebus rufus*, samples collected from clean cages during trapping (S. Zohdy, personal communication), suggesting they were not collected with the feces off the forest floor. 3) The larvae may be stage 1 larva of a parasite worm that hatches in the gastro-intestinal track. All three options remain possible at this time.

More information is needed about the phylogeny and life cycle of these parasites to better understand their relationship to each other and to their hosts. Future studies should focus on directly investigating mode of transmission, symptoms, life cycle, taxonomy, other potential hosts, and geographic distribution of these parasites. This information will elucidate the dynamic host-parasite relationship and allow for more detailed understanding of host disease risk.

3.6 Figures and Tables

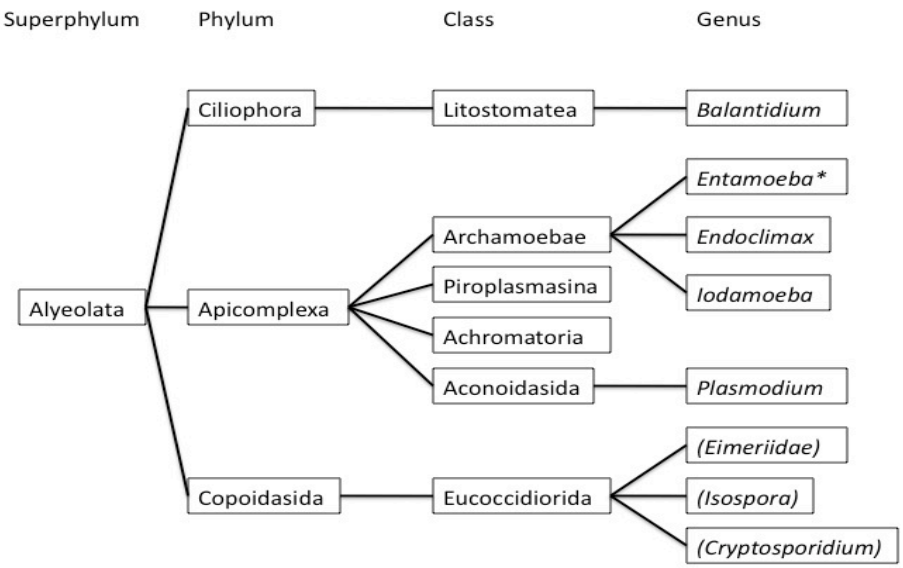

Figure 3.1. Selective phylogeny of protozoan parasites in *Eulemur* spp.
Information from: Bowman, 2003, Faulkner et al., 2004, Irwin and Raharison, 2009, Clough, 2010. Note taxa marked with (*) were found in this study. The taxa in parenthesis have not been identified in *Eulemur* but are included for clarity. Branches are not to scale.

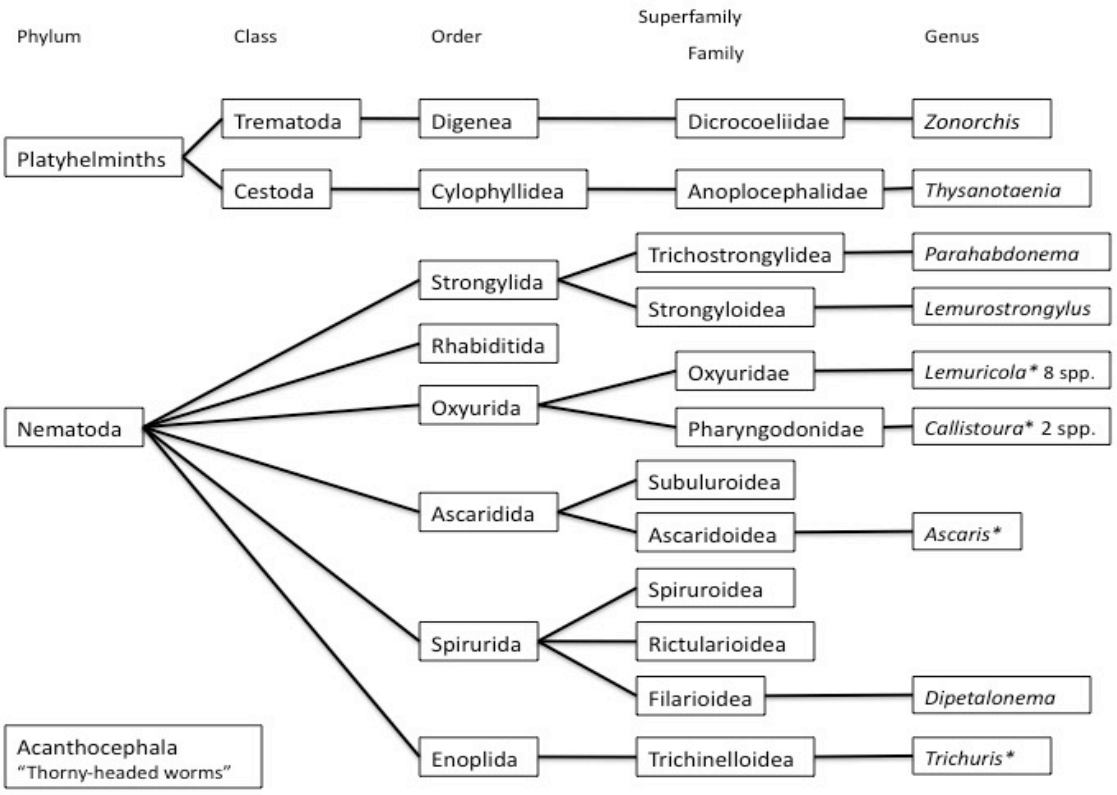

Figure 3.2. Selective phylogeny of helminth parasites in *Eulemur* spp.
Information from: Bowman, 2003, Faulkner et al., 2004, Irwin and Raharison, 2009, Clough, 2010. Note taxa marked with (*) were found in this study. Branches are not to scale.

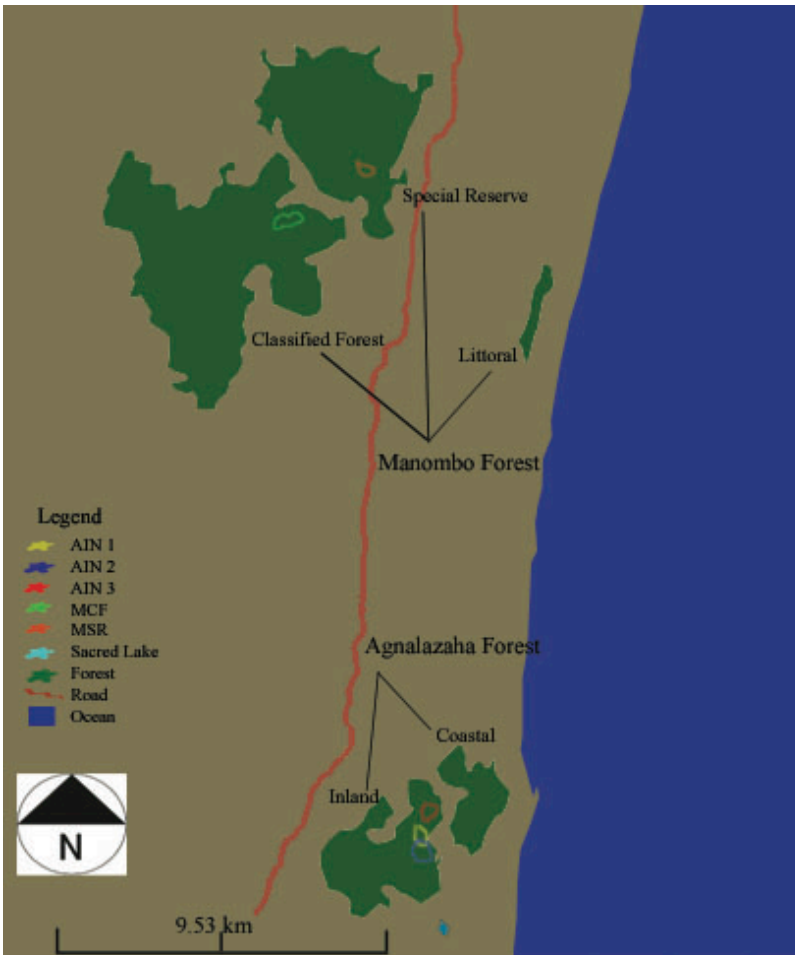

Figure 3.3 Map of study sites: Agnalazaha and Manombo, forest fragments, and study group home ranges.

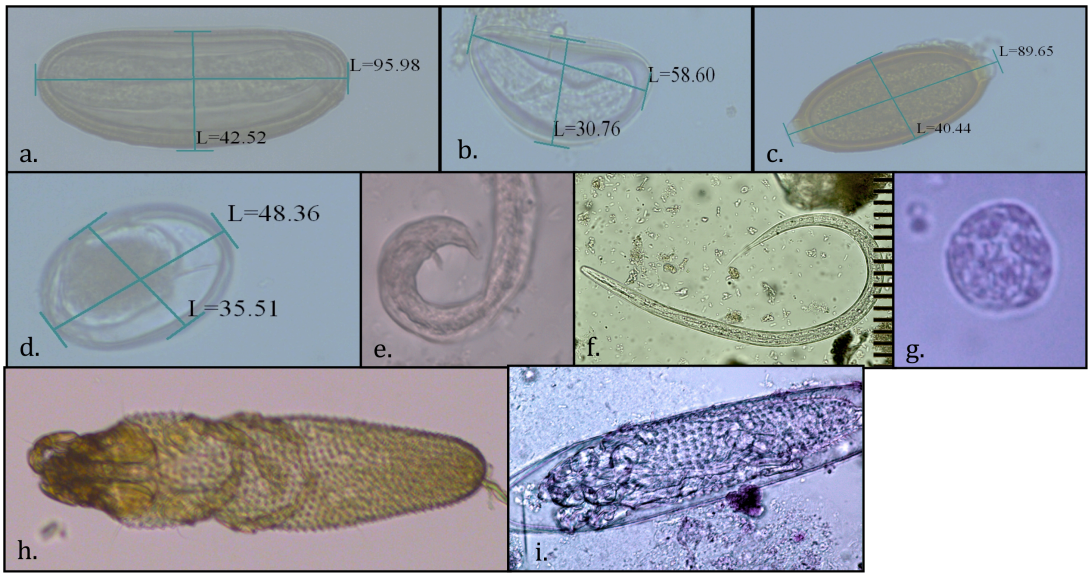

Figure 3.4 Endo- and ectoparasites from *Eulemur cinereiceps* at Manombo and Agnalazaha.
a. *Callistoura* sp. b. *Lemuricola* sp. c. *Trichuris* sp. d. Ascarid sp. e. Larva taxon 1 f. Larva taxon 2 g. *Entamoeba* sp. h. Lice found in feces i. Developing lice encased in egg, found in feces

Table 3.1 Study sites and forest fragments (Data from Ingraldi, 2010).

Forest Fragment	Site Management	Area (km^2)	*Eulemur cinereiceps* Population Size Estimate
Agnalazaha Fragment 1	Missouri Botanical Gardens	10.63	81.53
Agnalazaha Fragment 2	Missouri Botanical Gardens	3.36	24.19
Manombo Fragment 1	Madagascar National Parks	15.85	166.60
Manombo Fragment 2	Madagascar National Parks	1.70	7.00
Manombo Fragment 3	Ministry of Environment, Forests and Tourism	29.10	275.00

Table 3.2 Prevalence of parasites found in *E. cinereiceps* individuals in four field seasons.

	June-August 2006	July-August 2007	January-April 2008	September-December 2008
Pooled Samples	31 Samples and Individuals (10% formalin, fecal flotation)	18 Samples and Individuals (10% formalin, fecal flotation)	57 Samples, 31 Individuals[ψ] (90% ethanol, fecal sedimentation)	160 Samples, 45 Individuals (10% formalin, fecal flotation)
Callistoura sp.	61.29%	72.22%	36.67%	97.78%
Lemuricola sp.	9.68%	33.33%	16.67%	84.44%
Ascarid sp.	X	X	3.23%	17.78%
Trichuris sp.	0.00%	0.00%	0.00%	17.78%*
Entamoeba sp.	12.90%	X	X	26.67%

Note **Trichuris* is only observed in *E. cinereiceps* feces from ACS, and was found in 72.72% of the fecal samples from ACS individuals, although that only represents 17.78% of all individuals sampled during this field season. [ψ]Includes three individuals are from the Manombo Littoral Forest fragment.

Table 3.3 Parasites identified from *Eulemur cinereiceps*.

Parasite	Taxonomy	Common Name	Egg/Cyst Morphology	Average Egg/Cyst Size	Transmission	Symptoms	Other Lemur Hosts
Callistoura sp.	Family: Pharyngodonidae, likely *C. blanci*	Pinworm	*E*ggs are oval and laterally slightly concave with an operculum on one end *C. blanci* are larvated and without any polar plugs.	26.42µm x 20.40µm (n=110)	*Direct fecal-oral	*Relatively asymptomatic	*Eulemur albifrons*, *E. rufifrons*, *E. fulvus*, *Indri indri*, *Varecia variegata*
Lemuricola sp.	Family: Oxyuridae, likely *L. vauceli*	Pinworm	Eggs are clear and crescent shaped and often larvated.	60.12 µm x 27.23 µm (n=80)	*Direct fecal-oral	*Relatively asymptomatic, possible anal itching and female genital tract infection.	*L. vauceli* exclusively infects *Eulemur* and *Hapalemur* hosts. Other species of *Lemuricola* parasitize *Eulemur, Hapalemur, Cheirogaleus, Lepilemur,* and *Microcebus* species.
Trichuris sp.	Family: Trichuridae, possibly *T. lemuris*	Whipworm	Trichuris eggs are barrel shaped with polar caps on both ends. They tend to be orange or reddish in color.	88.87 µm x 39.93 µm (n=15)	*Direct fecal-oral	*Mild infection relatively asymptomatic, except in unhealthy host or heavy infection where may cause anemia, vitamin deficiency, impaired growth and rectal prolapse	*Daubentonia madagascariensis, Eulemur albifrons, E. fulvus, E. rufifrons, Cheirogaleus major, Lemur catta* and possibly *Microcebus murinus*.
Ascarid sp.	Family: Ascarididae, unknown genus and species	Roundworm	The ascarid eggs found in these samples are unlike ascarid eggs described from other lemur studies. Eggs are slightly elongated with a thick shell and large dark nucleus.	26.43 µm x 20.40 µm (n=7)	*Direct fecal-oral	* Mild infections relatively asymptomatic or cause a cough and bowl irritation. Heavy infections may cause intestinal blockage, impaired growth, and very rarely death.	*Ascaris petiti* is the only ascarid found in lemurs, found in *Daubentonia madagascariensis*, *Eulemur rufifrons.* However the eggs in this study do not resemble *A. petiti*.
Entamoeba sp.	Order: Amoebida	NA	Small circular cysts with multiple nuclei characterize this parasite.	19.32 µm x 19.32 µm on average (n=31)	*Direct fecal-oral	*Unknown, some species may be harmful, others are relatively asymptomatic	*Eulemur* spp., *Varecia variegata*, and *Lemur catta*

Note taxonomy, transmission, symptoms and other lemur hosts based on Chabaud et al., 1964; Chabaud et al., 1965; Faulkner et al. 2004; Junge and Louis, 2005 & 2007; Junge and Sauther, 2006; Irwin and Raharison, 2009; and the Centers for Disease Control and Prevention. Where information was not available *indicates information based on closely related species. Average egg and cyst sizes from current study.

Chapter 4. Environmental and Behavioral Correlates of *Eulemur cinereiceps* Fecal Parasite Burden

4.1 Abstract

Parasite burden is the result of both host susceptibility and host exposure to parasites. While susceptibility is determined by physiological and genetic mechanisms, exposure is expected to correlate with behavioral and environmental factors. This study investigates exposure correlates of parasites transmitted through fecal-oral contamination in a wild population of grey-headed lemurs, *Eulemur cinereiceps*. To determine the impact of behavioral and environmental variables on parasite exposure and infection, *Eulemur cinereiceps* from two study sites in southeastern Madagascar were studied during two field seasons. The following parasites were identified from *E. cinereiceps* feces: *Callistoura* sp., *Lemuricola sp., Entamoeba* sp., and an ascarid that could not be identified to the genus-level. As expected, behavioral and environmental correlates varied with each parasite: Behaviors such group size and physical contact best predicted *Callistoura* infection. Behaviors such as activity budget and time spent on the ground, likely the result of environmental variability, best predicted *Lemuricola* infection. Environmental factors such as fruit availability and the corresponding behaviors best predicted *Entamoeba* infection. Environmental factors, in particular home range microhabitat characteristics, best predicted the ascarid infection.

4.2 Introduction

Parasite burden is the combined result of host susceptibility and host exposure to parasites. This study investigates parasite exposure parameters by studying host parasite burden, host behavior and environmental variability. Hosts may be exposed to parasites directly from one

host to another through physical contact (such as louse infection), or directly through ingestion or inhalation of infective parasite eggs/larvae/cysts on contaminated soils, foods, or water (Loehle, 1995; Anderson, 2000). Parasite infections transmitted through fecal contamination are expected to correlate with both behavioral and environmental variables. Host exposure is the direct result of environmental factors, host behavior and parasite characteristics (Figure 1.1). Host exposure, in turn, affects an individual's disease risk, which can be measured by parasite burden (Figure 1.1).

Environmental factors such as seasonality, habitat quality, and food availability are expected to play a role in primate parasite burden. In particular, parasite prevalence is expected to increase in wetter seasons (*Alouatta fusca*: Stuart et al., 1993; *Alouatta palliata*: Stoner, 1996; *Mandrillus sphinx*: Setchell et al., 2007). Studies on primate parasite interactions suggest these results are both host and parasite species specific (*Pan troglodytes*: Huffman et al., 1997; *Lemur catta* and *Propithecus verreauxi*: Loudon, 2009).

Although habitat quality is expected to correlate with parasite burden, studies have yielded mixed results (*Colobus angolensis palliates*: Okanga et al., 2006; *Alouatta pigra*: Vitazkova and Wade, 2007; *Lemur catta* and *Propithecus verreauxi*: Loudon, 2009; *Microcebus murinus*: Rahariviololona and Ganzhorn, 2009). For example, in an island study on wild horses, *Equus ferus* microhabitat variability was expected to affect the potential for host parasite exposure (Rubenstein and Hohmann, 1989). Rubenstein and Hohmann (1989) reported that the most important environmental factor in *Parascaris* (nematode) infection was the group's home range, which may affect parasite exposure through soil/sand condition or through group home range overlap. However, studies on primate host and parasite species have not supported these findings (*Papio anubis*: Weyher et al., 2006; *Lemur* catta: Villers et al., 2008).

Foraging, food quality, and food availability, have been linked to parasite burden through both susceptibility and exposure. Parasite infection taxes a host's immune system, and even asymptomatic parasites may increase the effects of poor nutrition, further compromising overall fitness (Coop and Holmes, 1996). A study of *Procolobus rufromitratus* suggests there is an indirect relationship between food availability and nematode infection (Chapman et al., 2006). An increase in food availability may reduce group travel demands and limit contact with other groups, thereby indirectly decreasing parasite exposure.

In addition to environmental factors, host behavior is expected to affect parasite burden through increased exposure to parasites either by habitat use behaviors or social behaviors (Altizer et al., 2003, See chapters 1 and 4). It is likely that host behaviors are linked with environmental variability, and that as a host's environment varies, its behavior varies, and both these mechanisms affect parasite exposure.

Demographic variables such as age, sex, and group size, have been reported to affect parasite infection through differences in habitat use, social contact, and immune system efficiency (Rubenstein and Hohmann, 1989; Côté and Poulin, 1995; Arneberg et al., 1998; Altizer et al., 2003; Chapman et al., 2009a). The relationship between demographic variables such as age, sex, and group size, and parasite infection are likely a function of both parasite and host characteristics.

Social behavior is also expected to play a role in disease ecology through the potential for transmission between conspecifics (Anderson and May, 1979). Social barriers such as selective immigration, territorial behaviors, and intergroup encounters are expected to prevent contamination-associated parasite infection (Loehle, 1995). Association, closeness, or proximity between hosts is expected to positively increase parasite species richness (Altizer et al., 2003)

and parasite prevalence through increased transmission opportunities (Freeland, 1976; Loehle, 1995). A study on howler monkeys (*Alouatta pigra*) found that the most important factor in predicting individual parasite infections is whether his/her group members have a gastrointestinal parasite infection (Vitazkova and Wade, 2007), suggesting that proximity plays a role in endoparasite infection.

Host spatial behavior is expected to play a role in parasite burden due to varying transmission opportunities (Freeland, 1976; Ezenwa, 2004; Chapman et al., 2009a). For example, parasite burden differences in male and female red squirrels, *Tamiasciurus hudsonicus*, have been attributed to different habitat use patterns by each sex (Bertolino et al., 2003). Habitat use in arboreal hosts includes variables such as height in the forest and time spent on the ground. Height in the forest is predicted to correlate negatively with parasite burden, while time spent on the ground should increase the potential for obtaining contamination-transmitted parasites, particularly in hosts foraging on the ground (*Lemur catta* and *Propithecus verreauxi*: Loudon, 2009).

Here I investigate the differences in behavioral and environmental factors on the host exposure of *Eulemur cinereiceps* to three nematodes and one protozoan parasite. *E. cinereiceps* from two field sites in southeastern Madagascar were studied during two field seasons. The behavioral and environmental correlates of four parasites, *Callistoura* sp. (likely *C. blanci*), *Lemuricola* sp. (likely *L. vauceli*), *Entamoeba* sp., and an ascarid species, are investigated to help identify the individual and combined relationships between these factors, and to evaluate whether environmental variables or behavioral factors are a better predictor of the infection of fecal-contamination based parasites in wild populations.

4.3 Method

4.3.1 Host Study Species and Study Sites

The grey-headed lemur, *Eulemur cinereiceps* (= *E. albocollaris*, 1975 Rumpler), is a predominantly frugivorous, sexually dichromatic, and cathemeral lemur weighing around 2.0 kg found in the southeastern rainforests of Madagascar (Irwin et al., 2005; Ralainasolo et al., 2008; Ingraldi, 2010). *E. cinereiceps* is critically endangered with a decreasing trend and current threats are both natural and anthropogenic (Adrainarivo et al., 2010). The northern part of its range lies within Andringitra National Park where they hybridize with *Eulemur rufifrons* (Johnson and Wyner, 2000). Genetically pure *E. cinereiceps* are found in isolated coastal fragments between the Manampatrana and Mananara Rivers (Irwin et al., 2005).

This study took place at Agnalazaha (S 23° 11.175' E 47° 43.095') and Manombo (S 23° 01.697' E 47° 43.838') coastal sites in the Fianarantsoa province in southeastern Madagascar (Figure 4.2). Agnalazaha contains two fragments, the Agnalazaha Inland Forest and the Agnalazaha Coastal Forest, both managed by the Missouri Botanical Gardens. Manombo contains three fragments, two of them, the Manombo Special Reserve and the Manombo Littoral Forest, are managed by the National Parks Service. A third fragment, the Manombo Classified Forest, is managed by the Ministry of Environment, Forests and Tourism. Both the Manombo Special Reserve and Manombo Classified Forest were evaluated in this study. This region of southeastern Madagascar has a yearly average rainfall of between 2,400-2,600 mm (Ratsimbazafy, 2002) and a temperature range of 7.5-36˚C (Ratsimbazafy, 2002). During 2008, the spring/wet season yielded significantly higher rainfall than the fall/dry season (Andriamaharo and Ratsimbazafy, unpublished data). Cyclone Ivan hit the southeastern coast of Madagascar on February 21, 2008, north of the study sites and resulted in extensive rain, flooding, and tree fall.

4.3.2 Evaluation of Environmental Variables

The following data and analyses are from Ingraldi (2010) and were collected from June-November 2007 in four forest fragments at Manombo and Agnalazaha, and are recorded in Table 4.1: *E. cinereiceps* population size, *E. cinereiceps* population density, lemur species richness, fragment area, fragment perimeter, shape index, average temperature, relative humidity, number and size of clearings. Ingraldi (2010) also established phenology plots in all four forest fragments. Each plot was one hectare (20 m x 500 m) and divided into 25 subplots. Each plot was selected because it overlapped the home range of at least one study group in each fragment. Ingraldi (2010) recorded median subplot tree species richness, median subplot number of trees, median subplot DBH, and mean tree height. The current study measured and recalculated tree height at Manombo, and also recorded tree productivity using a 0-3 scale for young leaves, flowers and buds, and ripe and unripe fruit at Manombo and Agnalazaha (Table 4.1). The productivity of each tree was recorded in each category for October and November 2008. Human presence in the forest, evidence of logging, and the presence or evidence of domesticated animals in the forest were recorded by Ingraldi (2010) during census surveys in each fragment and were evaluated using a measure of incidence/km. The current study recorded each instance ad libitum while in the forest, and measures were evaluated as a daily rate. In both cases each forest fragment was ranked identically and are recorded in Table 4.1 by rank. Microhabitat home range area was assessed through vegetation and water classification using ArcGis and Google Earth Pro. Maps of each site are presented in figures 4.3 and 4.4.

4.3.3 Behavior Data Collection and Analysis

Three groups of *E. cinereiceps* including 20 individuals from forest fragments at Agnalazaha and Manombo were studied from January through April, 2008 for a total of 227 hours of observational data (Table 4.2). Five groups including 23 individuals from forest fragments at Agnalazaha and Manombo were studied from September through December 2008 for a total of 445 hours of observational data (Table 4.2). These five groups included the original three groups, plus an additional group from Agnalazaha, and a second from Manombo. For group locations see Figure 4.2. *E. cinereiceps* are sexually dichromatic, therefore sex was determined by pelage color. Age was estimated based on relative size and observation of the populations over several years. Group size was estimated based on the number of group members present during at least 80% of the focal follows.

During the spring/wet season (January – April) focal animal behavior was recorded instantaneously every five minutes during 30 minute focal follows on habituated individuals. During the fall/dry season (September –December) focal animal behavior was recorded instantaneously every two minutes during 30 minute focal follows on habituated individuals. During this time physical contact, time on the ground, and activity budget were recorded.

Physical contact was estimated using the total number of individuals a focal animal was in contact with during each instantaneous recording, divided by the total observations for the focal animal. In this way, each focal animal is given a physical contact value, which records the frequency he/she was in physical contact with conspecifics, while taking into account occurrences where the focal animal was in contact with more than one individual during an instantaneous field observation.

Activity budget was used to estimate travel time and foraging time and represent the frequency of time a focal animal spent in travel or foraging. Activity was recorded as *travel* when a focal animal was moving from one location to another. Only individuals with 450 or more activity budget observations (15 hours) were used for individual values of time spent in travel. Focal animals with 300 or more observations (10 hours) were used only to calculate the frequency of travel time for each group. Foraging time represents the frequency of time a focal animal spent in food manipulation or mastication. Only individuals with 450 or more activity budget observations (15 hours) were used for individual travel time and foraging time estimates. Focal animals with 300 or more observations (10 hours) were used only to calculate the group averages. Foraging food was classified as fruit, leaves, insects, flowers, other (includes bark and fungus), or unidentified.

The frequency of time spent on the ground was estimated using instantaneous focal animal observations. Because time spent on the ground was rare (0.0 – 3.34% of focal animal observations), this variable was only assessed for groups. Only one focal animal was observed in each group during a given time period. Therefore, a total number of records on the ground was summed for the entire group and divided by the total number of group observation hours. Although this estimate excludes occurrences when group members other than the focal animal were on the ground, it provides the best available assessment of time spent on the ground. During the spring/wet season time spent on the ground was assessed for individuals because only three groups were studied and a comparison by group could not be completed.

Height was assessed using instantaneous measurements recorded at 10 minute intervals during 30 minute individual focal follows. To assess individual height, only focal animals with more than 90 records (15 hours) were used. To assess group height focal animals with more than

60 records (10 hours) were used. In the field, height was assessed to the half meter using naked eye estimates. The primary observer used a clinometer during practices until estimates with the naked eye were 90% precise and accurate, and interobserver reliability was measured at 90%. All height estimates used in statistical analyses were divided by mean forest canopy height (Table 4.1) to enable comparisons between fragments and sites.

Group spread and GPS locations were assessed using group scan sampling and 30 minute instantaneous recording. Groups were followed for an entire day. Group spread was measured as the horizontal distance between the two farthest individuals present in the group. This expanse was a maximum of 10 meters, and was only recorded as >10 meters when necessary. For this reason, estimates may be low. A total of 618.5 hours of scan sampling were observed (1,237 records). Home range size was only assessed during the fall/dry season due to sampling problems during the spring/wet season.

Home range size was measured using GPS points taken during group follows. An average of five GPS points were taken each day over 20 non-consecutive days for each group. Only points with at least three satellites and ≤ 20 meter error were used. A total of 522 GPS points were used during the fall/dry season for all five groups. Home range estimates may show seasonal bias. Home range size was estimated in Google Earth Pro using both minimum convex polygon (MCP) and grid cell counts (GCC) methods. Grid cells were one hectare in size. Two groups, AIN 1 and AIN 2 had overlapping home ranges of 5.3 ha (Figure 4.1). A previous study by Ingraldi (2010) took place during the fall/dry season of 2007 and observed an overlap of 23.61 ha between the same two groups. Between the 2007 and the current 2008 study there were changes to adult male group membership. This suggests group home ranges are flexible and may change with group members. However, the overall location of the home ranges, as well as their

size, are similar between the current and previous study, and therefore home range size will still be used in this study.

4.3.4 Fecal Sample Collection and Evaluation

During the spring/wet season, fecal samples were collected in the field immediately following defecation, weighed, and preserved in 90% ethanol. A total of 52 fecal samples from 28 individuals were evaluated for parasite prevalence during the spring/wet season. Sample collection prioritized early morning defecation weighing at least four grams. Two samples were evaluated for 24 individuals. An additional four samples were collected from individuals classed by age and sex. Age categorized as adult and juvenile and was determined by size. Parasites were recovered using two grams of fecal sedimentation from each sample.

During the fall/dry season, fecal samples were collected in the field immediately following defecation, weighed, and preserved in 10% formalin. A total of six fecal samples were collected from each of 23 habituated individuals. Samples from a single individual were collected 5-7 days apart. Sample collection prioritized early morning defecation with feces weighing at least four grams. Fecal samples were not collected from infants because no solid feces were observed until juveniles were more than three months of age. An additional 22 fecal samples were collected from unhabituated individuals and were classed by age and sex. Fecal samples were analyzed in the lab using three trials of one gram fecal flotation. This method has been verified in a diagnostic study (Martin et al., 2009; see chapter 2). A total of 160 fecal samples were evaluated in a total of 480 laboratory trials using fecal flotation.

Fecal samples were stored in 90% ethanol during the first field season, however a diagnostic analysis indicated that 10% formalin is a significantly better preservative (see chapter

2). Further analysis suggests that a comparison of samples preserved in 10% formalin and evaluated using fecal flotation is comparable to samples stored in 90% ethanol and evaluated with fecal sedimentation recovery technique (see chapter 2).

4.3.5 Terminology and Data Analysis

All statistics were completed using SPSS 17.0 and Microsoft excel 12.2. Parasite infection was assessed using measures of *prevalence*, which include data from all individuals (habituated and unhabituated). *Prevalence* was defined as the presence of a parasite for all fecal samples from each individual. Infection was also assessed using the *frequency* of each parasite infection. *Frequency* measured how often a parasite was observed from the 6 fecal samples collected for an individual habituated host during the fall/dry season. Estimates of parasite frequency were used for all 23 habituated individuals during this field season and were not assessed for samples from the spring/wet season.

Normally distributed variables included time spent on the ground, travel time, group spread, home range size, group size, ascarid prevalence, ascarid frequency, *Entamoeba* prevalence, *Entamoeba* frequency, tree productivity, and fruit availability. These variables are assessed using t-tests for Independent Samples and Pearson's Correlation Analysis. Non-normally distributed variables included height, physical contact, *Callistoura* prevalence, *Callistoura* frequency, *Lemuricola* prevalence, and *Lemuricola* frequency. These variables were analyzed using nonparametric statistics, primarily Mann-Whitney U-Test, Kruskal-Wallis test, and Spearman's Correlation Analysis. Prevalence of individual parasites was analyzed using Chi-Square analyses.

4.3.6 Parasite Study Species

Eggs from three nematodes, and cysts from a protozoan parasite were recovered from *E. cinereiceps* fecal samples (Figure 4.5; see chapter 3). Two pinworms, *Callistoura* sp. (likely *C. blanci*, Chabaud et al., 1965; see chapter 3) and *Lemuricola* sp. (likely *L. vauceli*, Chabaud et al., 1965, see chapter 3) were the most commonly found parasite during both field seasons. Both pinworms are likely transmitted by fecal-oral contamination (Irwin and Raharison, 2009) and are likely to become infective within hours-several weeks. A third egg was identified as an ascarid, likely from the family Ascarididae, based on egg morphology. *Acaris petiti* (Chabaud et al., 1964) is the only currently identified ascarid to infect lemur hosts. Chabaud (1964) didn't describe eggs and thus the morphology cannot be matched from a fecal diagnosis. Egg appearance is different from those of other ascarids reported in lemurs (Anderson, 2000; Irwin and Raharison, 2009), as well as those known to infect humans and domesticated animals (see chapter 3). The ascarid egg is likely to become infective several months after deposition into the environment. An *Entamoeba* species was identified based on cyst morphology, following identification of similar cysts recovered from *Eulemur rufifrons* and classified through molecular analysis (Clough, 2010). *Entamoeba* is one of several Amoebida genera, and has several species that infect humans and other nonhuman primates (see chapter 3).

4.4 Results

Table 4.3 and 4.4 list group parasite indices for the spring/wet and fall/dry seasons. Lemur species and their potential parasites are listed by forest fragment in table 4.5. Tables 4.6, 4.7 and 4.8 list significant differences in parasite infection, behavior, demographic variables and environmental variables by field site and season. Tables 4.9 and 4.10 list the range, mean and

standard deviation for all group and individual behavioral variables. All significant statistical results referenced in the text are recorded in Table 4.11, and all non-significant results are recorded in Table 4.12. Study group home ranges are depicted in figures 4.2, 4.3, and 4.4.

4.4.1 Seasonal Differences in the Parasite Infection Parameters of Eulemur cinereiceps

Both *Callistoura* and *Lemuricola* were significantly more prevalent during the fall/dry season than the spring/wet season (*Callistoura*: $\chi^2 = 22.85$, $p \leq 0.001$; *Lemuricola*: $\chi^2 = 27.87$, $p \leq 0.001$). Due to an error in identification *Entamoeba* was not recorded for the spring/wet samples. The ascarid sp. was found in only one sample during the spring/wet season (see chapter 3).

4.4.2 Seasonal Differences in the Behavior of Eulemur cinereiceps

Behavior data was collected from 23 individuals, from five groups during the fall/dry season, and from 18 individuals from three groups during the spring/wet season. Height was significantly higher during the fall/dry season than the spring/wet season (U-statistic = 68.50, $p = 0.008$). Individuals spent significantly more time in travel during the fall/dry season than the spring/wet season ($t = -5.87$, $p \leq 0.001$). There was no difference in physical contact, or time spent on the ground between the fall/dry and spring/wet seasons.

4.4.3 Differences in the Environment at Manombo and Agnalazaha

Manombo and Agnalazaha are each made up of two main fragments. *E. cinereiceps* was found in a larger population and in greater population density at Manombo than at Agnalazaha (Table 4.1). Lemur species richness was highest at Manombo (Table 4.1).

The Agnalazaha fragments are smaller in size, have greater shape indices, and are more fragmented than the Manombo fragments (Table 4.1). The Manombo fragments had significantly greater tree species richness and tree diameter at breast height (Table 4.1). The number of trees and mean tree height did not vary significantly by site (Table 4.1). Young leaf, flower and fruit availability was significantly greater at Manombo; although when considering only fruit availability, Agnalazaha had significantly more fruit available than Manombo (All Availability: U-statistic = 134485.0, $p \leq 0.001$; Fruit Availability: U-statistic = 263586.0, $p \leq 0.001$; Table 4.1). Forest temperature was significantly higher at Agnalazaha than at Manombo, although relative humidity did not differ significantly between sites (Table 4.1). Significant differences between the two fragments at Agnalazaha were found in temperature, relative humidity, median number of trees, median tree diameter at breast height, and mean tree height (Table 4.1). Significant differences between the two fragments at Manombo were found in temperature, relative humidity, overall productivity and median tree diameter at breast height (Table 4.1).

Disturbance was assessed using categories of human presence in the forests, the presence of domesticated animals, clearings and evidence of logging. Each forest was ranked for each category, and in all cases the two fragments at Agnalazaha were more disturbed than the fragments at Manombo (Table 4.1).

4.4.4 Differences in the Parasite Infection Parameters of Eulemur cinereiceps *at Manombo and Agnalazaha*

During the fall/dry season, *Lemuricola* was significantly more common at Agnalazaha than at Manombo ($\chi^2 = 5.43$, $p = 0.020$), and the frequency of *Lemuricola* infection was higher at Agnalazaha than at Manombo (U-statistic = 25.50, $p = 0.038$). *Entamoeba* had a significantly greater prevalence and frequency from Manombo than from Agnalazaha (prevalence: $\chi^2 = 7.62$,

$p = 0.006$; frequency: U-statistic = 20.50, $p = 0.024$). There was no significant difference in parasite species richness, the prevalence or the frequency of Ascarid or *Callistoura* eggs found in fecal samples from each site.

During the spring/wet season, the presence of *Entamoeba* cysts were not recorded. Ascarid eggs were found in only one of 28 individuals during the spring/wet season while in the fall/dry season ascarid eggs were recovered from 8 out of 45 individuals. There was no significant difference in the prevalence of *Callistoura* or *Lemuricola* eggs found in samples from Agnalazaha and Manombo.

4.4.5 Differences in the Behavior of Eulemur cinereiceps *at Manombo and Agnalazaha*

During the fall/dry season, behavior data was recorded for seven individuals, in two groups at Manombo, and from 16 individuals in three groups at Agnalazaha. Height was significantly greater at Manombo than Agnalazaha (U-statistic = 11.00, $p \leq 0.001$). There is a trend towards greater time spent in travel at Agnalazaha than Manombo ($t = 1.95$, $p = 0.068$). Individuals at Agnalazaha spent significantly more time on the ground than at Manombo (U-statistic = 2.00, $p \leq 0.001$). No significant differences between sites were observed in physical contact.

During the fall/dry season, differences in foraging between the two Agnalazaha and the two Manombo fragments were assessed separately as well as together. Individuals at Agnalazaha foraged on fruit significantly more often than individuals at the Manombo Classified Forest (MCF), however the group at MCF has only three individuals ($t = 3.17$, $p = 0.006$). When both Manombo groups are combined (three from the Manombo Classified Forest and three from the

Manombo Special Reserve), time spent foraging on fruit was not significantly different between Manombo and Agnalazaha (t = 1.25, $p = 0.228$).

During the spring/wet season, behavior data was collected from 20 individuals, 14 individuals were from two groups at Agnalazaha and six were from one group at Manombo. The individuals at Manombo were observed significantly higher in the canopy than those at Agnalazaha (U-statistic = 0.00, $p = 0.005$). The individuals at Agnalazaha spent significantly more time on the ground than those at Manombo (U-statistic = 3.00, $p = 0.016$). Time spent in travel and physical contact did not vary between the sites.

4.4.6 Correlates of Callistoura *Infection*

During the fall/dry season, the frequency of *Callistoura* infection among habituated *E. cinereiceps* correlated significantly and positively by group size (rho = 0.900, $p = 0.037$) and group spread (rho = 1.000, $p = 0.001$). *Callistoura* infection did not correlate significantly with any other behavior or environmental variables during the fall/dry season. *Callistoura* prevalence was too high for behavioral comparisons between individuals with and without the presence of eggs in at least one stool sample.

During the spring/wet season, *Callistoura* prevalence was lower than during the fall/dry season, for this reason, comparisons were made between individuals with *Callistoura* and those without. Measures of physical contact were significantly greater among individuals with at least one occurrence of *Callistoura* infection (U-statistic = 15.50, $p = 0.029$). No other behavior or demographic variables varied significantly by *Callistoura* infection prevalence.

4.4.7 Correlates of Lemuricola *Infection*

During the fall/dry season, a positive trend was found between the frequency of *Lemuricola* infection by group overall group travel time (r = 0.857, $p = 0.064$). Group average *Lemuricola* frequency correlated significantly and positively with time spent on the ground (r = 0.941, $p = 0.017$). Individual *Lemuricola* prevalence does not vary with any behavioral variables.

During the spring/wet season, no individual behavioral or demographic variables varied with *Lemuricola* prevalence. Group measures from the spring/wet season were not used in statistical analyses because only three groups were evaluated. When combining average group *Lemuricola* prevalence across both seasons, prevalence correlates positively and significantly with time spent in travel (rho = 0.708, $p = 0.050$).

4.4.8 Correlates of Entamoeba *Infection*

E. cinereiceps individuals with an *Entamoeba* infection at least once during the fall/dry season were observed higher in the forest than individuals without an infection (U-statistic= 25.00, $p = 0.013$). Height also correlated with the frequency of individual *Entamoeba* infection (rho = 0.448, $p = 0.032$). Although the frequency of individual *Entamoeba* infection did not correlate with time spent foraging for fruit, the frequency of group *Entamoeba* infection had a negative trend with fruit foraging (r = -0.868, $p = 0.056$). Similarly, individuals with at least one *Entamoeba* infection did not spend less time foraging fruit, however, average group prevalence correlated negatively and significantly with average group time spent foraging fruit (r = -0.893, $p = 0.042$). *Entamoeba* infection prevalence does not vary by sex, age, physical contact, or time spent in travel. Group prevalence did not correlate with any other behavioral or demographic

variables. *Entamoeba* cysts were not recorded during fecal evaluations for the spring/wet season samples.

4.4.9 Correlates of Ascarid Infection

Ascarid infection prevalence did not correlate with any individual behavior or demographic variables. The frequency of ascarid infection, and the prevalence of ascarid infection correlated positively with home range size using grid cell counts (Frequency: $r = 0.888$, $p = 0.044$; Prevalence: $r = 0.863$, $p = 0.060$). However, when using multiple convex polygon method to assess home range size, the correlations were not significant. There is a trend towards higher Ascarid infection prevalence in groups with home ranges overlapping swampy areas ($\chi^2 = 3.16$, $p = 0.076$), although two cells have expected values less than five and when using a Yates correction for continuity, the difference is not significant ($\chi^2 = 1.65$, $p = 0.198$). Individuals with a home range that overlaps any water, a swampy area or river, were more likely to have an ascarid infection ($\chi^2 = 2.73$, $p = 0.099$). Ascarid eggs were only found from one individual during the spring/wet season. Eggs were found from eight individuals during the fall/dry season.

4.5 Discussion

4.5.1 Callistoura *Infection*

During both seasons *Callistoura* correlated with demographic and social behavior variables. During the fall/dry season, the frequency of *Callistoura* infection correlated positively with group size and group spread. Because group spread correlated perfectly with group size, group size is likely a mediating factor in group spread. During the spring/wet season, *Callistoura* infection prevalence correlated positively with physical contact.

Callistoura infection prevalence did not vary between sites, however, at both sites prevalence was significantly higher during the fall/dry season than the spring/wet season and no social behaviors or demographic variables varied by season. Although parasite burden was expected to increase during the wet season, prevalence was significantly lower than the fall/dry season. This may be the result of the preservation solution differences (90% ethanol used during the spring/wet season, and 10% formalin used during the fall/dry season) although different recovery techniques were used in the laboratory to minimize differences in parasite recovery.

If the data represent a seasonal variation, there are four possible explanations for the results: 1) Gregarious or social behaviors increase in the fall due to infant births, thus mediating an increase in *Callistoura* exposure through an increase in social behavior. 2) *Callistoura* infection is not only a function of host exposure through social behavior, but also of susceptibility due to hormonal variations between seasons. 3) Seasonal variations may be the result of natural cyclical fluctuations in the host-parasite relationship and the observation of seasonal variation coincidentally correlated with seasonal changes. 4) While some parasite ova require time after being shed to become infective, the fecal-oral transmission of *Callistoura* ova may occur directly following defecation, resulting in a seasonal difference in exposure patterns due to weather patterns. If the first hypothesis is correct, then measures of social behavior such physical contact should correspondingly increase with an increase in *Callistoura* prevalence during the fall/dry season. However, no social behavior variables varied by season. Therefore, hypothesis one is not a likely explanation for the observed seasonal variation in *Callistoura* prevalence. If the second hypothesis were true and *Callistoura* infection was also the result of increased susceptibility due to hormonal fluctuations during the birthing season, than birthing females would be expected to yield the highest prevalence and frequency of *Callistoura*

infections during the fall/dry season. However, there was no age or sex bias during either season in *Callistoura* prevalence or frequency. If the third hypothesis were true, then no patterns in seasonal variation from previous seasons would be expected. During the spring/wet season of 2008 *Callistoura* prevalence was 36.67%, and during the fall/dry season of 2008 *Callistoura* prevalence was at 97.78%. During the summer seasons in 2006 and 2007, *Callistoura* prevalence was between 61.29-72.22% (see chapter 3). Although no fall and spring samples were collected during these seasons, it is possible that the intermediary summer seasons also represent an intermediary *Callistoura* prevalence. If this were true, then fewer *Callistoura* infections may normally occur during the spring/wet season, and greater *Callistoura* infection prevalence may typically occur during the fall/dry season. However, this is extrapolating from an incomplete set of yearly data and should be considered cautiously at best. At this time, hypothesis three can be neither accepted nor rejected. If hypothesis four were true, eggs could be ingested from fecal contamination from conspecifics in the vicinity and there would likely be less frequent exposure during the spring/wet season due to the rain washing the fecal contamination from areas as they are used. This hypothesis not only explains the seasonal variation, but it also explains the correlation between physical contact and *Callistoura* infection during the spring/wet season, when fecal-oral ingestion would need to be more immediate due to more frequent rainfall.

Seasonal behavior and group size appear to be better predictors of *Callistoura* infection then habitat use behaviors, seasonality, and environmental variables. Similarly, Clough (2009) found that parasite species richness increased significantly with group size in *Eulemur rufifrons*, including the prevalence of *Callistoura* infections.

4.5.2 Lemuricola *Infection*

Lemuricola infection correlated with time spent in travel during the fall/dry season and time spent on the ground during both the fall/dry and spring/wet seasons. *Lemuricola* prevalence and frequency was higher at Agnalazaha than Manombo during the fall/dry season and during this season, time spent in travel and time spent on the ground were higher at Agnalazaha than Manombo. The Agnalazaha fragments are smaller, more fragmented, and have greater disturbance from domesticated animals and the local human populations. There were significantly more clearings at Agnalazaha than Manombo, which may result in animals descending to the ground to forage for flowers and fruit from ground foliage, and to cross over deforested areas within their home range.

Additionally, *Lemuricola* prevalence was significantly higher during the fall/dry season than the spring/wet season. Correspondingly, time spent in travel and time spent on the ground were also significantly higher during the fall/dry season than the spring/wet season. This suggests that habitat use behaviors, rather than seasonality, were the more important factors in *Lemuricola* infection.

Seasonality is expected to affect parasite burden, in particular, contamination based parasite infections are expected to increase during the wet season. During the spring/wet season, time spent on the ground was greater for individuals at Agnalazaha, but travel time and *Lemuricola* infection prevalence did not vary between sites. This further suggests that animals travel on the ground more frequently at Agnalazaha due to environmental disturbances. However, time spent on the ground is significantly greater during the fall/dry season, when the ground is less flooded. *Lemuricola* infection correlated during both seasons with time spent on the ground, and during the fall/dry season with time spent in travel, and when combing groups

from both seasons, average group time spent in travel correlated positively with group *Lemuricola* prevalence. Therefore, the reduction in *Lemuricola* prevalence during the spring/wet season may be the result of a reduction in both travel time and time spent on the ground, rather than the result of climate differences in parasite exposure.

Cross species contamination between host species is a concern in community ecology (Ezenwa, 2003). Although the other parasites are unlikely to present this problem (see chapter 3), the potential for *Lemuricola* cross species contamination should be considered. *Lemuricola* prevalence and frequency is higher at Agnalazaha than at Manombo during the fall/dry season. *Lemuricola* species were identified from other wild populations of *Hapalemur griseus*, *Cheirogaleus major* and *Daubentonia madagascariensis*, all of which are found at these study sites (Irwin and Raharison, 2009; Ingraldi, 2010; Table 4.5). *Lemuricola vauceli*, likely the same species as found in this study, was found in a wild *Hapalemur* from Ambavaniasy (Irwin and Raharison, 2009). And *Hapalemur griseus* was found in all four forest fragments at Agnalazaha and Manombo (Ingraldi, 2010; Table 4.5). Additionally, two other species of *Lemuricola, L. contagious* and *L. daubentoniae* were found in *Cheirogaleous major* and *Daubentonia madagascariensis* (Irwin and Raharison, 2009). *Cheirogaleous major* can be found only at Agnalazaha, and *Daubentonia madagascariensis* can be found only at the Manombo Special Reserve fragment (Ingraldi, 2010; Table 4.5). The eggs from both other species of *Lemuricola* are slightly larger than *L. vauceli* and it is unknown if *C. major* and *D. madagascariensis* may also harbor *L. vauceli* in addition to these other *Lemuricola* species. However this remains a possibility since it is known that another lemur, *Eulemur fulvus* from Ampijoroa, can harbor both *Lemuricola vauceli* and *L. baltazardi* (Chabaud et al., 1965). *Hapalemur griseus* are found in significantly higher densities at Manombo than Agnalazaha (Ingraldi, 2010) and are therefore

unlikely to represent a mitigating factor in the higher prevalence of *Lemuricola* infection at Agnalazaha. The same is true of *Daubentonia madagascariensis*. Only *Cheirogaleus major* represents the potential for cross-species transmission that would result in a significantly higher *Lemuricola* prevalence at Agnalazaha. *C. major* is found at very low densities (0.45/km^2 and 0.32/km^2) and when compared to the density of *E. cinereiceps* (7.67/km^2 and 7.20/km^2) at Agnalazaha (Ingraldi, 2010) and is therefore unlikely to be a mediating factor in *E. cinereiceps Lemuricola* infection.

Overall, *Lemuricola* infection clusters with both habitat use behaviors and forest structure variables that may impact habitat use such as clearings. Habitat use behavior and corresponding forest structure variables are more important predictors of *Lemuricola* infection than seasonality, forest productivity, and social behavior.

4.5.3 Entamoeba *Infection*

Entamoeba prevalence was significantly higher at Manombo than at Agnalazaha, and prevalence between the fragments at either site was not significant. *Entamoeba* prevalence and frequency did not correlate with any demographic or behavioral variables except fruit foraging. The frequency and prevalence of group *Entamoeba* infection decreased significantly with an increase in the percentage of foraging time spent on fruit. Significantly more fruit was available from phenology plots at Agnalazaha than at Manombo, and there was not a significant difference between the fruit availability from the two fragments at Manombo. When only taking the three individuals from the Manombo Classified Forest group into account, individuals at Agnalazaha spent more time foraging for fruit than those from the Manombo Classified Forest. When

combining additional individuals from the Manombo Special Reserve, the difference between fruit foraging at Agnalazaha and Manombo was not significant.

E. cinereiceps are a predominantly frugivorous lemur, with alternate food sources including flowers, young leaves, insects, fungus and nectar. Time spent foraging for fruit can be indirectly connected to fruit consumption. If this is the case, then individuals who consume more fruit are less likely to have an *Entamoeba* infection. This may be due to an increase in immune system efficiency due to an increased nutritional/medicinal value of fruit, or it may reflect an increase in the fruit availability in the forest, suggesting that a healthier environment may lead to healthier host inhabitants. The relationship between *Entamoeba* infection, fruit availability, and time spent foraging for fruit yields three possibilities: 1) Time spent foraging for fruit, and therefore potential fruit consumption, is the driving factor for *Entamoeba* infection. 2) Fruit availability, and by extension the productivity of the forest, is the driving factor for the relationship between fruit availability, time spent foraging fruit, and *Entamoeba* infection. 3) Alternate food sources, rather than time spent foraging for fruit, is the driving factor behind the relationship. *E. cinereiceps* spends the most time foraging for young leaves and flowers when not foraging for fruit (Ingraldi, 2010; Martin, unpublished data). While fruit availability is significantly lower at Manombo than Agnalazaha, young leaves and flowers are significantly more abundant at Manombo than Agnalazaha.

If the first hypothesis were true, as *Entamoeba* infection decreases, time spent foraging for fruit is expected to increase. This was true across group averages, but not for individuals. In addition, *Entamoeba* infection was lower overall at Agnalazaha, but fruit foraging time was not significantly greater. If the second hypothesis were true, as fruit availability increases, *Entamoeba* infection is expected to decrease. This hypothesis holds true for both sites, as well as

the fragments within each site. If the third hypothesis were true, as flower and young leaf availability increases, or foraging for alternate food sources increases, *Entamoeba* infection is expected to increase. When combining the fragments at both sites, the availability of flowers and young leaves is significantly greater at Manombo than at Agnalazaha, and *Entamoeba* infection is also significantly greater at Manombo than at Agnalazaha. However, when comparing the two fragments at Manombo, the availability of flowers and young leaves is significantly greater at the Manombo Classified Forest, but the *Entamoeba* infections do not vary significantly between the two fragments. However the sample size is small when separating the Manombo fragments; there are only three individuals in the fall/dry season group at the Manombo Classified Forest, and six in the Manombo Special Reserve. Therefore comparisons should be considered cautiously. Furthermore, foraging data on resources other than fruit were too rare to be compared in a statistical analysis.

Given these considerations, the most likely hypothesis is the second, that as fruit availability increases, *Entamoeba* infections decrease. As fruit availability increases, individuals tend to spend more time foraging for fruit, but this is unlikely to be the mediating variable in the relationship. It is more likely that fruit availability, as either an indicator of individual nutritional health, or forest health, is the best predictor of *Entamoeba* infection in *Eulemur cinereiceps*.

4.5.4 Ascarid Infection

Ascarid eggs were only found in the samples from one individual during the spring/wet season. During the fall/dry 2008 season, eggs were found in 17.39% of the 46 individuals sampled. Although the infection prevalence increased during the dry season, it is possible that the infection was encountered during the wet season. In human ascaris, *Ascaris lumbricoides*,

infective eggs require two to three months after ingestion before adult females oviposition eggs and the infection may be detected from feces (CDC, 2011).

Of the 23 habituated individuals with known home ranges, all ascarid infections occurred in the three groups whose home range overlaps water. Two of the groups from Agnalazaha had home ranges that overlapped a swamp-forest, and the group's home range from the Manombo Classified Forest crossed a river (approximately five feet wide). Human and pig ascaris are typically transmitted through soil contamination, and it may be that especially during the dry season, eggs are more likely to be picked up on food or the body in areas where surface ground water keeps the soil moist. At Agnalazaha, the soil frequently gives way to a sandy matrix. Sandy soils were more common within the home range of the third Agnalazaha group that did not have any ascarid infections, and whose home range did not overlap any water sources. Fertile *Ascaris lumbricoides* eggs embryonate and become infective after approximately two to three weeks in the soil, and its possible that a sandy soil is less compatible with this life cycle. Other studies have suggested that microhabitat, and in particular the presence of water, may have an affect on parasite infection parameters (Rubenstein and Hohmann, 1989; Stoner, 1996; Clough, 2009).

Overall ascarid infections did not correlate with any demographic, behavioral, or environmental variables except home range size and microhabitat characteristics. Therefore it can be determined that exposure to this ascarid species is likely mediated by microhabitat variables rather than large-scale environmental characteristics or host behavior.

4.6 Conclusion

Parasite exposure is the result of both behavioral and environmental variations in *Eulemur cinereiceps* at Agnalazaha and Manombo in southeastern Madagascar. As expected, correlates of parasite indices were parasite species specific. However, *Callistoura*, *Lemuricola*, and ascarid infections were all significantly more prevalent during the fall/dry season than the spring/wet season. This is contrary to expectations and previous studies on primate parasite ecology. However, a study by Loudon (2009) on *Lemur catta* parasite ecology found that while February (spring/wet season) was the peak month for parasite prevalence, March was the lowest, which may indicate a more detailed pattern in lemur parasite seasonality than those found in other primates.

Callistoura pinworm infections were best predicted by physical contact and group size, rather than habitat use behaviors or environmental characteristics. *Lemuricola* pinworms correlated with both behavioral and environmental factors. *Lemuricola* infection was better predicted by habitat use behaviors such as travel time and time spent on the ground, which were in turn mediated by environmental factors such as forest fragmentation and shape indices. Infection from protozoan *Entamoeba* was best predicted by environmental factors such as fruit availability, and indirectly by foraging behavior based on fruit availability. The ascarid parasite did not correlate with any individual behavior or demographics. However, the ascarid tentatively correlated with home range size, and with microhabitat variability. Ascarid was more common in groups with home ranges that overlapped swamps or rivers, suggesting that ascarid infections may be best predicted by environmental variables.

4.7 Figures and Tables

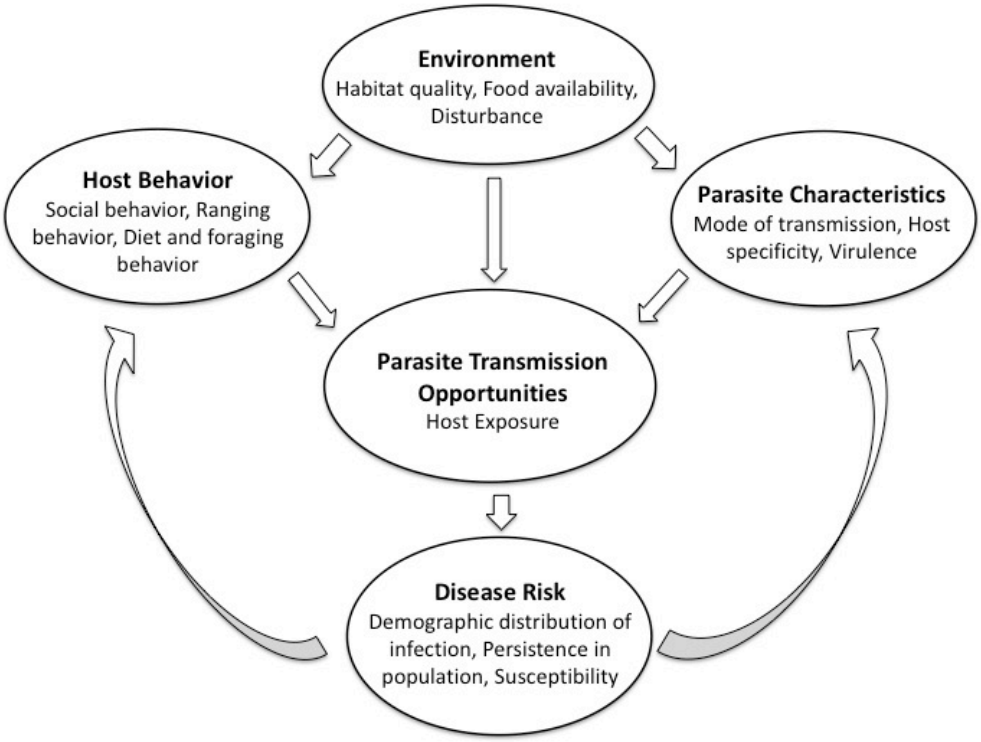

Figure 4.1 Parameters of primate parasite exposure and disease risk.
Adapted and altered from Altizer et al., 2003.

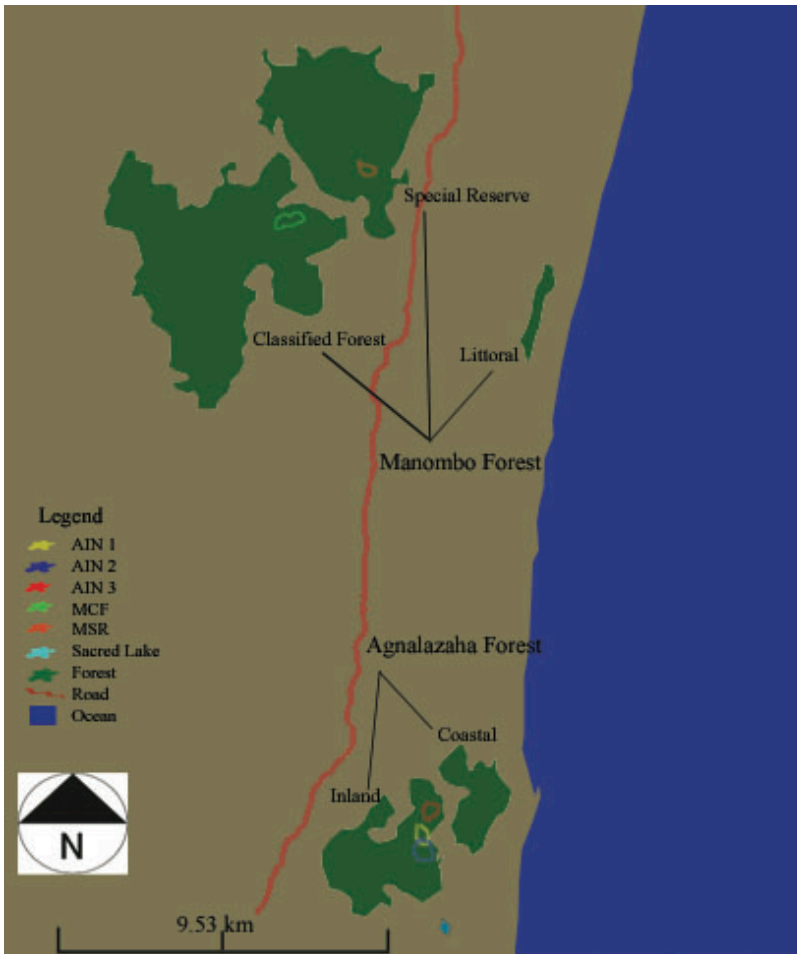

Figure 4.2 Map of study sites: Agnalazaha and Manombo forest fragments, and study group home ranges.

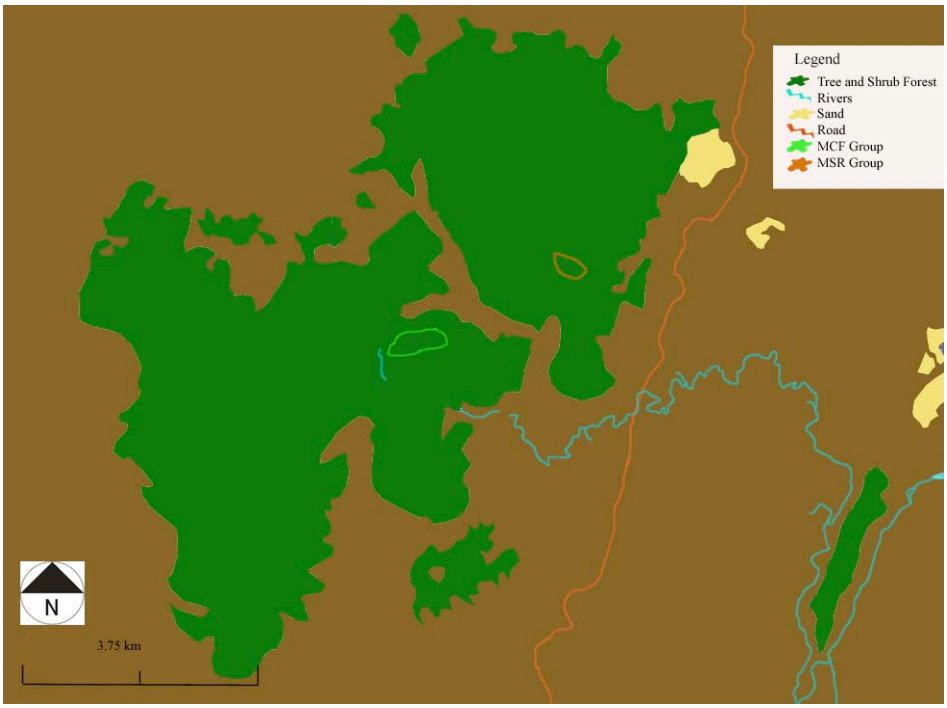

Figure 4.3 Map of Manombo vegetation classification with study group home ranges.

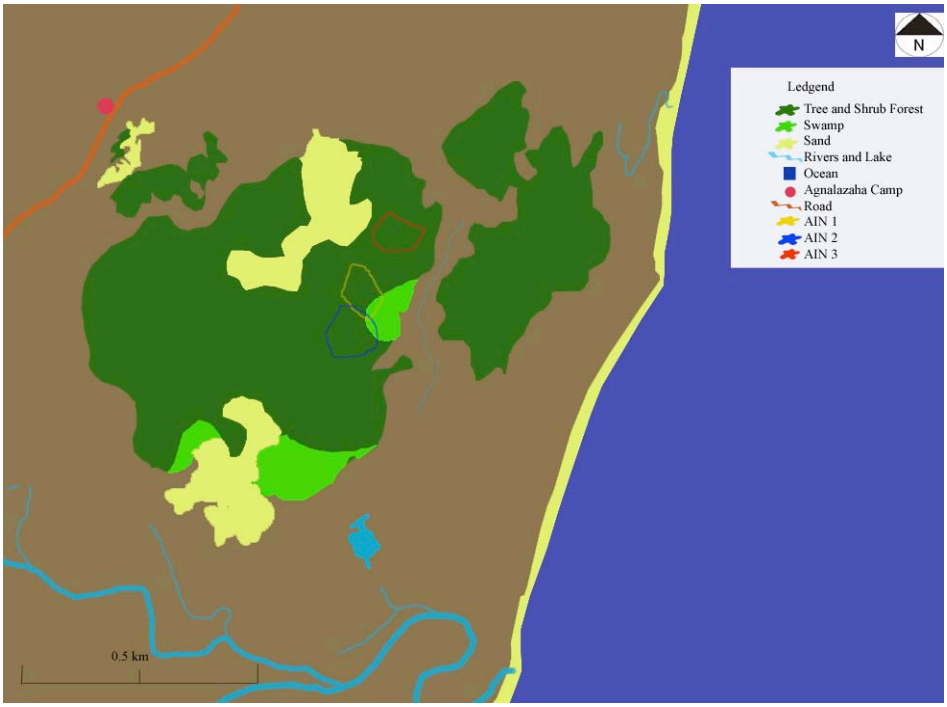

Figure 4.4 Map of Agnalazaha vegetation classification with study group home ranges.

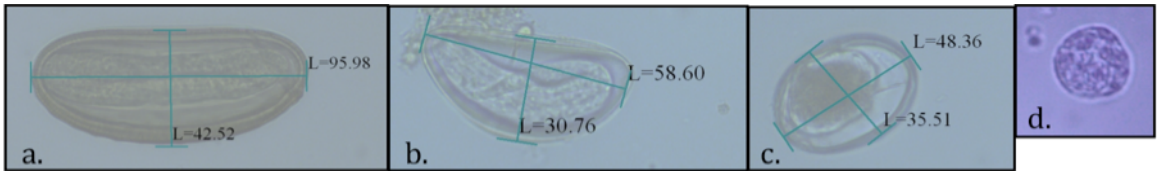

Figure 4.5 Fecal parasites recovered from *E. cinereiceps*.
Note a. *Callistoura* sp., b. *Lemuricola* sp., c. ascarid sp., d. *Entamoeba* sp.

Table 4.1 Forest Structure at Agnalazaha and Manombo. Data from Ingraldi, 2010, except where indicated.

	AIN	ACS	AGA	MSR	MCF	MAN
E. cinereiceps population size	81.53 (3)	24.19 (4)		166.58 (2)	275.48 (1)	
E. cinereiceps density (*E. cinereiceps*/km^2)	7.67 (3)	7.20 (4)	7.45	10.51 (1)	8.47 (2)	9.95
Lemur Species Richness	5 (3.5)	5 (3.5)		6 (2)	7 (1)	
Area (km^2)	10.63 (3)	3.36 (4)		15.85 (2)	29.09 (1)	
Perimeter (km)	70.24 (2)	34.79 (4)		52.53 (3)	109.14 (1)	
Shape Index	6.08 (2)	15.85 (1)		3.72 (4)	5.71 (3)	
Temperature (°C)	21.6* (2.5)	23.2* (1)	22.2†	21.6* (2.5)	21* (4)	21.4†
Relative Humidity (%)	85.2* (2)	80.4* (4)	83.3	81.8* (3)	90.0* (1)	84.5
Median Subplot Tree Species Richness	15.2 (3)	14.64 (4)	14.92†	18.64 (1)	16.28 (2)	17.46†
Median Subplot Number Trees	29.56* (4)	34.92* (1)	32.24	33.24 (3)	33.72 (2)	33.48
Median Subplot DBH (cm)	14.2* (4)	14.9* (3)	14.6†	16.0* (2)	21.0* (1)	18.5**
ΨΨMean Tree Height (m)	9.90* (1)	9.44* (2)	9.65	8.03 (4)	8.27 (3)	8.15
ΨΨAvailability Percent (Fruit, Flowers, Young Leaves)	37.48 (2)	29.29 (3)	33.09†	24.20* (4)	42.14* (1)	34.61†
ΨΨFruit Availability Percent	13.10 (1)	11.11 (2)	12.03†	4.43 (3)	4.00 (4)	4.18†
ΨHuman Presence (Rank)	(1)^	(2)^		(4)	(3)	
Clearings	0.55 (1)	0.25 (2)		0.00 (4)	0.06 (3)	
ΨEvidence of Logging	(1)	(2)		(4)	(3)	
ΨDomesticated Animals	(1)	(2)		(4)	(3)	

Note Ψindicates combined data from Ingraldi, 2010 and the current study. ΨΨindicates data from the current study only. *indicates significance between fragments at a single site where $p \leq 0.05$. †indicates significance between sites where $p \leq 0.05$. ^refers to a statistically significant difference from Ingraldi (2010) but not the current study. Availability refers to the phenology of tree plots and records to availability of food sources. Fruit includes both ripe and unripe fruit.

Table 4.2 Study groups at Agnalazaha and Manombo during the spring/wet and fall/dry seasons.

Forest Site	Forest Fragment	Group	Spring/Wet Season Group Size	Fall/Dry Season Group Size	Changes Between Seasons
Agnalazaha Forest	Agnalazaha Inland Forest	AIN 1	8	7 +2i	-1 +2i
Agnalazaha Forest	Agnalazaha Inland Forest	AIN 2	6	5 +1i	-2 +1 +1i
Agnalazaha Forest	Agnalazaha Inland Forest	AIN 3	NA	4 +1i	NA
Manombo Forest	Manombo Classified Forest	MCF	8	3	-5
Manombo Forest	Manombo Special Reserve	MSR	3	6 +2i	+3 +2i

Note i indicates infants born during the study season.

Table 4.3 Parasite Indices by Forest for Spring/West Season

	AIN	ACS	AGA	MCF	MSR	MAN
Prevalence Host Sample Size	14	8	22	6	0	6
Callistoura Prevalence	42.86%%	25.00%	36.36%	50.00%	NA	50.00%
Lemuricola Prevalence	21.43%	12.50%	18.18%	16.67%	NA	16.67%
Ascarid Prevalence	7.14%	0.00%	4.55%	0.00%	NA	0.00%

Table 4.4 Parasite Indices by Forest for Fall/Dry Season

	AIN	ACS	AGA	MCF	MSR	MAN
Frequency Host Sample Size	23	0	23	3	4	7
Prevalence Host Sample Size	25	10	35	4	6	10
Callistoura Frequency	0.86 (sd ± 0.29)		0.86 (sd ± 0.29)	0.56 (sd ± 0.42)	1.00 (sd ± 0.00)	0.81 (sd ± 0.34)
Callistoura Prevalence	96.00%	100.00%	97.22%	75.00%	100.00%	90.00%
Lemuricola Frequency	0.63 (sd ± 0.35)		0.63 (sd ± 0.35)†	0.33 (sd ± 0.16)	0.21 (sd ± 0.25)	0.26 (sd ± 0.21)†
Lemuricola Prevalence	92.00%	100.00%	94.44%†	50.00%	50.00%	50.00%†
Ascarid Frequency	0.16 (sd ± 0.27)		0.16 (sd ± 0.27)	0.06 (sd ± 0.10)	0.00	0.02 (sd ± 0.06)
Ascarid Prevalence	24.00%	9.09%	19.44%	25.00%	0.00%	10.00%
Entamoeba Frequency	0.10 (sd ± 0.21)		0.10 (sd ± 0.21)†	0.33 (sd ± 0.58)	0.26 (sd ± 0.21)	0.29 (sd ± 0.16)†
Entamoeba Prevalence	20.00%	9.09%	16.67%†	75.00%	50.00%	60.00%†

Note *indicates a significant difference between fragments (either AIN and ACS, or MCF and MSR) where $p \leq 0.05$. †Indicates a significant difference between sites (AGA and MAN) where $p \leq 0.05$.

Table 4.5 Agnalazaha and Manombo lemur species and their parasites.

Forest	Nematode Parasites of *E. cinereiceps*	Other Lemur Species	Documented Lemur Nematode Parasites from Other Wild Populations
Agnalazaha Inland Forest	*Callistoura*, *Lemuricola*, Ascarid	*Hapalemur griseus, Avahi ramantsoavani, Microcebus jollyae, Cheirogaleus major*	*Lemurostrongylus, Lemuricola, Protofilaria, Parahabdonema, Trichuris*
Agnalazaha Coastal Forest	*Callistoura*, *Lemuricola*, Ascarid, *Trichuris*	*Hapalemur griseus, Avahi ramantsoavani, Microcebus jollyae, Cheirogaleus major*	*Lemurostrongylus, Lemuricola, Protofilaria, Parahabdonema, Trichuris*
Manombo Special Reserve	*Callistoura*, *Lemuricola*	*Hapalemur griseus, Avahi ramantsoavani, Microcebus jollyae, Varecia variegata editorum, Lepilemur jamesi*	*Lemurostongylus, Callistoura, Protofilaria, Lemuricola*
Manombo Classified Forest	*Callistoura*, *Lemuricola*, Ascarid	*Hapalemur griseus, Avahi ramantsoavani, Microcebus jollyae, Varecia variegata editorum, Lepilemur jamesi, Daubentonia madagascariensis*	*Lemurostongylus, Callistoura* (found in *V. v. editorum*), *Protofilaria, Lemuricola, Ascaris, Trichuris*

Note lemur species from Ingraldi, 2010 census data, documented lemur nematodes from Irwin and Raharison, 2009.

4.6 Significant difference in the prevalence and frequency of parasites by site, habitat use, behavior and demographic variables.

	Field Sites: AGA, MAN	Habitat Use: Travel Time, Ground Time, Foraging, Homerange Size, Homerange Location, Height	Social Behavior: Physical Contact, Group Spread	Demographic Variables: Age, Sex, Group Size
Callistoura	AGA = MAN	None	+ Correlation with Physical Contact	+ Correlation with Group Size
Lemuricola	AGA > MAN	+ Correlation with Travel Time, Ground Time	None	None
Ascarid	AGA = MAN	+ Correlation with Group Size, + Correlation with Homerange Water Location	None	None
Entamoeba	AGA < MAN	None	+ Correlation with Height, - Correlation with Fruit Foraging	None

4.7 Field site difference by *Eulemur cinereiceps* population, habitat use, social behavior, demographic and environmental variables.

	E. cinereiceps: Density, Population Size	Habitat Use: Travel Time, Ground Time, Foraging, Homerange Size, Homerange Location, Height	Social Behavior: Physical Contact, Group Spread	Demographic Variables: Age, Sex, Group Size	Environmental Variables: Area, Shape Indices, Tree Species Richness, Tree DBH, Tree Density, Food Availability, Fruit Availability, Temperature, Humidity, Disturbance
Field Site: AGA, MAN	AGA < MAN: Density, Population Size	AGA < MAN: Height, AGA > MAN: Travel Time, Ground Time	None	None	AGA > MAN: Shape Index, Fruit Availability, Temperature, Disturbance AGA < MAN: Area, TSR, Tree DBH, Food Availability

4.8 Seasonal significant difference in parasites infection, social behavior, habitat use, demographic and environmental variables.

	Callistoura Infection	*Lemuricola* Infection	Ascarid Prevalence	Social Behavior	Habitat Use	Demographic Variables	Rainfall
Season	Fall/Dry > Spring/Wet	Fall/Dry > Spring/Wet	Fall/Dry > Spring/Wet	None	Fall/Dry > Spring/Wet: Height + Travel Time	None	Spring/Wet > Fall/Dry

Table 4.9 Individual and group measures during fall/dry season.

Behavior	Sample Size	Range	Mean	SD
Group Size	5	3-9	6.20	2.39
Group Spread (m)	5	3.71-8.49	6.99	1.98
Physical Contact (measure)	23	0.22-1.61	0.62	0.38
Individual Travel Time (%)	19	4.04-13.24	7.79	2.47
Adjusted Height (m)	23	0.22-1.61	0.62	0.38
Group Time on the Ground (%)	5	0.09-2.33	1.38	1.13
Home Range MCP (km^2)	5	10.70-24.00	18.27	5.58
Individual Fruit Foraging Time (%)	21	46.08-83.33	61.16	10.41
Actual Height (m)	23	0.00-13.00	6.14	1.23
Individual Time on Ground (%)	23	0.00-4.43	1.55	1.38

Table 4.10 Individual and group measures during the spring/wet season.

Behavior	Sample Size	Range	Mean	SD
Group Size	3	6-8	7.33	NA
Physical Contact (measure)	18	0.12-0.75	0.37	0.17
Individual Travel Time (%)	14	2.00-12.00	6.79	2.78
Adjusted Height (m)	13	0.37-0.99	0.51	0.20
Group Time on the Ground (%)	3	0.13-1.13	0.77	NA
Actual Mean Height (m)	13	3.70-8.20	4.74	1.37
Individual Time on Ground (%)	13	0.00-4.00	1.46	1.27

Table 4.11 Significant statistical analyses and values.

Variable 1	Variable 2	Statistical Analysis	Sample Size	Mean (Mean Rank)	Test Statistic	Significance	Test Assumption Violations
Ascarid Prevalence	Microhabitat: Water	Chi-Square	23		2.73	0.099	50% cells < 5
Average Group Ascarid Frequency	Home Range (GCC)	Pearson's Correlation Analysis	5		0.888	0.044	
Average Group Ascarid Prevalence	Home Range (GCC)	Pearson's Correlation Analysis	5		0.863	0.060	
Average Group Fall/Dry Season *Entamoeba* Frequency	Fruit Foraging	Pearson's Correlation Analysis	5		-0.868	0.056	
Average Group Fall/Dry Season *Entamoeba* Prevalence	Fruit Foraging	Pearson's Correlation Analysis	5		-0.893	0.042	
Average Group *Lemuricola* Prevalence	Time Spent on the Ground	Spearman's Correlation Analysis	8		0.415	0.037	
Average Group *Lemuricola* Prevalence	Travel Time	Spearman's Correlation Analysis	8		0.708	0.050	
Callistoura Prevalence	Season	Chi-Square	74		22.85	0.000	25% cell < 5
Fall/Dry Season Average Group Ascarid Prevalence	Average Group Physical Contact	Spearman's Correlation Analysis	5		0.872	0.054	
Fall/Dry Season Average Group Ascarid Prevalence	Home Range (GCC)	Pearson's Correlation Analysis	5		0.863	0.060	
Fall/Dry Season Average Group *Entamoeba* Frequency	Average Group Fruit Foraging	Pearson's Correlation Analysis	5		-0.868	0.056	
Fall/Dry Season Average Group *Entamoeba* Prevalence	Average Group Fruit Foraging	Pearson's Correlation Analysis	5		-0.893	0.042	
Fall/Dry Season *Entamoeba* Frequency	Forest Site: AGA, MAN	Mann-Whitney U-test	AGA = 16, MAN = 7	AGA = 10.09, MAN = 16.36	20.50	0.024	
Fall/Dry Season *Entamoeba* Frequency	Height	Spearman's Correlation Analysis	23		0.448	0.032	
Fall/Dry Season *Entamoeba* Frequency	Physical Contact	Spearman's Correlation Analysis	23		0.165	0.451	
Fall/Dry Season *Entamoeba* Prevalence	Forest Fragment: AIN, ACS, MAN	Chi-Square	23		6.74	0.034	
Fall/Dry Season *Entamoeba* Prevalence	Forest Site: AGA, MAN	Chi-Square	AGA = 36, MAN = 10		7.62	0.006	25% cell < 5

Fall/Dry Season *Entamoeba* Prevalence	Height	Mann-Whitney U-test	Presence = 10, Absence = 13	Presence = 16.00, Absence = 8.92	25.00	0.013	
Fall/Dry Season Fruit Foraging	Forest Fragment: AIN, MCF	Independent Samples t-test	AIN = 15, MCF = 3	AIN = 62.92, MCF = 47.06	3.17	0.006	
Fall/Dry Season Fruit Foraging	Forest Fragment: AIN, MCF, MSR	Kruskal-Wallis	AIN = 15, MCF = 3, MSR = 3	AIN = 12.27, MCF = 2.00, MSR = 13.67	7.49	0.024	
Fall/Dry Season Height	Forest Site: AIN. MAN	Mann-Whitney U-test	AIN = 16, MAN = 7	AIN = 8.63, MAN = 19.71	2.00	0.000	
Fall/Dry Season *Lemuricola* Frequency	Forest Site: AGA, MAN	Mann-Whitney U-test	AGA = 16, MAN = 7	AGA = 13.91, MAN = 7.64	25.50	0.038	
Fall/Dry Season *Lemuricola* Prevalence	Forest Site: AGA, MAN	Chi-Square, Correction	AGA = 36, MAN = 10		8.18	0.004	50% cells < 5
Fall/Dry Season Time Spent on the Ground	Forest Site: AIN, MAN	Mann-Whitney U-test	AIN = 16, MAN = 7	AIN = 15.38, MAN = 4.29	2.00	0.000	
Fall/Dry Season PSR	Fruit Foraging	Spearman's Correlation Analysis	21		-0.483	0.027	
Fall/Dry Season Travel Time	Forest Site: AIN, MAN	Independent Samples t-test	AIN = 16, MAN = 3	AIN = 12.75, MAIN = 9.67	1.95	0.068	
Fruit Availability	Forest Site: AGA, MAN	Mann-Whitney U-test	AGA = 1662, MAN = 1722	AGA = 1733.05, MAN = 1653.36	136358 6.00	0.000	
Fruit Foraging	MAN Forest Fragment: MCF, MSR	Independent Sample t-test	MCF = 3, MSR = 3	MCF = 47.06, MSR = 66.44	-2.26	0.087	
Fruit, Young Leaf, Flower Availability	Forest Site: AGA, MAN	Mann-Whitney U-test	AGA = 1662, MAN = 1722	AGA = 1640.70, MAN = 1742.50	134485. 00	0.000	
Fruit, Young Leaf, Flower Availability	MAN Forest Fragment: MCF, MSR	Mann-Whitney U-test	MCF = 999, MSR = 723	MCF = 901.78, MSR = 805.85	320900. 50	0.000	
Height	Season	Mann-Whitney U-test	Spring = 13, Fall = 23	Spring = 12.27, Fall = 22.02	68.50	0.008	
Lemuricola Prevalence	Season	Chi-Square	79		27.89	0.000	
Spring/Wet Season *Callistoura* Prevalence	Physical Contact	Mann-Whitney U-test	Presence = 8, Absence = 10	Presence = 12.56, Absence = 7.05	15.50	0.029	

Spring/Wet Season Height	Forest Site: AIN, MAN	Mann-Whitney U-test	AIN = 9, MAN = 4	AIN = 5.00, MAN = 11.50	0.00	0.005	
Spring/Wet Season Time Spent on the Ground	Forest Site: AIN, MAN	Mann-Whitney U-test	AIN = 9, MAN = 4	AIN = 8.67, MAN = 3.25	3.00	0.016	
Travel Time	Season	Independent Samples t-test	Spring = 17, Fall = 19	Spring = 7.04, Fall = 12.26	-5.87	0.000	
Fall/Dry Season Average Group *Callistoura* Frequency	Average Group Height	Spearman's Correlation Analysis	5		0.300	0.624	
Fall/Dry Season Average Group *Callistoura* Frequency	Group Size	Spearman's Correlation Analysis	5		0.900	0.037	
Fall/Dry Season Average Group *Callistoura* Frequency	Group Spread	Spearman's Correlation Analysis	5		1.000	<0.001	
Fall/Dry Season Average Group *Callistoura* Frequency	Sex Ratio	Spearman's Correlation Analysis	5		0.800	0.104	
Fall/Dry Season Average Group Height	Group Spread	Spearman's Correlation Analysis	5		0.800	0.104	
Fall/Dry Season Average Group *Lemuricola* Frequency	Average Group Travel Time	Pearson's Correlation Analysis	5		0.857	0.064	
Fall/Dry Season Average Group *Lemuricola* Frequency	Average Group Time on the Ground	Pearson's Correlation Analysis	5		0.941	0.017	
Fall/Dry Season Group Spread	Group Size	Spearman's Correlation Analysis	5		0.900	0.037	
Fall/Dry Season Fall/Dry Season Height	Age	Mann-Whitney U-test	23	Adult = 15.45, Juvenile = 8.83	33.00	0.042	
Fall/Dry Season Height	Physical Contact	Spearman's Correlation Analysis	23		0.564	0.005	

Fall/Dry Season Physical Contact	Age	Mann-Whitney U test	23	Adult = 16.09, Juvenile = 8.25	21.00	0.006	
Fall/Dry Season Physical Contact	Sex	Mann-Whitney U test	23	Male = 7.73, Female = 17.55	9.50	0.001	
Fall/Dry Season Average Group Time on the Ground	Age Ratio	Pearson's Correlation Analysis	5		-0.805	0.100	
Fall/Dry Season Average Group Time on the Ground	Average Group Travel Time	Pearson's Correlation Analysis	5		0.640	0.088	
Fall/Dry Season Travel Time	Physical Contact	Spearman's Correlation Analysis	19		-0.552	0.014	
Fall/Dry Season Travel Time	Sex	Independent Samples t-test	19		1.85	0.082	

Note AIN = Agnalazaha Inland Forest, ACS = Agnalazaha Coastal Forest, AGA = Agnalazaha Forest both fragments combined, MSR = Manombo Special Reserve, MCF = Manombo Classified Forest, MAN = Manombo Forest both fragments combined. PSR = parasite species richness.

Table 4.12 Non-significant statistical analyses and values.

Variable 1	Variable 2	Statistical Analysis	Sample Size	Mean (Mean Rank)	Test Statistic	Significance	Violations
Fall/Dry Season Ascarid Frequency	Forest Site: AGA, MAN	Mann-Whitney U-test	AGA = 16, MAN = 7	AGA – 12,75, MAN – 10.29	32.50	0.114	
Fall/Dry Season Ascarid Prevalence	Forest Site: AGA, MAN	Chi-Square	AGA = 36, MAN = 10		0.49	0.469	25% cell < 5
Fall/Dry Season Ascarid Prevalence	Height	Mann-Whitney U-test	Presence = 6, Absence = 17	Presence = 10.00, Absence = 12.71	39.00	0.400	
Fall/Dry Season Ascarid Prevalence	Physical Contact	Mann-Whitney U-test	Presence = 6, Absence = 17	Presence = 10.83, Absence = 12.41	44.00	0.624	
Fall/Dry Season Ascarid Prevalence	Travel Time	Independent Sample t-test	Presence = 6, Absence = 13	Presence = 11.50, Absence = 12.62	-0.83	0.419	
Fall/Dry Season *Callistoura* Frequency	Forest Site: AGA, MAN	Mann-Whitney U-test	AGA = 16, MAN = 7	AGA = 12.19, MAN = 11.57	53.00	0.795	
Fall/Dry Season *Callistoura* Prevalence	Forest Site: AGA, MAN	Chi-Square	AGA = 36, MAN = 10		0.98	0.332	50% cells < 5
Fall/Dry Season *Entamoeba* Prevalence	Physical Contact	Mann-Whitney U-test	Presence = 10, Absence = 13	Presence = 12.85, Absence = 11.35	56.50	0.598	
Fall/Dry Season *Entamoeba* Prevalence	Travel Time	Independent Sample t-test	Presence = 7, Absence = 12	Presence = 11.57, Absence = 12.67	-0.85	0.410	
Fall/Dry Season Parasite Species Richness	Forest Site: AGA, MAN	Mann-Whitney U-test	AGA = 36, MAN = 10	AGA = 24.10, MAN = 21.35	158.50	0.539	
Fall/Dry Season Average Group Ascarid Prevalence	Height	Spearman's Correlation Analysis	5		0.103	0.870	
Fall/Dry Season Average Group Ascarid Prevalence	Average Time on the Ground	Pearson's Correlation Analysis	5		0.241	0.692	
Fall/Dry Season Average Group Ascarid Prevalence	Average Group Travel Time	Pearson's Correlation Analysis	5		0.698	0.190	
Fall/Dry Season Average Group Ascarid Prevalence	Group Spread	Pearson's Correlation Analysis	5		-0.116	0.852	
Fall/Dry Season Average Group Ascarid Prevalence	Home Range (MCP)	Pearson's Correlation Analysis	5		0.745	0.148	

Fall/Dry Season Average Group *Entamoeba* Prevalence	Average Group Physical Contact	Spearman's Correlation Analysis	5		0.300	0.624	
Fall/Dry Season Average Group *Entamoeba* Prevalence	Height	Spearman's Correlation Analysis	5		0.000	1.000	
Fall/Dry Season Average Group *Entamoeba* Prevalence	Average Time Spent on the Ground	Pearson's Correlation Analysis	5		-0.783	0.117	
Fall/Dry Season Average Group *Entamoeba* Prevalence	Average Group Travel Time	Pearson's Correlation Analysis	5		-0.479	0.414	
Fall/Dry Season Average Group *Entamoeba* Prevalence	Group Spread	Pearson's Correlation Analysis	5		-0.680	0.207	
Fall/Dry Season Average Group *Entamoeba* Prevalence	Home Range (MCP)	Pearson's Correlation Analysis	5		0.111	0.859	
Fall/Dry Season Average Group *Entamoeba* Prevalence	Home Range (GCC)	Pearson's Correlation Analysis	5		-0.242	0.695	
Fall/Dry Season Ascarid Prevalence	Microhabitat: Swamp	Chi-Square	23		1.66	0.198	50% cells < 5
Fall/Dry Season *Entamoeba* Prevalence	Microhabitat: Swamp	Chi-Square	23		1.81	0.179	25% cell < 5
Fall/Dry Season Average Group *Lemuricola* Prevalence	Average Group Physical Contact	Spearman's Correlation Analysis	5		0.335	0.581	
Fall/Dry Season Average Group *Lemuricola* Prevalence	Average Group Height	Spearman's Correlation Analysis	5		-0.671	0.215	
Fall/Dry Season Average Group *Lemuricola* Prevalence	Travel Time	Pearson's Correlation Analysis	5		0.457	0.439	
Fall/Dry Season Average Group *Lemuricola* Prevalence	Time Spent on the Ground	Pearson's Correlation Analysis	5		0.472	0.422	
Fall/Dry Season Average Group *Lemuricola* Prevalence	Group Spread	Pearson's Correlation Analysis	5		-0.506	0.384	
Fall/Dry Season Average Group *Lemuricola* Prevalence	Home Range (MCP)	Pearson's Correlation Analysis	5		0.560	0.326	
Fall/Dry Season Average Group *Lemuricola* Prevalence	Home Range (GCC)	Pearson's Correlation Analysis	5		0.375	0.534	
Fall/Dry Season Physical Contact	Forest Site: AIN, MAN	Mann-Whitney U-test	AIN = 16, MAN = 7	AIN = 11.41, MAN = 13.36	46.50	0.525	
Fall/Dry Season *Callistoura* Frequency	Fruit Foraging	Spearman's Correlation Analysis	18		0.170	0.501	
Fall/Dry Season *Lemuricola* Frequency	Fruit Foraging	Spearman's Correlation Analysis	18		0.283	0.225	

Fall/Dry Season Fruit Foraging	Forest Site: AIN, MAN	Independent Samples t-test	AIN = 15, MAN = 6	AIN = 62.92, MAN = 56.75	1.25	0.228	
Physical Contact	Season	Mann-Whitney U-test	Spring = 20, Fall = 23	Spring = 18.50, Fall = 18.50	162.00	1.000	
Time Spent on the Ground	Season	Independent Samples t-test	Spring = 13, Fall = 23	Spring = 1.46, Fall = 1.54	-0.18	0.859	
Spring/Wet Season Physical Contact	Forest Site: AIN, MAN	Mann-Whitney U-test	AIN = 12, MAN = 6	AIN = 9.79, MAN = 8.92	32.50	0.743	
Spring/Wet Season Travel Time	Forest Site: AIN, MAN	Independent Samples t-test	AIN = 14, MAN = 5	AIN = 2.62, MAN = 2.64	1.00	0.335	
Spring/Wet Season *Lemuricola* Prevalence	Forest Site: AGA, MAN	Chi-Square	28		0.00	1.000	50% cells < 5
Spring/Wet Season *Callistoura* Prevalence	Forest Site: AGA, MAN	Chi-Square	28		0.00	1.000	
Fall/Dry Season *Callistoura* Prevalence	Forest Site: AGA, MAN	Chi-Square	45		0.01	0.909	
Spring/Wet Season *Callistoura* Prevalence	Height	Mann-Whitney U-test	Presence = 6, Absence = 7	Presence = 8.00, Absence = 6.14	15.00	0.389	
Spring/Wet Season *Callistoura* Prevalence	Time Spent on the Ground	Mann-Whitney U-test	Presence = 6, Absence = 7	Presence = 6.25, Absence = 7.64	16.50	0.502	
Spring/Wet Season *Callistoura* Prevalence	Travel Time	Independent Samples t-test	Presence = 7, Absence = 10	Presence = 6.27, Absence = 7.58	-1.01	0.327	
Spring/Wet Season *Lemuricola* Prevalence	Physical Contact	Independent Samples t-test	Presence = 4, Absence = 14	Presence = 11.25, Absence = 9.00	21.00	0.457	
Spring/Wet Season *Lemuricola* Prevalence	Travel Time	Independent Samples t-test	Presence = 4, Absence = 14	Presence = 5.84, Absence = 7.17	-0.87	0.389	
Fruit, Young Leaf, Flower Availability	AGA Forest Fragment: AIN, ACS	Mann-Whitney U-test	AIN = 771, ACS = 890	AIN = 839.98, ACS = 823.22	336168.00	0.248	
Fruit Availability	AGA Forest Fragment: AIN, ACS	Mann-Whitney U-test	AIN = 771, ACS = 890	AIN = 826.39, ACS = 835.00	339538.00	0.414	
Fruit Availability	MAN Forest Fragment: MSR, MCF	Mann-Whitney U-test	MCF = 999, MSR = 723	MCF = 861.99, MSR = 860.83	360653.50	0.859	
Fall/Dry Season *Entamoeba* Prevalence	Age: Adult, Juvenile	Chi-Square	46		0.00	1.000	25% cell < 5
Fall/Dry Season *Entamoeba* Prevalence	Sex	Chi-Square	46	1.05	0.305		
Fall/Dry Season *Entamoeba* Frequency	Travel Time	Person's Correlation Analysis	19	-0.004	0.988		

Fall/Dry Season Average Group *Entamoeba* Frequency	Average Group Travel Time	Person's Correlation Analysis	5	-0.393	0.512		
Fall/Dry Season Average Group *Entamoeba* Frequency	Time Spent on the Ground	Person's Correlation Analysis	5	-0.776	0.123		
Fall/Dry Season Average Group *Entamoeba* Frequency	Home Range (GCC)	Person's Correlation Analysis	5	-0.187	0.640		
Fall/Dry Season Average Group *Entamoeba* Frequency	Home Range (MCP)	Person's Correlation Analysis	5	0.017	0.979		
Fall/Dry Season Average Group *Entamoeba* Frequency	Group Spread	Person's Correlation Analysis	5	-0.553	0.334		
Fall/Dry Season Average Group *Entamoeba* Frequency	Group Size	Person's Correlation Analysis	5	-0.235	0.704		
Fall/Dry Season Average Group *Entamoeba* Frequency	Average Group Height	Spearman's Correlation Analysis	5	0.600	0.285		
Fall/Dry Season Average Group *Entamoeba* Frequency	Average Group Physical Contact	Spearman's Correlation Analysis	5	0.300	0.624		
Fall/Dry Season *Entamoeba* Prevalence	Fruit Foraging	Independent Sample t-test	Presence = 10, Absence = 11	Presence = 58.39, Absence = 63.68	-1.17	0.256	
Fall/Dry Season *Entamoeba* Frequency	Fruit Foraging	Pearson's Correlation Analysis	21		-0.148	0.523	
Average Group Ascarid Prevalence	Home Range (MCP)	Pearson's Correlation Analysis	5		0.745	0.148	
Average Group Ascarid Frequency	Home Range (MCP)	Pearson's Correlation Analysis	5		0.684	0.203	
Fall/Dry Season *Entamoeba* Prevalence	Fruit Foraging	Independent Samples t-test	Presence = 10, Absence = 11	Presence = 58.39, Absence= 63.68	-1.17	0.256	
Fall/Dry Season Ascarid Prevalence	Fruit Foraging	Independent Samples t-test	Presence = 6, Absence = 15	Presence = 56.47, Absence = 63.04	-1.33	0.199	
Fall/Dry Season Average Group *Callistoura* Frequency	Average Group Fruit Foraging	Spearman's Correlation Analysis	5		0.600	0.285	
Fall/Dry Season Average Group *Lemuricola* Frequency	Average Group Fruit Foraging	Spearman's Correlation Analysis	5		0.500	0.391	
Fall/Dry Season Average Group Ascarid Frequency	Average Group Fruit Foraging	Pearson's Correlation Analysis	5		-0.154	0.805	
Fall/Dry Season Average Group *Callistoura* Prevalence	Average Group Fruit Foraging	Spearman's Correlation Analysis	5		0.000	1.000	

Fall/Dry Season Average Group *Lemuricola* Prevalence	Average Group Fruit Foraging	Spearman's Correlation Analysis	5		-0.783	0.118	
Fall/Dry Season Average Group Ascarid Prevalence	Average Group Fruit Foraging	Pearson's Correlation Analysis	5		-0.364	0.547	
Fall/Dry Season PSR	Sex	Mann-Whitney U-test	Male = 25, Female = 21	Male = 22.86, Female = 24.26	246.50	0.705	
Fall/Dry Season PSR	Age	Mann-Whitney U-test	Adult = 31, Juvenile = 15	Adult = 23.06, Juvenile = 24.40	219.00	0.734	
Fall/Dry Season PSR	Forest Site	Mann-Whitney U-test	AGA = 36, MAN = 10	AGA = 24.10, MAN = 21.35	158.50	0.539	
Fall/Dry Season FPI	Sex	Independent Samples t-test	Male = 13, Female = 10	Male = 1.77, Female = 1.47	1.26	0.135	
Fall/Dry Season FPI	Age	Independent Samples t-test	Adult = 11, Juvenile = 12	Male = 1.76, Female = 1.53	0.95	0.841	
Fall/Dry Season FPI	Forest Site	Independent Samples t-test	AGA = 16, MAN = 7	AGA = 1.75, MAN = 1.38	1.45	0.163	
Fall/Dry Season FPI	Fruit Foraging	Pearson's Correlation Analysis	21		0.047	0.840	
Fall/Dry Season FPI	Travel Time	Pearson's Correlation Analysis	19		0.130	0.595	
Fall/Dry Season FPI	Height	Spearman's Correlation Analysis	23		-0.043	0.845	
Fall/Dry Season Average Group FPI	Average Group Travel Time	Pearson's Correlation Analysis	5		0.788	0.113	
Fall/Dry Season Average Group FPI	Time Spent on the Ground	Pearson's Correlation Analysis	5		0.341	0.574	
Fall/Dry Season Average Group FPI	Group Spread		5		0.678	0.209	
Fall/Dry Season Average Group FPI	Home Range Size (GCC)	Pearson's Correlation Analysis	5		0.543	0.443	
Fall/Dry Season Average Group FPI	Home Range Size (MCP)	Pearson's Correlation Analysis	5		0.047	0.941	
Fall/Dry Season Average Group FPI	Average Group Fruit Foraging	Pearson's Correlation Analysis	5		0.292	0.633	
Fall/Dry Season Average Group FPI	Average Group Height	Spearman's Correlation Analysis	5		0.100	0.873	
Fall/Dry Season Average Group FPI	Average Group Physical Contact	Spearman's Correlation Analysis	5		0.700	0.188	

Fall/Dry Season Average Group FPI	Local Density	Pearson's Correlation Analysis	5		0.347	0.568	
Fall/Dry Season Average Group FPI	Group Size	Pearson's Correlation Analysis	5		0.753	0.142	
Fall/Dry Season PSR	Travel Time	Spearman's Correlation Analysis	19		-0.204	0.402	
Fall/Dry Season PSR	Physical Contact	Spearman's Correlation Analysis	23		-0.083	0.706	
Fall/Dry Season PSR	Height	Spearman's Correlation Analysis	23		-0.058	0.793	
Fall/Dry Season Average Group Callistoura Frequency	Age Ratio	Spearman's Correlation Analysis	5		0.001	1.000	
Fall/Dry Season Average Group Callistoura Frequency	Average Group Height	Spearman's Correlation Analysis	5		0.300	0.624	
Fall/Dry Season Average Group Callistoura Frequency	Average Group Physical Contact	Spearman's Correlation Analysis	5		0.001	1.000	
Fall/Dry Season Average Group Callistoura Frequency	Average Time on the Ground	Spearman's Correlation Analysis	5		-0.400	0.505	
Fall/Dry Season Average Group Callistoura Frequency	Home Range (GCC)	Spearman's Correlation Analysis	5		-0.500	0.391	
Fall/Dry Season Average Group Callistoura Frequency	Home Range (MCP)	Spearman's Correlation Analysis	5		-0.700	0.188	
Fall/Dry Season Average Group Callistoura Frequency	Travel Time	Spearman's Correlation Analysis	5		0.000	1.000	
Fall/Dry Season Average Group Height	Average Group Physical Contact	Spearman's Correlation Analysis	5		0.100	0.873	
Fall/Dry Season Average Group Height	Average Group Travel	Spearman's Correlation Analysis	5		-0.200	0.747	
Fall/Dry Season Average Group Height	Average Time on the Ground	Spearman's Correlation Analysis	5		-0.600	0.285	
Fall/Dry Season Average Group Height	Group Size	Spearman's Correlation Analysis	5		0.600	0.285	
Fall/Dry Season Average Group Height	Group Spread	Pearson's Correlation Analysis	5		0.461	0.435	
Fall/Dry Season Average Group Lemuricola	Average Group Height	Spearman's Correlation Analysis	5		0.200	0.747	
Fall/Dry Season Average Group Lemuricola Frequency	Age Ratio	Spearman's Correlation Analysis	5		-0.700	0.188	

Fall/Dry Season Average Group Lemuricola Frequency	Average Group Physical Contact	Spearman's Correlation Analysis	5		0.100	0.873	
Fall/Dry Season Average Group Lemuricola Frequency	Group Size	Pearson's Correlation Analysis	5		0.398	0.507	
Fall/Dry Season Average Group Lemuricola Frequency	Group Spread	Pearson's Correlation Analysis	5		0.546	0.341	
Fall/Dry Season Average Group Lemuricola Frequency	Home Range (GCC)	Pearson's Correlation Analysis	5		0.167	0.788	
Fall/Dry Season Average Group Lemuricola Frequency	Home Range (MCP)	Pearson's Correlation Analysis	5		0.502	0.389	
Fall/Dry Season Average Group Lemuricola Frequency	Physical Contact	Spearman's Correlation Analysis	23	Male: 7.44, Female: 8.24	0.001	1.000	
Fall/Dry Season Average Group Lemuricola Frequency	Sex Ratio	Spearman's Correlation Analysis	5		0.300	0.624	
Fall/Dry Season Average Group Travel Time	Group Size	Pearson's Correlation Analysis	5		0.474	0.419	
Fall/Dry Season Average Time on the Ground	Average Group Physical Contact	Pearson's Correlation Analysis	5		-0.243	0.694	
Fall/Dry Season Average Time on the Ground	Group Spread	Pearson's Correlation Analysis	5		0.346	0.568	
Fall/Dry Season Average Time on the Ground	Home Range (MCP)	Pearson's Correlation Analysis	5		0.190	0.759	
Fall/Dry Season Average Time on the Ground	Sex Ratio	Spearman's Correlation Analysis	5		0.100	0.873	
Fall/Dry Season Callistoura Prevalence	Age	Chi-Square	46		0.288	0.592	50% cells < 5
Fall/Dry Season Callistoura Prevalence	Sex	Chi-Square	46		0.0`6	0.900	50% cells < 5
Fall/Dry Season Callistoura Frequency	Age	Mann-Whitney U test	23	Adult: 13.90, Juvenile: 11.47	44.00	0.110	
Fall/Dry Season Callistoura Frequency	Height	Spearman's Correlation Analysis	23		0.343	0.109	
Fall/Dry Season Callistoura Frequency	Physical Contact	Spearman's Correlation Analysis	23		0.140	0.523	
Fall/Dry Season Lemuricola Prevalence	Sex	Chi-Square	46		0.84	0.359	50% cells < 5

Note AIN = Agnalazaha Inland Forest, ACS = Agnalazaha Coastal Forest, AGA = Agnalazaha Forest both fragments combined, MSR = Manombo Special Reserve, MCF = Manombo Classified Forest, MAN = Manombo Forest both fragments combined. PSR = parasite species richness, FPI = frequency of parasite infection for all parasite species.

Chapter 5. Parasite Burden and Exposure in a Wild Population of *Eulemur cinereiceps*: Conclusion

Primate parasite ecology plays a role in community ecology, conservation biology, and biodiversity (Altizer et al., 2003). Understanding the relationship between primate behavior and disease risk is critical for the conservation of primates and their ecosystems (Dobson and Lyles, 2000). Extinction concerns have led to conservation management plans for many global parks and reserves with endangered primate species. A key element to biodiversity and primate conservation is understanding host-parasite infection patterns (Nunn et al., 2004). The grey-headed lemur, *Eulemur cinereiceps*, is a critically endangered species, and among the top 25 most endangered primates in the world (Mittermeier et al., 2009). Among primates, Lemurifomes have been underrepresented in host-parasite studies (Irwin and Raharison, 2009) and no other studies to date have investigated the parasites of *E. cinereiceps*.

The spread of disease within a population or community is the result of both host susceptibility and host exposure. Susceptibility refers to physiological, genetic, or environmental constraints on immune system strength (Nunn and Altizer, 2006). Exposure refers to an individual, group or population's encounter with pathogens (Fenton et al., 2002). This work investigated behavioral and environmental correlates of parasite exposure through the evaluation of parasite burden in two wild metapopulations of *E. cinereiceps* in southeastern Madagascar.

The objectives of this study were to: 1) Identify the most efficient and effective way to evaluate *E. cinereiceps* fecal parasite burden. 2) Identify the gastro-intestinal parasites recovered from coastal populations of *E. cinereiceps* populations. 3) Identify demographic, social behavior, habitat use behavior and environmental correlates of parasite burden in *E. cinereiceps*. 4) Determine if social behaviors or habitat use behaviors better predict parasite burden in *E.*

cinereiceps. 5) Determine if host behavior or environmental factors better predict parasite burden in *E. cinereiceps*.

This study investigated fecal parasite burden in the grey-headed lemur, *Eulemur cinereiceps*, formally the white-collared lemur, *Eulemur albocollaris*. *E. cinereiceps* is a sexually dichromatic, frugivorous, arboreal, cathemeral primate weighing approximately 2.0 kg (Johnson et al., 2005; Irwin et al., 2005). Isolated populations of *E. cinereiceps* can be found in coastal fragments between the Mananara and Manampatrana Rivers (Irwin et al., 2005). The study sites for this research included two of these small forest fragments at Agnalazaha, and 12.0 kilometers to the north, another 3 fragments located at Manombo.

During two study seasons, the spring/wet season (January-April) and the fall/dry season (September – December) habituated study groups were followed from one fragment at Agnalazaha and two fragments at Manombo. Repetitive fecal samples were collected and social and habitat use behaviors were recorded for all habituated individuals. Additional fecal samples were collected from all forest fragments. Research questions, predictions, and results are given in table 5.1.

5.1 Field and Laboratory Method Validation

Field and laboratory fecal parasite recovery techniques were evaluated for precision, cost, and efficiency. This is one of the few critical analyses of fecal parasite recovery techniques for wild primates. Preservation solution, recovery technique, aspects of fecal flotation, and quantity of feces were evaluated. Ten percent formalin provided a more reliable preservative than 90% ethanol. Fecal sedimentation was a more sensitive method for parasite egg/cyst recovery than fecal flotation. Fecal flotation can be improved with repeated floats, and when using 10%

formalin it approaches similar results to fecal sedimentation. When using fecal flotation, homogeneity of the sample did not impact results. Overall, the results from trials using more 'solid' and less digested portions of feces did not vary significantly from those using the more 'liquid' and homogenous mixture of feces and preservation solution. The results from trials using a common fecal flotation solution, Sheather's solution, with two different specific gravities did not vary. When assessing parasite species richness, at least two grams of feces, or 60% of the fecal sample, should be evaluated for maximum results. When assessing nematode egg counts, at least 3 grams of feces, or 77% of the fecal sample should be evaluated to maximize efficiency.

Although these results are specific to the feces and parasite species from *E. cinereiceps*, the results are likely to remain true for other *Eulemur* species. Furthermore, this research demonstrates the need for standardization across primate parasitology studies, as well as the need for validation for specific fecal parasite recovery techniques. The late 20th and early 21st century has seen an increase in primate-parasite studies. More recent ethical concerns prohibit the use of former experimental clinical studies, as well as frequent necroscopy of wild primates often seen in earlier studies. This forces researcher, particularly those studying wild populations, to rely on less direct methods for assessing and investigating pathogenic diseases. Non-invasive fecal sampling has become common practice in many areas of wild primate research (molecular biology, endocrinology, reproductive biology) as well as parsitology. However, there has been a lack of standardization in the field, the laboratory, and in statistical analyses. This has lead to difficulties interpreting data and comparing results between studies, even those completed on the same host-parasite groups at a single field site. This chapter highlights the need to standardize and validate both laboratory and field parasitology work in a growing field.

5.2 Fecal Parasites Identified from Coastal Populations of *Eulemur cinereiceps*

The fecal parasites of *E. cinereiceps* were identified for the first time in this study. Four nematodes, one protozoan and several unidentifiable larvae were recovered. This is the first study to identify the parasites infecting *E. cinereiceps*, and is among the first studies investigating detailed patterns in lemur-parasite interactions (*Lemur catta*: Villers et al., 2008; *Eulemur rufifrons*: Clough, 2009; *Microcebus murinus*: Raharivololona and Ganzhorn, 2009; *Lemur catta* and *Propithecus verrreauxi*: Loudon, 2009; *Propithecus edwardsi*: Wright et al., 2009).

Two pinworms belonging to the order Oxyurida, *Callistoura* (likely *C. blanci*; Chabaud et al., 1965) and *Lemuricola* (likely *L. vauceli*; Chabaud et al., 1965), were the most commonly found parasites. A species of *Trichuris*, an Ascarididae, and a species of *Entamoeba* were also identified in *Eulemur cinereiceps* fecal samples from Agnalazaha and Manombo.

5.3 *Callistoura* Infection

Callistoura is a member of the Pharynogodonidae family, a group of parasites typically infecting reptiles and amphibians (Faulkner et al., 2004). *Callistoura* may have arrived on Madagascar with the first adapids, and may represent a several million year old example of lemur-parasite coevoluation (Faulkner et al., 2004). It is unknown if *Callistoura* parasitizes any non-lemur hosts (Irwin and Raharison, 2009). Little is known about the lifecycle of *Callistoura*, but based on other species from the Pharynogodonidae family, it is likely transmitted through fecal-oral contamination, and is expected to be relatively asymptomatic in healthy hosts. The *Callistoura* eggs (likely *C. blanci*) found in this study were larvated, therefore is likely they become infective within hours to days after defecation. This key information in life-cycle

patterns is likely to play a role in host exposure patterns, and the results from this study correspond with the above supposition.

Previous literature investigating *Callistoura* infection in primates have not indicated any seasonal, behavioral, or demographic relationships (See Table 1.1). In the current study, *Callistoura* infection frequency and prevalence in the *E. cinereiceps* populations at Agnalazaha and Manombo clustered with social behavior variables such as group size, group spread, and physical contact. Parameters of *Callistoura* infection did not correlate with any environmental variables except seasonality. Prevalence of *Callistoura* infection was higher during the fall/dry season than the spring/wet season. This result was unexpected and not easily explained. Overall, *Callistoura* infection in *E. cinereiceps* correlated with social behavior variables rather than habitat use or environmental variables.

5.4 *Lemuricola* Infection

Lemuricola is a pinworm belonging to the Oxyuridae family, and is one of eight known species, all of which infect lemur hosts (Irwin and Raharison, 2009). This parasite is closely related to the Oxyuridae *Enterobius*, which infects human and nonhuman primates (Hugot, 1997). Based on this relationship, *Lemuricola* is expected to transfer directly between hosts through fecal-oral contamination and is expected to be relatively asymptomatic in healthy hosts. Like *Callistoura*, *Lemuricola* eggs from this study were often larvated. However, this was not universally the case, and the larvae appeared to be found at different stages of development. *Lemuricola* adult female oviposit their eggs on the perianal area of their host. Eggs attach to the host, likely in the hosts fur and may become larvated during this time. *Lemuricola* is transferred between hosts when 1) eggs are passed in feces either when females do not oviposit directly on

perianal area, or when they have become loosened from the anal region, 2) individuals scent mark branches using anal glands, and this action rubs the eggs onto branches, 3) during grooming, in particular dental grooming of the lower back, upper tail and anal region.

In the *E. cinereiceps* study groups, *Lemuricola* infection clustered with the habitat use behaviors travel time and time spent on the ground, and was also more prevalent at Agnalazaha, where these behaviors were more frequent. The Agnalazaha forest fragments have higher shape indices and more clearings than the Manombo fragments, which may have facilitated the greater travel time and time spent on the ground at Agnalazaha. *Lemuricola* prevalence was higher during the fall/dry season than the spring/wet season, which corresponds with higher seasonal rates of travel time and time spent on the ground.

The current results on *Lemuricola* infection in *E. cinereiceps* largely support previous research on *Lemuricola* prevalence in four lemur species, *Microcebus murinus, Lemur catta, Propithecus verreauxi,* and *Eulemur rufifrons* (Clough, 2009; Loudon, 2009; Raharivololona and Ganzhorn, 2009). A study of *Lemuricola* in *E. rufifrons* found that infection rates did not correlate with group size or age, although contrary to the current study, *Lemuricola* prevalence was higher in male than in female individuals (Clough, 2009). Whether this difference in *Lemuricola* prevalence is connected to a difference in habitat use behaviors between male and female individuals was not accessed as no behavioral data was collected to support the parasite infection study. A study on *L. catta* found a positive correlation with forest quality (Loudon, 2009), although the opposite results were found in this study, since *L. catta* are highly terrestrial in both low and high quality forests, the difference between the Loudon and current study may not represent overall different patterns in host exposure. In addition, *Lemuricola* was found to be more prevalent in *L. catta* than sympatric *P. verreauxi* (Loudon, 2009), suggesting that time

spent on the ground may play a role in *Lemuricola* infection exposure. Similarly, a study on *M. murinus* found that an Oxyuridae parasite (likely *Lemuricola*) did not correlate with forest quality (Rahariviololololona and Ganzhorn, 2009). However, since *M. murinus* rarely travel on the ground, and travel comparatively small distances (than brown lemurs), it is possible that habitat disturbance did not affect habitat use behaviors, and therefore no relationship between habitat quality and *Lemuricola* infection was found. The current study is the only study that used habituated focus groups from more than one forest to study parasite infection rates, host behavior, as well as including a comparison of environmental factors.

Although there is the potential for cross species transmission between other lemur hosts at both sites, this is unlikely to be a mediating factor in *E. cinereiceps Lemuricola* infection. Overall, *Lemuricola* infection was best predicted by habitat use behaviors, likely as a function of environmental variability.

5.5 *Trichuris* Infection

Trichuris eggs were found from *E. cinereiceps* in one forest fragment, the Agnalazaha Coastal Forest fragment (ACS), only during the fall/dry season of 2008. They were present in the fecal samples from eight out of 11 individuals sampled at this time. *Trichuris* is a whipworm with species infecting a variety of mammals globally. Egg morphology from different species are nearly indistinguishable and it was impossible to determine if these *Trichuris* belong to a *Trichuris lemuris*, a species know to infect other lemur hosts, or if the eggs belong to a *Trichuris* species infecting other mammals such as cattle, pigs, mice, or humans. *Trichuris* may have been present in very low frequencies in the ACS population during previous field seasons (2006, 2007, spring 2008) and did not show up in fecal sample collection. If this species of *Trichuris*

represents cross species transmission from another mammalian host, then *Trichuris* may also have been introduced to the population between the spring and fall 2008 field research seasons through local human populations or their domesticated animals. Further information is needed about the potential spread of *Trichuris* between Agnalazaha forest fragments, and about *Trichuris* infections in other lemur species and non-lemur mammals in the forest.

The ACS is the smallest forest fragment in this study. It has the smallest population of *E. cinereiceps* (~24), the lowest population density of *E. cinereiceps*, and the highest shape index of all the studied forest fragments (Ingraldi, 2010). At this time, environmental variables can not be sufficiently linked to the presence of *Trichuris* in this forest fragment. In particular, if the *Trichuris* is a newly introduced species, then it may spread to the second forest fragment at Agnalazaha (Agnalazaha Inland Forest).

Trichuris was not found from individuals in any of the habituated study groups and no behavior data can be linked to their presence. In the ACS, where *Trichuris* was recovered, prevalence did not correlate with age or sex. *Trichuris* was found to correlate positively with age but not with sex in *Eulemur rufifrons* (Clough, 2009). Although in *E. rufifrons Trichuris* infections did not correlate with group size, there was a negative relationship between *Trichuris* infection and group size in *Procolobus rufomitratus* (Chapman et al., 2009b; Clough, 2009). In addition, *Trichuris* infection was seen to correlate negatively with forest quality in *Colobus angolensis palliates*, while no relationship was observed in *Microcebus murinus* (Okanga et al., 2006; Raharivololona and Ganzhorn, 2009). There are two conclusions that can be drawn from comparing results from the current study with those from previous studies: 1) Although *Trichuris* is a commonly found parasite, it may include many different species with different life cycles qualities that affect host exposure differently, 2) more information is needed on the behavioral

correlates of *Trichuris* infection to assist with the interpretation of demographic and environmental variables.

5.6 Ascarid Infection

Eggs from a fourth nematode were identified as roundworm eggs most likely from the family Ascarididae. An adult ascarid, *Ascaris petiti*, was described from *Daubentonia madagascarensis* by Chabaud (1964), however no eggs were described. The ascarid eggs in this study do not closely resemble eggs reported from other lemurs (Anderson, 2000), or those known to infect domesticated animals or humans. This may represent a new species, or they may be the eggs belonging to the adult worm described by Chabaud (1964). Ascarididae are typically transmitted through fecal-oral contamination, and small infections are relatively harmless to their hosts. Unlike the other nematode eggs found in this study, Ascarididae eggs often do not become infective for several months after defecation. Therefore it is likely that infected individuals may have been exposed two-three months before the current study.

Ascarid infection was significantly more prevalent during the fall/dry season than the spring/wet season, however, based on the lifecycle information of other Ascarididae, it is possible that infective eggs were ingested during the spring/wet season and did not mature in their hosts to pass eggs through the feces until the fall/dry season. Ascarid prevalence and frequency did not correlate with any behavior variables other than home range size, where it approached significance. Ascarid prevalence was also present only in the three study groups whose home range overlapped water sources (swamp or river). Overall, ascarid infection in *E. cinereiceps* was best predicted by home range size and location.

Similarly, a study on *Gorilla gorilla* did not find that age or sex correlated with Ascarid infection (Lilly et al., 2002). A previous study on *Microcebus murinus* found that *Trichuris* infection prevalence was greater in low quality forests (Raharivololona and Ganzhorn, 2009). Although there was no relationship between forest quality and ascarid infection in the current study, it possible that the lower quality forests in Raharivololona and Ganzhorn were prone to greater flooding or occurred in swampy forests. More research into the links between habitat location, quality and home range size should be investigated in lemur ascarid infections.

5.7 *Entamoeba* Infection

An *Entamoeba* species was identified from cyst morphology in the fecal samples from *Eulemur cinereiceps*. A molecular analysis on similar cysts from *Eulemur ruffifrons* identified the genus *Entamoeba*, but was unable to further identify the species (Clough, 2010). It is likely that these parasites, as well as the nematodes, are transmitted through fecal-oral contamination of food and water sources.

Entamoeba infections were more prevalent at Manombo than Agnalazaha. Fruit availability and correspondingly *E. cinereiceps* time spent foraging for fruit was lower at Manombo than at Agnalazaha. Overall, as fruit availability increased, *Entamoeba* infections decreased, although not as a direct result of individual time spent foraging for fruit. Therefore, fruit availability is likely a predictor of *Entamoeba* infection either as an indicator of individual health, or habitat/forest health.

5.8 Behavioral and Environmental Correlates of *Eulemur cinereiceps* Parasite Burden

The behavioral and environmental correlates of *Eulemur cinereiceps* parasite infection parameters varied by parasite species. Overall, neither behavior, nor environmental variables were a better predictor of parasite burden in the Agnalazaha and Manombo *E. cinereiceps* populations. *Callistoura* infection was best predicted by social behaviors. *Lemuricola* infection was best predicted by habitat use behaviors likely mediated by environmental variables. *Entamoeba* infection was best predicted by environmental variables and the corresponding habitat use behaviors. And ascarid infection was best predicted by environmental variables.

Overall parasite burden, measured by parasite species richness and the frequency of having any parasite infection, did not correlate with any demographic, behavioral, or environmental variables (see chapter 4, Table 4.12). Only *Trichuris* was not found in all four forest fragments which makes the parasite species richness at the Agnalazaha Coastal Forest significantly higher than the parasite species richness found in the other three forest fragments in this study. However, at this time, the presence of *Trichuris* at the Agnalazaha Coastal Forest can not be convincingly attributed to environmental variables.

5.9 Seasonal Differences in *Eulemur cinereiceps* Parasite Burden

Parasite burden is expected to increase during warm and wet seasons (Nunn and Altizer, 2006). However, the opposite was found during this study. This may be the result of varied field and laboratory techniques between the two seasons. During the spring/wet season, all samples were preserved in 90% ethanol and evaluated with fecal sedimentation. During the fall/dry season, all samples were preserved in 10% formalin and evaluated with fecal flotation. As a part of this dissertation, a study of preservation solution and recovery technique indicated that

although 10% formalin yields higher egg counts and parasite species richness, the results approach those found when using 90% ethanol preservative with a fecal sedimentation fecal recovery technique. A second explanation for the increase in parasite burden during the fall/dry season are hormone and social behavior variation. Social behavior is an unlikely antecedent of seasonal variation in parasite burden since social behavior did not significantly change between the two seasons. Seasonal variation in hormone levels may affect host susceptibility and therefore parasite burden. A third alternative hypothesis is that frequent rain during the spring/wet season washed fecal contamination away from frequently used areas, minimizing a host's chances to encounter infective parasites through fecal contamination. At this time, the seasonal differences in parasite burden cannot be conclusively attributed to any of these hypotheses.

5.10 Conclusions

This research investigated host exposure to parasites transferred through fecal contamination in wild populations of *Eulemur cinereiceps*. This research is important for several reasons:

1) This was the first study to identify the fecal parasites of *E. cinereiceps*.

2) Identifying the parasite species of *E. cinereiceps* allows for the development of more detailed studies on their host-parasite relationships and for monitoring their disease risk.

3) As a critically endangered species, this study identifies several important risk factors in *E. cinereiceps*. The parasite infections of the ascarid and *Entamoeba* are likely to pose the greatest potential threat to the population. Because the ascarid was found only in groups whose home range overlapped water sources, park management should ensure that other

areas of the home range remain intact to prevent a higher prevalence and/or frequency of these infections throughout the population. In addition, water sources are used by human and domesticated animal populations and should be treated as potentially harmful due to cross-species contamination (zoonoses). *Entamoeba* infection correlated with fruit availability within each forest fragment. This suggests that *Entamoeba* infection may represent an indicator of *E. cinereiceps* health and habitat/forest health. To better preserve both the *E. cinereiceps* and the forest, park management should prioritize protection of fruit-baring trees.

4) Several of the parasites found in this study are found in other lemur species. Identifying all of a parasite's potential hosts allows for further examination of the spread of disease in wild populations, possible cross-species contamination, and the co-evolution of hosts and parasites.

5) This research indicates several environmental and host behavioral correlates for the infection of four parasite species.

6) This research investigates relatively asymptomatic parasites in *E. cinereiceps* metapopulations. The pinworms in particular may serve as a model system for parasite exposure in more virulent parasites transmitted through fecal contamination.

7) All the parasites identified in this study are expected to transfer to new hosts through fecal-oral contamination. However, the host behavioral and environmental variables mediating host exposure and parasite burden varies for each parasite species. This suggests that even parasites with similar modes of transmission may transfer between hosts and spread through populations using different mechanisms.

5.11 Recommendations for Future Research

Future primate-parasite research at Agnalazaha and Manombo should focus on a community ecology approach. This should include identifying the parasites infecting other lemur species, other mammal species, and domesticated animals found in and around the forest. This will further illuminate the potential for cross-species transmission of the parasites from *E. cinereiceps*. A molecular analysis of the parasites found in *E. cinereiceps* samples will allow for better identification of the parasites. Detailed information is needed about the parasites' lifecycles, symptoms, and mode of transmission. Studies investigating host-parasite interactions require understanding of both organisms and their environment, and the current lack of information about most lemur parasites hinders better interpretations of available data. Furthermore, the new presence of *Trichuris* in the Agnalazaha Coastal Forest fragment is an area of interest that could be further pursued.

5.12 Tables

Table 5.1 Research questions, predictions, and results.

	Research Question	Prediction	Result
1.	What is the most efficient and effective way to evaluate fecal parasite burden for *E. cinereiceps*?	The following will affect the precision of fecal parasite recovery: 1) preservation solution, 2) recovery technique, 3) quantity of feces, and when using fecal flotation, 4) flotation solution specific gravity and 5) consistency of the fecal sample.	1) 10% formalin was a better preservation solution than 90% ethanol. 2) Fecal sedimentation was more sensitive to egg counts than fecal flotation. Fecal flotation can be improved with repeated trials on the same sample. 3) At least 2 grams of feces or 60% of the sample should be used for parasite species richness, and at least 3 grams of feces or 77% of the sample should be used for egg counts. 4) Sheather's solution with 1.32 or 1.27 specific gravity did not affect results. 5) Consistency of the fecal sample did not affect results.
2.	What are the fecal parasites infecting the coastal populations of *E. cinereiceps*?	Nematode and protozoan parasites will be identified.	*Callistoura*, *Lemuricola*, *Trichuris*, *Entamoeba*, an ascarid species, and two unidentified larvae were recovered. Additionally a number of ectoparasites were also recovered.
3.	What are the demographic variables associated with parasite burden in *E. cinereiceps*?	Parasite burden will not vary with age, sex and group size.	Parasite burden did not vary with age and sex. Group size correlated with the average group frequency of *Callistoura* infection.
4.	What are the social behavior variables associated with parasite burden in *E. cinereiceps*?	Group spread and physical contact will correlate with parasite burden.	Group spread and physical contact correlated with *Callistoura* infection.
5.	What are the habitat use behavior variables associated with parasite burden in *E. cinereiceps*?	Travel time, foraging time, height in the canopy, time spent on the ground, home range size, and local density will correlate with parasite burden.	Average group ravel time and time spent on the ground correlated with *Lemuricola* infection. Local density correlated with average group *Callistoura* frequency. Average group foraging time correlated with *Entamoeba* infection. Home range size correlated with ascarid infection prevalence and frequency.

	Research Question	Prediction	Result
6.	What are the environmental variables associated with parasite burden in *E. cinereiceps*?	Seasonality, food availability, fruit availability, forest quality (assessed by height of the canopy, tree species richness, DBH, shape indices, clearings, logging, presence of human and domesticated animals in the forest), lemur species richness, *E. cinereiceps* population density and home range microhabitat will correlate with parasite burden.	*Lemuricola* infection varied by forest disturbance. *Entamoeba* infection varied by fruit availability. Ascarid infection varied by home range microhabitat, specifically by the presence of water within the home range. *Callistoura*, *Lemuricola*, and ascarid infection prevalence were higher during the fall/dry season than the spring/wet season.
7.	Are social behaviors, habitat use behaviors, or environmental variables a better predictor of parasite burden in *E. cinereiceps*?	Host behaviors will better predict parasite burden than environmental variables.	The results were parasite species specific. *Callistoura* infection was best predicted by host social behavior. *Lemuricola* infection was best predicted by habitat use behaviors mediated by environmental variables. Ascarid infection was best predicted by home range size and location. *Entamoeba* was best predicted by environmental variables and corresponding host behaviors.

References

Altizer S., Nunn C., Thrall P., Gittleman J., Antonovics J., Cunningham A., Dobson A., Ezenwa V., Jones K., Poss A., Pulliam J. 2003. Social organization and parasite risk in mammals: integrating theory and empirical studies. *Annual Review of Ecology, Evolution, and Systems*, 34: 517-547.

Altizer S., Nunn C., and Lindenfors P. 2007. Do threatened hosts have fewer parasites? A comparative study in primates. *Journal of Animal Ecology,* 76: 304-314.

Anderson R. 2000. *Nematode parasites of vertebrates: their development and transmission*. Wallingford: CABI Publishing.

Anderson R., May R. 1979. Population biology of infectious diseases: Part 1. *Nature,* 280: 361–367.

Andrainarivo C., Andriaholinirina V., Feistner A., Felix T., Ganzhorn J., Garbutt N., Golden C., Konstant B., Louis E., Meyers D., Mittermeier R., Perieras A., Princee F., Rabarivola J., Rakotosamimanana B., Rasamimanana H., Ratsimbazafy J., Raveloarinoro G., Razafimanantsoa A., Rumpler Y., Schwitzer C., Thalmann U., Wilmé L., Wright P. 2008. *Eulemur cinereiceps*. In: IUCN 2010. IUCN Red List of Threatened Species. Version 2010.4.

Arneberg P. 2001. Ecological law and its macroecological consequences as revealed by studies of relationships between host densities and parasite prevalence. *Ecography*, 24: 352–359.

Arneberg P., Skorping A., Grenfell B., Read A. 1998. Host densities as determinants of abundance in parasite communities. *Proc R Soc B*, 265: 1283-1289.

Ashford R., Reid G., Butynski T. 1990. The intestinal faunas of man and mountain gorillas in a shared habitat. *Annuals of Tropical Medical Parasitology*, 84: 337-340.

Bertolino S., Wauters L., Bruyn L., Canestri-Trotti G. 2003. Prevalence of coccidian parasites (Protozoa) in red squirrels (*Sciurus vulgaris*): effects of host phenotype and environmental factors. *Oecologia*, 137: 286-295.

Bollen A., Donati G. 2006. Conservation status of the littoral forest of south-eastern Madagascar: a review. *Oryx*, 40: 1-10.

Bonhomme M., Blancher A., Jalil M., Crouau-Roy B. 2007. Factors shaping genetic variation in the MHC of natural non-human primate populations. *Tissue Antigens* 70, 398-411.

Bowman D. 2003. *Georgis' Parasitology for Veterinarians*. 8th edition. Philadelphia: W.B. Saunders Company.

Bowman D., Lynn R. 1995. *George's Parasitology for Veterinarians*, 6th edition. Sydney: W.B. Saunders company.

Branda J., Lin T., Rosenberg E., Halpern E., Ferraro M. 2006. A rational approach to the stool ova and parasite examination. *Clinical Infectious Diseases,* 42: 972-978.

Brenneman R., Johnson S., Bailey C., Ingraldi C., Delmore K., Wyman T., Andriamaharoa H., Ralainasolo F., Ratismbazafy J., Louis E. In review. Population genetics and abundance of gray-headed lemurs (*Eulemur cinereiceps*) in southeastern Madagascar. *Oryx*.

Bush A., Lafferty K., Lotz J., Shostak A. 1996. Parasitology meets ecology on its own terms: Margolis et al. revisited. *Journal of Parasitology* 83, 575-583.

Centers for Disease Control and Prevention. 2011. Parasites. CDC, Global Health. Atlanta, GA: cdcinfo@cdc.gov.

Chabaud A., Brygoo E. 1964. L'endémisme chez les Helminthes de Madagascar. *C.R.Soc. Biogéogr.,* 356: 3-13.

Chabaud A., Brygoo E., Petter A. 1965. Les nematodes parasites de lemuriens malgaches: description de six especes nouvelles et conclusions generales. *Annales de Parasitologie*, 40: 181-214.

Chapman C., Gillespie T., Speirs M. 2005. Parasite prevalence and richness in sympatric colobines: effects of host density. *American Journal of Primatology,* 67: 259-266.

Chapman C., Hodder S., Rothman J. 2009a. Host-parasite dynamics: connecting primate field data to theory. In: *Primate Parasite Ecology*. Huffman M., Chapman C., editors. Cambridge: Cambridge University Press. pp. 463-483.
Chapman C., Rothman J., Hodder S. 2009b. Can parasite infections be a selective force influencing primate group size? In: *Primate Parasite Ecology*. Huffman M., Chapman C., editors. Cambridge: Cambridge University Press. pp. 423-440.
Chapman C., Wasserman M., Gillespie T., Speirs M., Lawes M., Saj T., Ziegler T. 2006. Do food availability, parasitism, and stress have synergistic effects on red colobus populations living in forest fragments? *American Journal of Physical Anthropology*, 131: 525-534.
Cheney D., Seyfarth R., Andelman S., Lee P. 1988. Reproductive success in vervet monkeys. In T. Clutton-Brock, editor, *Reproductive Success*. Chicago: University of Chicago Press. pp. 384–402.
Cleveland L. 1926. Symbiosis among animals with special references to termintes and their intestinal flagellates. *Quarterly Review of Biology*, 1: 51-60.
Clough D. 2009. Variation in susceptibility to parasite infection: patterns, determinants and consequences in red-fronted lemurs. Dissertation from Gottingen University.
Clough D. 2010. Gastro-intestinal parasites of red-fronted lemurs in Kirindy Forest in western Madagascar. *Journal of Parasitology,* 96: 245-251.
Coop R., Holmes P. 1996. Nutrition and parasite interaction. *International Journal of Parasitology*, 26: 951–962.
Coop R., Kyriazakis I. 1999. Nutrition-parasite interaction. *Veterinary Parasitology*, 84: 187–204.
Coop R., Kyriazakis I. 2001. Influence of host nutrition on the development and consequences of nematode parasitism in ruminants. *Trends in Parasitology*, 17: 325–330.
Cort W. 1942. Human factors in parasite ecology. *The American Naturalist*, 76: 113-128.
Côté I., Poulin R. 1995. Parasitism and group size in social animals: a meta-analysis. *Behavioral Ecology*, 6: 159-165.
Cote S., Stien A., Irvine J., Dallas J., Marshall F., Halvorsen O., Langvatn R., Albon S. 2005. Resistance to abomasal nematodes and individual genetic variability in reindeer. *Molecular Ecology*, 14: 4159-4168.
Cowen D., Wolf A. 1945. Toxoplasmosis in the monkey: acute fatal infection experimentally produced in a young *Macaca mulatta*. *The Journal of Infectious Diseases*, 77: 144-157.
Cuvier F., St. Hilaire G. 1829. Histoire Naturelle des Mammiferes. *Bulletin Museum Naturelle Histoire Paris* p. 218. In: Stein R. 1936. The myth of the lemur's comb. *The American Naturalist*, 70:19-28.
Da Silva A., Gillard J., Yoccoz N., Hewison A., Galan M., Coulson T., Allaine D., Vial L., Delorme D., Van Laere G., Klein F., Luikart G. 2009. Heterozygosity-fitness correlations revealed by neutral and candidate gene markers in roe deer from a long-term study. *Evolution*, 63: 403-417.
Di Bitetti M., Vidal E., Baldovino M., Benesovsky V. 2000. Sleeping site preferences in tufted capuchin monkeys (*Cebus apella nigritus*). *American Journal of Primatology*, 50: 257-274.
Dobson A., Lyles A. 2000. Black-footed ferret recovery. *Science*, 288: 985-988.
Dumetz N. 1999. High plant diversity of lowland rainforest vestiges in eastern Madagascar. *Biodiversity and Conservation*, 8: 273-315.
Duszynski D., Wilson W., Upton S., Levine N. 1999. Coccidia (Apicomplexa: Eimeriidae) in the primates and the scandentia. *International Journal of Primatology*, 20: 763-797.
Ebert D., Lipsitch M., Mangin K. 2000. The effect of parasites on host population density and extinction: experimental epidemiology with daphnia and six microparasites. *The American Naturalist*, 156: 459-477.
Ezenwa, V. 2003. Habitat overlap and gastrointestinal parasitism in sympatric African bovids. *Parasitology*, 126: 379-388.

Ezenwa V. 2004. Host social behavior and parasite infection: a multifactorial approach. *Behavioral Ecology*, 15: 446-454.
Ezenwa V., Price S., Altizer S., Vitone N., Cook K. 2006. Host traits and parasite species richness in even and odd-toed hoofed mammals, Artiodactyla and Perissodactyla.. *Oikos*, 115: 526-536.
Faulkner C., Britt A., Welch C. 2004. Endoparasitic infections of Malagasy Lemurids. Paper presented at *American Society of Parasitology*, Philadelphia: July, 2004.
Fleagle J. 1999. *Primate Adaptations and Evolution*. Academic Press, Inc.: New York, New York.
Freeland W. 1976. Pathogens and the evolution of primate sociality. *Biotropica*, 8:12-24.
Freeland W. 1979. Primate social groups as biological islands. *Ecology*, 60: 719–728.
Freeland, W. 1980. Mangabey (*Cercocebus albigena*) movement patterns in relation to food availability and fecal contaminations. *Ecology*, 61: 1297-1303.
Gillespie T. 2006. Noninvasive assessment of gastrointestinal parasite infections in free-ranging primates. *International Journal of Primatology*. 27, 1129-1143.
Gillespie T., Chapman C. 2004. Forest fragmentation alters primate parasite dynamics: implications for primate health and conservation. *Folia Primatologica*, 75: 267.
Global Mammal Parasite Database. 2008. www.mammalparasites.org/. February 2010.
Gregory W. 1920. *American Museum of Natural History*, 3: 203. In: Stein R. 1936. The myth of the lemur's comb. *The American Naturalist*, 70:19-28.
Greiner E., McIntosh A. 2009. Collection methods and diagnostic procedures for primate parasitology. In: *Primate Parasite Ecology*. Huffman M., Chapman C., editors. Cambridge: Cambridge University Press. pp. 3-28.
Grompper M., Williams E. 1998. Parasites and conservation: insights from the black-footed ferret recovery program. *Conservation Biology*, 12: 730–732.
Grompper M., Wright A. 2005. Altered prevalence of raccoon roundworm (*Baylisascaris procyonis*) owing to manipulated contact rates of hosts. *Journal of Zoology (London)*, 266: 215-219.
Hamilton W., Zuk M. 1982. Heritable true fitness and bright birds: a role for parasites? *Science*, 218: 384-387.
Hegner R. 1928. The evolutionary significance of the protozoan parasites of monkey and man. *The Quarterly Review of Biology*, 3: 225-244.
Hegner R. 1937. Parasite reactions to host modifications. *The Journal of Parasitology*, 23: 1-12.
Hinde K. 2007. Milk composition varies in relation to the presence and abundance of *Balatidium coli* in the mother in captive rhesus macaques (*Macaca mulatta*). *American Journal of Primatology*, 69: 625-634.
Hoogland J. 1979. Aggression, ectoparasitism, and other possible costs of prairie dog (Sciuridae, *Cynomys* spp.) coloniality. *Behaviour*, 69: 1–35
Hoste H., Torres-Acosta J., Paolini V., Aguilar-Cabbellero A., Etter E., Lefrileux Y., Chartier C., Broqua C. 2005. Interactions between nutrition and gastrointestinal infections with parasitic nematodes in goats. *Small Ruminant Research* 60, 141-151.
Huffman M., Chapman C. 2009. *Primate Parasite Ecology: The dynamics and study of host-parasite relationships*. Cambridge: Cambridge University Press.
Huffman M., Gotoh S., Turner L., Hamai M., Toshida K. 1997. Seasonal trends in intestinal nematode infection and medicinal plant use among chimpanzees in the Mahale Mountains, Tanzania. *Primates,* 38: 111-125.
Hugot J. 1999. Primates and their pinworm parasites: The Cameron hypothesis revisited. *Systematic Biology*, 48: 523-546.
Hugot J., Demanche C., Barriel V., Dei-Cas E., Guillot J. 2003. Phylogenetic systematics and evolution of primate-derived *Pneumocystis* based on mitochondrial or nuclear DNA sequence comparison. *Systematic Biology,* 52: 735-744.
Hugot J., Gardner S., and Morand S. 1996. The Enterobiinae fam. nov. (Nematoda, Oxyurida), parasites of primates and rodents. *International Journal of Parasitology*, 26: 147–159.

Ingale S., Mulik S., Suryawanshi A., Zadbuke S. 2010. Nutrition-Parasite Interaction- A Review. *Agricultural Review*, 31: 48-55.

Ingraldi C. 2010. Forest fragmentation and edge effects on eight sympatric lemur species in southeast Madagscar. Masters thesis, University of Calgary, Calgary.

Irwin M., Johnson S., and Wright P. 2005 The state of lemur conservation in south-eastern Madagascar: population and habitat assessments for diurnal and cathemeral lemurs using surveys, satellite imagery and GIS. *Oryx*, 39: 204-218.

Irwin M., Raharison J. 2009. A review of the endoparasites of the lemurs of Madagascar. *Malagasy Nature*, 2: 66-93.

Johnson S. 2002. Ecology and speciation in brown lemurs: white collared lemur (*Eulemur albocollaris*) and hybrids (*Eulemur albocollaris* x *Eulemur fulvus rufrus*) in southeastern Madagascar. Dissertation, University of Texas, Austin, Tx.

Johnson S. 2006 Evolutionary divergence in the brown lemur species complex. In: Gould L., Sauther M., editors: *Lemurs: Ecology and Adaptation*. New York: Springer, pp. 189-212.

Johnson S., Overdorff D. 1999. A census of brown lemurs (*Eulemur fulvus* sspp.) in southeastern Madagascar: methods-testing and conservation implications. *American Journal of Primatology*, 47: 51-60.

Johnson S., Ingraldi C., Ralainasolo F., Andriamaharoa H., Ludovic R., Birkinshaw C., Wright P., and Ratsimbazafy J. 2011, In Press. Gray-headed lemur (*Eulemur cinereiceps*) abundance and forest structure dynamics at Manombo, Madagascar. Biotropica.

Johnson S., Lei R,, Martin S., Irwin M., and Louis E. 2008. Does *Eulemur cinereiceps* exist? Preliminary evidence from genetics and ground surveys in southeastern Madagascar. *American Journal of Primatology*, 70: 372-385.

Johnson S., Wyner Y. 2000. Notes on the biogeography of *Eulemur fulvus albocollaris*. *Lemur News*, 5: 25–28.

Jones W. 1918. *Journal of Anatomy*, 52: 345-346. In: Stein R. 1936. The myth of the lemur's comb. *The American Naturalist*, 70:19-28.

Junge R., Louis E. 2005. Biomedical evaluation of two sympatric lemur species (*Propithecus verreauxi deckeni* and *Eulemur fulvus rufus*) in Tsiombokibo Classified Forest, Madagascar. *Journal of Zoo and Wildlife Medicine* 36, 581-589.

Kellogg V. 1914. Ectoparasites of mammals. *The American Naturalist*, 48: 257-279.

Lilly A., Mehlman P., Doran D. 2002. Intestinal parasites in gorillas, chimpanzees, and humans at Mondika Research Site, Dzanga-Ndoki National Park, Central African Repulic. *International Journal of Primatology*, 23: 555-573.

Lindenfors P., Nunn C., Jones K., Cunningham A., Sechrest W., Gittleman J. 2007. Parasite species richness in carnivores: effects of host body mass, latitude, geographic range and population density. *Global Ecology and Biogeography*, 16: 496-509.

Loehle C. 1995. Social barriers to pathogen transmission in wild animal populations. *Ecology*, 76: 326-335.

Loudon J. 2009. The parasite ecology and socioecology of ring-tailed lemurs (*Lemur catta*) and Verreaux's sifaka (*Propithecus verreauxi*) inhabiting the Beza Mahafaly Special Reserve. Dissertation from Central Washington University.

Loudon J., Sauther M., Fish K., Hunter-Ishikawa M., Ibrahim Y. 2006. One reserve, three primates: Applying a holistic approach to understand the interconnections among ring-tailed lemurs (*Lemur catta*), Verreaux's sifaka (*Propithecus verreauxi*), and humans (*Homo sapiens*) at Beza Mahafaly Special Reserve, Madagascar. *Ecological and Environmental Anthropology*, 2: 54-74.

Margolis L., Esch G., Holmes J., Kuris, A., Schad, G. 1982. The use of ecological terms in parasitology. *Journal of Parasitology*, 68: 131-133.

Martin R. 1972. Adaptive radiation and behaviour of the Malagasy lemurs. *Philosophical Transactions of the Royal Society of London Series B. Biological Sciences*, 264: 295-352.

Martin S., Rakotoarivlo H., Kutz S. 2009. Challenges associated with preservation and isolation of parasite stages in non-human primate species: words of caution (poster presentation). American Association of Physical Anthropologists (AAPA) Annual Meeting, April 2009. Abstract in *American Journal of Physical Anthropology* Supplement, 138: 185.

Martinsen E., Paperna I., Schall J. 2006. Morphological versus molecular identification of avian Haemosporidia: an exploration of three species concepts. *Parasitology*, 133: 279-288.

Mathews F. 2009. Zoonoses in wildlife: integrating ecology into management. *Advances in Parasitology*, 68: 185-209.

May R. 1988. Conservation and disease. *Conservation Biology*, 2: 28-30.

May R., Anderson R. 1978. Regulation and stability of host-parasite population interactions. 2. Destabilizing processes. *Journal of Animal Ecology*, 47: 249-267.

Mbora D., McPeek M. 2009. Host density and human activities mediate increased parasite prevalence and richness in primates threatened by habitat loss and fragmentation. *Journal of Animal Ecology* 78: 210-218.

Meagher S. 1999. Genetic diversity and *Capillaria hepatica* (Nematoda) prevalence in Michigan deer mouse populations. *Evolution*, 54: 1318-1324.

Michaud C., Tantalean M., Ique C., Montoya E., Gozalo A. 2003. A survey for helminth parasites in feral New World non-human primate populations and comparison with parasitological data from man in the region. *Journal of Medical Primatological*, 32: 341-345.

Milton K. 1996. Effects of bot fly (*Alouattamyia baeri*) parasitism on a free-ranging howler monkey (*Alouatta palliata*) population in Panama. *Journal of Zoology (London)*, 239: 39-63.

Mittermeier R., Konstant W., Hawkins F., Louis E., Langrand O., Ratsimbazafy J., Rasoloarison R., Ganzhorn J., Rajaobelina S., Tattersall I., and Meyers D. 2006. Lemurs of Madagascar. Washington, D. C.: Conservation International.

Mittermeier R., Wallis J., Rylands A., Ganzhorn J., Oates J., Williamson E., Palacios E., Heymann E., Kierulff M., Long Y., Supriatna J., Roos C., Walker S., Cortés-Ortiz L., Schwitzer C. (eds.). 2009. *Primates in Peril: The World's 25 Most Endangered Primates 2008–2010*. Arlington, VA: IUCN/SSC Primate Specialist Group (PSG), International Primatological Society (IPS), and Conservation International (CI). pp. 84.

Morand S., Poulin R. 1998. Density, body mass and parasite species richness of terrestrial mammals. *Evolutionary Ecology*, 12: 717–727.

Muehlenbein M. 2005. Parasitological analyses of the male chimpanzees (*Pan troglodytes schweinfurthii*) at Ngogo, Kibale National Park, Uganda. *American Journal of Primatology*, 65: 167-179.

Muehlenbein M., Schwartz M., Richard A. 2003. Parasitologic analyses of the sifaka (*Propithecus verreauxi verreauxi*) at Beza Mahafaly, Madagascar. *Journal of Zoo and Wildlife Medicine,* 34: 274-277.

Negre A., Tarnaud L., Roblot J., Gantier J., Guillot J. 2006. Plants consumed by *Eulemur fulvus* in Comoros Islands (Mayotte) and potential effects on intestinal parasites. *International Journal of Primatology*, 27: 1495-1517.

Nunn C., Altizer S. 2006. *Infectious Diseases in Primates: Behavior, Ecology and Evolution*. Oxford University Press, New York.

Nunn C., Altizer S., Jones K., Sechrest W. 2003. Comparative tests of parasite species richness in primates. *The American Naturalist,* 162: 597-614.

Nunn C., Thrall P., Leendertz F., Boesch C. 2011. The spread of fecally transmitted parasites in structured populations. *PLos ONE* 6, e21677. Doi: 10.1371/journal.pone.0021677

Nunn C., Altizer S., Sechrest W., Jones K., Barton R., Gittleman J. 2004. Parasites and the evolutionary diversification of primate clades. *The American Naturalist*, 164: 90-103.

Odoi A., Gathuma J., Gachuiri C., Omore A. 2007. Risk factors of gastrointestinal nematode parasite infections in small ruminants kept in smallholder mixed famrs in Kenya. *BMC Veterinary Research*, 3: 1-6.

Okanga S., Muchemi G., Maingi N., Mogoa E., Munene E. 2006. Gastrointestinal parasites of free-ranging Colobus monkeys (*Colobus angolensis palliatus*) in Kwale district, Kenya coast. *African Journal of Ecology*, 44: 410-412.

Oliver M., Telfer S., Piertney S. 2009. Major histocompatibility complex (MHC) heterozygote superiority to natural multi-parasite infections in the water vole (*Arvicola terrestris*). *Proc R Soc B*, 276: 1119-1128.

Overdorff D., Johnson S. 2003. *Eulemur*, True Lemurs, In: *The Natural History of Madagascar*, Chicago: University of Chicago Press.

Pedersen A., Jones K., Nunn C., Altizer S. 2007. Infection diseases and extinction risk in wild mammals. *Conservation Biology*, 21: 1269-1279.

Poulin R., Morand S. 2000. Diversity of parasites. *The Quarterly Review of Biology*, 75: 277-293.

Raharivololona B. 2009. Parasites gastro-intestinaux de *Microcebus murinus* de la foret littorale de Mandena,Madagascar. *Madagascar, Conservation and Development*, 4: 52-62.

Raharivololona B., Ganzhorn J. 2009. Gastrointestinal parasite infection of the gray mouse lemur (*Microcebus murinus*) in the littoral forest of Mandena, Madagascar: effects of forest fragmentation and degradation. *Madagascar, Conservation and Development*, 4: 103-112.

Ralainasolo F., Ratsimbacafy J., Stevens N. 2008. Behavior and diet of critically endangered *Eulemur cinereiceps* in Manombo forest, southeast Madagascar. *Madagascar Conservation and Development*, 3: 38-43.

Ratsimbazafy J. 2002. On the brink of extinction and the process of recovery: Responses of black-and-white ruffed lemurs (*Varecia variegata variegata*) to disturbance in Manombo Forest, Madagascar. Ph.D. Dissertation, State University of New York at Stony Brook, Stony Brook, New York.

Ratsimbazafy J. 2006. Diet composition, foraging, and feeding behavior in relation to habitat disturbance: implications for the adaptability of ruffed lemurs (*Varecia v. editorum*) in Manombo forest, Madagascar. In: Gould L., Sauther M., editors: *Lemurs: Ecology and Adaptation*. New York: Springer, 403-422.

Ratsimbazafy J., Ramarosandratana H., Zaonarivelo R. 2002. How do black-and-white ruffed lemurs still survive in a highly disturbed habitat? *Lemur News,* 7: 7-10.

Read A., Allen J. 2000. Evolution and immunology: the economics of immunity. *Science*, 290: 1104-1105.

Reed D., Light J., Allen J., Kirchman J. 2007. Pair of lice lost or parasites regained: the evolutionary history of anthropoid primate lice. *BMC Biology* 5.

Rothman J., Pell A., Bowman D. 2009. How does diet quality affect the parasite ecology of mountain gorillas? In: *Primate Parasite Ecology*. Huffman M., Chapman C., editors. Cambridge: Cambridge University Press. pp. 441-462.

Rubel D., Wisnivesky C. 2005. Magnitude and distribution of canine fecal contamination and helminth eggs in two areas of different urban structure, Greater Buenos Aires, Argentina. *Veterinary Parasitology*, 133: 339-347.

Rubenstein D., Hohmann M. 1989. Parasites and social behavior in island feral horses. *Oikos,* 55: 312–320.

Schrag S., Wiener P. 1995. Emerging infectious disease: what are the relative roles of ecology and evolution? *Trends in Ecology and Evolution*, 10: 319–324.

Schwensow N., Fietz J., Dausmann K., Sommer S. 2007. Neutral versus adaptive genetic variation in parasite resistance: importance of major histocompatibility complex supertypes in a free-ranging primate. *Heredity,* 99: 265-277.

Semple S., Cowlishaw G., Bennett P. 2002. Immune system evolution among anthropoid primates: parasites, injuries and predators. *Proceedings: Biological Sciences*, 269: 1031-1037.

Setchell J., Bedjabaga I., Goossens B., Reed P., Wickings E., Knapp L. 2007. Parasite prevalence, abundance, and diversity in a semi-free-ranging colony of *Mandrillus sphinx*. *International Journal of Primatology,* 28: 1345-1362.

Sorci G., Skarstein F., Morand S., Hugot J. 2003. Correlated evolution between host immunity and parasite life histories in primates and oxyurid parasites. *Proceedings: Biological Sciences*, 270: 2481-2484.
Spehn S., Ganzhorn J. 2000. Influence of seed dispersal by brown lemurs on removal rates of three *Grewia* species (Tiliaceae) in the dry deciduous forest of Madagascar. *Ecotropica*, 6: 13-21.
Stein R. 1936. The myth of the lemur's comb. *The American Naturalist*, 70:19-28.
Stoner K. 1996. Prevalence and intensity of intestinal parasites in mantled howling monkeys (*Alouatta palliata*) in northeastern Costa Rica: implications for conservation biology. *Conservation Biology*, 10: 539-546.
Stuart M., Greenspan L., Glander K., Clarke M. 1990. A carpological survey of parasites of wild mantled howling monkeys, *Alouatta palliata palliata*. *Journal of Wildlife Diseases* 26, 547-549.
Stuart M., Strier K. 1995. Primates and parasites: a case for a multidisciplinary approach. *International Journal of Primatology*, 15: 577-593.
Stuart M., Strier K., Pierberg S. 1993. A coprological survey of parasites of wild muriquis, *Brachyteles arachnoids*, and brown howling monkeys, *Alouatta fusca*. *Journal of Helminth Society Washington,* 60: 111-115.
Stuart S., Cowlishaw G., Bennett P. 2002. Immune system evolution among anthropoid primates: parasites, injuries and predators. *The Proceedings of Biological Sciences*, 269: 1031-1037.
Sykes A. 2008. Manipulating host immunity to improve nematode parasite control- quo vadit. *Parasite Immunology*, 30: 71-77.
System Imaging Database. 2002. sid.zoology.gla.ac.uk. February 2010.
Tattersall I. 1982. *The Primates of Madagascar*. Columbia Univeristy Press, New York.
Vaughn S., McGee M. 2009. Association of *Allobosca crassipes* (Diptera: Hippoboscidae) with the black and white ruffed lemur (*Varecia variegata variegata*) and Milne-Edwards' sifaka (*Propithecus edwadsi*) in southeastern Madagascar. *Pan-Pacific Entomologist,* 85:162-166.
Villers L., Jang S., Lent C., Lewin-Koh S., Norosoarinaivo J. 2008. Survey and comparison of major intestinal flora in captive and wild ring-tailed lemur (*Lemur catta*) populations. *American Journal of Primatology*, 70: 175-184.
Vitazkova S., Wade S. 2007. Effects of ecology on the gastrointestinal parasites of *Alouatta pigra*. *International Journal of Primatology,* 28: 1327-1343.
Vitone N., Altizer S., Nunn C. 2004. Body size, diet and sociality influence the species richness of parasite worms in anthropoid primates. *Evolutionary Ecology Research,* 6: 183-199.
Watve M., Sukumar R. 1995. Parasite abundance and diversity in mammals: correlates with host ecology. *Proceedings of the National Academy of Sciences*, 92: 8945-8949.
Weyher A. 2009. Crop raiding: the influence of behavioral and nutritional changes on primate-parasite relationship. In: *Primate Parasite Ecology*. Huffman M., Chapman C. editors. Cambridge University Press, Cambridge, UK. Pages 403-422.
Weyher A., Ross C., Semple S. 2006. Gastrointestinal parasites in crop raiding and wild foraging *Papio anubis* in Nigeria. *International Journal of Primatology*, 27: 1519-1537.
Wilson K., Bjørnstad O., Dobson A., Merler S., Poglayen G., Randolph S., Read A., Skorping, A. 2002. Heterogeneities in macroparasite infections: patterns and processes. In Hudson P., Rizzoli A., Grenfell B., Heesterbeek H., and Dobson A., editors. *The Ecology of Wildlife Diseases*. Oxford: Oxford University Press. pp. 6–44.
Woolhouse M., Taylor L., Haydon D. 2001. Population biology of multihost pathogens. *Science,* 292: 1109-1113.
Wright P. 1999. Lemur traits and Madagascar ecology: coping with an island environment. *Yearbook of Physical Anthropology,* 42: 31-72.

Wright P., Arrigo-Nelson S., Hogg K., Bannon B., Morelli T., Wyatt J., Harivelo A., Ratelolahy F. 2009. Habitat disturbance and seasonal fluctuations of lemur parasites in the rainforest of Ranomanfana National Park, Madagascar. In: *Primate Parasite Ecology*. Huffman M., Chapman C., editors. Cambridge: Cambridge University Press. pp. 311-330.

Glossary of Terms

Direct transmission includes parasite transmission due to physical contact with a parasite by inhalation (such as the common cold), ingestion (such as pinworms) or penetration of the skin (such as hookworms).

Disease ecology is the interaction of the behavior and ecology of hosts with the biology of pathogens, as it relates to the impact of diseases on populations.

Endoparasite is a parasite that spends a large portion of its lifecycle within another organism.

Ectoparasite is a parasite that spends a large portion of its lifecycle on the exterior or surface of another organism.

Fecal-oral contamination is a form of parasite transmission that occurs when contaminated feces from an infected individual are ingested by a second individual either by eating the feces itself, or from fecal contamination of food or water sources.

Frequency of parasite infection is the number of samples from an individual host (or group of hosts) infected with a given parasite out of all samples analyzed from the individual host (or group of hosts) during a particular season. For example: if at least one whipworm egg is found in three out of six fecal samples evaluated for an individual, then the **frequency** of whipworm infection for that individual is .50, or 50% during that season.

Gastro-intestinal parasites are parasites that inhabit the gastro-intestinal tract in their hosts. They can live throughout the body during some stages of infection.

Indices of parasite infection is a general term referring to more specific measures including prevalence, intensity, and parasite species richness.

Indirect transmission includes parasite transmission from intermediate hosts such ingestion of an intermediate host, parent to unborn offspring, biting vectors (such as mosquitoes and ticks), and penetration by free-living transmission stages produced by intermediate hosts (such as mollusks).

Intensity refers to the number of parasite individuals within a single host individual. With some parasite species this can be determined by egg/cyst/larva counts.

MAPE is the mean absolute percentage error. **MAPE** is typically used to forecast error and was used in chapter 2 as a measure of error from average egg counts. See section 2.4.2 for the formula and detailed explanation of use.

Parasites are broadly defined as viruses, bacteria, protozoa, helminthes and arthropods that spend the greatest part of their life cycle in or on another organism.

Parasite burden is a general term referring to the parasite(s) infecting and individual host, host population, or host species.

Parasite infection parameter is a general term, also known as an index of parasite infection, referring to a number of more specific measures including prevalence, intensity, and parasite species richness.

Parasite species richness (PSR) is the number of different parasite species in a given host individual or in a single fecal sample.

Presence is the observation of at least one egg/cyst/larva of a particular parasite species (or parasite taxonomic group) in a single host sample or set of samples. In Chapter 2 **presence** refers to the occurrence of at least one egg/cyst in a single sample. For example: if either one or 150 whipworm eggs are found in a single sample, then whipworm is **present** in that sample. If no whipworm eggs are found, then whipworm is not present, or absent.

Prevalence is the number of hosts infected with one or more individuals of a particular parasite species (or parasite taxonomic group) divided by the number of hosts examined for that parasite species. This is a descriptive statistic for presence-absence data considering all samples from an individual during one season. For example: if at least one whipworm egg was found in the samples of 12 out of 20 individuals, then whipworm **prevalence** would be .60, or 60%.

Species area curve represents the number of species within a given area through a power curve. This is based on MacArthur and Wilson's equilibrium model of island biogeography. The **species area curve** is used in Chapter 2 as a method of evaluating parasite species richness from a given fecal sample.