PRIMATE-PARASITE INTERACTIONS IN A SEMI-ARID ENVIRONMENT

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It has been said that everything everywhere affects everything else. This may be true.

Or perhaps the world is just full of patterns.

- Terry Pratchett, *Wings*

DEDICATION

For my parents, Penny and Peter, who instilled in me a deep love for the natural world at a very early age. By fostering and encouraging my curiosity, they have guided my journey from a barefoot child with unbrushed hair, collecting bugs in my bug box, to where I am today—a naturalist with a deep appreciation of and respect for wildlife.

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ABSTRACT

I combined physiological, environmental, behavioural and parasite data to investigate the correlates of infection in wild vervet monkeys (*Chlorocebus pygerythrus*) living in a semi-arid region of South Africa. I aimed to assess whether our well-established assumptions about primate-parasite interactions hold true in the context of severe ecological stress and how these external stressors may impact how monkeys respond to infection. I found that environmental conditions were the primary drivers of parasitism in the population, with individual-level characteristics playing a diminished role. I also found that while there were links between aspects of behaviour and parasitism, ecological conditions constrained behavioural flexibility. These results highlight the difficulty of generalising across primate populations, and point to the importance of expanding primate-parasite ecology to include animals in more extreme environments. Doing so will allow them to serve as a window into how animals confront climate change-induced environmental changes.

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CHAPTER ONE: INTRODUCTION

This thesis presents the results of a broad study on the gastrointestinal parasites of wild vervet monkeys (*Chlorocebus pygerythrus*) living in a semi-arid environment. First, I provide a description of gastrointestinal helminths in a vervet population living in a drought-prone, semi-arid environment. I then explore seasonal variation and the multi-scale, socioecological predictors of gastrointestinal helminth infection in the study population. Next, I investigate how parasite infection shapes behaviour in a gregarious mammal coping with chronic ecological stress. The overarching theme of the thesis is how approaching these questions in the context of a more extreme ecological environment overturns well-established assumptions about primate-parasite interactions and points to a need to (i) continue to expand primate-parasite ecology research beyond tropical and sub-tropical regions and (ii) strive to include more diverse, comprehensive datasets in primate parasitological research.

In what follows, I present an overview of the factors that drive host-parasite dynamics in the wild and the challenges that wildlife epidemiologists face. Next, I discuss the relevant literature on primate-parasite ecology and how this field has expanded in recent years. I then discuss the links between parasites and host behaviour more generally before discussing the somewhat limited field of primate-parasite behavioural interactions. Lastly, I provide an outline of the structure of this thesis.

1.1 Host-parasite dynamics

1.1.1 *The basics of a system*

Host-parasite dynamics and transmission dynamics are inherently complex given that they are a function of host, pathogen and environmental ecology (Cable et al., 2017). Simply put,

parasitism is an ecological relationship between the populations of at least two different species of organisms - host and parasite – where a parasite is defined as an organism that lives in or on a another organism (the host), feeding on it, showing structural adaptation to it and causing it some degree of harm (Crofton, 1971; Poulin, 2007b). Several characteristics of hosts shape their individual susceptibility to disease, as well as parasite richness and diversity, within a population. Characteristics of the host such as age and sex (Nunn et al., 2003; Willis et al., 2015), body mass (Arneberg, 2002), life-history traits (Johnson et al., 2012) and behaviour (Hart, 1990; Ezenwa et al., 2016) all affect parasite prevalence, richness and/or transmission. Additionally, population level characteristics such as group size (Davies et al., 1991; Nunn et al., 2015), social organisation (Altizer et al., 2003), ranging patterns (Nunn & Dokey, 2006), and community structure (Johnson et al., 2008) also affect parasite assemblages and transmission. Pathogen or parasite characteristics vary mostly in terms of phenotype/genotype and clinical properties (McCallum et al., 2017). Properties such as infectivity, within-host multiplication, pathogenicity and translocation of pathogens vary across species and ultimately determine the success and fitness of a pathogen or parasite (Antolin, 2008).

Aside from host and pathogen characteristics, the environment in which the host-parasite system exists also shapes its dynamics. Naturally occurring patterns such as rainfall, temperature and latitude (Chapman et al., 2009; Nunn et al., 2005) all affect parasite prevalence, richness and/or transmission dynamics. In addition, anthropogenic disturbance, such as urbanisation or agricultural land use, can affect both abundance and transmission of parasites (Bradley & Altizer, 2007).

1.1.2 *Parasites and wildlife: Why the interest?*

Wildlife disease research has increased dramatically, incorporating numerous parasite and host species across a broad range on environments over the past 20 years, for several reasons. First, much of the interest in wildlife epidemiology is a result of the ever-expanding human-wildlife interface and the problems it poses for human health. Emerging infectious diseases (EIDs) are becoming an increasing threat to global health (Warren et al., 2008) where between 60 and 80% of EIDs are presumed to be of animal origin (Hassell et al., 2017). Thus, zoonotic diseases are becoming increasingly important given their impact on human health and economics, and understanding the host-parasite systems and transmission dynamics of these diseases is essential.

Second, large-scale human migration and anthropogenic disturbance is linked to the current decline in global biodiversity. Given the complex nature of host-parasite systems, the potential response of parasites to biodiversity loss is of interest to ecologists, epidemiologists and conservationists alike (Civitello et al., 2015; Lafferty, 2012). Parasites have been implicated in parasite-driven extinctions (Best et al., 2012) and decreased diversity of other hosts in a system can increase transmission of pathogens to species of concern (Keesing et al., 2006), thus increasing the likelihood of parasite-driven population decline or extinction. Conversely, biodiversity loss has been linked to parasite diversity loss and, when viewing parasites as part of an ecosystem rather than a purely undesirable component, this results in numerous "unseen" species losses (Lafferty, 2012). Regardless of perspective, biodiversity loss appears to have significant detrimental impacts on host populations and has thus garnered increased interest in an effort to understand and potentially mitigate these effects.

Third, on a more practical level, the development and use of non-invasive monitoring techniques have allowed parasitologists to study parasite and pathogen dynamics in wild populations with minimal interference (Gillespie, 2006). This has allowed for a departure from captive or experimental studies and increased the diversity of wild study species and systems.

Lastly, building on the convenience of non-invasive sampling techniques and more robust statistical analyses, wildlife epidemiologists are increasingly collecting parasite and pathogen-specific physiological and behavioural data, as well as monitoring long-term environmental conditions. This has opened the door for more complex analyses of the environmental factors that shape parasitism, as well as the use of social network analyses to investigate and model transmission dynamics (Silk et al., 2017). Additionally, this research now stretches beyond simple measures of community interactions to include multiple-host-multiple-parasite systems, sequential hosts interacting on different trophic levels or stochastic effects resulting from small population size (Collinge & Ray, 2006).

1.1.3 *Challenges to the understanding host-parasite systems in the wild*

Infectious disease and parasitological research in animals has historically been experimental, particularly when concerned with transmission dynamics, because fully understanding transmission ideally requires the introduction of a single infected individual. While the breadth and detail of data now being collected in the wild has greatly increased our understanding of wildlife-parasite dynamics, parasitologists and wildlife epidemiologists face several challenges when studying host-parasite relationships and transmission dynamics in wild populations given the nature of the complex physical and social environments in which the host-parasite system exists.

The first challenge facing wildlife epidemiologists is the presence of social heterogeneity in social groups, a challenge not typically encountered in captive studies and more easily quantified in human studies. In a wild population of social animals, individuals typically structure their space preferentially either to overlap with mates or family members, or to avoid competitive interactions with individuals of the same sex, resulting in smaller social subgroups within a population (Godfrey, 2013). This means that contact between individuals in a social group is not uniform and individuals are more likely to be infected by a neighbour or member of their social group than by others more socially distant. This results in some individuals being at higher risk of both transmitting parasites and becoming infected (Best et al., 2012). It has also been shown that, in a social group, relatively few individuals will harbour the majority of the parasite assemblage (Crofton, 1971). The use of social network analysis, discussed below, has emerged as a promising tool to both control for social heterogeneity and to investigate its consequences (Silk et al., 2017).

The second challenge, closely linked to the first, is the presence of spatial heterogeneity in wild populations (i.e. variation across geographical ranges). This can arise from intrinsic differences in locations, such as habitat quality, or emerge from processes within populations, such as individual movement (White et al., 2018). When combined with the characteristics and ecology of the host, vector and pathogen, spatial heterogeneity can result in different parasite assemblages and patterns of spread within a population. For example, pathogens may spread uniformly from an epicentre (West Nile Virus), via jump dispersal on a network (foot- and-mouth disease), through short- or long-range dispersal events (rabies), or through combination of all these processes, as has been found in the case of Sudden oak death (Maher et al., 2012; White

et al., 2018). The presence of spatial heterogeneity points to a need to study wildlife diseases on multiple scales ranging from the individual to the ecosystem (Tompkins et al., 2011).

Third, host behaviour can play a key role in transmission dynamics and, subsequently, any wild studies require detailed, often difficult to collect behavioural data to capture host-parasite processes. Hosts may adjust their behaviour in order to lower parasite acquisition and transmission, while parasites may manipulate hosts to increase their own fitness. These processes are complex and are often difficult to observe but they affect all components of transmission dynamics and have been observed in almost all transmission modes (Moore, 2002). Behaviour is considered by some to be the first line of defence against pathogens and parasites (Hart, 1990). Such behaviours include avoidance strategies, controlled exposure to potentiate the development of immunological competence, sickness behaviour, helping sick or injured animals, mate choice directed to those that are disease-free and sexual selection for genes that confer immunological, physiological and behavioural resistance to microparasites and macroparasites (Hart, 1990). Furthermore, interactions between hosts and parasites are often reciprocal; thus, changes in host behaviour are likely to result in behaviour-parasite feedback loops creating further complexity in transmission dynamics processes (Ezenwa et al., 2016). Given that behavioural change can often be subtle or hard to quantify, this source of variation is often overlooked. However, research in non-human primates has shown that individual behaviour, particularly grooming and proximity measures, supersedes the effects of any host individual characteristics on parasite measures (Friant et al., 2016a), highlighting the need for continued investigation into the behavioural correlates of parasitism in the population.

Lastly, it has become increasingly clear that host-parasite systems are context- dependent where global environmental change (reviewed: Harvell et al., 2002) or more local anthropogenic

disturbance, such as urbanisation or deforestation (reviewed: Bradley & Altizer, 2007) alter host, pathogen and environmental ecology. This has been shown to be the case for both transmission dynamics (Poteet, 2006) and parasite prevalence, richness and diversity (e.g. Lafferty, 1997; Lane et al., 2011; Thatcher et al., 2018). This poses problems for direct comparison of parasite assemblages of the same host species between study sites or even within the same study site across time. Further, this highlights the importance of the collection of detailed, long-term environmental data when studying wildlife epidemiology and the links between parasitism and behaviour in order to make informed and accurate conclusions about any host-parasite system.

These challenges are important to consider when studying any host-parasite system in the wild. However, they can be, and have been, largely overcome through the collection of long-term, detailed physiological, environmental and behavioural data. Further, advances in analytical methods have greatly increased the breadth, flexibility and robustness of statistical analysis.

1.2 Primates and Their Parasites: Ecology

1.2.1 *The story so far*

Decades of primatological research has cemented our understanding of non-human primate behaviour, ecology and evolution across a diverse range of species and geographic locations. There was considerable early interest in how parasites might shape the evolution of primate sociality (see: Freeland et al., 1976), with studies documenting the presence of infections in various primates (Kalter & Heberling, 1990; Ruch, 1959). Although both parasite and primate ecologists began, in parallel, to move away from biomedical settings and incorporate ecological and evolutionary variables in their research (Sukhdeo & Hernandez, 2005), the integrative field of primate disease ecology has emerged only recently (Altizer et al., 2006; Chapman et al., 2005;

Gillespie & Chapman, 2006; Huffman, 1997; Nunn & Altizer, 2006). Since the mid 2000s, primate-parasite ecology research has increased exponentially, covering a broad range of ecological, social and environmental topics, and has been identified as a crucial component of primate conservation initiatives (Nunn & Gillespie, 2016).

1.2.2 *Primate parasite ecology: why the increased interest?*

Interest in primate disease ecology has increased for several reasons. Perhaps the most widely discussed is the disease risk to humans due to our close phylogenetic links to other primates. Emerging infectious diseases are increasing rapidly, largely due to more frequent contact between wild primates and humans as a consequence both of habitat destruction/fragmentation and the bush meat trade (Chapman et al., 2005; Daszak et al., 2000). Some the most virulent EIDs found in humans, such as AIDS or Ebola, have resulted from zoonotic transmission between wild primate populations and humans (Pedersen & Davies, 2009). Furthermore, disease spillover may be bi-directional, with some primate species, particularly apes, being highly susceptible to human infections (Woodford et al., 2002). Thus, understanding diseases present in wild primate populations, and how to mitigate transmission risks, is hugely important for the health of both humans and other primates.

Primates also harbour an exceptionally high diversity of parasites from all taxonomic groups, including bacteria, protozoa, viruses and helminths (Nunn et al., 2003). Primates are likely to be favourable hosts for parasites given that most primate species live in social groups, which has been shown to be positively associated with higher parasite diversity and abundance (Cote & Poulin, 1995; Nunn et al., 2003). Primates are also highly diverse with regard to phylogeny, demography, ontogeny, sociality, and physiology (Strier, 1994), and parasitism is

thought to have played a role in both the evolution of sociality (Freeland et al., 1976) and phylogenetic diversity (Nunn et al., 2004). In combination, this provides primate-parasite researchers with a rich and diverse range of topics and allows for extensive comparative research.

Parasite infection can also have detrimental effects on the host, in both the short- and long-term. Although parasite infections are often non-lethal, heavy parasitic infection can have detrimental consequences for the host by, for example, altering general metabolism, immune functions, food intake, or by increasing predation risk (Gulland, 1995; Hudson & Dobson, 1992; Morand & Harvey, 2000). Given this, parasites can have a significant impact on population dynamics, which can put small populations of endangered species at risk (Nunn et al., 2015). Parasites are therefore considered a major component of primate conservation biology, and ongoing efforts are being made to understand the processes and risks of primate pathogens and parasites.

Lastly, primates employ a range of behaviours to mitigate their considerable parasite burdens. These range from more common behaviours, such as grooming, which serves as a means of ectoparasite removal (Nunn & Altizer, 2006), to the use of self-medication (Huffman, 1997; Huffman et al., 1997; Nunn & Altizer, 2006). The links between primate behaviour and parasites are discussed in more depth in section 1.3.

1.2.3 *Socio-ecological correlates of primate parasitism*

Much of primate parasite ecology research focuses on two primary themes: how the environment shapes parasitism and how intrinsic host characteristics shape parasitism. The influence of host environment, whether natural or degraded, on parasite diversity, richness, and

prevalence in wild primate populations is perhaps the most extensively documented (e.g. Gillespie et al., 2005; Gillespie & Chapman, 2006; Mbora & McPeek, 2009; Schwitzer et al., 2010). Environmental conditions, including rainfall, temperature and humidity, all have an impact on parasite diversity, richness, prevalence as well as, in some cases, transmission dynamics (Appleton & Henzi, 1993; Benavides et al., 2012; Blersch et al., 2021; Nunn et al., 2005; Poirotte et al., 2016). For example, Parr et al. (2013) found that white-faced capuchins (*Cebus capucinus*) were more likely to be infected with *Strongyloides* sp. during the dry season, while Martínez-Mota et al. (2017) found that both parasite richness and prevalence in black howler monkeys (*Alouatta pigra*) decreased during months of increased rainfall. On a broader scale, latitudinal gradients in parasite richness have been found for protozoans but not viruses or helminths (Nunn et al., 2005). Specifically, vector-borne protozoa diversity is higher in the tropics. Consequently, detailing a taxon's pathogen or parasite diversity and intensity across all the habitats it occupies provides a better understanding of its vulnerability, both locally and globally, to shifts in environmental conditions.

Numerous host characteristics have also been explored as possible predictors of parasitism in primates, with varying results for all predictors. These include age, sex, dominance rank, stress biomarkers, reproductive state and physical condition (For wild primates, see: Parr et al., 2013; chacma baboons: Benavides et al., 2012; Japanese macaques: MacIntosh et al., 2010; red colobus monkeys: Chapman et al., 2006). Across studies, results vary widely for all host intrinsic factors, and within studies, results differ according to the parasite species under consideration. For example, MacIntosh et al. (2010) found that, in Japanese macaques (*Macaca fuscata yakui*), prevalence and EPG of *Strongyloides fuelleborni* and *Trichuris trichiura* were significantly higher among juveniles than adults, while no clear age differences were found in

the prevalence of either *Streptopharagus pigmentatus* or *Gongylonema pulchrum*. Conversely, no relationship was found between *Strongyloides* sp. and age in white-faced capuchins, but age was the primary predictor of *Filariopsis barretoi* infection (Parr et al., 2013). Similarly, for dominance rank, Muehlenbein and Watts (2010) found that dominance rank was positively associated with helminth parasites richness in male chimpanzees (*Pan troglodytes schweinfurthii*) whereas there was no association between rank and parasite richness in chacma baboons (*Papio ursinus*) (Benavides et al., 2012).

Recently, primate researchers have begun to explore the links between parasites and variation in hormonal levels. While extensively covered in other vertebrate literature, the links between hormones and parasitism in wild primates remains limited (see: Chapman et al., 2006; Clough et al., 2010; Foerster et al., 2015; Friant et al., 2016a; Muehlenbein, 2006). In addition, this work focuses disproportionately on faecal glucocorticoid metabolites and, to a lesser extent, on sex hormones (reviewed: Beehner & Bergman, 2017). This is likely a result of both the difficulties of non-invasive assessment of hormones in wild populations and because glucocorticoids offer an insight into the energetic demands associated with environmental challenges (Sapolsky et al., 2000). When considering glucocorticoids only, Chapman et al. (2006) found a positive relationship between elevated cortisol levels and parasite infections in red colobus monkeys (*Procolobus rufomitratus*), while Friant et al. (2016b) found a positive association between parasite abundance and cortisol levels in semi-free-ranging red-capped mangabeys (*Cercocebus torquatus*). When considering both cortisol and sex hormones, a positive relationship was found between both cortisol and testosterone, and total parasite species richness in wild chimpanzees (Muehlenbein, 2006), while Clough et al. (2010) found that both male androgen and glucocorticoid levels during mating season were associated with a time-

lagged increase in nematode infection in red-fronted lemurs (*Eulemur fulvus rufus*). Despite these associations, links between fGCMs and any socio-ecological variables should be interpreted with caution (addressed further in section 1.2.4).

1.2.4 *Progress and Pitfalls in Primate-parasite Ecology*

Although we now have a decent grasp on the fundamental associations between environmental, social and physiological factors, and parasitism in wild primates, there are still several areas of concern, gaps in our knowledge, and potentially problematic conclusions.

One of the fundamental difficulties in understanding any host-parasite system is that of establishing causality between external variables and parasite infection. This is inherently difficult, particularly in the wild where environmental conditions are uncontrolled, and social and spatial heterogeneity are prevalent (Hudson et al., 2002). Neither primate host nor parasite live independently of their environment, which means that any environmental variation can affect host, parasite, or both. Additionally, many variables can be both the predictor and the result of parasitism. For example, temperature and rainfall have well-established effects on the longevity and persistence of parasites, particularly those that are environmentally transmitted or transmitted by a vector (Nunn & Altizer, 2006). However, a reduction in water availability as a result of decreased rainfall has been linked to increased mortality in vervet monkeys, presumably underpinned by an overall deterioration in body condition (Young et al., 2019). Reduced body condition increases parasite susceptibility. Thus, rainfall can influence both parasite and host making establishing the causality of a parasite-rainfall association is problematic. Unfortunately, causality cannot be easily teased out without the use of experimental manipulation and, given that this is unfeasible in most field studies, these problems can be mitigated only by taking more

care when interpreting and reporting results. Despite these drawbacks, it is essential first to explore the environmental correlates of any parasite measure before any other behaviour-parasite relationships are considered.

Another general concern is the loose use and interpretation of glucocorticoid measures, or faecal glucocorticoid metabolites (fGCMs). There is an extensive body of literature on the correlates of glucocorticoids in wild primates including rank-related effects, anthropogenic predictors, non-rank social predictors and, to a lesser extent, parasite measures (reviewed: Beehner & Bergman, 2017). The majority of this research focuses on the *causes* of hormonal variation rather than on any fitness *consequences* (Bonier et al., 2009). Primate stress research is largely embedded in earlier frameworks where the overarching view of elevated fGCMs has been that stress is simply bad for an animal rather than an adaptive coping mechanism. That is, individuals with higher fGCMs concentrations are routinely assumed to be in worse condition and subsequently will have reduced fitness (Bonier et al., 2009). Given the actual adaptive nature of the stress response, and that glucocorticoids are a metabolic hormone first and a stress hormone second (MacDougall-Shackleton et al., 2019), the way that we think about the links between fGCMs and socio-ecological variables needs to shift. There has been progress in this regard in other areas of animal research where frameworks like the Reactive Scope and Allostatic Load Models have been used to better explain how animals react to unpredictable environmental stimuli (DuRant et al., 2016; Romero et al., 2009; Romero, 2012). However, the use of such frameworks is uncommon in wild primate research (but see: Hämäläinen et al., 2015; Young et al., 2019 for exceptions), and is not a feature of primate-parasite research. Given the broad range of available stress data (Beehner & Bergman, 2017), the field of primatology is primed to shift to the use of newer stress frameworks and focus less on the causes of hormonal

variation and more on its fitness consequences. This is of particular interest to primate-parasite ecologists given that parasites may serve as the predictor or outcome of hormonal variation and play a key role in the immune system.

On a more practical note, in many cases primate-parasite ecologists have lagged behind other ecologists in embracing the use of new analytical and modelling techniques. For example, primate-parasite ecologists have a propensity to focus on linear variation in parasite measures, particularly when considering environmental variables, yet non-linear environmental fluctuation is inherent in nature and there are analytical techniques commonly used in other fields of ecology to address this (e.g. hierarchical generalized additive models: Pedersen et al., 2019). Data are also often split into categorical variables (e.g. wet versus dry season). This leads to a loss of valuable, more fine-grained information on potential non-linear variation across a study period and creates a tempting opportunity for over-generalisation of results, such as a direct comparison of wet and dry seasons across studies. Furthermore, non-linear variation in parasite measures themselves may be missed. While categorical variables are, of course, useful, primate-parasite ecology would benefit from the application of newer analytical and modelling techniques to take best advantage of the valuable, long-term climatic data that is often collected at field sites.

Another concern for primate parasitologists is the impact that systems changes, both broader climatic change and more local anthropogenic disturbances, have on primate-parasite dynamics. These are likely to affect multiple aspects of host-parasite systems and a significant effort is being made to understand and anticipate how parasite and pathogen dynamics might shift in response to system changes (Barrett et al., 2013; Behie et al., 2014; Bonnell et al., 2010). In this regard, primate-parasite ecologists have embraced more advanced predictive modelling techniques that allow the manipulation of environmental factors to simulate spread of an

infectious agent (e.g. Bonnell et al., 2010). Efforts have also been made with real-world data to anticipate how parasite assemblages might change in the face of more extreme weather events. For example, Behie et al. (2014) assessed the prevalence and intensity of parasite infection in black howler monkeys following a hurricane, a weather pattern likely to increase as a result of global climate change. They considered parasite measures in relation to susceptibility (fruit consumption and faecal cortisol) and exposure (population density, group size and ingestion of *Cecropia peltata*). They found that directly transmitted parasites were positively associated with host density only, while the indirectly transmitted parasite—*Controrchis* sp.—was positively associated with the percentage of *C. peltata* in their diet. These results suggest that there is a cascading effect of climate change on plant-animal interactions and disease ecology. Despite the clear importance of understanding primate-parasite dynamics under diverse conditions, primate-parasite research in Africa, where there is a large latitudinal distribution of host species, has largely concentrated on tropical and subtropical regions. However, primates in semi-arid regions provide us with a way of understanding how both hosts and parasites might change and adapt in response to climate change-induced environmental changes.

1.2.5 *Conclusion*

Despite the growing field of primate-parasite research and in-depth parasitological surveys conducted in numerous primate populations, relatively little is known about parasites in primates living in semi-arid regions. These regions are significantly affected by climate change (Jury, 2013), and animals in these regions are central to a better understanding of how animals might cope and adapt. In this regard, my research provides the first report of the gastrointestinal parasite assemblage in vervet monkeys living in a semi-arid environment. It also aims to explore

how more conventional parasite-environment relationships may differ in more extreme environments, particularly when monkeys are subject to both chronic parasite infection and chronic food stress.

1.3 Primates and Their parasites: Behaviour

1.3.1 *Parasites and behaviour*

While the links between the environment and host-intrinsic factors have been relatively extensively researched, the links between behaviour and parasitism in primates have only come into focus more recently. Exposure to parasites is linked to almost every aspect of host behaviour (Ezenwa et al., 2016). Foraging behaviour is associated with exposure to environmentally acquired parasites; social behaviour results in the acquisition and transmission of a variety of contact-transmitted parasites and pathogens, and mating behaviour results in the transmission of sexually transmitted diseases (Altizer et al., 2003; Moore, 2002). At the same time, behaviour also plays a fundamental role in how hosts both cope with infection and avoid transmission and, as noted above, is often considered the primary line of defence (Ezenwa et al., 2016; Hart, 1990; Moore, 2002).

Behavioural change in primates in response to parasitism takes several forms and can be viewed as a "behavioural immune system" that complements their physiological immune system (Poirotte et al., 2017; Schaller, 2006). First, individuals may opt to alter their behaviour to avoid or reduce parasite acquisition. This comprises a specific set of avoidance strategies to decrease parasite encounters. For environmentally transmitted parasites, faecal avoidance through changing sleep sites (Hausfater & Meade, 1982) or avoidance of contaminated food (Poirotte et al., 2019) may reduce the likelihood of infection. For directly transmitted parasites, including

ectoparasites, changing social grooming patterns or avoiding parasitised individuals can decrease the likelihood of transmission (Duboscq et al., 2016). Second, if infected, individuals may modulate their behaviour to cope with an infection. This strategy often involves reducing time spent on energetically costly behaviours and favouring those that help with recovery such as resting (Ghai et al., 2015). Behavioural strategies employed by primates are contingent on several measures including parasite transmission mode, group structure and the external environment.

1.3.2 *Avoiding infection*

Although often non-lethal, infection can be costly and have negative effects for fitness. Ideally, therefore, animals should act so as to avoid getting infected at all. Avoidance strategies are largely contingent on the parasite in question, where animals employ varying strategies to avoid macro- and micro-parasites respectively (Hart, 1990). Given the topic of my thesis, I will focus primarily on the avoidance of macro-parasites which are primarily helminths (internal worm-like parasites) and arthropods (ectoparasites).

One of the primary ways to avoid infection with intestinal parasites is through selective foraging, which reduces exposure to infectious stages of internal parasites. This has been well-documented in ungulates where individuals avoid grazing in areas adjacent to recently dropped faeces irrespective of food quality (Ezenwa, 2004a; Gunn & Irvine, 2003). In primates, Poirotte et al. (2019) experimentally investigated whether mandrills (*Mandrillus sphinx*) exhibited behavioural strategies to avoid nematode infection. They found evidence of faecal avoidance where individuals avoided eating contaminated food items but showed no avoidance behaviours in non-feeding contexts. Moreover, the age of the faeces and the presence of nematodes did not

influence the level of avoidance, which suggests that, when feeding, mandrills avoid contaminated material in general rather than in response to nematodes specifically. In an earlier study, Freeland (1980) found that mangabeys (*Cerocebus albigena*) travelled further, used a larger home range and exhibited less day-to-day overlap in home range use during dry weather compared to period of wet weather when rain could remove faecal contamination from leaves. This suggested that mangabeys avoided using contaminated areas.

A second strategy is the rotation and choice of sleep sites. The area beneath sleep trees is believed to be a primary cause of contact between primates and infected stages of macro-parasites given frequent defecation in that area. Given this, varying the location of sleeping sites would substantially reduce the risk of infection (Hausfater & Meade, 1982). Yellow baboons (*Papio cynocephalus*) were found to not use a sleeping grove for any longer than two consecutive nights and to only return to a grove after an average of nine nights (Hausfater & Meade, 1982). In a comparative study, Nunn and Heymann (2005) found preliminary evidence that sleeping in closed sleeping sites, such as holes, reduced the risks of vector-borne disease, particularly malaria, in owl monkeys (*Aotus* sp.). However, it should be noted that there are numerous reasons for sleep site selection in primates and these may override any parasite-avoidance benefits.

Aside from environmental transmission, social living poses particular challenges to pathogen and parasite transmission and avoidance. Social behaviour is a key component that facilitates the acquisition and spread of parasites (Briard & Ezenwa, 2021). A primary strategy employed by social animals to reduce transmission is the alteration of social interactions, often by modulating the number of grooming partners or grooming frequency. Aside from any social benefit, grooming facilitates the removal of ecto-parasites and serves a role in reducing infection.

For example, Akinyi et al. (2013) found that, in yellow baboons, individuals who received more grooming had lower tick loads and higher haematocrit, which is a general measure of health status. Similarly, in Japanese macaques, females who had more grooming partners in fall and spring had lower lice loads (Duboscq et al., 2016). However, grooming and social proximity have also been identified as primary mediators of the transmission of intestinal macro-parasites, and variation in grooming patterns has been associated with non-directly transmitted parasites. For example, Macintosh et al. (2012) investigated the links between grooming, social rank and directly transmitted helminths in Japanese macaques. They found no relationship between grooming measures and parasite richness but did find that high ranking females had increased infection with *Oesophagostomum aculeatum* (EPG) and *S. fuelleborni* (probability of occurring). As high-ranking females had more grooming partners overall, this suggests rank-mediated social contact increases parasite transmission. In gorillas (*Gorilla gorilla*), individuals in social groups, who were assumed to groom more, had higher Ebola-linked mortality than did solitary males, which again points to a disease transmission cost of social interactions (Caillaud et al., 2006). For non-directly transmitted parasites, Wren et al. (2016) found that, in vervet monkeys, individuals infected with hookworm had significantly more grooming partners than uninfected individuals, while individuals infected with *Trichuris sp*. spent significantly less time grooming others than those who were not infected (Wren et al., 2021). These studies suggest that while grooming appears to be linked to parasitism, the mode of transmission and life cycle of the specific parasite is relevant when considering the links between sociality and parasitism.

Lastly, while mating behaviour serves as the primary transmission mode of sexually transmitted infection (STI), it is also necessarily linked to reproductive fitness, resulting in a mating-parasite avoidance trade-off. STIs can be costly and can result in reduced offspring

survival, sterility and costly immune defences (Paciência et al., 2019). Although less well studied in primates, Paciência et al. (2019) found that female olive baboons (*Papio anubis*) were more likely to avoid copulation when approached by males with visible STI infection (ulcerated genitals), which indicates behavioural avoidance of diseased individuals.

1.3.3 *Coping with infection*

While avoidance strategies can mitigate and reduce the likelihood of transmission, infection in the wild is still highly likely. Given this, primates employ several behavioural coping strategies to reduce the impact of parasite infections.

First, primates show evidence of sickness behaviour, an adaptive suite of behaviours that occur in response to infection. These behaviours typically include anorexia, lethargy, somnolence, and a reduction in grooming (Dantzer & Kelley, 2007; Hart, 1988). Previously, sickness behaviours were viewed as a by-product of illness, but sickness behaviour is now considered to be a part of a highly organised strategy to combat infection by reallocating resources away from non-essential activities towards the immune system (Hart, 1988). Sickness behaviour has been extensively documented in captive populations (Lopes et al., 2016; Stockmaier et al., 2020; Weary et al., 2009). For example, in an experimental study of highly social, captive common vampire bats (*Desmodus rotundus*), animals who were immune-challenged had fewer grooming partners than did healthy bats and reduced their grooming of non-kin Stockmaier et al. (2020). However, sickness did not influence food sharing behaviour, which suggests that the effects of sickness are lower for social behaviours that confer greater fitness benefits. Less is known about sickness behaviour in wild mammals (Ghai et al., 2015; Hamilton et al., 2020; Krief et al., 2005), perhaps as a consequence of the challenges associated

with long-term environmental and physiological monitoring. Given this, sickness behaviour research in the wild has focused almost exclusively on the relationship between parasite infection and behaviour independent of other concurrent environmental or physiological stressors. For example, Ghai et al. (2015) found that when red colobus monkeys were whipworm-positive, they rested more and moved, groomed and copulated less. In a study on the links between a transmissible cancer, devil facial tumour disease (DFTD), and social behaviour in Tasmanian devils (*Sarcophilus harrisii*), Hamilton et al. (2020) found that an individual's probability of interaction declined progressively as DFTD load increased, thus decreasing their connectivity in their social network.

When simultaneously exposed to other environmental or social stressors, the reorganisation of behaviour becomes more complex (Cohn & de Sá-Rocha, 2006; Moyers et al., 2015) and the expression of sickness behaviour should vary accordingly. While we have some understanding of the social factors that may influence investment in sickness behaviours (reviewed: Lopes, 2014), the influence of environmental stressors on the expression of sickness behaviour remains poorly understood. Understanding the interactions between environmental stress and behavioural changes is essential when attempting to quantify the impact that sickness behaviour has on long-term fitness in wild populations.

Another strategy used to cope with infection by some primate species is self-medication via plant secondary compounds or other non-nutritional substances (Huffman, 1997). The benefits can be derived either pharmacologically (Pebsworth et al., 2006) or physically (e.g. leaf swallowing: Huffman et al., 1996). Although expected to occur in other non-human primates, self-medication has been best documented in the great apes, particularly chimpanzees (*Pan troglodytes*). For example, Huffman and Seifu (1989) documented a female chimpanzee in ill

health consuming *Vernonia amygdalina*, a naturally occurring plant of known ethnomedicinal value. Given that this plant is not frequently consumed, this suggests that consumption of this plant is primarily medicinal. In red colobus monkeys, Ghai (2014) found that when individuals were whip-worm positive, they increased their consumption of *Albizia grandibracteata* and *Albizia gummifera*, two plant species that are used by local people as anti-parasitics. However, these results are largely correlative given an inability to control for other factors such as seasonal patterns in plant availability. Leaf swallowing behaviour in chimpanzees—the deliberate swallowing of leaves without chewing—has been linked to the expulsion of the nematode *Oesophagostomum stephanostomum* (Mahale, Tanzania: Huffman et al., 1996) and tapeworm, *Bertiella studeri* (Kibale, Uganda: Wrangham, 1995) suggesting that leaf-swallowing serves as a strategy to control infection with both cestodes and nematodes. Lastly, geophagy—the consumption of soil—has also been linked to parasitism in great apes, where clay is thought to treat gastrointestinal discomfort or illness (Huffman, 1997). However, geophagy may also increase the risk of acquiring soil-transmitted helminths (Pebsworth et al., 2012) which calls into question whether the potential medicinal benefits of geophagy outweigh the risk of acquiring infection. While self-medication is thought to occur in non-ape species, it is a challenge to identify its occurrence in primates that have lower food diversity or who consume non-nutritional food for other reasons, such as the consumption of succulents during periods of reduced water availability.

Although not well-understood, changes in vigilance behaviour have been linked to parasitism in some mammals. Vigilance serves as a protective mechanism against both predators and conspecific competition (Treves, 2000), and individual investment in vigilance may increase with increasing vulnerability. For example, in an experimental study on Grant's gazelles (*Nanger*

granti), Worsley-Tonks and Ezenwa (2015) found that vigilance decreased, and foraging increased, following anti-helminthic treatment. This suggests that parasite-infected individuals may invest more time in vigilance to compensate for greater vulnerability. In adult marmots (*Marmota flaviventris*), vigilance decreased as body condition improved (Chmura et al., 2016). However, links between vigilance and parasite infection were dependent on the specific parasite. Vigilance decreased when marmots were infected with *Trypanosome lewisi* but *Ascaris* spp. infection was not linked to vigilance. Further, any changes in vigilance were traded off with foraging time. Thus, while vigilance does appear to be linked to parasite infection, it is likely that any changes in vigilance when infected are indirectly mediated through changes in foraging, a result of the variable energetic demands posed by different parasite species. Links between vigilance and parasite infection have been largely unreported in primates. In semi-free-ranging red-capped mangabeys, vigilance levels decreased following anti-helminthic treatment (Friant et al., 2016b). However, as with the marmots, this decrease was concurrent with an increase in foraging and may not reflect a true reallocation of time in response to infection. Further, vigilance may increase simply because animals are resting more, and vigilance serves as a low energy activity. Nevertheless, vigilance serves a fundamental role in survival and understanding how parasites influence vigilance provides an insight into the potential fitness consequences of infection.

1.3.4 *Parasites in Social groups: new tools for old problems*

Living in groups poses particular challenges for both host and parasite. In the primate literature, group size has been the key metric for assessing the impact that social behaviour has on parasite dynamics and is used as a proxy for the number of social contacts that are likely to

occur between group members (Nunn & Altizer, 2006). However, evidence of a relationship between group size and parasitism is mixed. In three meta-analyses, Patterson and Ruckstuhl (2013) found a positive relationship between parasite intensity, prevalence, and group size, but no relationship between group size and parasite richness; Cote and Poulin (1995) found consistent positive relationships between group size and the prevalence and intensity of contagious parasites, although mobile parasites that required an intermediate host decreased with increasing group size; and Rifkin et al. (2012) found a weak but overall positive association between group size and parasite risk. In primates specifically, group size and group spread were both negatively related to parasite prevalence in red colobus monkeys, with larger groups harbouring fewer parasites (Snaith et al., 2008). In black howler monkeys, group size was positively associated with *Trichuris* sp. prevalence and intensity, but no association was found for other parasite genera (Behie et al., 2014). Thus, while there is some evidence of parasite-group size links in primates, these links depend on other factors such as the transmission mode and the parasite measure used (abundance, prevalence or richness), suggesting that group size may not adequately capture the nature of social associations relevant to transmission (Briard & Ezenwa, 2021). Subsequently, researchers have begun using new tools to better describe and quantify social associations and social network analysis has emerged as a powerful tool to understand primate social interactions and more accurately assess how these might influence parasitism.

Broadly, social network analysis provides a framework with which to visualise the social structure and connectedness of a social group and capture the structural complexity of a population on an individual level (Godfrey, 2013). Individuals are represented as “nodes” that are connected by “edges” which, in an epidemiological context, represent likely pathways of

parasite transmission. The use of social networks to understand parasite dynamics in social groups addresses two primary problems not captured by group size measures: social and spatial heterogeneity. Heterogeneity in the structure of spatial and social interactions exists in all social groups. Traditional epidemiological models assume that contact or proximity is random within the group and that all group members therefore have an equal opportunity to become infected (May & Andersen, 1979). However, animals often structure their space preferentially to avoid competitive interactions or to overlap with mates or family members (Godfrey, 2013). Members may also vary in the degree to which they interact with others in the group or change the number or identity of their social partners (Briard & Ezenwa, 2021). This results in some individuals being at higher risk of both transmitting parasites and becoming infected (Best et al., 2012), especially given that relatively few members tend to harbour the majority of the parasite assemblage (Crofton, 1971). Coupled with this, social groups themselves are also often spatially heterogenous across geographical ranges (White et al., 2018). For example, host density may vary according to habitat type or quality and individual movement can influence host contact rates (White et al., 2018). The flexibility and individual-level nature of social network analysis allows for the incorporation of both social and spatial heterogeneity into disease modelling.

Social network analysis in primate-parasite research has focused on three main themes: to simulate pathogen spread, to examine how contact networks affect individual infection status, and in comparative studies that identify interspecific differences in contact and transmission patterns (Rushmore et al., 2017). In primate-parasite research both spatial proximity and contact/grooming networks have been constructed. While often used to investigate real or theoretical viruses, protozoa and bacteria (e.g. Romano et al., 2016; Rushmore et al., 2014; Springer et al., 2017; Tung et al., 2015), network analysis has also been used with some macro-

parasites to examine how social networks affect individual infection status. For example, in brown spider monkeys (*Ateles hybridus*), central monkeys—those with higher connectivity to others in the network—had higher parasite species richness than did individuals with lower connectivity Rimbach et al. (2015). Female rank centrality was linked to *Oesophagostomum aculeatum* infection intensity and *Strongyloides fuelleborni* probability in Japanese macaques (Macintosh et al., 2012). Given that higher-ranking females generally occupy more central positions, it is possible that exposure to infective stages via rank-mediated social contact can be an important mechanism for the transmission of some parasites (Macintosh et al., 2012). For ecto-parasites, Duboscq et al. (2016) found that females who associated with more grooming partners (higher in-degree) harboured lower lice loads than those with few grooming partners suggesting that grooming serves an anti-parasite function. However, this pattern was seasonal and only occurred during the birth and mating seasons.

While now used extensively in epidemiology, network analyses have been most widely applied to the transmission of contagious parasites that require direct physical contact between hosts and has largely neglected parasites that have more complex life cycles, involving either a vector, intermediate host, or free-living infectious stages residing in the off-host environment (Godfrey, 2013). Despite this, some efforts have been made to apply network analysis to parasites that employ indirect transmission and, rather than more traditional transmission modelling, have aimed to use networks to understand the ecology of parasite transmission (Godfrey, 2013). For example, Fenner et al. (2011) used spatial proximity to assess the spread of ticks and a nematode *Pharyngodon wandillahensis* in pygmy blue lizards while other researchers have used food webs and networks to model parasite transmission (reviewed: Lafferty et al., 2008).

1.3.5 *Conclusion*

A growing body of research is shedding light on the complex links between behaviour, sociality and parasitism in non-human primates. However, the links between behaviour and parasitism are often considered independently of the environment, despite the progress and understanding stemming from primate behavioural ecology research. Furthermore, comparatively little attention has been given to parasites with indirect lifecycles or intermediate hosts. New analytical methods are providing us with the necessary tools to address primate-parasite relationships in an ecological context, which is essential for understanding parasite-induced behavioural change.

1.4 Thesis outline

The overarching aims of my thesis were to conduct the first gastrointestinal parasitological assessment of vervet monkeys living in a semi-arid environment, to determine how the environment shapes their parasite assemblage and, in turn, how the parasites they harbour shape their behaviour. Given their habitat, these interactions take place in the context of prevailing environmental stress, particularly food stress. The extreme seasonal temperatures coupled with declining annual rainfall and severe periodic drought of the semi-arid Karoo poses distinct challenges to both primate hosts and parasites and serve as a window into how animals confront climate change-induced environmental changes.

To achieve this, my thesis is divided into two parts. In part I, I identify and quantify parasite prevalence, richness and diversity of three troops of wild vervet monkeys at Samara Private Game Reserve, South Africa, establish seasonal effects in parasite measures and

investigate socio-ecological predictors of parasitism. This allows me to identify any important underlying relationships in the primate-parasite system. In part II, I investigate the impact that non-lethal parasite infection has on the primate host and the behavioural strategies they employ to cope with infection.

1.4.1 *Chapter 2*

In chapter 2, I provide information on the field site and study species as well as details on behavioural, physiological and environmental data collection methods. I also discuss the suitability of this vervet population for my study. Additionally, I provide a more detailed description of the parasite and faecal glucocorticoid metabolite extraction procedures. Analytical methodologies are described separately in the relevant data chapters.

1.4.2 *Part I: Chapters 3, 4 & 5*

Part I addresses five broad questions:

1. What is the prevalence, diversity and intensity of parasites in vervet monkeys living in a semi-arid region?
2. How does this compare to other vervet populations?
3. Is there evidence of seasonal variation in parasite measures and what environmental factors drive this variation?
4. How do host and environmental factors affect parasite prevalence, intensity and richness?
5. To what extent does inter-individual variation play a role in parasitism?

In chapter 3, I provide a summary of the gastrointestinal helminths recovered from faecal samples in both a short pilot season and the 12-month period that this thesis focuses on. I provide a general description of the parasites recovered, and compare and contrast these results with those from other vervet monkey populations. I also investigate whether there is evidence of a latitudinal gradient in parasite richness in vervet monkeys given that my study population lives very close to their southerly latitudinal limit.

In chapter 4, I quantify annual variation in parasite richness, prevalence and intensity and determine the climatic variables driving these changes. I consider non-linear variation in all parasite measure across the year and investigate whether temperature, rainfall and food availability are predictors of parasitism. I also assess whether there is long-term temporal dependence in intra-individual faecal egg counts and whether egg shedding served as a reliable indicator of infection.

In chapter 5, I consider the multi-scale (individual-, group- and population-level) predictors of gastrointestinal parasite richness, prevalence and intensity. Sex, rank and faecal glucocorticoid concentrations are included as individual-level predictors, food availability and troop membership as group-level predictors and total bi-weekly precipitation, daily minimum temperature and daily maximum temperature as population-level predictors. I aim to assess whether conventional thinking on the predictors of primate-parasite relationships hold true for populations in more extreme environments.

1.4.3 *Part II: Chapters 6 & 7*

Part II addresses five primary questions:

1. How do non-lethal parasites affect primate behaviour and what role does co-infection play?
2. What strategies do primates employ to cope with infection when also coping with food stress?
3. Are there links between parasitism and grooming networks?
4. Does parasite infection shape socio-spatial associations?
5. Do parasites with different transmission modes shape behaviour in different ways?

In chapter 6, I combine physiological, environmental, behavioural and parasite measures to assess the behavioural responses of the vervets to non-lethal nematode infection. I quantify both activity budget and behavioural predictability to investigate the occurrence of sickness behaviour in response to high parasite load and richness. I also consider the impact of co-infection on behaviour.

In chapter 7, I build on the results of chapter 6, and investigate the links between parasite infection and other aspects of sociality. I use social network analysis to explore the links between grooming, spatial association and parasite infection to assess whether, in an area where environmental conditions already constrain behaviour, animals still modulate their grooming behaviour in response to infection as has been shown in other primate populations.

CHAPTER TWO: GENERAL METHODOLOGY

Here I present background data on the study site, study species and data collection protocol. General methodologies are presented here while specific analytical methods are addressed separately in each chapter.

2.1 Study site

Samara Private Game Reserve (~27 000 ha) is situated in the Karoo, Eastern Cape, South Africa (Figure 2.1) where the study population has been the focus of extensive research since 2008 (Pasternak et al., 2013). Situated in the semi-arid karoo biome, the reserve comprises of mountains and nama-karoo grassland intersected by the Milk River and its tributaries. The study area is situated in the north of the reserve where the river flows intermittently, and where the vervet monkeys do not have access to artificial water sources. The study area is characterised by Acacia (*Vachellia karroo*) woodland along the river and floodplain as well as open dwarf shrubland in the drier areas dominated by *Grevia robusta*, *Rhus longispina* and the dwarf shrub *Penzia globosa* (Figure 2.1).

The study area is subject to the effects of anthropogenic climate change (Hoffman et al., 2009; Jury, 2013), which typically manifests itself in unpredictable and often reduced rainfall in an already low rainfall area. There are large spatio-temporal fluctuations in both food and water availability and there are severe, periodic droughts (McDougall et al., 2010). This population is also subject to high predation from a well-established terrestrial predator guild (Ducheminsky et al., 2014), that includes caracal (*Caracal caracal*), black-backed jackal (*Canis mesomelas*) and reintroduced cheetah (*Acinonyx jubatus*). Aerial predation is less common in this study area and

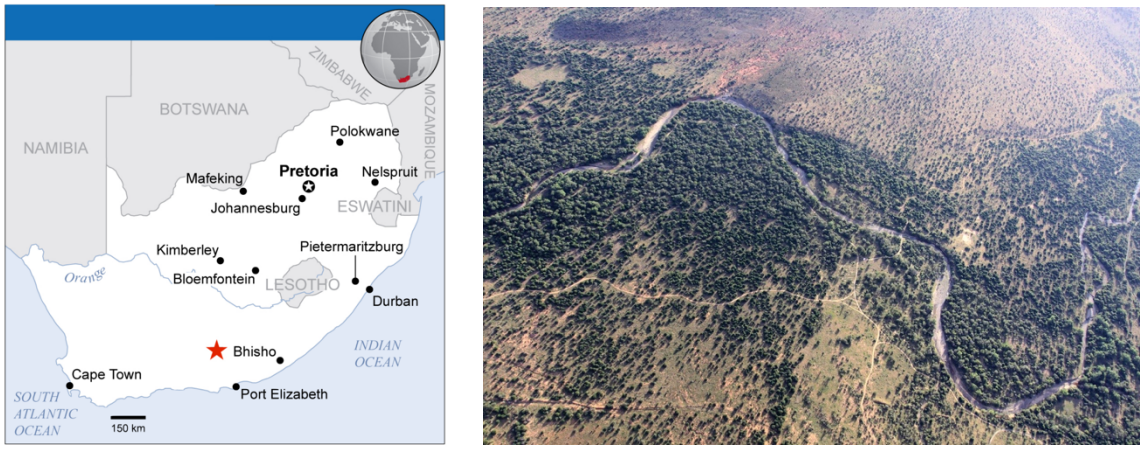

Figure 2.1 Map of South Africa with the study site indicated with a star (left). Aerial view of the study area that includes the home ranges of the three adjacent study troops (right). Map source: UNGIS, ESRI (Sept. 2019)

aerial predators include Verroux's eagle-owl (*Bubo lacteus*) and Verroux's eagle (*Aquila verreauxii*). While not predators, vervet monkey deaths have been attributed to venomous snake bites. Puff adders (*Bitis arietans*), cape cobra (*Naja nivea*) and boomslang (*Dispholidus typus*) are the primary venomous snakes in the region.

2.1.1 *Climate*

The area receives a declining average of 330 mm rain per annum with October to March constituting the wet season and April to September the dry season. The area experiences a mean annual maximum temperature of 27°C and a mean annual minimum temperature of 10°C (Pasternak et al., 2013).

Climate data for the study period were recorded at the field site. Information on the daily minimum and maximum temperatures was taken from a centralized weather station (Hobo U30-NRC, Onset Computer Corporation, USA), while daily records of precipitation during the study period were recorded using a standard rain gauge situated in the field. For the study period, the daily minimum temperature ranged from -3.9 ºC to 20 ºC while daily maximum temperature

ranged from 11.8 ºC to 49.6 ºC. Monthly rainfall was low except for two distinct peaks in October 2017 (69 mm) and January 2018 (67 mm). Total precipitation for the study period was 317 mm (Figure 2.2).

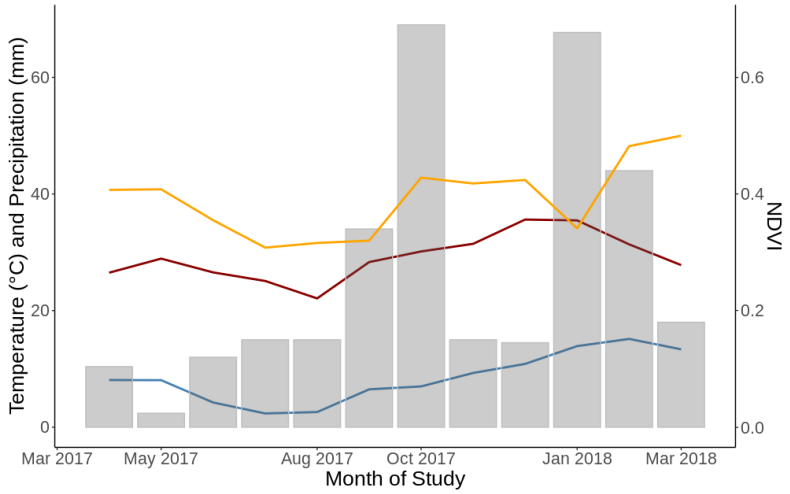

Figure 2.2 Graph showing overall relationships between average monthly minimum temperature (blue line), average monthly maximum temperature (red line) and total monthly precipitation (grey bars) and average monthly NDVI (orange line) across the study period (April 2017 – March 2018). The y-axis (left) shows temperature in degrees Celcius and the y-axis (right) shows Normalized Difference Vegetation Index (NDVI) score on a 0-1

2.1.2 *Resource availability*

2.1.2.1 Water availability

Water availability was recorded daily for each of the three troops on all 234 days of data collection and classified as river flowing, pools in territories (standing water) or no water available. During the study period, RST had no access to water on 120 days, RBM on 105 days and PT on 111 days (Table 2.1).

2.1.2.2 Food availability

The study site is characterised by both distinct inter- and intra-annual variation in rainfall and temperature. This variation results in temporal shifts in habitat productivity and subsequent changes in food availability (Figure 2.2). To quantify this, I calculated the Normalized Difference Vegetation Index (NDVI) every 16 days (Dostie 2020, in progress) for each of the three troops. Using Moderate Resolution Imaging Spectroradiometer MOD13Q1 vegetation indices at a 250-meter resolution (Didan, 2015), NDVI measures the amount of biomass or chlorophyll activity by calculating the difference between the visible red and near-infrared bands divided by their sum. Given the generalist, largely plant-based nature of vervet diet (Pasternak et al., 2013), the synoptic view of NDVI is a reliable measure of food availability in this species (Jarrett et al., 2020; Willems et al., 2009).

Table 2.1 The number of study days on which each study Troop had no access to drinking water.

Year	**Month**	**Number of Study days**	**Days without water**		
			RST	**PT**	**RBM**
2017	April	20	1	1	0
	May	23	15	9	8
	June	12	11	11	11
	July	21	20	20	20
	August	23	16	16	15
	September	21	14	16	15
	October	23	12	13	11
	November	19	0	0	0
	December	23	15	3	3
2018	January	21	15	15	14
	February	26	0	0	0
	March	25	1	7	8
Total			120	111	105

MODIS NDVI data were downloaded from NASA's Reverb|ECHO site (NASA) from MODIS data collected by Earth Observing System (EOS) satellites Terra (EOS AM-1) and Aqua (EOS PM-1) with a return-to-site periodicity of 16 days. These MODIS data were imported into "ArcGIS" version 1.6.1 and overlayed onto the territories of the three troops, with each territory represented as a regular series of points 10-m apart. NDVI values were then extracted from the MODIS rasters at each point. Area-weighted averages for each territory were generated every 16 days by averaging all NDVI values for points lying within the territory's 95% isopleth weighted by the troop's use of its territory during that period. The resultant estimate ranges between -1 and 1, where negative values indicate an absence of vegetation and positive values approaching 1 indicate larger concentrations of green vegetation (Pettorelli et al., 2005).

2.2 Study species

2.2.1 *Distribution and habitat*

Vervet monkeys (*Chlorocebus spp.*) are a widely distributed, semi-terrestrial, African cercopithecine (Wolfheim, 1983). Although associated with primarily riparian habitat (Isbell et al., 2002), they occupy a variety of habitats, from tropical woodland to semi-desert (Pasternak et al., 2013) and, after the baboon, are the most widely distributed African primate (Figure 2.3). As such, populations are exposed to a wide range of predators, resource richness, thermal regimes and pathogens, and, with the publishing of the vervet genome (Warren et al., 2015), on their way to being the most comprehensively phenotypically and genomically characterised non-human primate.

The *Chlorocebus* genus is generally considered to comprise five widely distributed species, *Ch. aethiops, Ch. cynosuros, Ch. pygerythrus, Ch. sabaeus* and *Ch. tantalus*, as well a

sixth species (*Ch. djamdjamensis*) that is confined to the Bale Mountains in Ethiopia (Groves, 2001). My study species—*Ch. pygerythrus*—has the largest latitudinal distribution of the genus and has been the most studied in the wild. My study animals form part of a high latitude population that is considered to be living close to its latitudinal limit.

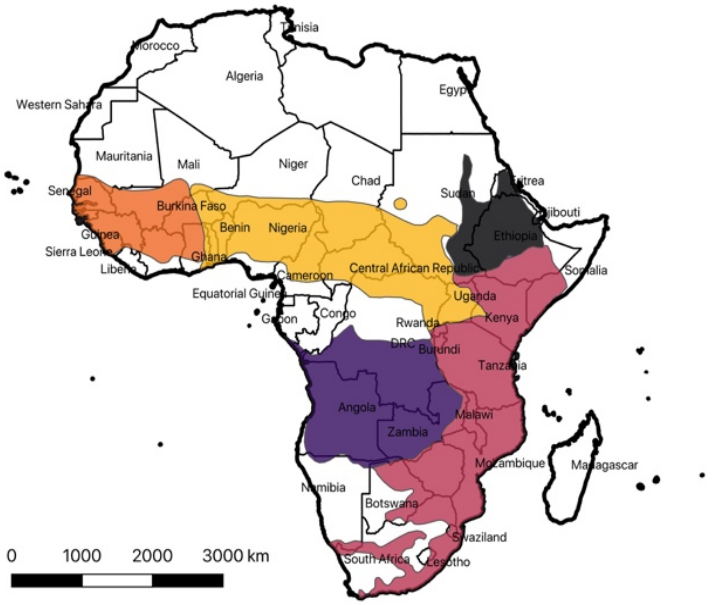

Figure 2.3 Map showing the distribution of the five widely distributed species of the genus Chlorocebus. Ch. aethiops (black), Ch. cynosuros (purple), Ch. pygerythrus (pink: study species), Ch. sabaeus (orange) and Ch. tantalus (yellow).

2.2.2 *Physical characteristics*

Vervet males and females are sexually dimorphic with wild adult males weighing between 3.9 and 8.0 kg and females weighing between 3.4 and 5.3 kg (for comparison across regions, see: Pasternak et al., 2013). Average length from the top of the head to the base of the tail is 490 mm for adult males and 426 mm for adult females. Adult males are distinguished by their blue scrota and red penises.

Vervet monkeys are seasonal breeders with mating in southern populations occurring primarily in the austral winter, with the birth season in the austral spring (Baldellou & Adan, 1997). Females give birth to one infant at a time with an interbirth interval of one to two years dependent on ecological conditions and the survival of the offspring from the previous season. Infants are characterised by black coats and pink faces and develop their grey pelage at approximately three months old (Lee, 1984; Lee, 1987).

2.2.3 *Feeding Ecology*

Vervet monkeys are semi-terrestrial omnivores. They eat a primarily herbivorous diet, feeding on leaves, seeds, flowers, gum and fruit. Insects, including grasshoppers and termites, are also eaten frequently, as are different fungi. Eggs and nestlings are eaten opportunistically.

While these vervets are known to eat at least 26 different plant species (Pasternak et al., 2013), the six most frequently eaten foods accounted for approximately 70% of their diet during the study period. These vervets rely heavily on the leaves, flowers, seeds and gum of *Vachellia karroo* which accounted for 32.7% of all feeding events over the study period. Other primary plant species eaten during the study period included grasses (*Cynodon incompletus*; *Panicum maximum*), *Schinus molle*, *Lycium oxycarpum and Lycium cinereum*.

During periods of drought, the Samara vervet monkeys have long periods without access to drinking water. At such times, they rely solely on moisture obtained from succulents and feed extensively on the roots and leaves of mother-in-law's tongue (*Sansevieria aethiopica*), and the roots of *Asparagus retrofractus* (Figure 2.4). In the driest months of the study period, July and August 2017, these succulents accounted for 16% and 10% of all feeding events, respectively. Vervets typically do not feed on these plants when water is available.

Figure 2.4 Adult female feeding on *Asparagus retrofractus* roots (left) and adult male feeding on a *Sansevieria aethiopica* root (right) during a period of no water availability.

2.2.4 *Social Organisation*

Vervet monkeys are gregarious, social primates that live in multi-male/multi-female groups (Pasternak et al., 2013; Struhsaker, 1967). Females are philopatric and males typically migrate from their natal groups at or around the age of sexual maturity (Henzi & Lucas, 1980). After that, they migrate between troops roughly every 2.5 to 3 years (Bonnell et al., 2020). In my study population, emigrations occurred between the 3 overlapping study troops as well as to more distant neighbouring groups.

Vervet monkey troops generally consist of 20 individuals or less (Fedigan & Fedigan, 1988). Group size is commonly constrained in vervet monkey populations regardless of habitat productivity (Hall & Gartlan, 1965; Harrison, 1983; Henzi et al., 2013). Troop sizes at Samara are considerably larger than the average troop size, even after a drought-induced period of high mortality in 2016 (Table 2.2) and population density is high along the Milk River (Pasternak et al., 2013). Larger troop sizes have been attributed to the consistently high availability of

Vachellia karroo leaves, flowers, seeds and sap, as well as to the constraints placed on troop fission due to low habitat quality away from the river.

Table 2.2 Mean troop sizes (± SD) across the study periods. Adults are individuals who have reached sexual maturity. Infants were born between August 2017 and February 2018.

Troop	RBM	PT	RST
Adults	14 (1.5)	16 (0.95)	14 (0.6)
Juveniles/yearlings	27 (1.25)	21 (0.43)	23(0)
Infants (born)	2	5	2
Annual mean	42 (2.3)	39 (1.63)	38 (1.4)

The size of non-human primate groups has social, physiological and behavioral consequences and, amongst others, increased group size has been attributed to underpin changes in parasite prevalence, faecal glucocorticoid concentrations, group spread, rank steepness and social dynamics (Cote & Poulin, 1995; Henzi et al., 2013; Josephs et al., 2016; Snaith et al., 2008). Dominance hierarchies are relatively stable for females while male dominance hierarchies are more variable over time (Bramblett et al., 1982). Male and female hierarchies are comprehensively interdigitated with instances of one or more females in a group outranking all males regardless of troop demographics (Young et al., 2017b).

2.3 Study groups

Data were collected from three troops of fully habituated troops of vervet monkeys from March 2017 to April 2018. Picnic troop (PT), Riverbend Mob (RBM) and Riverside troop (RST) live in adjacent, overlapping home ranges along the Milk River. RST and RBM have been habituated to human observers since 2008, while data collection on PT started in 2012. All individuals are identified based on individual markings including variation in facial colouration, breaks or shortening of the tail and ear nicks.

While my study troop sizes were still generally larger than other study sites, there were significant mortalities in the two years prior to my study period (N = 25) owing to extensive drought. Troop demographics are provided in Table 2.2. My study troops had considerable home range overlaps (estimated to be up to 54% in an earlier study), significantly larger than at other sites (Pasternak et al., 2013). Home range sizes varied across troops and across the year. The home range sizes were 0.22 – 0.55 km^2 (mean = 0.31 ±0.1SD) for RST, 0.36 – 0.89 km^2 (mean = 0.42, ± 0.1SD) for RBM and 0.41 – 0.74 km^2 (mean = 0.42 ± 0.11SD) for PT.

2.4 Behavioural data collection

Each group was followed for five days each week, and data collected for 10 hours each day by 3 to 5 observers split over the 3 troops. Day length differs substantially between summer and winter. In summer we alternated between data collection beginning at dawn (5:00) and data collection finishing at dusk (19:00), whereas, in winter, daylight hours (07:30-17:30) generally corresponded with the 10-hour sampling period. Data were collected from a subset of 27 adult individuals (PT: 4 males, 6 females from 16 adults; RBM: 2 males, 6 females from 14 adults; RST: 3 males, 6 females from 14 adults), selected to be representative of adult demography and to reflect the full range of dominance ranks. Three methods of behavioural data collection were used: scan sampling, continuous focal sampling and ad libitum data collection (Altmann, 1974).

2.4.1 *Scan sampling*

Ten-minute scans were conducted every 30 minutes during which the behaviour, location (height above ground and tree/shrub/ground), nearest neighbours (male, female and juvenile), posture and vigilance were recorded for all visible individuals. Behaviours were mutually

exclusive, and definitions are outlined in Table 2.3. When an individual was foraging, the type of food was also recorded. A total of 55 154 individual-level scans were collected during my study period.

2.4.2 *Focal sampling*

Ten-minute continuous focal samples were collected bi-weekly for each of the 27 subjects (N_{total} =1614 focal samples). Randomised focal times were generated for each day. During these focal samples, a single individual was followed, and a continuous, timed record of its behaviour was obtained using an electronic data logger fitted with proprietary software. The same mutually exclusive behaviours were identified. I also continuously recorded whether a focal individual was in the sun or shade, as well as avoidance behaviours (approaches and departs).

2.4.3 *Ad libitum*

Ad libitum observations were used to compile data on dyadic agonistic interactions, copulations, inter-troop encounters (ITEs) and predator calls. For this study, I focused only on dyadic agonistic interactions where participants and outcomes were recorded for the subsequent assessment of dominance hierarchies. Given the open nature of the site's vegetation, visibility was generally very good, and I am confident that there was no systematic bias in the likelihood of observing encounters.

Table 2.3 Behavioural ethogram for the primary behaviours recorded during scan, focal and ad libitum data collection

Behaviour	Definition	Additional Details Recorded
Rest	Stationary (eyes closed or eyes open) and not engaged in any other active behaviour	
Locomote	Travelling including short bouts of movement then sitting while looking for next steps	
Forage	Ingesting food and actively looking for food	Food type
Groom	Individual is receiving or giving grooming	Give or receive Partner ID
Aggression	Individual gives or receives any form of aggression	Give or receive Partner ID Maximum level of aggression (displace, supplant, physical contact, vocal threat, facial threat, lunge, charge, chase, physical contact) Outcome
Autogroom	Monkey is grooming itself	
Nurse	Mother is nursing	
Play	Playing with another individual	Partner ID
Swim	Swimming but does not include locomotion through water such as crossing the river	
Vigilance	Individual alert and actively scanning the social or physical environment	Reason for vigilance: predator, social, human, unknown

2.5 Faecal sample collection and analysis

2.5.1 *Faecal sample collection*

Faecal samples were collected by four to five observers spread over the three troops during each of the 234 10h study days. Faecal samples were collected non-invasively once every two-weeks from each of the 27 individually-identifiable adults. Two corresponding faecal samples—one for parasite analysis and one for faecal glucocorticoid metabolites (fGCM)

analysis—were collected from the same defecation event. A total of 573 faecal samples was analysed and included in this thesis (mean/individual = 21 ± 3.1SD).

2.5.1.1 Parasite sample analysis

For each sample, approximately 1 g of fresh faeces was weighed in the field immediately after defecation and directly placed into 10% neutral, buffered formalin. Samples were stored in a field lab and transported to the University of Lethbridge, Canada, where faecal flotation and sedimentation techniques were used to identify parasites. A modified zinc sulphate flotation was used to isolate helminth eggs, whereby an additional washing step was included in the faecal flotation to avoid egg damage, which had been evident in the initial samples that were analysed (Blersch et al., 2019).

Briefly, faecal samples suspended in formalin were placed in 15 ml Falcon tubes and centrifuged at 1,389 g for 6 min after which the supernatant was discarded. The Falcon tube was filled with water, mixed with the faecal material, centrifuged at 1389 g for 6 min, and the supernatant was discarded. The deposit was resuspended in $ZnSO_4$ (specific gravity 1.3), vortexed to mix, and centrifuged at 617 g for 8 min. The supernatant was pipetted into 4x15 ml tubes and combined with water. The pellet that remained after flotation was kept aside for sedimentation. This step reduced the specific gravity of the $ZnSO_4$ after flotation, thus preventing egg damage and allowing the eggs to deposit upon sedimentation. These supernatant-water tubes were centrifuged at 964 g for 6 min. The supernatant was discarded, and the deposits were combined into 1 test tube, which was filled with water and centrifuged at 964 g for 6 min. The supernatant was discarded, and the entire pellet was examined under the microscope. Parasites were identified to genus-level based on egg shape, size, colour, and contents, and all eggs were counted (Gillespie, 2006). Representative eggs were photographed. Ethyl-acetate

sedimentation was used to isolate potential trematodes that were too heavy to float during ZnSO4 flotation. Here, the deposit from the flotation was suspended in water, vortexed, and centrifuged at 964 g for 6 min. The supernatant was discarded, and the sample was rewashed. Water was added to the pellet to the 7 ml mark of the centrifuge tube and vortexed. Then, 3 ml of ethyl-acetate was added to the tube, mixed thoroughly, and centrifuged at 1389 g for 6 min, and the supernatant was then discarded. The entire pellet was examined under the microscope. As with the zinc-sulphate flotation, parasites were identified to genus- level based on egg shape, size, colour, and contents, and all eggs were counted (Gillespie, 2006). Representative eggs were photographed. We have previously established that there is evidence that faecal egg counts are not stochastic events and point to an underlying infection in our population (Blersch et al., 2019; Blersch et al., 2021) and thus use egg counts as a proxy for helminth infection.

2.5.1.2 fGCM concentrations

Samples were collected following the protocol of Young et al. (2017a; 2019). Within 15min of defecation, a 2-5 g piece of faecal material was transferred into a plastic vial following physical homogenization of the full faecal sample. Prior to collection, faecal samples were checked to ensure there was no contamination with urine during excretion or one the substrate where the sample landed. Vials were stored on ice in a thermos in the field before transfer to a −20°C freezer at the end of the day. Samples were stored until transport on dry ice to the Endocrine Research Laboratory, University of Pretoria, for analysis.

Young et al. (2017a) previously confirmed the most appropriate enzyme immunoassay (cortisol assay) for vervet monkey faecal sample analysis of fGCM concentrations in the study population and the full extraction process. Briefly, samples were lyophilized and then pulverized and sieved to remove seeds and fibrous matter. The resulting faecal power was weighed and 0.10

g was extracted. For extraction, samples were vortexed for 15 min with 80% ethanol in water (3 ml) followed by 10 min of 1500 g centrifugation. 1.5 ml of the resultant supernatants were transferred into microcentrifuge tubes for hormone analysis. Hormone analysis was conducted following the standard procedures of the Endocrine Research Laboratory, University of Pretoria (Ganswindt et al., 2002) using the cortisol enzyme immunoassay (EIA) (Young et al., 2017a). Inter- and intra-assay coefficients of variation of high- and low-value quality controls were: 4.64–5.96 and 8.13–11.60% respectively. All steroid concentrations are given as ng g−1 faecal dry weight.

2.6 Study species suitability

Vervet monkey populations are exposed to a wide range of predators, resource richness, thermal regimes and pathogens, and, with the publishing of the vervet genome (Warren et al., 2015), on their way to being the most comprehensively phenotypically and genomically characterised non-human primate. Thus, making them a model organism for studies into the biogeography and evolutionary ecology of health (Jasinska et al., 2012; Jasinska et al., 2013). Vervets are a highly social, group-living species living in multimale, female-philopatric groups (Henzi & Lucas, 1980), which allows for both broad and detailed social analysis as described above.

The study troops are subject to the effects of anthropogenic climate change (Hoffman et al., 2009) which typically manifests itself in unpredictable and often reduced rainfall in an already low rainfall area. There are large spatio-temporal fluctuations in food availability and they suffer long periods without access to drinking water, surviving solely on water derived from succulents (McDougall et al., 2010). Additionally, seasonal temperature variation is wide with

cold winter nights being particularly physiologically stressful as shown by a decline in the ability to thermoregulate efficiently as winter progresses (Henzi et al., 2017; McFarland et al., 2015). This population is also subject to high predation from a well- established predator guild (Ducheminsky et al., 2014) thus making it an ideal population for the investigation into environmental stressors, parasitism and health.

In terms of the proposed social network analysis, it has been shown that dominant females are neither socially (Henzi et al., 2013), nor spatially (Josephs et al., 2016), central, despite linear dominance hierarchies. This will allow me potentially to infer that dominant females have higher parasite loads by virtue of their network centrality (Macintosh et al., 2012). In another vervet population, it has been found that network degree is associated with increased levels of hookworm infection (Wren et al., 2016). Coupled with the finding at my study site that network degree is positively correlated with both improved thermoregulation and reduced predation risk (Josephs et al., 2016; McFarland et al., 2015), I am in a good position to accurately assess the positive and negative consequences of sociability in the context of the trade-offs involved in obligate sociality.

It has also been demonstrated that network transitivity, a global network property that measures the extent to which animals in the network are clustered fluctuates over moderate time scales (Vilette et al. in prep.). This may serve as a potentially important index of the probability of socially mediated infection. Additionally, during times of fever, as identified from abdominal temperature logger data, animals show signs of sickness behaviour and that this behaviour is detected by other group members (Hetem et al. in prep.). These findings bring to light the possibility that sifts in global network structure may be due to realignments in response to disease.

Lastly, social disease transmission is evident in our population as shown by an outbreak of herpes virus SA8 being positively associated with reach (Murphy, 2016). Combined, all these findings show the excellent suitability of the population for all aspects of my proposed study.

PART A: GASTROINTESTINAL HELMINTHS AND THEIR MULTI-SCALE PREDICTORS IN VERVET MONKEYS

CHAPTER THREE: GASTROINTESTINAL PARASITES IN VERVET MONKEYS

Parts of this chapter have been published in the Journal of Parasitology (19 August, 2019) under the title "Gastrointestinal Parasites of Vervet Monkeys (Chlorocebus pygerythrus) in a High Latitude, Semi-Arid Region of South Africa". The version presented here includes additional details and results from long-term work (April 2018 – March 2019) that expand on the results included in the publication.

3.1 Abstract

Parasitism is fundamentally linked to the environment of both host and pathogen (Nunn & Altizer, 2006). Given global climate changes and large-scale human migration, understanding parasites and pathogens of wildlife and the factors that drive the spread of these is becoming increasingly important. Primate parasites are of particular interest due to potential zoonotic transmission as the human-wildlife interface expands. There is a wide range of social and environmental factors that influence parasite transmission and prevalence and understanding these is crucial to understanding both population-level consequences of climate change for animals living in social groups. Here, I present data on the gastrointestinal parasites of vervet monkeys in a high latitude, semi-arid region of South Africa. Vervet monkeys are a widely-distributed African cercopithecine (Wolfheim, 1983) that occupy a broad range of habitats and thus serve as a good species for studying how environment shapes parasite transmission, prevalence and intensity. I identified the gastrointestinal parasites in the population and compare these to vervet populations found in tropical and sub-tropical regions. I found that this population has a distinctively high prevalence of parasites with an insect intermediate host compared to all other vervet populations suggesting a strong environmental driver of parasitism in this

population. Additionally, the presence of both *Trichostrongylus* sp. and *Ternidens* sp. as well as the absence of *Trichuris* and *Strongyloides* sp. points to a unique parasite assemblage in these vervets.

3.2 Introduction

Parasites and pathogens are ubiquitous in nature where they typically exist at the expense of the host. While severe infection can lead to blood loss, tissue damage, spontaneous abortion, congenital malformations, and death (Tompkins et al., 2011), non-lethal or sub-clinical infections are more common in wildlife, but equally important. These infections can have severe consequences and can influence feeding, predator response, nutrition, travel, reproductive potential, and competition for resources or mates (Hudson & Dobson, 1992; Packer et al., 2003; Tompkins et al., 2011).

Owing to the close phylogenetic relationship between non-human primates and humans, and the increase in zoonotic transmission, primate parasites and pathogens are of particular interest to epidemiologists and conservationists alike (Wolfe et al., 1998). Given the need for non-invasive methods in wildlife studies, much disease research in non-human primates has focused on gastrointestinal parasites that can be assessed using faecal sampling (Gillespie et al., 2004), however, advances in genetic testing have resulted in a recent surge in reports on the gut microbiome (Clayton et al., 2018a; Clayton et al., 2018b). Historically, the gastrointestinal parasites of great apes, baboons and howler monkeys have been extensively studied (Gillespie et al., 2004), and although the focus has often been on parasites of significance to humans (Gulland, 1995) a recent, significant rise in research across many primate taxa means that the drivers of parasite infections in non-human primates are increasingly better understood.

A well-documented driver of parasitism and parasite transmission is host environment, whether natural or degraded (Gillespie et al., 2005; Gillespie & Chapman, 2006; Mbora & McPeek, 2009; Schwitzer et al., 2010). Strong evidence suggests that both parasite species richness and diversity, as well as parasite transmission, are affected by aspects of host environment. These environmental characteristics include temperature, humidity and rainfall, all of which may vary along latitudinal gradients (for primates, see: Appleton & Henzi, 1993; Nunn et al., 2005; Nunn & Dokey, 2006). Consequently, detailing a taxon's pathogen or parasite diversity and intensity across all the habitats it occupies provides a better understanding of its vulnerability both locally and globally to shifts in environmental conditions.

Vervet monkeys of the genus *Chlorocebus* are a widely-distributed African cercopithecine (Wolfheim, 1983), and a highly social, group-living taxon that lives in multi-male, female-philopatric groups (Henzi & Lucas, 1980). Although associated primarily with riparian woodland (Isbell et al., 2002) they occupy a range of habitats, from tropical woodland to semi-desert (Pasternak et al., 2013). As such, vervets are exposed to a wide range of predators, resource availability, thermal regimes and pathogens. Despite their broad distribution, knowledge of their pathogens and parasites is currently restricted to data from five tropical sites (Amenu et al., 2015; Gillespie et al., 2004; Kooriyama et al., 2012; Legesse & Erko, 2004; McGrew, 1989; Muriuki et al., 1998; Petrášová et al., 2010; Valenta et al., 2017) and one subtropical zone, represented by four sites in South Africa (Appleton, 1989; Kaschula et al., 1978; Pitchford et al., 1973; Wren et al., 2015). Further, several of these studies only report parasites of zoonotic interest and not the full assemblage of gastrointestinal parasites, hampering comparison between studies (see: Appleton, 1989; Legesse & Erko, 2004; Muriuki et al., 1998).

While there is a growing body of literature on the gastrointestinal parasites found in wild primate populations, relatively little is known about how these parasites affect their hosts. This is in part due to the nature of data collection and the need for methods of assessment to be non-invasive and that pathological assessment is typically conducted on opportunistically found carcasses (Gulland, 1995). Parasite infections are also often sub-clinical and non-lethal, limiting non-invasive assessment of pathology in wild populations (Ghai et al., 2015). Thus, much of the parasite life cycle and clinical manifestation reports provided here stem from studies of human or non-human species of veterinary importance.

Here I present gastrointestinal parasite data from a study population close to its latitudinal limits in the semi-arid, temperate Karoo biome of South Africa. This is a region under escalating risk from climate change (Jury, 2013), and is distinctive in its low annual rainfall, very high summer temperatures, and very cold winters (McFarland et al., 2015; Pasternak et al., 2013). My aim is to describe the primary gastrointestinal parasites recorded from this population and to compare these results to a short-term dataset from our study population and other vervet populations. As part of this, given that there is evidence of a link between latitude and parasite richness in primates in general (Nunn et al., 2005), I also assessed parasite species richness across a latitudinal gradient for vervet monkeys.

3.3 Methods

3.3.1 *Study Site and Study Subjects*

Data were collected from three fully habituated groups (PT, RBM, and RST) of wild vervet monkeys that have been the subject of intensive data collection since 2009 at Samara Private Game Reserve, South Africa (32º22’S, 24º52’E). All group members were individually

identified based on natural markings. The study area is semi-arid riverine woodland (Pasternak et al., 2013), with a declining annual average rainfall of 386 mm, and average minimum and maximum temperatures of 6.1 C and 21.2 C respectively. Jury (2013) estimated a rainfall decline of -0.6 mm/day with larger interannual variation in Southern Africa.

There were two distinct data collection periods. Pilot study data reported in Blersch et al. (2019) were collected during the austral winter (May – July 2016) on 55 study days from 56 individually-identifiable adults of both sexes: RBM (M = 4, F = 11), PT (M = 6, F = 8) and RST (M = 11, F = 16). Following this, further data were collected across 12 consecutive months – April 2017 to March 2018 – on 232 study days from a subset of 27 adult individuals across the three troops. These individuals were selected to be representative of adult demography and to reflect the full range of dominance ranks (PT: 4 males, 6 females from 16 adults; RBM: 2 males, 6 females from 14 adults; RST: 3 males, 6 females from 14 adults).

3.3.2 *Faecal sampling and analysis*

Faecal samples were collected by four or five observers spread over the three troops during each of the 234 10h study days. Faecal samples were collected non-invasively twice per month (once during each two-week period) from each of the 27 subjects. I analysed a total of 573 faecal samples (mean/individual = 21 ± 3.1 SD).

For each sample, approximately 1 g of fresh faeces was weighed in the field immediately after defecation and directly placed into 10% neutral, buffered formalin. Samples were stored in a field lab and transported to the University of Lethbridge, Canada, where faecal flotation and sedimentation techniques were used to identify parasites.

A modified zinc sulphate flotation was used to isolate helminth eggs, whereby an additional washing step was included in the faecal flotation to avoid egg damage, which had been evident in the initial samples that were analysed (Blersch et al., 2019). Briefly, faecal samples suspended in formalin were placed in 15 ml Falcon tubes and centrifuged at 1389 g for 6 min after which the supernatant was discarded. The Falcon tube was filled with water, mixed with the faecal material, centrifuged at 1389 g for 6 min, and the supernatant was discarded. The deposit was resuspended in $ZnSO_4$ (specific gravity 1.3), vortexed to mix, and centrifuged at 617 g for 8 min. The supernatant was pipetted into 4x15 ml tubes and combined with water. The pellet that remained after flotation was kept aside for sedimentation. This step reduced the specific gravity of the $ZnSO_4$ after flotation, thus preventing egg damage and allowing the eggs to deposit upon sedimentation. These supernatant-water tubes were centrifuged at 964 g for 6 min. The supernatant was discarded, and the deposits were combined into 1 test tube, which was filled with water and centrifuged at 964 g for 6 min. The supernatant was discarded, and the entire pellet was examined under the microscope.

Ethyl-acetate sedimentation was used to isolate potential trematodes that were too heavy to float during ZnSO4 flotation. Here, the deposit from the flotation was suspended in water, vortexed, and centrifuged at 964 g for 6 min. The supernatant was discarded, and the sample was rewashed. Water was added to the pellet to the 7 ml mark of the centrifuge tube and vortexed. Then, 3 ml of ethyl-acetate was added to the tube, mixed thoroughly, and centrifuged at 1,389 g for 6 min, and the supernatant was then discarded. The entire pellet was examined under the microscope.

For both methods, parasites were identified to genus-level based on egg shape, size, colour, and contents, and all eggs were counted. Representative eggs were photographed.

3.4 Results

I recovered five helminth taxa morphologically similar to *Trichostrongylus* sp., *Subulura* sp., *Ternidens* sp., *Oesophagastomum* sp. and one spirurid with 98.6% of samples being positive for one or more parasite genera (Figure 3.1). The spirurid could not be identified to species- or genus- level based on microscopy alone, as the eggs of *Physaloptera* sp. and *Protospirura* sp. are too similar to differentiate. However, based on the morphological characteristics of the eggs, including their size and the presence of a hyaline substance (Brumpt, 1931; Petrželková et al., 2006), I consider it most likely to be *Protospirura* sp. (hereafter referred to as ?*Protospirura* sp.). I recovered one other spirurid in the short-term study that was not subsequently recovered in the long-term study. A conclusive identification could not be made but it is likely *Streptopharagus pigmentatus*. Eggs of two unidentified parasites were recovered. These were likely free-living nematodes resulting from soil contamination during faecal sample collection. I recovered no protozoans.

I compared and contrasted our results to other vervet populations (Table 1.1). Notably absent but common in other vervet populations was *Strongyloides* sp. and *Trichuris* sp. Strongyles indicates the presence of unidentified strongyle eggs recovered.

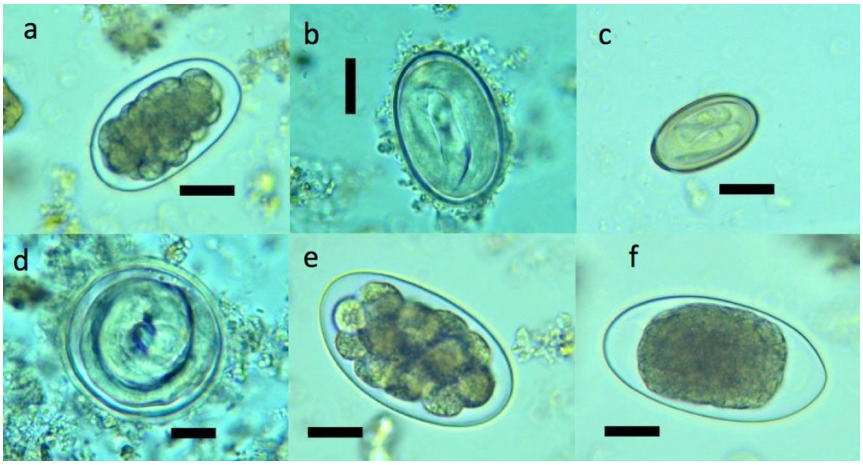

Figure 3.1 Helminth eggs from vervet monkey faeces in the karoo, South Africa. (a) *Oesophagostomum* sp. (b) Spirurid 1 (considered to be *Protospirura* sp.). (c) Spirurid 2 (likely *Streptopharagus pigmentatus*). (d) *Subulura* sp. (e) *Ternidens* sp. (f) *Trichostrongylus* sp. Scale bar: 20µm.

3.4.1 *Helminths with indirect life cycles*

The most prevalent parasite genera in our study population were two spirurids with an arthropod intermediate host. i. ?*Protospirura* sp. eggs were colourless, thick-shelled, ellipsoidal and surrounded by a hyaline substance. Prevalence of ?*Protospirura* sp. was unusually high in our population with a mean annual sample prevalence of 98.7%, and present in all 27 individuals.

Table 3.1 Presence of gastrointestinal helminths recovered in this study compared to other vervet studies including two common helminths of vervet monkeys not recovered here.

	This study [1]	**South Africa**	**Senegal**	**Kenya**	**Uganda**	**Ethiopia**	**Tanzania**	**Tanzania**	**Ethiopia**	**South Africa**	**Kenya**	**Uganda**
Oesophagostomum sp.	X					X		X		X		X
Trichostrongylus sp.	X	X										
Subulura sp.	X						X					
Ternidens sp.	X											
Protospirura sp.	X						X					
Strongyle			X	X	X		X		X		X	X
Strongyloides sp.			X	X	X		X	X	X	X		X
Trichuris sp.		X	X	X	X		X	X	X	X	X	X

[1] Data, in column order, from: Blersch et al., 2019, 2020; Appleton et al., 1989; McGrew et al., 1989; Muriuki et al., 1998; Gillespie et al., 2004; Legesse and Erko, 2004; Petrášová et al., 2010; Kooriyama et al., 2012; Amenu et al., 2015; Wren et al., 2015; Obanda et al., 2015; Valenta et al., 2017.

Egg counts varied widely. Annual minimum and maximum egg counts from positive samples (ps) were 2 eggs per gram (EPG) and 5841 EPG respectively ($\overline{x}_{ps}$= 752.22 ± 861.33 sd), while mean annual egg counts for individuals ranged from 93.84 ± 90.00 sd to 1862.94 ±1521.61 sd. There was also evidence of individual variation in *?Protospirura* sp. egg shedding with some individuals having consistently low egg counts (e.g. 7 – 400 EPG) while others had consistently high counts across the year (e.g. 600-2500 EPG).

ii. *Subulura* sp. eggs were rounded, relatively thick-shelled and contained coiled larvae. Mean annual sample prevalence was 3.83% recovered from 12 individuals and egg counts ranged from 2 – 10 EPG ($\overline{x}_{ps}$ = 5.6 ± 2.03 sd). A third spirurid, likely *Streptopharagus pigmentatus*, was recovered during my short-term field season but not recorded the following year.

3.4.2 *Soil transmitted helminths*

I recovered three soil transmitted helminths. i. Eggs of *Trichostrongylus* sp. from 21 individuals, with a mean annual sample prevalence of 22%. Eggs were non-larvated, colourless, thin-shelled and pointed at either one or both ends. Egg counts ranged from 2 to 47 EPG ($\overline{x}_{ps}$ = 6.5 ± 5.2 sd).

ii. *Oesophagastomum* sp. eggs were ovular in shape with rounded extremes, thin-shelled and non-larvated. Eggs contained numerous blastules. Given the morphological similarity between strongyle eggs and the possibility of size overlap, a definitive identification could not be made pending confirmation via molecular analysis. Prevalence in our population was low with a mean annul sample prevalence of 1.7% recovered from 8 individuals. Egg counts ranged from 1 – 17 EPG ($\overline{x}_{ps}$ = 4.08 ± 2.17 sd).

iii. Mean annual sample prevalence of *Ternidens* sp. eggs was 2.42%, recovered from 13 individuals. Ternidens sp. eggs were ellipsoidal in shape, thin-shelled, non-larvated and contained numerous blastules. Eggs were differentiated from *Oesophagastomum* sp. based on size, shape, and the larger width to length ratio. However, owing to the similarities between strongyle eggs, a definitive identification could not be made pending the results of molecular diagnostics. Egg counts ranged from 1 to 8 EPG ($\overline{x}_{ps}$ = 3.10 ± 2.22 sd). Sample prevalence was lower than previously reported in a short-term study (20.4%) suggesting there is seasonal or annual variation in our population.

3.4.3 *Parasite species richness*

Helminth richness in this study was comparable to other vervet monkey populations (Table 3.2).

Table 3.2 Number of gastrointestinal parasite genera identified in vervet monkey parasite studies. Counts include unidentified strongyles as one genus.

Location	Author	Helminths	Protozoa	Cestodes	Trematodes
South Africa (this study)	Blersch *et al*. (2019/2020)	6/5	0	0	0
South Africa	Appleton (1989) [1]	4	0	0	0
Senegal	McGrew *et al*. (1989) [2]	3	2	0	0
Kenya	Muriuki *et al*. (1998) [1]	4	2	0	1
Western Uganda	Gillespie *et al*. (2004)	4	0	0	1
Ethiopia	Legesse & Erko (2004) [1]	2	5	0	0
Tanzania	Petrášová *et al*. (2010)	6	5	0	0
Tanzania	Kooriyama *et al*. (2012)	6	1	0	1
Ethiopia	Amenu *et al*. (2015)	6	6	1	0
South Africa	Wren *et al*. (2015)	5	1	0	0
Kenya	Obanda (2015)	5	0	0	0
Uganda	Valenta *et al*. (2017)	9	0	0	2

[1] Only parasites of zoonotic importance considered
[2] Host species: *Cercopithecus (aethiops) sabeus*

Overall, I found that helminth diversity did not change across a latitudinal gradient (Figure 3.2A). However, in studies that examined protozoa, protozoan species diversity was generally found to be higher closer to the equator (Table 3.2, Figure 3.2B). Given that some studies did not examine protozoa and others only considered gastrointestinal parasites of zoonotic importance, this cannot be definitively confirmed.

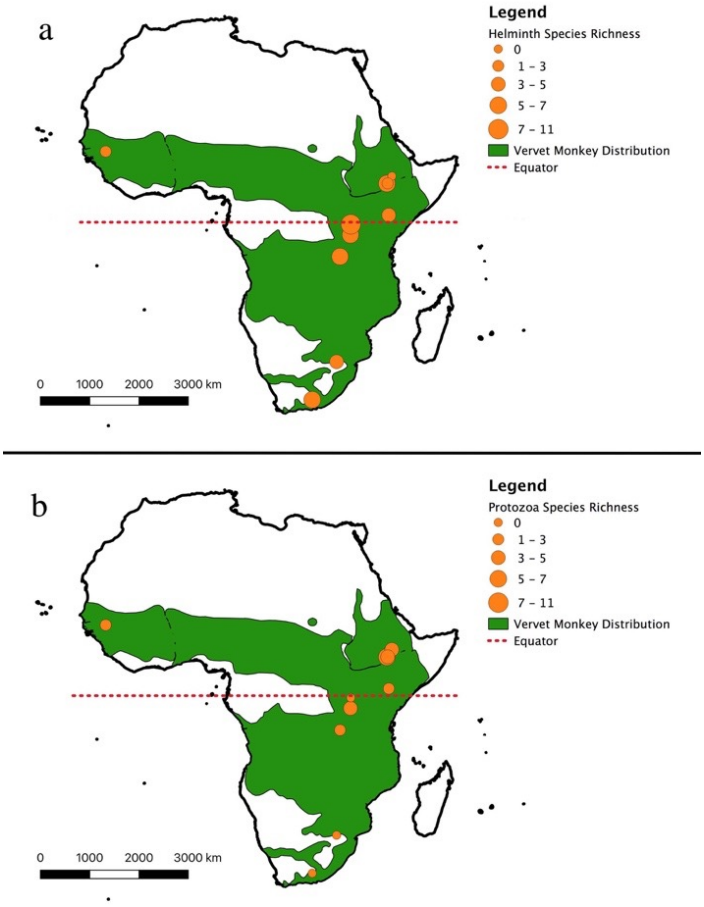

Figure 3.2 Parasitic taxon richness in wild vervet monkey populations in Africa. (A) Helminths. (B) Protozoa. (Data from: Pitchford et al., 1973; Appleton, 1989; Muriuki et al., 1998; Gillespie et al., 2004; Legesse and Erko, 2004; Petrášová et al., 2010; Kooriyama et al., 2012; Amenu et al., 2015; Wren et al., 2015; Valenta et al., 2017; Blersch et al., 2019).

3.4.4 *Discussion*

To the best of my knowledge, this is the first report on the gastrointestinal parasites of vervet monkeys living in a semi-arid, temperate region. Several of the parasites reported here have been identified in other vervet populations (Appleton, 1989; Kooriyama et al., 2012; Legesse & Erko, 2004; Petrášová et al., 2010; Valenta et al., 2017; Wren et al., 2015). At the same time, however, the high prevalence of spirurids with an arthropod intermediate host (98%) is distinctive of my study population. The highest host group prevalence of spirurids in other populations was 68% when eggs resembling *Physaloptera* sp. and *Streptopharagus pigmentatus* were combined (Wren et al., 2015).

?*Protospirura* sp. has been recorded in a wide range of mammal hosts including rodents, carnivores and primates across Africa, Asia, Central America and South America (Petrželková et al., 2010; Smales et al., 2009). Although infections in rodents appear non-pathogenic, *P. muricola* infection can cause severe disease in some captive primates (Ruch, 1959). Arthropods and reptiles serve as the intermediate host for spirurid transmission, and research has shown that primates increase their insect intake under dry conditions (Chapman, 1988; Garber, 1987). Monkeys become infected through eating arthropods that have cysts containing infective larvae after which the adult worms develop and reside in the oesophagus and stomach (Foster & Johnson, 1939). The high prevalence of ?*Protospirura* sp. differs strongly from the other vervet population it was recovered in. Petrášová et al. (2010) recorded a sample prevalence of only 9.1% in the wet season and 4.5% in the dry season. Given the semi-arid conditions at this study site, increased dietary intake of intermediate hosts could be responsible for the unusually high prevalence of ?*Protospirura* sp. in our population.

The second arthropod transmitted helminth, *Subulura* sp., is a genus that typically infects poultry and wild birds. However, nine species have been identified that infect primates, with *Subulura distans* reported in *Cercopithecus* spp. (Cameron, 1930; Yamashita, 1963). *Subulura* sp. is also transmitted via an intermediate arthropod host. Intermediate hosts ingest eggs that release the larvae which develop into infective stage L3 larvae. Primates then eat contaminated insects and the ingested larvae develop into adult worms. Worms are not—or only mildly—pathogenic but this is not well-studied in primates. *Subulura* sp. has only been reported in one wild vervet population where sample prevalence was 1.1% (Petrášová et al., 2010).

Trichostrongylus sp. is most commonly a parasite of sheep and goats but can infect both humans and non-human primates (Munene et al., 1998). Infection occurs either through the skin or through the mouth from contaminated food or water (Brooker & Bundy, 2013). Infections are typically asymptomatic or mild. Heavy infections can cause gastrointestinal problems that include abdominal pain, diarrhoea and anorexia. While common among other non-human primates, *Trichostrongylus* sp. has only been reported in one population of wild vervets (Appleton, 1989) but has been reported in sympatric baboons (Legesse & Erko, 2004) and other guenons (Gillespie et al., 2004). Prevalence in my population was comparable to that described by Appleton (1989) but lower than in baboon populations (Munene et al., 1998; Müller-Graf et al., 1996).

Oesophagostomum spp. are nematodes common in a variety of mammals including livestock, non-human primates and humans (Ghai et al., 2014). Eggs are shed in the faeces and hatch into infected larvae. Infection then occurs through the ingestion of infective larvae from contaminated food. Infections in wild primates appear to be largely asymptomatic, and clinical signs and mortality have only been reported in captive populations (Stewart & Gasbarre, 1989).

Host group prevalence from other South African vervets was 84% (Wren et al., 2015). *Oesophagastomum* sp. thrives in warm and humid tropical and subtropical regions (Rose & Small, 1980). In chimpanzees, Huffman et al. (1997) found that the incidence of *Oesophagostomum stephanostomum* was significantly higher in the rainy season. Thus, it is likely that the drier climate at the field site reduces encounter probability and susceptibility to *Oesophagostomum* sp. infection. Ghai et al. (2014) also found links between host group size, travel distance, and *Oesophagostomum* sp. prevalence. Although group size was not a direct predictor of *Oesophagostomum* sp. prevalence, prevalence was higher in primates that travel large distances in smaller groups (Ghai et al., 2014). Given the larger than average group sizes in our study population and their relatively short travel (Pasternak et al., 2013), this may combine with the drier climate to reduce susceptibility to *Oesophagostomum* sp. infection.

Often referred to as false-hookworm, *Ternidens* sp. is an intestinal helminth common in non-human primates in Africa and Asia. Infection occurs through ingestion of infective larvae which then reside in the large intestine (Bradbury, 2019). *Ternidens* sp. infection in humans is thought to be a zoonosis. Infection appears to be largely asymptomatic but has not been well-studied and heavy worm loads have been associated with malaise and obstipation (Bradbury, 2019). Sample prevalence was lower than previously reported in a short-term study (20.4%) suggesting there is seasonal or annual variation in our population. Vervet monkeys are frequently cited as a common host of *Ternidens deminutus* (Kouassi, Roland Wa et al., 2015), although it has only been reported in one study, where Blackie (1932) found that three of the five vervet monkeys examined were positive for *T. deminutus.* It has been found in sympatric baboons (Obanda, 2015) and other cercopithecids (Kouassi et al., 2015).

There were several notable genera absent from our population. The first was *Trichuris* which has been recorded from vervets elsewhere, although McGrew (1989), in Senegal, also found no evidence of *Trichuris* infection. He did, however, find *Trichuris* in the closely-related and sympatric patas monkeys (*Erythrocebus patas*). Along with being widely geographically distributed, the prevalence of *Trichuris* in vervet monkey populations varies widely, with host group prevalence as high as 92% in another South African vervet population (Wren et al., 2015). The absence of *Trichuris* is likely also to be due to the semi-arid conditions of our study site, where ground cover can be limited, since *Trichuris* egg development in the environment is sensitive to direct sunlight and relative humidity (Nolf, 1932).

Also absent from our population was *Strongyloides* sp. *Strongyloides* is a common parasite of non-human primates and humans. *Strongyloides* is widely distributed and most common in warm, moist areas worldwide (Brooker & Bundy, 2013). Only two other studies, one in Ethiopia (Legesse & Erko, 2004) and the other, in South Africa, Appleton (1989) did not report *Strongyloides* sp. infection. However, both these studies only reported parasites of zoonotic importance and it is possible that *Strongyloides* sp. was present but unreported. It was found, but at relatively low prevalence, by Wren (2013) in their study on vervets in South Africa. Given the complexity of its lifecycle involving a free-living stage, it is possible that the dry and hot environmental conditions in our study area are unfavourable and free-living worms are unable to survive (Brooker & Bundy, 2013).

Further, I did not detect any protozoans in our study population in both the short- and long-term study. While highly diverse in some vervet studies across Africa (see: Amenu et al., 2015; Legesse & Erko, 2004), identifiable protozoans were also not recorded in some other studies (Valenta et al., 2017), or were not considered (Appleton, 1989; Gillespie et al., 2004). In

South Africa, Wren (2013) reported finding only *Entamoeba coli*. While it is certainly possible that no protozoans are present in the population, long term storage in formalin can damage protozoans and, given the time between collection and analysis, it may be that any protozoans in the faecal matter were not detected due to damage.

Latitudinal gradients of parasite species richness have been found in primates with vector-borne protozoa showing a significant latitudinal gradient. Richness was highest near the tropics for vector-borne protozoa but no latitudinal effect was found for helminths or viruses (Nunn et al., 2005). Similarly, for vervets, no relationship was found between latitude and helminth richness, however, there appeared to be a relationship trend between overall protozoa richness and latitude. Due to low sample size, this is a trend but could not be quantitively confirmed. Given the increase in zoonotic transmission, characterising the global distribution of parasites is essential for understanding human health and wildlife conservation (Wolfe et al., 1998). Further, understanding the geographical variation in parasite diversity is important for predicting how climate change may influence disease risk (Nunn et al., 2005). Combined, this highlights the importance of considering parasites within a host species across a wide geographical range.

It is clear that, while there are some common parasite species, there is large variation in parasite prevalence and richness across vervet populations. This variation is likely due to environmental conditions, both natural and human-induced, which can affect parasites, hosts and intermediate vectors. The higher prevalence of parasites that have an intermediate insect host vector in our population suggests a strong environmental driver of parasitism in an omnivore living in a low rainfall area. Further, the relative rarity of *Trichostrongylus* sp. and *Ternidens* sp. in other vervet populations, as well as the absence of *Trichuris* and *Strongyloides*, highlights an unusual parasite assemblage in our population. These results pave the way for further research

into the social, environmental and physiological factors that shape parasite richness, intensity and prevalence in this semi-arid region and how those compare to tropical and sub-tropical regions. Further, the results serve as a foundation to investigate how chronic infection with non-lethal parasite infections might shape host behaviour, survival and fitness.

CHAPTER FOUR: SEASONAL EFFECTS IN GASTROINTESTINAL PARASITE PREVALENCE, RICHNESS AND INTENSITY IN VERVET MONKEYS LIVING IN A SEMI-ARID ENVIRONMENT

A version of this chapter is published under the same title in the Journal of Zoology (2021), doi.org/10.1111/jzo.12877.

4.1 Abstract

Parasite and pathogen incidence and prevalence is driven by both periodic variation in environmental conditions and host characteristics. Given the increasing risk of zoonotic transmission to humans, and the close phylogenetic relationship between humans and non-human primates, understanding this variation in parasite dynamics is becoming essential for epidemiologists and conservationists alike. The extreme seasonal temperatures coupled with declining annual rainfall and severe periodic drought of the semi-arid Karoo poses distinct challenges to both hosts and pathogens and serves as a window into how animals confront climate change-induced environmental changes. Here I quantified annual variation in gastrointestinal parasite prevalence, intensity and richness in three troops of wild vervet monkeys and determined what climatic variables were driving these changes. Further, I assessed whether there is long-term temporal dependence in intra-individual faecal egg counts. I found variation in the prevalence of 5 genera of helminths identified in the study population, but little variation in parasite richness across the year. Such variation was driven primarily by precipitation and maximum daily temperature. Finally, I found structure in faecal egg counts, suggesting that contrary to previous findings, egg shedding of *Trichostrongylus* sp. and ?*Protospirura* sp. are not stochastic processes and may serve as an indicator of individual levels of infection in our

population. Combined, these results provide the first report of seasonal effects in gastrointestinal parasites of vervet monkeys living in an extreme environment.

4.2 Introduction

Seasonality in the incidence of pathogens and parasites has been well-documented across several host and parasite genera (Altizer et al., 2006), yet the underlying causes for this variation are often unknown. Seasonality in pathogens may be driven by external environmental factors, host characteristics, or features of the host-pathogen system (Lass & Ebert, 2006). Host environment is a well-documented driver of both species richness and diversity across genera (Barrett et al., 2013) and seasonal climatic changes can generate periodic variation in the biology and behaviour of both pathogens and their hosts (Altizer et al., 2006). Environmental conditions, including rainfall, temperature and humidity, all have an impact on parasite diversity, richness, prevalence as well as, in some cases, transmission dynamics (Altizer et al., 2006; Harvell et al., 2002). This can result from a seasonal influence on the pathogen's ability to survive and proliferate in the environment, presence or absence of invertebrate vectors, or from seasonal changes in host characteristics and behaviour, such as reproductive seasonality or grouping patterns (Gulland, 1995; Benavides et al., 2012).

In addition to the external environment, parasite prevalence and intensity are driven by host characteristics, including age, sex, stress-related and immunosuppressive hormones and behaviour (Altizer et al., 2006; MacIntosh et al., 2010; Nunn & Dokey, 2006). Sex differences are evident in many populations ranging from sexual dimorphism to more complex physiological mechanisms and energetic needs (Key & Ross, 1999), including parasite prevalence and intensity. Sex has been shown to predict helminth infections in both human and animal models

(Monteiro et al., 2007). These sex differences are often an interaction between the effects of sex hormones on immunosuppression, as well as the physiological demands of pregnancy and lactation (Moore & Wilson, 2002; Klein, 2004). In general, it is postulated that female mammals are more resistant to parasitic infections than are males, and that this is likely to be due to sex-associated differences in exposure to parasites as well as the immunosuppressive properties of testosterone (Morales-Montor et al., 2004). There is mixed evidence for sex bias in parasite prevalence in non-human primates. Against expectation, Benavides et al. (2012) found that parasite species richness was higher in female chacma baboons than in males, while Wren et al. (2015) and Valenta et al. (2017) found no evidence of sex differences in parasite infection in vervet monkeys. Sex differences have also been shown to vary across age classes (golden lion tamarins, *Leontopithecus rosalia*: Monteiro et al., 2007) and according to reproductive season where males show higher parasite prevalence during the mating season and females harbour more parasites during the birth season (Japanese macaques: MacIntosh et al., 2010).

Here, I investigate whether variation in gastrointestinal parasites of vervet monkeys living in the semi-arid Karoo biome in South Africa is linked to environmental conditions and sex. Specifically, I (i) assess whether rainfall, temperature, food availability/ground cover (NDVI) and sex predict parasite richness, prevalence and intensity across the annual cycle. I have shown previously that, over the short-term, this population exhibited an unusually high parasite prevalence, with an infection rate of 98% for parasites with an arthropod intermediate host (Blersch et al., 2019). Here I investigate (ii) whether this trend, and the trends found for other parasite genera, are sustained across the year. Given that faecal egg counts are often considered to be an unreliable index of adult parasite burden (Coadwell & Ward, 1982; Gillespie, 2006; Roepstorff et al., 1996; Stear et al., 1995; Vidya & Sukumar, 2002), I also (iii) investigate

whether egg shedding is a purely stochastic process or whether there is evidence of structure in egg shedding across the year.

4.3 Methods

4.3.1 *Study Subjects*

Data were collected across 12 consecutive months – April 2017 to March 2018 – from three fully habituated groups (PT, RBM, and RST) of wild vervet monkeys at Samara Private Game Reserve, South Africa (32º22'S, 24º52'E). These monkeys have been the subject of continuous data collection since 2009 and are individually identifiable from natural markings. Data were collected from a subset of 27 adult individuals (PT: 4 males, 6 females from 16 adults; RBM: 2 males, 6 females from 14 adults; RST: 3 males, 6 females from 14 adults), selected to be representative of adult demography and to reflect the full range of dominance ranks.

4.3.2 *Study Site and Environmental Conditions*

The study area is semi-arid riverine woodland, an area characterised by low rainfall, very hot summers and cold winters (McFarland et al., 2015; Pasternak et al., 2013). The area is under escalating risk from climate change (Jury, 2013) and has a declining annual average rainfall of 386 mm, and average minimum and maximum temperatures of 6.1º C and 21.2 º C, respectively (Pasternak et al., 2013). Climate data were recorded at the field site. Information on the daily minimum and maximum temperatures was taken from a centralized weather station at the field site (Hobo U30-NRC, Onset Computer Corporation, USA), while daily measurements of precipitation during the study period were recorded using a standard rain gauge.

For the study period, the daily minimum temperature ranged from -3.9ºC to 20ºC while daily maximum temperature ranged from 11.8ºC to 49.6ºC. Monthly rainfall was low except for two distinct peaks in October 2017 (69mm) and January 2018 (67mm). Total precipitation for the study period was 317mm. Mean monthly NDVI ranged from a low of 0.3 in July 2017 to a high of 0.5 in March 2018 (Figure 4.1).

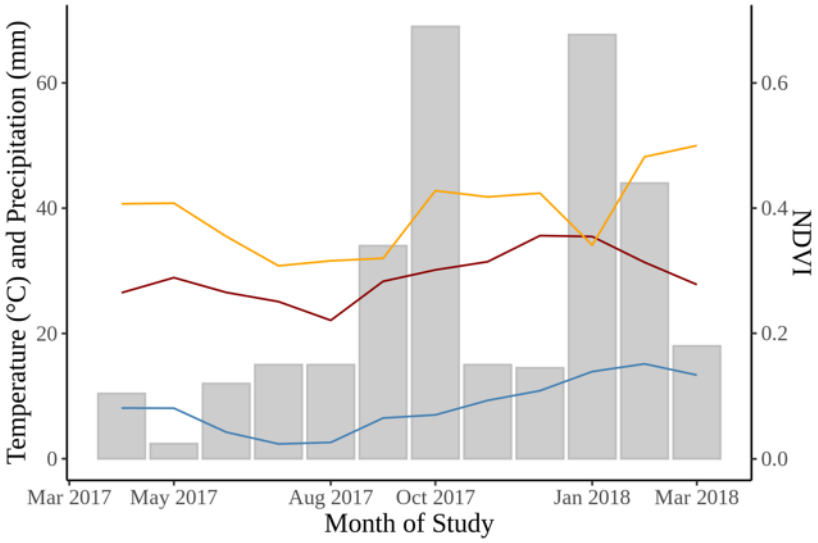

Figure 4.1 Graph showing overall relationships between average monthly minimum temperature (blue line), average monthly maximum temperature (red line), total monthly precipitation (grey bars) and average monthly normalized difference vegetation index (orange line) across the study period (April 2017–March 2018). The y-axis (left) shows temperature in degrees Celsius and precipitation in millimetres. The y-axis (right) shows NDVI score on a 0–1 scale.

4.3.3 *Faecal Sampling and Analysis*

Faecal samples were collected by four to five observers spread across the three troops during each of the 234 10h study days. Faecal samples were collected ad libitum non-invasively twice per month from each of the study subjects (N=573 samples; $\bar{x}$ = 21/subject ± 3.1 SD).

For each sample, approximately 1 g of fresh faeces was weighed in the field immediately after defecation and directly placed into 10% neutral, buffered formalin. Individual identity (ID), date, time, troop and exact faecal weight (mean = 1.21g ± 0.3SD) were recorded. Samples were stored in a field laboratory before being transported to the University of Lethbridge, Canada, where faecal flotation and sedimentation techniques were used to identify parasites. A zinc sulphate centrifugal flotation technique was used to isolate helminth eggs, after modification to include an additional washing step. This washing step reduced the specific gravity of the $ZnSO_4$, preventing egg damage and allowing the eggs to deposit. Full methods are provided in Blersch et al. (2019).

Ethyl-acetate sedimentation was used to isolate potential trematodes that were too heavy to float during ZnSO4 flotation using the deposit from the flotation. Following sedimentation, the entire pellet was examined under the microscope (for full methods see: Blersch et al., 2019). For both methods, parasite eggs were identified to genus level based on egg shape, shell thickness, colour and contents, and all eggs were counted. Representative eggs were photographed.

4.3.4 *Food Availability (NDVI)*

I quantified the food available in each troop's home range by calculating the Normalized Difference Vegetation Index (NDVI) every 16 days (Dostie, 2020, in progress) from MODIS data collected by Earth Observing System (EOS) satellites Terra (EOS AM-1) and Aqua (EOS PM-1). Using Moderate Resolution Imaging Spectroradiometer MOD13Q1 vegetation indices at a 250-meter resolution (Didan, 2015), NDVI measures the amount of biomass or chlorophyll activity by calculating the difference between the visible red and near-infrared bands divided by

their sum. The resultant estimate ranges between -1 and 1, where negative values indicate an absence of vegetation and positive values approaching 1 indicate larger concentrations of green vegetation (Pettorelli et al., 2005). Given the generalist, largely plant-based nature of vervet diet (Pasternak et al., 2013), the synoptic view of NDVI is a reliable measure of food availability for this species (Jarrett et al., 2020; Willems et al., 2009).

4.3.5 *Statistical Analysis*

All statistical analyses were undertaken in a Bayesian framework, using the 'brms' package (Bürkner, 2017; Bürkner, 2018) in R version 3.5.2 (R Core Team, 2018). I constructed hierarchical generalized additive mixed models to allow for non-linear relationships between explanatory and response variables (Pedersen et al., 2019).

I present summary statistics and posterior density plots ("bayesplot" package: Gabry et al., 2019) for posterior means, standard errors and 95% credible intervals (CIs) for the main effects, and for individual variance within the random effects. For the smooth terms, I modelled both global and individual-level smooths and present summary statistics of the spline variance parameter ("wiggliness") for the global smooth and each individual's smooth. I conducted prior predictive checks (Gabry et al., 2019) for each model and specified weakly informative priors (normal (0, 1)), unless otherwise indicated. I ran models with 4 chains and 2000 iterations, which provided me with a large enough sampling pool to conduct posterior sampling and achieve model convergence (Bürkner, 2018; McElreath, 2016). Chain convergence was confirmed by $\hat{R}$ values ≤ 1.01, and model goodness-of-fit was assessed using the 'posterior predictive check' (pp_check) function from the "bayesplot" package (Gabry et al., 2019). I assessed potential collinearity of fixed and random effects visually using pairs plots which produce univariate

histograms and bivariate scatterplots for each parameter (mcmc_pairs function: "bayesplot" package). Collinearity would manifest as narrow bivariate plots, which were not observed between our predictor variables. I used the "bayes_R2" function to generate conditional R^2 values for each model (Gelman et al., 2019).

4.3.5.1 Hierarchical generalized additive mixed effects models

I constructed four GAMMs to assess whether environmental variables and sex predicted parasite prevalence, richness or intensity across the year. Table 4.1 provides a summary of the model parameters. For all models, fixed effects and random effects were constant. To assess whether there was non-linearity in parasite prevalence and richness across the year, and to account for samples not being an equal number of days apart, I included a spline on date.

For parasite intensity (egg count), I was interested in individual-level annual variation, in addition to population level annual variation, and structure in egg count. Thus, I specified the thin plate regression spline (k=15, m=1) on date of collection by individual ID as a fixed effect which allows each individual to have its own smoothing parameter and "wiggliness" (GI model: Pedersen et al., 2019). I also included a global smoother (thin plate regression spline: k=12, m=2) on date to assess population-level variation in parasite intensity across the year (GI model: Pedersen et al., 2019). Given that not all samples were exactly 1g, I included log faecal weight as an offset variable. All continuous predictor variables were mean-centered and standardized by two standard deviations to allow for effect size comparisons across continuous and dichotomous variables (Gelman, 2008).

Table 4.1 Model parameters used in the specification of generalised additive mixed effects models (GAMMs) to assess the influence of environmental factors and sex of parasite prevalence, richness and intensity. Log of faecal weight was included in all models as an offset variable to account for variation in faecal weight.

Measure	Species	Response variable	Fixed Effects	Splines (fixed effects)	Random effects	Distribution
Parasite prevalence	*Trichostrongylus* sp.	Presence/absence	Maximum daily temperature Minimum Daily temperature Bi-weekly precipitation NDVI Sex	Date	Individual Troop	Bernoulli
Parasite richness	All (combined)	Count: number of species	Same as above	Date	Same as above	Poisson (hurdle)
Parasite intensity	*Trichostrongylus* sp.	Egg count	Same as above	Date Date by individual	Same as above	Poisson (hurdle)
	?Protospirura sp.	Egg count	Same as above	Date Date by individual	Same as above	Negative binomial

4.3.5.2 Parasite prevalence

I could not fit statistical models for three genera, owing to low frequency and resultant small sample size (<5% annual sample prevalence), and I therefore present only descriptive statistics. One genus, where sample prevalence was 98%, could not be modelled given that it was present on all sample collection dates. For the remaining genus with high prevalence (>20% annual sample prevalence), I classified the genus as present or absent in each fecal sample and constructed a Bayesian GAMM with a Bernoulli distribution. I specified presence/absence as the binary response variable and included fixed effects, random effects and individual-level spline (Table 4.1).

4.3.5.3 Species richness

To determine whether parasite richness (number of parasite genera) was influenced by environmental factors and sex, I first constructed a GAMM with a Poisson distribution. Given

98% of samples were positive for one of the parasite genera, I used a non-parametric dispersion test using the "DHARMa" package (Hartig, 2020) to confirm underdispersion (for these results, see appendix A.2). To account for the lower than expected variance, I used a hurdle model with a Poisson distribution. This allows for the assumption of fewer (or greater) zeroes than expected for a count distribution (Min & Agresti, 2005; Hilbe, 2017). Our response variable was the number of parasite genera recovered from each fecal sample with the predictors as specified in Table 4.1.

4.3.5.4 Parasite intensity and stochasticity in fecal egg counts

This model set served two purposes: to establish if there was annual variation in eggs related to environmental conditions and sex, and to assess whether there was detectable structure in egg shedding. As I have shown that total fecal egg counts between successive individual samples are correlated, I have suggested that egg counts may be a reliable indicator of an underlying infection in these vervet monkeys, rather than reflecting some stochastic process (Blersch et al., 2019). I tested this for each parasite genus across an annual cycle. If egg shedding were not stochastic, I would have expected date of collection to explain variation in egg count.

As with model set 1, I present descriptive statistics only for the three genera with low sample prevalence (<5% sample prevalence) and low egg count, and modelled the genera with higher prevalence (>20%). For the genus with the highest prevalence (>98%), I constructed a GAMM, specifying a negative binomial distribution, with fecal egg count as our response variable. For the remaining genus, I used a GAM hurdle model with a Poisson distribution to account for the large number of zeroes (Table 4.1). I used prior predictive checks to first assess the suitability of the default priors set for the model parameters (Gabry et al., 2019) and assess whether predicted egg counts using a prior-only are reasonable. Using a weakly informative

prior, normal(0,1), for model parameters resulted in predicted infinity values suggesting unreasonably high egg counts. Thus, I constrained two priors, hu and sds, to normal(0,0.5) for the *Trichostrongylus* sp. model and one prior, sds, for ?*Protospirura* sp. model. Hu is the parameter for the hurdle portion of the model and is the probability that the response variable will be zero, and sds is the variance parameter for the spline which controls the wiggliness of the smooth.

4.4 Results

4.4.1 *Model Set 1: Do environmental variation and sex predict parasite presence/absence and richness?*

I identified 5 helminth taxa, namely, *Trichostrongylus* sp., *Ternidens* sp., *Oesophagastomum* sp., *Subulura* sp., and a spirurid, with 98.6% of samples being positive for one or more parasite genera. All genera were recovered in both the flotation and sedimentation and egg counts reported are combined (for egg morphology, appendix A.1). The spirurid could not be identified to species- or genus- level based on microscopy alone, as the eggs of *Physaloptera* sp. and *Protospirura* sp. are too similar to differentiate. However, based on the morphological characteristics of the eggs, including their size and the presence of a hyaline substance (Brumpt, 1931; Petrželková et al., 2006), I consider it most likely to be *Protospirura* sp. (hereafter referred to as ?*Protospirura* sp.). Given the morphological similarities between strongyle eggs and potential size overlap, there is a degree of uncertainty in distinguishing between *Ternidens* sp. and *Oesophagastomum* sp. based solely on morphology. Molecular analysis is underway to confirm all identified genera. I recovered no protozoa from fecal samples.

I found annual variation in parasite prevalence across all identified genera (Table 4.2). While host group prevalence is the preferred indication of parasite prevalence in a population (Bush et al., 1997), I present both percentage host group and sample prevalence for comparison to other literature (Table 4.2).

The most prevalent parasite in our population was ?*Protospirura* sp., with a mean annual sample prevalence of 98.7%, and present in all 27 individuals. Second was *Trichostrongylus* sp. which was recovered from 21 individuals, with a mean annual sample prevalence of 22%. Mean annual sample prevalence for the remaining nematodes was considerably lower: 3.83% for *Subulura* sp. recovered from 12 individuals, 2.42% for *Ternidens* sp., recovered from 13 individuals, and 1.7% for *Oesophagastomum* sp., recovered from 8 individuals.

4.4.1.1 GAMM results

I present model results for *Trichostrongylus* sp. only. Sample size was too small to model *Ternidens* sp., *Subulura* sp. and *Oesophagastomum* sp. and ?*Protospirura* sp. eggs were recovered on every collection date and thus equally probable across the year, regardless of host and environmental characteristics.

Model results showed evidence of non-linear variation in parasite prevalence across the year for *Trichostrongylus* sp. as indicated by the date spline parameter (Table 4.3: smooth-term). The probability of *Trichostrongylus* sp. occurring was predicted by maximum daily temperature, with occurrence being highest when temperatures were higher (Estimate = 0.74, Estimate error = 0.33, l-CI = 0.09, u-CI = 1.4). No other environmental variables predicted presence or absence of *Trichostrongylus* sp., and no sex differences were found (Table 4.3). I found evidence of inter-individual variation in the prevalence of *Trichostrongylus* sp. and, while there was evidence of inter-troop differences, credible intervals were wide, suggesting that the estimate was very

Table 4.2 Monthly sample prevalence (SP: number of samples positive for the parasite genus/number of samples collected) and host group prevalence (HG: number of positive hosts/number of sampled hosts) for identified species. Lines show austral seasons: summer (December – March), autumn (March – June), winter (June – September) and spring (September – December).

Year	Month	Prevalence	*?Protospirura* sp.	*Trichostrongylus* sp.	*Subulura* sp.	*Ternidens* sp.	*Oesophagostomum* sp.
2017	Apr	SP (%)	98.18	16.36	1.82	14.55	1.82
		HG (%)	100	22.22	3.7	22.22	3.7
	May	SP (%)	96.83	46.03	0	4.76	0
		HG (%)	100	51.85	0	11.11	0
	Jun	SP (%)	100	53.85	0	0	3.85
		HG (%)	100	50	0	0	4.55
	Jul	SP (%)	100	43.4	5.66	5.66	1.89
		HG (%)	100	50	11.54	11.54	3.85
	Aug	SP (%)	100	35.56	2.22	2.22	6.67
		HG (%)	100	38.46	3.85	3.85	11.54
	Sept	SP (%)	100	14.81	1.85	1.85	0
		HG (%)	100	25.93	3.7	3.7	0
	Oct	SP (%)	100	23.33	3.33	0	0
		HG (%)	100	26.92	7.69	0	0
	Nov	SP (%)	100	4.88	7.72	0	0
		HG (%)	100	8	12	0	0
	Dec	SP (%)	94.34	9.43	3.77	0	3.77
		HG (%)	96.3	14.81	7.41	0	7.41
2018	Jan	SP (%)	100	14.71	8.82	0	2.94
		HG (%)	100	15.79	10.53	0	5.26
	Feb	SP (%)	97.62	0	4.76	0	0
		HG (%)	95.65	0	8.7	0	0
	Mar	SP (%)	97.87	2.13	6.38	0	0
		HG (%)	100	4.35	8.7	0	0
Mean		SP (%)	98.74 (±1.74 SD)	22.04 (± 17.56 SD)	3.83 (±2.71 SD)	2.42 (±4.13 SD)	1.74 (±2.09 SD)
		HG (%)	99.33 (±1.51 SD)	25.69 (±17.53 SD)	6.48 (±3.99 SD)	4.37 (±6.77 SD)	3.03 (±3.59 SD)

uncertain (Table 4.3). The full model explained 41% of variance ($R^2_{conditional}$ = 0.41, Est. Error = 0.03, l-CI = 0.35, u-CI = 0.46).

Table 4.3 Summary statistics of Bayesian mixed-effects model for parasite prevalence (present/absent) of *Trichostrongylus* sp. CI = credible interval; SD = standard deviation. Smooth-term sds() = spline "wiggliness"(spline variance parameter). Estimates for fixed effects where credible intervals do not cross zero are in bold.

	Effect	Estimate	Est.Error	l-95% CI	u-95% CI	$\widehat{R}$
Fixed effects						
Population-Level	Intercept	-1.95	0.75	-3.35	-0.38	1
	Bi-weekly precip	-0.4	0.33	-1.06	0.24	1
	Minimum temp	-0.5	0.34	-1.17	0.17	1
	Maximum temp	**0.74**	**0.32**	**0.1**	**1.38**	**1**
	NDVI	-0.25	0.48	-1.19	0.71	1
	Sex (ref:M)	-0.75	0.65	-2	0.59	1
	s(Date)	0.65	0.78	-0.92	2.12	1
Smooth Terms	sds(date)	5.15	2.58	1.71	11.75	1
Random Effects	sds(Troop)	0.77	0.78	0.02	2.91	1
	sds(ID)	1.88	0.41	1.21	2.82	1

4.4.1.2 Parasite richness

While individual species prevalence varied across the year (Table 4.2), model results showed low annual variation in parasite species richness (Figure 4.2). Neither environmental variables nor sex strongly predicted variation in parasite species richness (Table 4.4). There was weak evidence of a negative relationship between parasite species richness and bi-weekly precipitation and NDVI (Table 4.4). However, these estimates were small with high estimate uncertainty and should be interpreted with caution. The full model explained 27% of variance ($R^2_{conditional}$ = 0.27, Est. Error = 0.05, l-CI = 0.17, u-CI = 0.38).

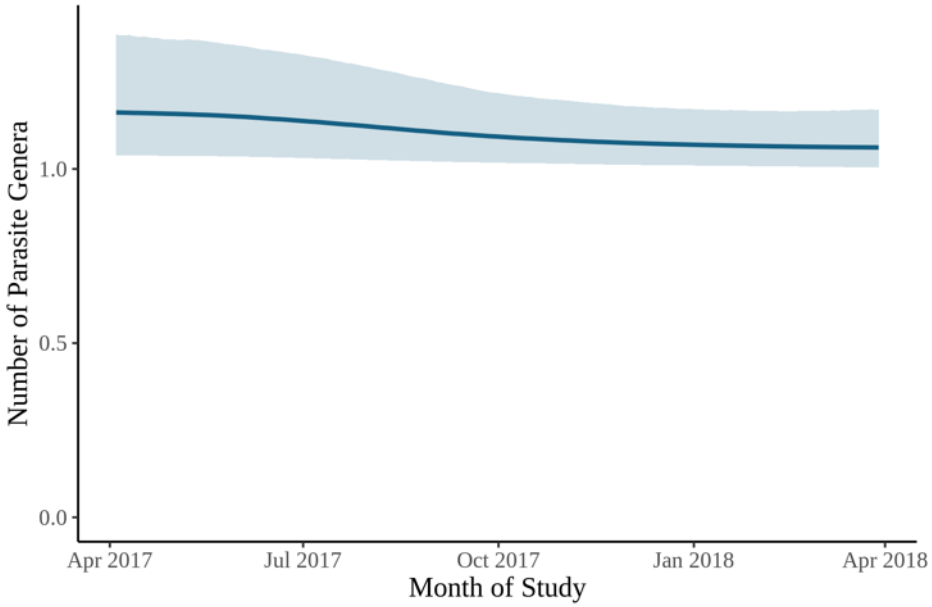

Figure 4.2 Estimate of parasite species richness across the study period derived from the fitted Bayesian mixed-effects hurdle model with a Poisson distribution. Upper and lower 95% credible intervals (bands) were derived from the fitted model.

Table 4.4 Summary statistics of Bayesian mixed-effects model for parasite richness (number of genera) CI = credible interval; SD = standard deviation. Smooth-term sds() = spline "wiggliness"(spline variance parameter).

	Effect	**Estimate**	**Est.Error**	**l-95% CI**	**u-95% CI**	$\widehat{R}$
Fixed effects						
Population-Level	Intercept	-0.86	0.3	-1.42	-0.19	1
	Bi-weekly precip	-0.38	0.22	-0.85	0.03	1
	Minimum temp	-0.23	0.19	-0.61	0.13	1
	Maximum temp	0.3	0.17	-0.03	0.65	1
	NDVI	-0.39	0.23	-0.83	0.06	1
	Sex (ref:M)	-0.47	0.32	-1.09	0.14	1
	s(Date)	-0.54	0.93	-2.18	1.39	1
Smooth Terms	sds(date)	0.64	0.43	0.04	1.68	1
Random Effects	sds(Troop)	0.31	0.32	0.01	1.21	1
	sds(ID)	0.65	0.16	0.39	1.02	1
Family	hu	0.02	0	0.01	0.03	1

4.5 Model set 2: Do faecal egg counts vary stochastically across samples?

4.5.1 *Overall parasite intensity*

There was large variation in parasite intensity (faecal egg count) within and between individuals. For *?Protospirura* sp., annual minimum and maximum egg counts from positive samples (ps) were 2 EPG and 5841 EPG respectively ($mean_{ps}$ = 752.22, ±861.33 sd), while mean annual egg counts for individuals ranged from 93.84 (± 90.00 sd) EPG to 1862.94 (±1521.61 sd). There was also evidence of individual variation in *?Protospirura* sp. egg shedding with some individuals having consistently low egg counts (e.g. 7 – 400 EPG) and some individuals having consistently high egg counts across the year (e.g. 600-2500 EPG). For *Trichostrongylus* sp., egg counts ranged from 2 to 47 EPG ($mean_{ps}$ = 6.5, ±5.2). Overall, there was lower variation in egg counts for the other identified genera. For Ternidens sp. egg counts recovered in positive samples ranged from 1 to 8 EPG ($mean_{ps}$ = 3.10, ±2.22 sd), *Oesophagastomum* sp. ranged from 1 – 17 EPG ($mean_{ps}$ = 4.08, ±2.17sd) and Subulura sp. ranged from 1.92 – 10.3 EPG ($mean_{ps}$ = 5.6, ±2.03sd).

Using a hierarchical generalized additive model and individual-level splines, I found evidence of temporal dependence, or structure, in egg counts (Figure 4.3). If egg shedding were a stochastic process, the spline would fail to capture any pattern in the faecal egg counts. I found inter-individual variation in the magnitude of change (e.g. lowest versus highest egg count), how quickly egg counts changed across an individual's range ("wiggliness") and in overall mean egg counts, with some individuals showing consistently high egg counts and others consistently low egg counts across the year (Figure 4.3). Notably, credible intervals varied across individuals, regardless of spline "wiggliness", suggesting lower certainty in the structure of consecutive egg counts for some individuals (Full results: Appendix A.4). That is, while there was a relationship

between consecutive egg counts for all individuals, model results suggest it was less structured for some individuals than for others, and this was not a function of egg count being more variable for that individual.

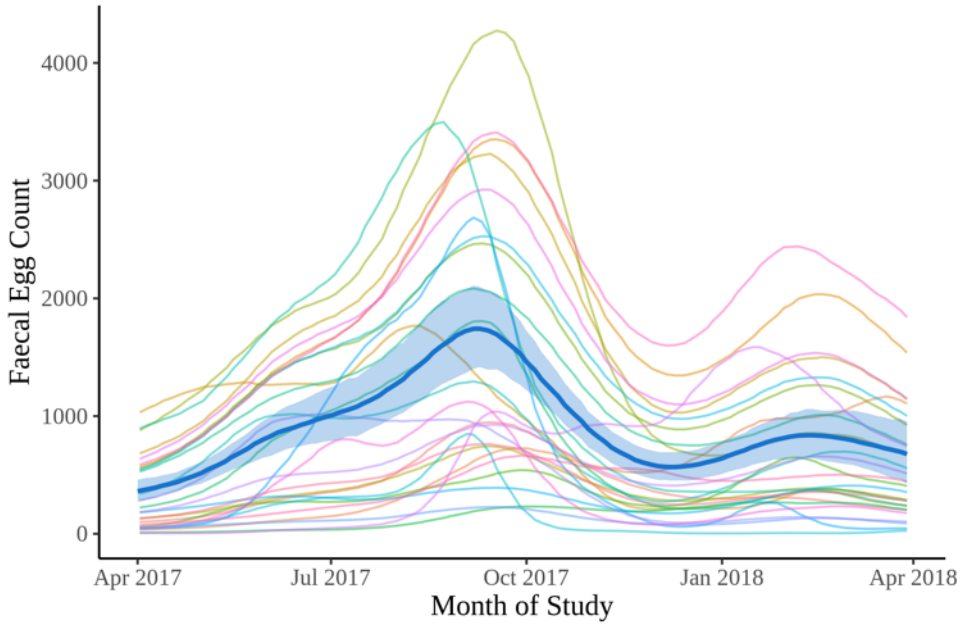

Figure 4.3 Estimate of mean faecal egg count of ?*Protospirura* sp. across the study period derived from the fitted Bayesian GAMM. The blue line shows the global smooth for all individuals with upper and 95% credible intervals (bands) derived from the fitted model. Coloured lines are the estimates of individual-level faecal egg counts across the study period (individual- level smooths). Individual-level credible intervals are not shown to allow for clarity (see Appendix A.4 for CIs).

I found evidence for overall annual variation in ?*Protospirura* sp. egg counts where intensity peaked in austral spring (Figure 4.3) and individual variation in egg counts (Figure 4.3). There was some evidence that parasite intensity was predicted by bi-weekly precipitation where higher precipitation resulted in lower ?*Protospirura* sp. egg counts (Estimate = -0.19, Estimate error = 0.07, $lower_{ci}$ = -0.33, $upper_{ci}$ = -0.05) however, the estimate was small. No other environmental variables affected parasite intensity ?*Protospirura* sp. and no sex differences were

found (Table 4.5). The full model explained 68% of variance ($R^2_{conditional}$ = 0.68, Est. Error = 0.03, l-CI = 0.63, u-CI = 0.73).

Table 4.5 Summary statistics of Bayesian mixed-effects model for parasite intensity of ?*Protospirura* sp. CI = credible interval; SD = standard deviation. Smooth-term sds() = spline "wiggliness"(spline variance parameter). Estimates for fixed effects where credible intervals do not cross zero are in bold.

	Effect	Estimate	Est.Error	l-95% CI	u-95% CI	$\widehat{R}$
Fixed effects						
Population-Level	Intercept	5.97	0.28	5.41	6.52	1
	Bi-weekly precip.	**-0.19**	**0.07**	**-0.34**	**-0.05**	**1**
	Minimum temp	-0.06	0.09	-0.23	0.12	1
	Maximum temp	0.01	0.08	-0.16	0.18	1
	NDVI	0.3	0.35	-0.38	0.96	1
	Sex (ref:M)	0	0.12	-0.25	0.24	1
	s(Date)	0.83	0.95	-1.03	2.73	1
Smooth Terms	sds(date)	2.04	0.34	1.39	2.71	1
Random Effects	Troop	0.25	0.21	0.01	0.78	1
	ID	0.93	0.12	0.72	1.2	1
Family	shape	2.3	0.17	1.97	2.66	1

Trichostrongylus sp. was both less prevalent and had lower egg counts than *?Protospirura* sp. and I found some evidence of temporal dependence, or structure, in *Trichostrongylus* sp. egg counts although uncertainty was high (Figure 4.4). Additionally, there was lower inter-individual variation in both spline wiggliness and mean *Trichostrongylus* sp. egg count (Figure 4.4) and wider credible intervals (full results: appendix A.5).

Trichostrongylus sp. egg counts did not vary meaningfully over the year (Figure 4.4) and none of the environmental variables included in the model predicted *Trichostrongylus* sp. parasite intensity, and no sex differences were found (Table 4.6). The full model only explained 10% of variance suggesting there are other predictors of *Trichostrongylus* sp. parasite intensity not accounted for in our models ($R^2_{conditional}$ = 0.09, Est. Error = 0.07, l-CI = 0.03, u-CI = 0.31).

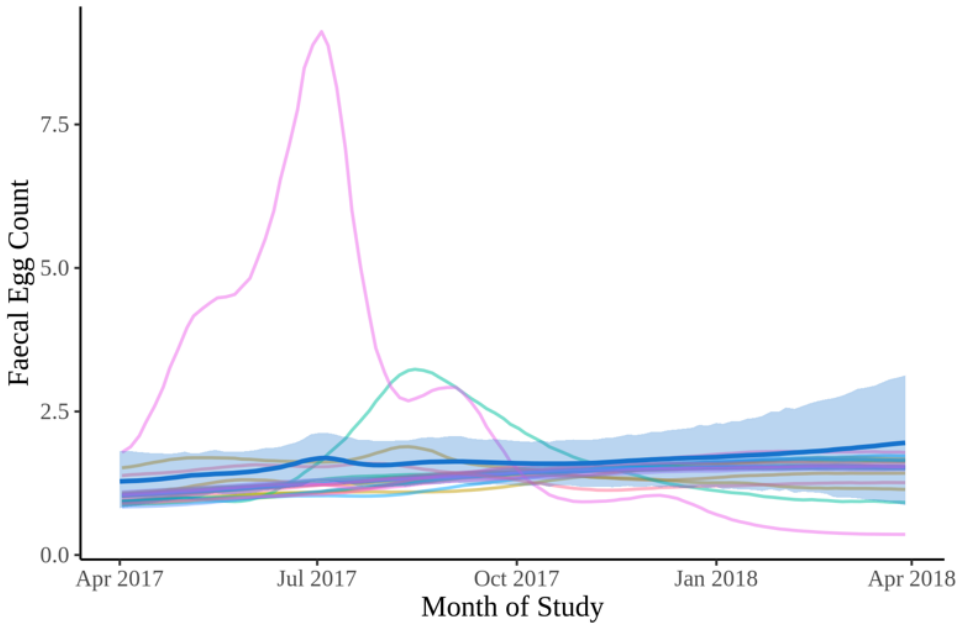

Figure 4.4 Estimate of mean faecal egg count of *Trichostrongylus* sp. across the study period derived from the fitted Bayesian GAMM. The blue line shows the global smooth for all individuals with upper and 95% credible intervals (bands) derived from the fitted model. Coloured lines are the estimates of individual-level faecal egg counts across the study period (individual- level smooths). Individual-level credible intervals not shown to allow for clarity (see Appendix A.5 for CIs).

Table 4.6 Summary statistics of Bayesian mixed-effects model for parasite intensity of *Trichostrongylus* sp. CI = credible interval; SD = standard deviation. Smooth-term sds() = spline "wiggliness"(spline variance parameter). Estimates for fixed effects where credible intervals do not cross zero are in bold.

	Effect	**Estimate**	**Est.Error**	**l-95% CI**	**u-95% CI**	$\widehat{R}$
Fixed effects						
Population-Level	Intercept	1.63	0.17	1.21	1.92	1
	Bi-weekly precip	-0.1	0.12	-0.33	0.11	1
	Minimum temp	-0.08	0.11	-0.3	0.13	1
	Maximum temp	0.04	0.12	-0.19	0.27	1
	NDVI	-0.01	0.16	-0.33	0.29	1
	Sex (ref:M)	-0.04	0.16	-0.35	0.29	1
	s(Date)	0.66	0.75	-0.88	2.1	1
Smooth Terms	sds(date)	0.33	0.25	0.01	0.93	1
Random Effects	Troop	0.19	0.17	0.01	0.65	1
	ID	0.11	0.08	0.01	0.31	1
Family	hu	0.78	0.02	0.74	0.81	1

4.6 Discussion

Our population had a distinctively high annual sample prevalence of 98.74% for their primary parasite genus, ?*Protospirura* sp. This level of infection is in line with previous, short-term research conducted on this population (Blersch et al., 2019), and is considerably higher than gastrointestinal parasite infection in other non-human primate populations. The highest infection proportion reported in vervet monkeys in South Africa was a combined *Physaloptera* sp. and *S. pigmentatus* host group prevalence of 68% (Wren et al., 2015). Given that ?*Protospirura* sp. is transmitted via an insect intermediate host, and that primates increase their insect intake under dry conditions when seasonally available or when food is more scarce (Chapman, 1988; Garber, 1987), it seems likely that the semi-arid conditions of the study site result in increased insect consumption, with a resultant increase in spirurid prevalence. Invertebrate foraging accounted for an average of 5.1% of all foraging events across the study period peaking at 10.34% which is higher than reported in some other vervets (Barrett, 2005).

Owing to the small sample size, statistical analysis could not be used to predict the presence or absence of *Oesophagastomum* sp., *Subulura* sp., and *Ternidens* sp. across the year and sample prevalence was presented as a general indication of prevalence in the population. I found only weak evidence of seasonal variation in parasite richness but did find variation in the occurrence of each genus across the year. I found that *Ternidens* sp. was only present from April to September, *Oesophagastomum* sp. was not recovered during spring while *Subulura* sp. was recovered throughout the year except for May and June, and prevalence was highest in summer. Further, sample prevalence for these genera in the current study was lower than reported in a short-term study the previous year (Blersch et al., 2019). This suggests that, while there may be variation in nematode prevalence across the year, it may not be strictly seasonal or predicted by

the environmental conditions at the time and that prevalence may oscillate biannually or even sporadically (Altizer et al., 2006). This also highlights the importance of conducting long-term, seasonal studies when assessing parasite prevalence in a population as short-term studies may fail to accurately capture the full patterns of parasitism in the population.

The probability of *Trichostrongylus* sp. being present varied across the year but was only predicted by maximum daily temperature, i.e., it was slightly more likely to occur when the maximum daily temperature was higher. Sample prevalence from May to July was lower (46%) than reported for the same period the previous year (63%). Experimentally, *Trichostrongylus* sp. infective larvae have been shown to be sensitive to both extremely high and low temperatures (Andersen et al., 1966). Given that summer temperatures can exceed 40ºC, and that this would likely decrease infective stage larvae survival, our results suggest that there are other underlying drivers of *Trichostrongylus* sp. infection in the population and point to the need for additional host and environmental conditions to be considered. ?*Protospirura* sp. was present across the year and not predicted by environmental conditions. Given that ?*Protospirura* sp. transmission requires an intermediate host, this suggests that the monkeys either consume that host consistently across the year or that monkeys continue to be infected even if not actively consuming the intermediate host. This highlights the need to better understand intermediate host biology and has led to ongoing molecular analysis to identify the intermediate host of both ?*Protospirura* sp. and *Subulura* sp.

While commonly used in parasite studies, there is significant controversy regarding whether faecal egg counts can serve as a measure of parasite intensity or load (Gillespie, 2006). Host immunity, density-dependent factors and environmental cues affect worm ovulation (Christensen et al., 1995; Roepstorff et al., 1996; Stear et al., 1995). Parasite age, fecundity and

sex ratio also affect worm ovulation (Coadwell & Ward, 1982; Roepstorff et al., 1996; Stear et al., 1995). I have previously suggested that, in the short-term, the number of eggs shed in vervet monkey faeces points to an underlying infection rather than a stochastic event (Blersch et al., 2019). Here, using hierarchical generalized additive models, I showed that, for *Trichostrongylus* sp. and, to a greater extent ?*Protospirura* sp., this inference holds over the longer-term. Our models showed that there is global temporal dependence across an annual cycle and that this varies between individuals, with some individuals showing larger variation in egg shedding across the year than others. While it is possible that some individuals may vary more stochastically in egg shedding than others, inter-individual variation in estimate certainty is possibly a result of intervals between samples being larger in some individuals than others. These results suggest that there is underlying structure in faecal egg counts and highlights the importance of considering egg counts on an individual level as well as a population level. These patterns can be more carefully studied with more frequent sample collection.

Parasite intensity of *?Protospirura* sp. varied across the year, peaking in austral spring whereas, *Trichostrongylus* sp. egg counts did not vary widely across the year. Rainfall was the primary predictor of variation in *?Protospirura* sp. parasite intensity with egg counts being lower when precipitation was higher. Periodic low food and water availability at the field-site have been linked to higher faecal glucocorticoid metabolite (fGCM) concentrations (Young et al., 2019). Low parasite intensity during periods of high rainfall may be a result of improved host condition when environmental conditions are more favourable or may be a function of the effect of rainfall on the intermediate arthropod vector. This further points to the need to identify the insect intermediate host for this parasite and molecular analysis is underway to achieve that.

I found no evidence of sex differences in overall parasite richness, prevalence or intensity. This is consistent with the findings of two previous studies on vervet monkeys (Valenta et al., 2017; Wren et al., 2015), as well as in an earlier short-term study at our field site (Blersch et al., 2019). Given the mixed evidence in non-human primates, it is possible that sex-bias in parasite infection is not prominent in vervet monkey populations. However, sex differences in parasite infections are thought to be the product of the physiological demands of pregnancy and lactation as well as the effects of sex hormones on immunosuppression (Klein, 2004; Moore & Wilson, 2002). As males and females have physiological demands that vary between the sexes across the year, there may be sex differences in seasonal variation of parasite intensity, richness and prevalence that are not captured by our models. This points to a need for more sex-specific seasonal measures, such as considering periods of lactation or pregnancy, to thoroughly assess the presence or absence of sex differences in parasite measures.

In summary, our results show that there is seasonality in both parasite prevalence and intensity in our study population predicted by precipitation and the maximum daily temperature. These vervets have a distinctively high overall parasite prevalence and intensity compared to other vervet populations living in tropical and sub-tropical zones. This study area is characterised by very high summer temperatures and very low winter temperatures with low annual rainfall (McFarland et al., 2015; Pasternak et al., 2013). With additional periodic, severe drought, this environment provides unique challenges to both host and parasites. This paves the way for more detailed research on how a primate living in an extreme environment copes with both environmental challenges and chronically high parasitic infection.

CHAPTER FIVE: THE MULTI-SCALE, SOCIO-ECOLOGICAL DRIVERS OF PARASITE SPECIES RICHNESS, PREVALENCE AND INTENSITY

5.1 Abstract

The drivers of parasitism in wild populations operate on multiple spatial scales that can influence both parasite and host. While efforts to identify these drivers in non-human primates is increasing, establishing causal relationships between environment, host and parasite is inherently difficult and connections should be interpreted with caution. Nevertheless, understanding underlying host-parasite relationships in a population is essential for accurate interpretation of the influence parasites may have on host behaviour. Here, I investigated the multi-scale predictors of gastrointestinal parasite richness, prevalence and intensity in wild vervet monkeys. Total Bi-weekly precipitation, daily minimum temperature and daily maximum temperature were included as population-level predictors, NDVI and troop membership as group-level predictors, and sex, rank and faecal glucocorticoid concentrations as individual-level predictors. Further, I considered whether environmental conditions at the estimated time of infection predicted parasite prevalence and richness at the time of faecal sampling. I found that while population-level effects (temperature and rainfall) were the primary drivers of parasite intensity and prevalence in the population, interindividual variation in mean parasite infection was high. I found no evidence of that group- or individual-level characteristics included in the study were linked to any parasite measures but did find some variation between sexes in ?*Protospirura* sp. intensity across the year. There was evidence that minimum daily temperature and bi-weekly rainfall at the estimated time of infection influenced the likelihood of *Trichostrongylus* sp. occurring, but no relationships were found between parasite richness and lagged environmental variables. These findings indicate that host environment strongly influences parasitism in these

vervets on multiple temporal scales but that there are individual-level processes occurring that were not captured in these analyses. This paves the way for more detailed analysis on how these individual characteristics, such as host behaviour, can shape parasitism.

5.2 Introduction

The drivers of parasitism operate on multiple scales - individual level, group level and population level - and can influence both the likelihood of exposure to parasites and susceptibility to infection (Benavides et al., 2012; Stuart & Strier, 1995). However, causal relationships between parasite infections and their drivers are inherently difficult to parse out given that host and environmental characteristics influence both the host and parasite. Further, these interactions between host and parasite are likely to be reciprocal (Ezenwa et al., 2016). As such, results on the likely correlates of parasitism in wildlife across spatial scales are mixed, interactions are often not considered and inferences sometimes problematic. Nevertheless, exploring the possible links between host, environment and parasite is necessary to facilitate more informed interpretation of the relationships between parasites and host behaviour.

At the population level, host environment serves as the primary, and perhaps most documented, driver of individual-level parasite prevalence, intensity, and richness. In primate research, the links between environmental factors such as temperature, rainfall, humidity, latitudinal gradients and forest fragmentation, and parasite species richness and diversity have been investigated (e.g. Altizer et al., 2006; Appleton & Henzi, 1993; Nunn et al., 2005; Schwitzer et al., 2010). Here, strong evidence suggests that both host environment and changes in the host environment, affect species richness and diversity, and play a role in efficient disease transmission. Further, host environment shapes both parasite and host, from parasite survival and

proliferation (Benavides et al., 2012; Gulland, 1995) to the thermal gradient a host needs to thrive. Thus, any parasitological study needs to first establish the environmental drivers of parasitism.

At the group-level, four aspects have been identified as correlates of parasitism: group size, home-range size, ranging behaviour, and home-range productivity. However, evidence for each differs across studies, and is largely dependent on the parasite metric (richness, prevalence or intensity) used. For example, Snaith et al. (2008) found a negative relationship between group size and parasite infection prevalence in red colobus monkeys, and Appleton et al. (1986) found that smaller troops of chacma baboons had lower parasite prevalence. In contrast, Stuart et al. (2011) found that, when comparing across groups, the largest group of muriquis *(Brachyteles arachnoides*) had the lowest level of intestinal helminth infections. Home-range size, ranging behaviours, and more intensively used home ranges increase the probability of exposure to parasites within the home range (Nunn & Dokey, 2006; Nunn et al., 2011). For primates, longer travel distances, but not home range size, are linked to higher parasite species richness in chacma baboons (Benavides et al., 2012) while, in mantled howling monkeys (*Alouatta palliata*), Stoner (1996), parasite intensity was found to be higher in a troop with a smaller, more intensively used home range. Finally, home-range productivity can influence both host body condition and susceptibility to infection, as well as parasite survival in the environment. The amount of vegetation present can serve as breeding or sheltering sites for a parasite (Ceccato et al., 2005) as well as function as a surrogate measure of thermal conditions and environmental moisture (Bavia et al., 2001). The influence of home-range productivity on primate parasite dynamics is poorly understood. In a study on chacma baboons, Benavides et al. (2012) found no relationship between NDVI and parasite species richness, concluding that species richness is not tied to the

density of parasite infectious stages in the environment, but rather to movement patterns and range use intensity.

Several individual-level characteristics, including host age, sex, social rank, body condition, endocrinology and diet, may influence an animal's susceptibly to parasite infection, as well as to prevalence, intensity, and richness of the parasites they harbour. Sex differences in parasite measures are perhaps most widely documented in mammals, and in non-human primates specifically. In mammals, sex differences in parasite susceptibility and infection are generally presumed to be a consequence of immunosuppressive sex hormones, principally testosterone, resulting in females being more resistant to parasite infections than males (Morales-Montor et al., 2004). This pattern, in general, does not hold true across non-human primate species (Benavides et al., 2012; Valenta et al., 2017) and often interacts with other variables such as age (golden lion tamarins: Monteiro et al., 2007) or season (Japanese macaques: MacIntosh et al., 2010). To the best of my knowledge, no previous studies on vervet monkeys have considered the links between social rank and endocrinology, and parasite infection measures.

Host social rank is considered to be a predictor of parasitism for three main reasons that—to an extent—depend on the mode of transmission of the parasite. First, dominant individuals may have more social interactions, thus increasing their likelihood of exposure to parasites, possibly including those that are not directly transmitted (Nunn & Altizer, 2006). Second, high-ranking individuals may have increased exposure risk to parasites that are transmitted trophically as they have better access to food, water and other resources (Nunn & Altizer, 2006). Third, when sufficiently high, cortisol can have immunosuppressive effects. Cortisol and rank have been linked in some non-human primates (although the direction of this relationship varies with species and hierarchy stability), thus I might expect individuals with higher cortisol levels to be

more likely to experience parasite infection. Higher rank has been linked to greater helminth burden in wild male chimpanzees (Muehlenbein & Watts, 2010) and higher infection intensity of *O. actuleatum* in female Japanese macaques (*Macaca fuscata fuscata*) (Hernandez et al., 2009). While no overall link between male rank and parasite richness was found in ursine colobus monkeys (*Colobus vellerosus*), males that changed rank had higher parasite richness than those whose rank did not change during the study period (Teichroeb et al., 2009). Further, it is possible that there is an interaction between sex and rank, particularly in species where rank acquisition varies (stable and heritable ranks among females versus varying ranks determined by fighting ability among males), given that rank has been associated with higher fGCM concentrations in some primate species (review: Cavigelli & Caruso, 2015).

In addition to potential rank-related differences in steroid hormones, steroid hormones can have an immunosuppressive effect and have been shown to underpin increased parasite infection in several taxa (Alexander & Stimson, 1988; Klein, 2004; Zuk, 1996). Other studies have either found neutral relationships between hormones and immune system functioning (Astheimer et al., 2000; Hasselquist et al., 1999; Tschirren et al., 2005) or, in some cases, enhancing effects (Bilbo & Nelson, 2001; Gross et al., 1980).

In primates, evidence of the link between steroid hormones and parasite infection is limited. Muehlenbein (2006) found a positive relationship between both cortisol and testosterone, and total parasite species richness in wild chimpanzees, while Chapman et al. (2006) found positive correlations between elevated cortisol levels and parasite infections in red colobus monkeys. In red-fronted lemurs, both male androgen and glucocorticoid levels during mating periods were associated with a time-lagged increase in nematode infection (Clough et al., 2010). However, long-term changes in males' steroid hormone levels across years were

negatively associated with parasite species richness and nematode infection, suggesting a potential immune-enhancing relationship (Clough et al., 2010). Lastly, Friant et al. (2016b) found a positive association between parasite abundance and cortisol levels in semi-free-ranging red-capped mangabeys. It should be noted, however, that glucocorticoids serve multiple functions, largely related to metabolism, and are only partly involved in the stress response (MacDougall-Shackleton et al., 2019). Thus, interpretation of any parasite-cortisol links should be approached with caution.

Lastly, although less well studied, diet may also play a role in both risk of parasite acquisition or exposure to parasites as well as coping with infection (Kowalzik et al., 2010; Vitone et al., 2004). This is likely to occur across all three scales: population-, group and individual-level and can range from the plant species consumed to the specific part of the plant consumed (e.g., leaves versus fruit). For example, at the group level, Kowalzik et al. (2010) found that the fewest eggs of the trematode *Controrchis* sp. were recovered in groups that foraged the least on trumpet trees (*Cecropia peltata*) while, at the individual level, monkeys that consumed the least *C. peltate* also had the lowest intensity of *Controrchis* sp. In a comparative study 69 anthropoid primate species, Vitone et al. (2004) found that primates that consume more leaves had a higher diversity of nematodes parasites. This is likely a result of leaves posing a higher risk of encounter for faecal-orally transmitted parasites. Conversely, comparative work across three neotropical primates (*Alouatta seniculus, Ateles hybridus and Cebus versicolor*) found that *A. hybridus*, the species that is predominately frugivorous, had a higher parasite prevalence than *A. seniculus* which is mainly folivorous (Rondon et al., 2017). Additionally, Rondon et al. (2017) found that primates with more diverse diet have the highest parasite prevalence. It has also been documented that primates may take an active role in coping with

infection by means of self-medication, via plant secondary compounds or other non-nutritional substances (Huffman, 1997). The benefits can be derived either pharmacologically (Pebsworth et al., 2006) or physically (e.g. Huffman et al., 1996). Although expected to occur in other non-human primates, self-medication has been best documented in the Great Apes, particularly chimpanzees (for example, see: Ghai, 2014; Huffman & Seifu, 1989; Huffman et al., 1996). These studies show that, as is the case with other potential predictors of parasitism in primates, diet is an inconsistent predictor of parasitism but does appear to play an important role in some species.

Given the potential significance of these multi-scale predictors for both parasite and host, quantifying any such links is essential for understanding parasite-host dynamics. Here, I investigate the multiscale predictors of parasitism in wild vervet monkeys living in the semi-arid karoo. Bi-weekly precipitation (total rainfall within the two-week period), daily minimum temperature and daily maximum temperature were included as population-level predictors, NDVI and troop membership as group-level predictors, and sex, rank and faecal glucocorticoid concentrations as individual-level predictors.

At the population level, I have shown previously that bi-weekly precipitation and maximum daily temperature, but not minimum daily temperature, predict parasite intensity and richness (Chapter 4, Blersch et al., 2021) and I would expect those trends to remain largely constant.

At the group level, group size and structure are thought to strongly influence parasite establishment, spread and infection intensity (Nunn et al., 2015). However, this is not consistently supported for helminths that are not directly transmitted (Appleton et al., 1986; Snaith et al., 2008; Stuart, 2011). Nevertheless, given the relative similarity in troop size and

composition across the three study troops, and the extent of their home range overlap, I predict that parasite intensity, richness and prevalence will not be linked to troop size. Further, at the group level, I predict that previous findings on the lack of relationship between NDVI and the parasite measures under consideration will remain constant (Chapter 4, Blersch et al., 2021), consistent with the findings of (Benavides et al., 2012).

At the individual level, although I found no mean sex difference in parasite measures (Chapter 4, Blersch et al., in press) it is nevertheless the case that males and females are placed under different physiological pressures across the year related to the mating and birth seasons (Klein, 2004; Moore & Wilson, 2002), I therefore investigated whether there are sex differences in variation in parasite measures across the year. Given that vervet monkeys are seasonal breeders and that males and females predictably differ in their energetic needs across the year (Andelman, 1987; Lee, 1984), I predict that parasite intensity will vary across the year in parallel to these changing needs (i.e., I expect males to have higher parasite loads during times of increased aggression (mating season) while females will be more susceptible to parasite infection during pregnancy and/or birth season). Similarly, I also considered rank and an interaction between sex and rank to account for differential susceptibility to infection that may arise from sex differences in rank acquisition or maintenance. That is, females have relatively stable and heritable ranks while males acquire rank through aggressive interactions. However, both rank and sex-rank variation in parasite measures are largely contingent on differential rank acquisition eliciting other physiological responses, such as an increase in fGCMs, rather than a direct relationship (Cavigelli & Caruso, 2015); this may hinder the ability of establishing a clear relationship between rank and parasite measures. Finally, I considered the possible links between fGCMs and parasite measures. fGCMs can serve as proxy for animals' underlying physiological

response to environmental stressors and so I predict that, while fGCMs are unlikely to influence environmental parasite acquisition and possible establishment in the host, the ability to cope with parasite infection is likely to decrease when individuals are dealing with other stressors, resulting in a positive relationship between fGCMs and parasite intensity.

I also conducted an exploratory analysis to assess whether environmental conditions at the estimated time of parasite acquisition influenced parasite measures at the time of sample collection. In this regard, a lagged response of parasite species richness has been shown in other primates (Benavides et al., 2012). Given that parasite larvae survival in the environment is contingent on environmental conditions (Andersen et al., 1966), it is possible that the likelihood of infection with faecal-oral parasites depends on the environmental conditions at the time of infection.

5.3 Methods

5.3.1 *Study Subjects and site*

Data were collected across 12 consecutive months – April 2017 to March 2018 – from three fully habituated groups (PT, RBM, and RST) of wild vervet monkeys at Samara Private Game Reserve, South Africa (32º22'S, 24º52'E). These monkeys have been the subject of continuous data collection since 2009 and are individually identifiable from natural markings. Data were collected from a subset of 27 adult individuals (PT: 4 males, 6 females from 16 adults; RBM: 2 males, 6 females from 14 adults; RST: 3 males, 6 females from 14 adults), selected to be representative of adult demography and to reflect the full range of dominance ranks. Table 5.1 shows troop demographics across the study period.

Table 5.1 Mean troop sizes (± SD) across the study periods. Adults are individuals who have reached sexual maturity. Infants were born between August 2017 and February 2018

Troop	RBM	PT	RST
Adults	14 (1.5)	16 (0.95)	14 (0.6)
Juveniles/yearlings	27 (1.25)	21 (0.43)	23 (0)
Infants (born)	2	5	2
Annual mean	42 (2.3)	39 (1.63)	38 (1.4)

The study area is semi-arid riverine woodland, an area characterised by low rainfall, very hot summers and cold winters (McFarland et al., 2015; Pasternak et al., 2013). The area is under escalating risk from climate change (Jury, 2013) and has a declining annual average rainfall of 386 mm, and average minimum and maximum temperatures of 6.1 ºC and 21.2 ºC, respectively (Pasternak et al., 2013).

5.3.2 *Faecal sampling*

Faecal samples were collected by four to five observers spread over three troops during each of the 234 10h study days. Faecal samples were collected non-invasively twice per month (once during each two-week period) from each of the 27 subjects. Two corresponding faecal samples, one for parasite analysis and one for faecal glucocorticoid metabolites (fGCM) analysis, were collected from the same defecation event. I analysed a total of 573 faecal samples ($mean_{individual}$ = 21 ± 3.1 SD).

Eight possible predictors of parasitism were included in all models. The links between these and parasite measures are visualised in Figure 5.1.

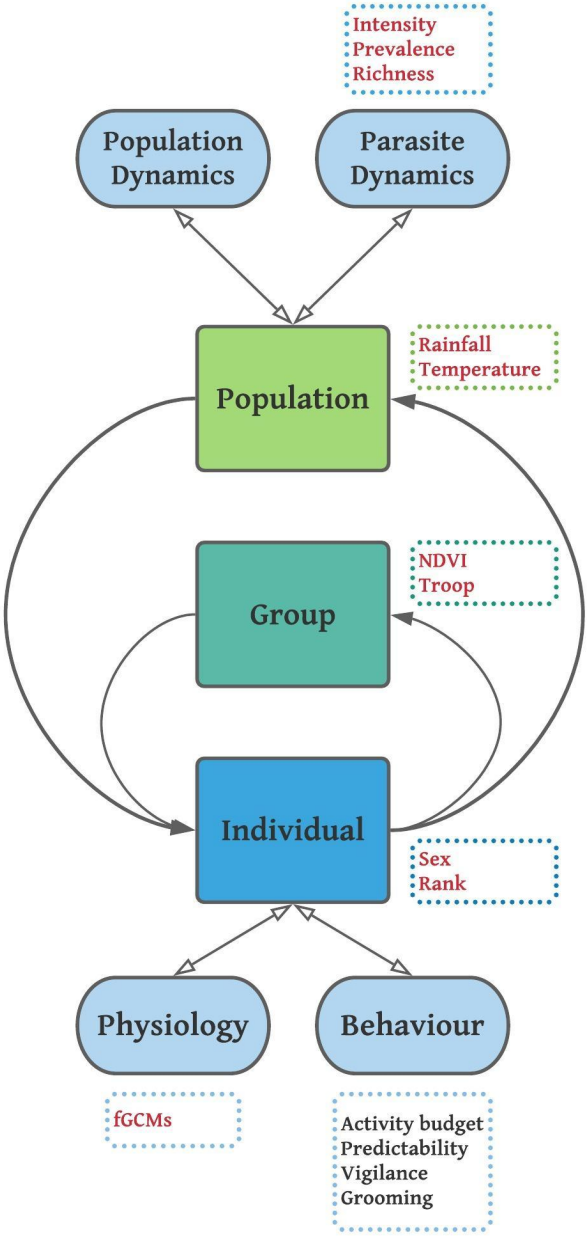

Figure 5.1 The links between population- level, group-level and individual-level variables and their role in determining parasite dynamics (richness, prevalence and intensity). Variables given in red represent model predictors.

5.3.3 *Population-level predictors: temperature and rainfall*

Climate data were recorded at the field site. Information on the daily minimum and maximum temperatures was taken from a centralized weather station at the field site (Hobo U30-NRC, Onset Computer Corporation, USA), while daily measurements of precipitation during the study period were recorded using a standard, cumulative rain gauge.

For the study period, the daily minimum temperature ranged from -3.9 ºC to 20 ºC while daily maximum temperature ranged from 11.8 ºC to 49.6 ºC. Monthly rainfall was low except for two distinct peaks in October 2017 (69 mm) and January 2018 (67 mm). Total precipitation for the study period was 317 mm.

5.3.3.1 Group-level predictors: Home range productivity (NDVI) and troop membership

I collected data on three troops living in adjacent home ranges. Troop size was similar across the study period with a range of 32-43 for RBM ($\bar{x}$ = 42 ±2.3 SD), 31-39 for RST ($\bar{x}$ = 38 ±1.4 SD) and 33-40 ($\bar{x}$ = 39, ±1.63 SD) for PT. RBM had a larger number of juveniles ($\bar{x}$ = 27 ±1.25 SD) compared to both PT ($\bar{x}$ =21 ±0.43SD) and RST ($\bar{x}$ =23 ±0).

I quantified the food available in each troop's home range by calculating the Normalized Difference Vegetation Index (NDVI) every 16 days (Dostie, 2020, in progress) from MODIS data collected by Earth Observing System (EOS) satellites Terra (EOS AM-1) and Aqua (EOS PM-1). Using Moderate Resolution Imaging Spectroradiometer MOD13Q1 vegetation indices at a 250-meter resolution (Didan, 2015), NDVI measures the amount of biomass or chlorophyll activity by calculating the difference between the visible red and near-infrared bands divided by their sum. The resultant estimate ranges between -1 and 1, where negative values indicate an absence of vegetation and positive values approaching 1 indicate larger concentrations of green vegetation (Pettorelli et al., 2005). Given the generalist, largely

plant-based nature of vervet diet (Pasternak et al., 2013), the synoptic view of NDVI is a reliable measure of food availability in this species (Willems et al., 2009; Jarrett et al., 2020)

5.3.4 *Individual-level predictors: Sex, social rank and faecal glucocorticoid metabolites*

5.3.4.1 Social rank

I collected *ad libitum* data on dyadic agonistic interactions, for which I identified participants and outcomes. These agonistic data were used to construct dominance hierarchies using David's Scores (de Vries et al., 2006). I divided the study period into four 3-month blocks: April – June 2017, July – September 2017, October – December 2017 and January – March 2018. I used *ad libitum* observations of agonistic interactions to construct hierarchies for each period (N_{RBM}: 963; N_{RST}: 810; N_{PT}: 1135) for all adults in each troop and not only the subset of study subjects. Given male-female co-dominance in this population (Young et al., 2017b), I generated a single matrix that included all decided agonistic interactions regardless of the sex of participants and created a single interdigitated hierarchy. Dominance ranks in each troop and for each three-month block were expressed as a standardized David's score using the package 'compete' (Curley, 2016). David's scores were standardized to enable direct comparison across groups of different size and interaction rates (Henzi et al., 2013).

5.3.4.2 FGCM concentration

Samples were collected following the protocol of Young et al. (2017a; 2019). Within 15min of defecation, a 2-5 g piece of faecal material was transferred into a plastic vial following physical homogenization of the full faecal sample. Prior to collection, faecal samples were checked to ensure there was no contamination with urine during excretion or on the substrate where the sample landed. Vials were stored on ice in a thermos in the field before transfer to a

−20 °C freezer at the end of the day. Samples were stored until transport on dry ice to the Endocrine Research Laboratory, University of Pretoria, for analysis.

Samples were lyophilized and then pulverized and sieved to remove seeds and fibrous matter (Young et al., 2017a). The resulting faecal powder was weighed, and 0.10 g was extracted. For extraction, samples were vortexed for 15 min with 80% ethanol in water (3 ml) followed by 10 min of 1500 g centrifugation. 1.5 ml of the resultant supernatants were transferred into microcentrifuge tubes for analysis. Hormone analysis was conducted following the standard procedures of the Endocrine Research Laboratory, University of Pretoria (Ganswindt et al., 2002) using the cortisol enzyme immunoassay (EIA) (Young et al., 2017a). Inter- and intra-assay coefficients of variation of high- and low-value quality controls were: 4.64–5.96 and 8.13–11.60% respectively. All steroid concentrations are given as ng g−1 faecal dry weight.

5.3.4.3 Parasite Sample Extraction Methodology

For each sample, approximately 1 g of fresh faeces was weighed in the field immediately after defecation and directly placed into 10% neutral, buffered formalin. Samples were stored in the field lab and transported to the University of Lethbridge, Canada, where faecal flotation and sedimentation techniques were used to identify parasites (Blersch et al., 2019).

A modified zinc sulphate flotation was used to isolate helminth eggs, whereby an additional washing step was included in the faecal flotation to avoid egg damage, which had been evident in the initial samples that were analysed (Blersch et al., 2019). Briefly, faecal samples suspended in formalin were placed in 15 ml Falcon tubes and centrifuged at 1,389 g for 6 min after which the supernatant was discarded. The Falcon tube was filled with water, mixed with the faecal material, centrifuged at 1,389 g for 6 min, and the supernatant was discarded. The

deposit was resuspended in $ZnSO_4$ (specific gravity 1.3), vortexed to mix, and centrifuged at 617 g for 8 min. The supernatant was pipetted into 4x15 ml tubes and combined with water. The pellet that remained after flotation was kept aside for sedimentation. This step reduced the specific gravity of the $ZnSO_4$ after flotation, thus preventing egg damage and allowing the eggs to deposit upon sedimentation. These supernatant-water tubes were centrifuged at 964 g for 6 min. The supernatant was discarded, and the deposits were combined into 1 test tube, which was filled with water and centrifuged at 964 g for 6 min. The supernatant was discarded, and the entire pellet was examined under the microscope.

Ethyl-acetate sedimentation was used to isolate potential trematodes that were too heavy to float during ZnSO4 flotation. Here, the deposit from the flotation was suspended in water, vortexed, and centrifuged at 964 g for 6 min. The supernatant was discarded, and the sample was rewashed. Water was added to the pellet to the 7 ml mark of the centrifuge tube and vortexed. Then, 3 ml of ethyl-acetate was added to the tube, mixed thoroughly, and centrifuged at 1,389 g for 6 min, and the supernatant was then discarded. The entire pellet was examined under the microscope. For both methods, parasites were identified to genus- level based on egg shape, size, colour, and contents, and all eggs were counted, Representative eggs were photographed.

5.3.5 *Statistical Analysis*

All statistical analyses were conducted in a Bayesian framework, using the 'brms' package (Bürkner, 2017; Bürkner, 2018) in R version 3.5.2 (R Core Team, 2018). I constructed hierarchical generalized additive mixed models to allow for non-linear relationships between explanatory and response variables (Pedersen et al., 2019).

I present summary statistics and posterior density plots ("bayesplot" package: Gabry et al., 2019) for posterior means, standard errors and 95% credible intervals (CIs) for the main effects, and for individual variance within the random effects. For the smooth terms, I modelled both global and sex-level smooths and present summary statistics of the spline variance parameter ("wiggliness") for the global smooth, and male and female smooths. I conducted prior predictive checks (Gabry et al., 2019) for each model and specified weakly informative priors (normal (0, 1)), unless otherwise indicated. I ran models with 4 chains and 2000 iterations, which provided me with a large enough sampling pool to conduct posterior sampling and achieve model convergence (McElreath, 2016; Bürkner, 2018). Chain convergence was confirmed by $\hat{R}$ values ≤ 1.01, and model goodness-of-fit was assessed using the 'posterior predictive check' (pp_check) function from the "bayesplot" package (Gabry et al., 2019). I assessed potential collinearity of fixed and random effects visually using pairs plots which produce univariate histograms and bivariate scatterplots for each parameter (mcmc_pairs function: "bayesplot" package). Collinearity would manifest as narrow bivariate plots in predictor variable comparisons, but I did not detect any. I used the "bayes_R2" function to generate conditional R^2 values for each model (Gelman et al., 2019).

5.3.5.1 Hierarchical generalized additive mixed effects models

I constructed four GAMMs to assess whether population-, troop- and individual-level variables predicted (1) parasite prevalence (presence/absence) of *Trichostrongylus* sp., (2) richness (number of genera) or intensity (eggs per gram) of both (3) *Trichostrongylus* sp. And (4) ?*Protospirura* sp. across the year. I used parasite egg count as a measure of infection intensity. As I have shown that total faecal egg counts between successive individual samples are correlated, I have suggested that egg counts may be a reliable indicator of an underlying

infection in these vervet monkeys, rather than reflecting some stochastic process (Blersch et al., 2021; Blersch et al., 2019). Owing to low sample size for other genera (<5%), models were fitted for ?*Protospirura* sp. and *Trichostrongylus* sp. prevalence and intensity only, while other genera were included in the richness count.

For all models, fixed effects and random effects were constant. Fixed effects included date, maximum daily temperature, minimum daily temperature, total bi-weekly precipitation, NDVI, sex, rank and fGCMs while Troop and ID were specified as random effects. Given that the influence of rank on parasite intensity may be sex specific, I included an interaction term between rank and sex. To assess whether there was non-linearity in parasite prevalence, richness and intensity across the year, and to account for samples not being an equal number of days apart, I included a spline on date.

In addition to mean sex differences in parasite measures, I assessed whether there was variation between the sexes across the year. I fitted a model that allowed for variation in parasite measures across the year (spline on date) as well as sex-level variation (GS model: Pedersen et al., 2019) similar to a GLMM model with varying slopes. That is, each grouping variable (sex) has its own functional response but is penalised if that response varies too far from the average. To do so, I specified a factor-smooth interaction term (GS model: Pedersen et al., 2019). This is a more conservative approach than allowing each group-level (sex) smoother to have its own smoothing parameter. I also included a global smoother (thin plate regression spline: k=12, m=2) on date to assess population-level variation in parasite intensity across the year (GS model: Pedersen et al., 2019). Given that not all samples were exactly 1g, I entered log faecal weight as an offset variable. All continuous predictor variables were mean-centred and standardized by two

standard deviations (SD) to allow for effect size comparisons across continuous and dichotomous variables (Gelman, 2008).

I used a negative binomial distribution for the ?*Protospirura* sp. intensity model and, owing to the large number of zeroes, I fitted a hurdle model with a Poisson distribution for *Trichostrongylus* sp. intensity. I used a Bernoulli distribution for the *Trichostrongylus* sp. Prevalence model and a hurdle-Poisson model for species richness.

For the ?*Protospirura* sp. and *Trichostrongylus* sp. intensity models, I was not explicitly interested in individual-level annual variation, in addition to population-level annual variation, and structure in egg count. However, I have previously shown a high level of individual-level variation in egg counts across the study period (Blersch et al., 2021). Thus, I ran these models twice for each genus: once with a thin plate regression spline (k=15, m=1) on date of collection by individual ID as a fixed effect, which allows each individual to have its own smoothing parameter and "wiggliness" (GI model: Pedersen et al., 2019) and once without the individual by ID spline. I then applied the widely applicable information criterion (WAIC: Gelman et al., 2014) to determine which of the models gave the best fit to my data, where the lowest WAIC score indicates the better fitting model. I found that, for both genera, the WAIC was lower—indicating better model fit—when the individual-level spline was used.

5.3.5.2 Exploratory analysis with lagged environmental variables

I considered the possibility that environmental variables may play a role in acquisition or establishment of parasite infection at the time of infection, rather than the time of sampling. Given that *Trichostrongylus* sp. is a soil-transmitted helminth with larvae survival sensitive to environmental conditions, as well as the potential influence of environmental conditions host susceptibility to infection, I constructed two models with lagged environmental variables. While

precise information is not readily available for the life cycle of *Trichostrongylus* sp. in primates, it takes approximately 15 days for *Trichostrongylus* sp. larvae to mature in other vertebrate hosts (reviewed in: Andersen, 2000). I used this as an approximation and calculated the mean Tmin, Tmax, NDVI and total rainfall for the 7-day period before and after the 15-day approximation, i.e. From 8 to 21 days before faecal sampling. I then constructed models for *Trichostrongylus* sp. prevalence and intensity using these lagged environmental variables as fixed effects and ID and troop as random effects.

Given both the more complex life cycle of *Protospirura* sp., which involves an intermediate insect host, and the lack of detail on the prepatent period, I did not conduct these exploratory analyses for *Protospirura* sp.

5.3.5.3 Posterior plots

Given the nature of the statistical models used, as well as the inclusion of variable interactions, direct interpretation of model estimates is not straightforward. To aid in interpretation, I generated whole model predictions using the fitted() function from the brms package. These predictions were then used to construct posterior density plots. Importantly, egg count was entered into our models as a continuous variable, but they are categorised as "high" and "low" for visualization purposes in the plots. This was to aid in interpretation only, and no assumptions are made as to the biological significance of what is designated as "high" or "low".

5.4 Results

5.4.1 *Parasite Richness*

There was a no evidence of a relationship between parasite richness and any socio-ecological variables included in the model (Table 5.2). While the full model explained 28.4% of

variance ($R^2_{conditional}$= 0.28, ± 0.05 SE), the main effects accounted for 13.5% of variance ($R^2_{marginal}$= 0.14, ± 0.09 SE).

Table 5.2 Summary statistics of Bayesian mixed-effects model for parasite richness (number of genera). CI = credible interval; SD = standard deviation. Smooth-term sds() = spline "wiggliness"(spline variance parameter). Estimates for fixed effects where credible intervals do not cross zero are in bold.

	Effect	**Estimate**	**Est.Error**	**l-95% CI**	**u-95% CI**	$\widehat{R}$
Fixed Effects						
Population-Level	Intercept	-0.84	0.35	-1.48	-0.03	1
	Bi-weekly precip	-0.37	0.21	-0.81	0.02	1
	Minimum temp	-0.24	0.19	-0.6	0.15	1
	Maximum temp	0.31	0.18	-0.05	0.65	1
	NDVI	-0.41	0.23	-0.84	0.03	1
	Sex (ref:M)	-0.29	0.41	-1.06	0.51	1
	Rank	0.15	0.32	-0.47	0.77	1
	fGCMs	0.03	0.15	-0.27	0.3	1
	Sex*Rank	-0.87	0.57	-1.99	0.27	1
	s(Date)	-0.44	0.9	-2.09	1.39	1
Smooth Terms	sds(date)	0.6	0.44	0.02	1.68	1
	sds(Date-Sex1)	0.71	0.53	0.03	2.02	1
	sds(Date-Sex2)	0.79	0.61	0.03	2.25	1
	sds(Date-Sex3)	0.83	0.62	0.03	2.28	1
Random Effects	Troop	0.40	0.49	0.01	1.76	1
	ID	0.65	0.16	0.37	1.01	1
Family	hu	0.02	0.01	0.01	0.03	1

I found no evidence of sex differences in the number of parasite genera across the year (Figure 5.2).

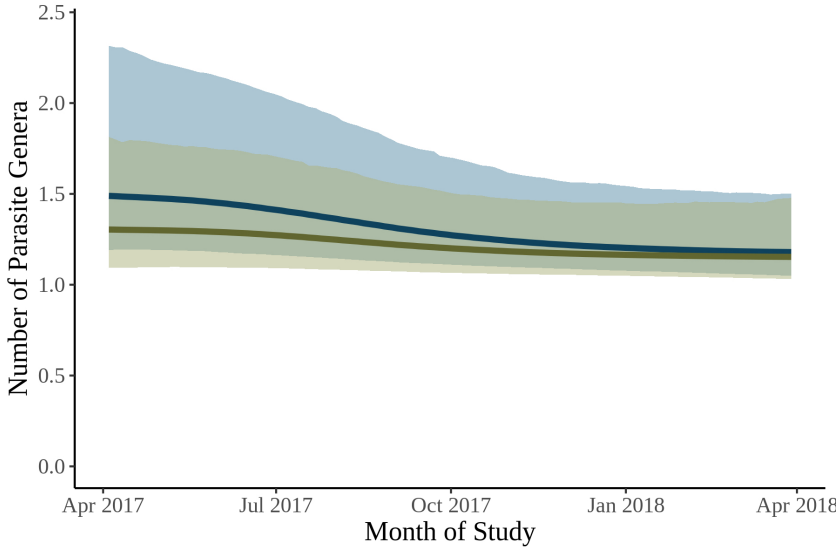

Figure 5.2 Estimate of parasite richness in males (blue line) and females (green line) across the study period derived from the fitted Bayesian mixed-effects hurdle model with a Poisson distribution. Upper and lower 95% credible intervals (bands) were derived from the fitted model

There was also no evidence of an interaction between rank and sex. Specifically, no relationship was found between rank and parasite richness for either males or females (Figure 5.3).

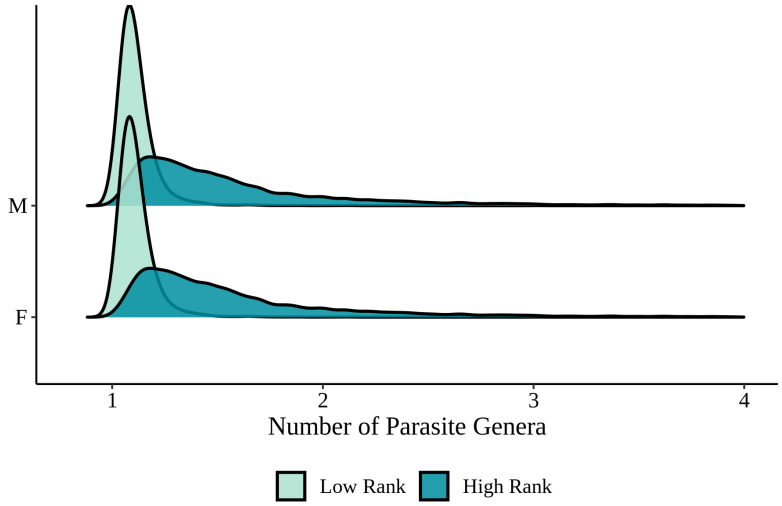

Figure 5.3 Changes in the parasite richness in males and females when individuals are high ranked and low ranked. Density plots show the range of parasite richness predicted by the model, with the height of the density curve indicating the probability of parasite richness. The spread of the curve indicates the uncertainty

There was some evidence of inter-individual variation in parasite richness (Table 5.2. β = 0.65 ± 0.16 SE, l-CI = 0.37, u-CI = 1.01) however, this was not meaningful given that individual means did not exceed one parasite genus. No differences were found between troops (Figure 5.4).

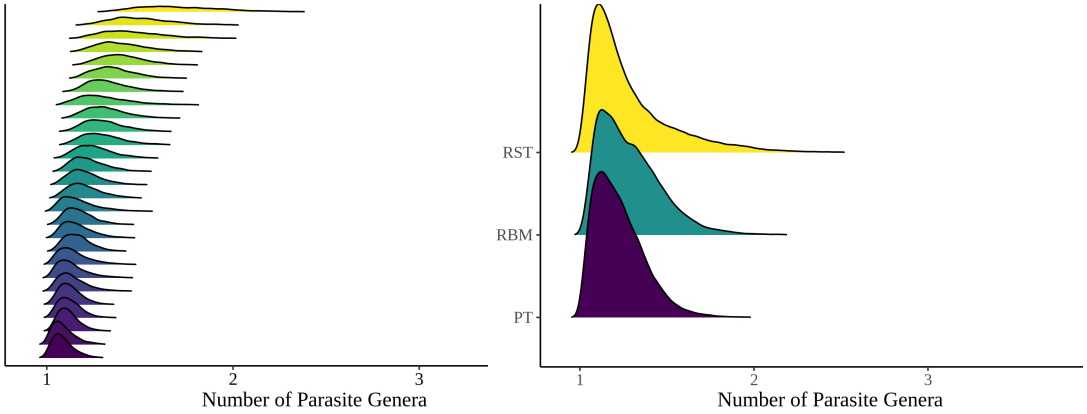

Figure 5.4 Density plots showing mean parasite richness by ID (left) and troop (right). Density plots show the range of parasite richness predicted by the model, with the height of the density curve indicating the probability the number of parasite genera. The spread of the curve indicates the uncertainty.

5.4.2 *Parasite Prevalence*

There was a positive relationship between the probability of *Trichostrongylus* sp. occurring and maximum daily temperature, with occurrence being highest when temperatures were higher (Table 5.3. β = 0.74 ± 0.33 SE, l-CI = 0.08, u-CI = 1.42). No other socio-ecological fixed effects predicted presence or absence of *Trichostrongylus* sp. (Table 5.3). The full model explained 42.3% of variance ($R^2_{conditional}$= 0.42, ± 0.02 SE) while the main effects accounted for 21.1% of variance ($R^2_{marginal}$= 0.21, ± 0.08 SE).

There was no evidence of sex differences in the probability of infection with *Trichostrongylus* sp. across the study period (Figure 5.5).

Table 5.3 Summary statistics of Bayesian mixed-effects model for parasite prevalence (present/absent) of *Trichostrongylus* sp. CI = credible interval; SD = standard deviation. Smooth-term sds() = spline "wiggliness"(spline variance parameter). Estimates for fixed effects where credible intervals do not cross zero are in bold.

	Effect	**Estimate**	**Est.Error**	**l-95% CI**	**u-95% CI**	$\widehat{R}$
Fixed effects						
Population-Level	Intercept	-2.15	0.92	-3.92	-0.11	1
	Bi-weekly precip	-0.41	0.32	-1.06	0.20	1
	Minimum temp	-0.52	0.35	-1.22	0.15	1
	Maximum temp	0.74	0.33	0.08	1.42	1
	NDVI	-0.17	0.52	-1.20	0.85	1
	Sex (ref:M)	-0.33	0.77	-1.81	1.24	1
	Rank	-0.03	0.6	-1.20	1.10	1
	fGCMs	0.36	0.27	-0.18	0.88	1
	Sex*Rank	-0.56	0.81	-2.11	1.01	1
	s(Date)	0.15	1.02	-1.83	2.14	1
Smooth Terms	sds(date)	5.38	2.64	1.73	11.68	1
	sds(Date-Sex1)	2.73	2.40	0.11	8.77	1
	sds(Date-Sex2)	2.67	2.91	0.09	9.99	1
	sds(Date-Sex3)	2.56	2.61	0.09	9.00	1
Random Effects	Troop	0.79	0.85	0.02	2.97	1
	ID	1.97	0.43	1.26	2.95	1
Family	hu	0.02	0.01	0.01	0.03	1

There was also no evidence of an interaction between rank and sex: there was no relationship between rank and *Trichostrongylus* sp. prevalence for either males and females (Figure 5.6).

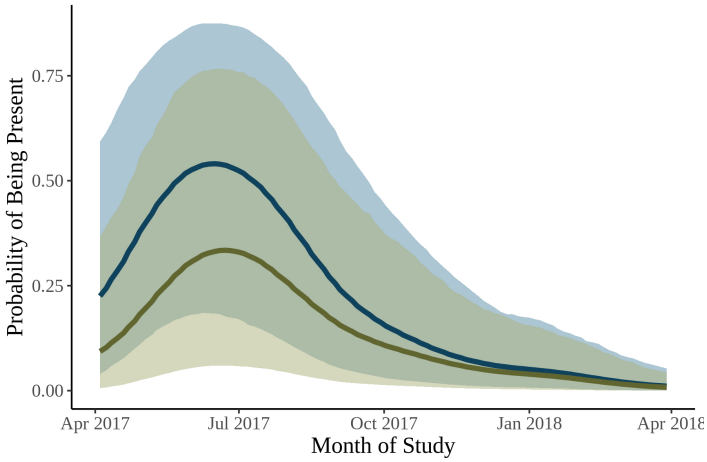

Figure 5.5 Estimate of *Trichostrongylus* sp. prevalence in males (blue line) and females (green line) across the study period derived from the fitted Bayesian mixed-effects hurdle model with a Poisson distribution. Upper and lower 95% credible intervals (bands) were derived from the fitted model

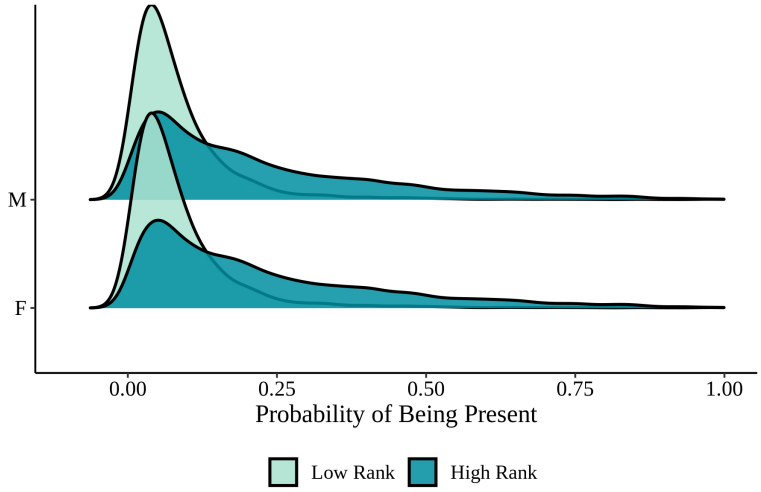

Figure 5.6 Changes in the *Trichostrongylus* sp. prevalence in males and females when individuals are high ranked and low ranked. Density plots show the range of likelihoods of *Trichostrongylus* sp. occurring predicted by the model, with the height of the density curve indicating the probability of *Trichostrongylus* sp. occurring. The spread of the curve indicates the uncertainty.

There was strong evidence of inter-individual variation in mean *Trichostrongylus* sp. Prevalence, but uncertainty was high for some individuals (Figure 5.7. β = 1.97± 0.43 SE, l-CI = 1.26, u-CI = 2.95). No differences were found between troops (Figure 5.7). The full model explained 42.3% of variance ($R^2_{conditional}$ = 0.42, Est. Error = 0.03, l-CI = 0.36, u-CI = 0.47).

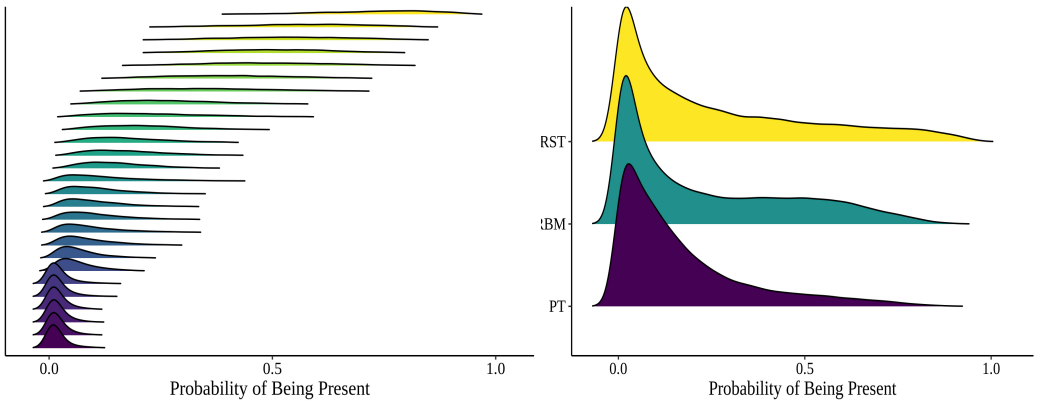

Figure 5.7 Density plots showing mean *Trichostrongylus* sp. prevalence by ID (left) and troop (right). Density plots show the range of likelihoods of *Trichostrongylus* sp. occurring predicted by the model, with the height of the density curve indicating the probability of *Trichostrongylus* sp. occurring. The spread of the curve indicates the uncertainty.

5.4.3 *Parasite Intensity*

5.4.3.1 ?*Protospirura* sp.

I found a negative relationship between *Protospirura* sp. egg count and total bi-weekly precipitation, where egg count increased with decreasing rainfall (Table 5.4). I found no relationships between the other socio-ecological variables included in the model and *Protospirura* sp. intensity (Table 5.4). The full model explained 68.3% of variance ($R^2_{conditional}$= 0.68, ± 0.03 SE), while the main effects accounted for 34.3% of variance ($R^2_{marginal}$= 0.34, ± 0.10 SE). Table 5.4 shows main effects only. For full model results showing individual level splines, see appendix Table B1.

While there were no mean sex differences in *Protospirura* sp. egg counts (Table 5.4), there was some evidence of sex differences across the year with females showing more variation across the year than males (Figure 5.8). However, credible intervals were wide and the estimates uncertain.

Table 5.4 Summary statistics of Bayesian mixed-effects model for parasite intensity (EPG) of ?*Protospirura* sp. CI = credible interval; SD = standard deviation. Smooth-term sds() = spline "wiggliness"(spline variance parameter). Estimates for fixed effects where credible intervals do not cross zero are in bold.

	Effect	**Estimate**	**Est.Error**	**l-95% CI**	**u-95% CI**	$\widehat{R}$
Fixed effects						
Population-Level Effects	Intercept	6.14	0.49	5.18	7.18	1.01
	Bi-weekly precip.	-0.19	0.07	-0.34	-0.05	1
	Minimum temp	-0.09	0.09	-0.27	0.09	1
	Maximum temp	0.01	0.08	-0.16	0.18	1
	NDVI	-0.03	0.13	-0.28	0.21	1
	Sex (ref:M)	0.18	0.42	-0.65	1.01	1
	Rank	-0.08	0.32	-0.72	0.55	1
	fGCMs	0	0.07	-0.13	0.14	1
	Sex*Rank	0.37	0.5	-0.61	1.35	1
	s(Date)	1	0.98	-0.94	2.92	1
Random Effects	Troop	0.54	0.65	0.01	2.45	1
	ID	1.02	0.16	0.77	1.38	1
Smooth Terms	sds(date)	2.02	0.34	1.38	2.7	1
	sds(Date-Sex1)	0.43	0.31	0.02	1.16	1
	sds(Date-Sex2)	0.40	0.3	0.01	1.13	1
	sds(Date-Sex3)	0.40	0.31	0.02	1.15	1
Family	shape	2.29	0.17	1.98	2.62	1

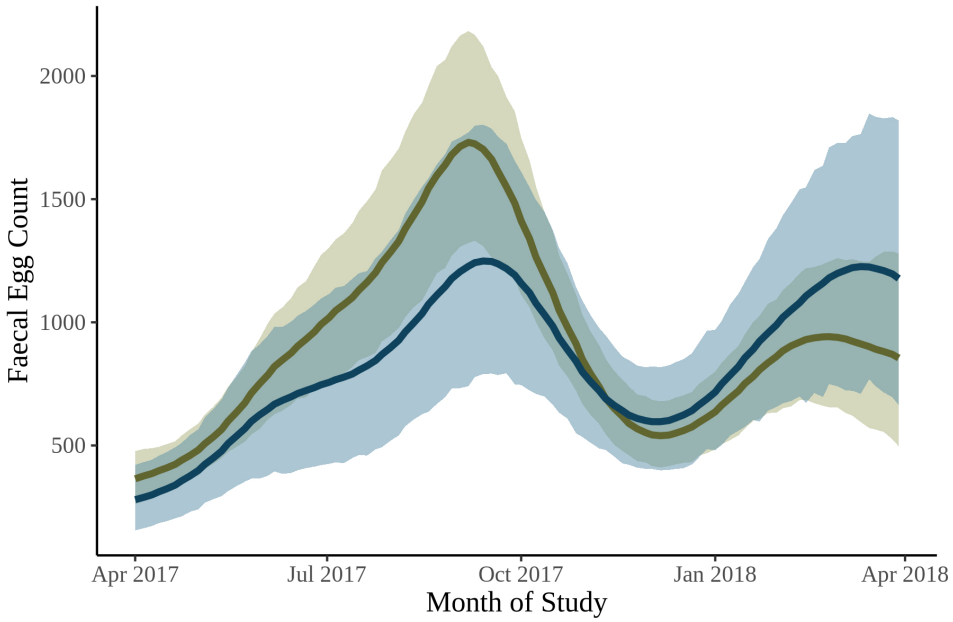

Figure 5.8 Estimate of ?*Protospirura* sp. intensity in males (blue line) and females (green line) across the study period derived from the fitted Bayesian mixed-effects hurdle model with a negative binomial distribution. Upper and lower 95% credible intervals (bands) were derived from the fitted model.

There was no evidence of an interaction between rank and sex, with no relationship found between rank and ?*Protospirura* sp. intensity for either males or females (Figure 5.9).

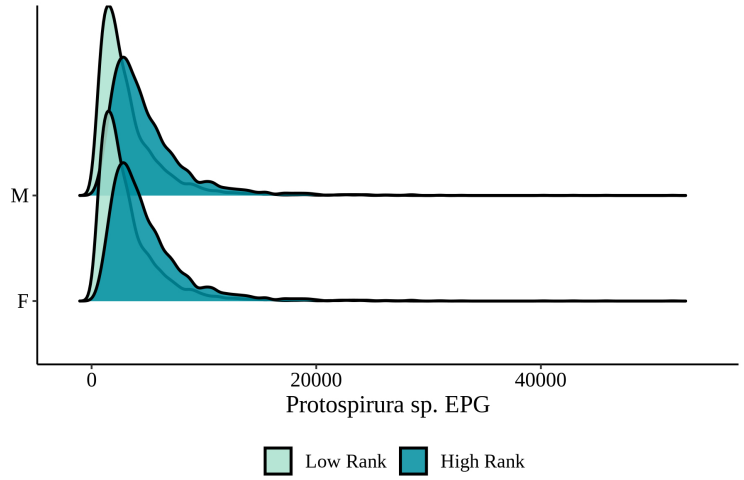

Figure 5.9 Changes in the ?*Protospirura* sp. intensity (EPG) in males and females when individuals are high ranked and low ranked. Density plots show the range of ?*Protospirura* sp. intensity (EPG) predicted by the model, with the height of the density curve indicating the probability of ?*Protospirura* sp. intensity (EPG). The spread of the curve indicates the uncertainty.

There was strong evidence of inter-individual variation in mean ?*Protospirura* sp. egg count (Figure 5.10, β = 1.02 ± 0.16 SE, l-CI = 0.77, u-CI = 1.38) but no differences were found between troops (Table 5.4, β = 0.49 ± 0.58 SE, l-CI = 0.01, u-CI = 2.09).

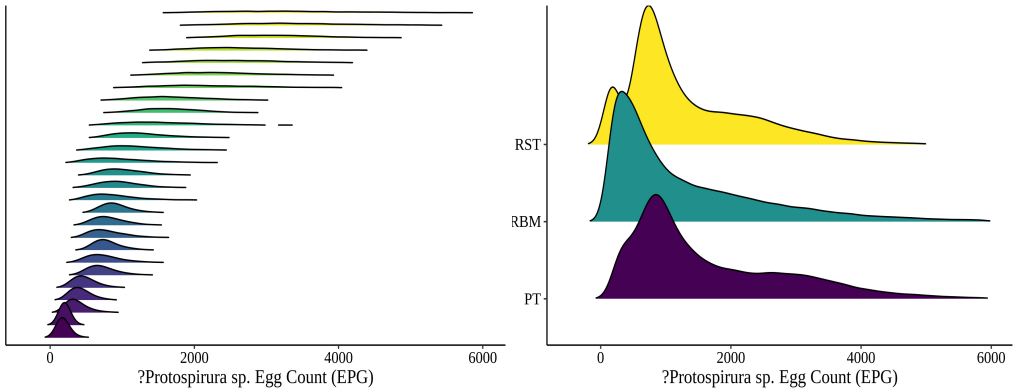

Figure 5.10 Density plots showing mean ?*Protospirura* sp. egg count (EPG) by ID (left) and troop (right). Density plots show the range of ?*Protospirura* sp. egg count predicted by the model, with the height of the density curve indicating the probability of ?*Protospirura* sp. The spread of the curve indicates the uncertainty.

5.4.3.2 *Trichostrongylus* sp.

None of the social-ecological variables included in the model predicted *Trichostrongylus* sp. egg count (Table 5.5). The full model only explained 10.1% of variance ($R^2_{conditional}$= 0.10, ± 0.06 SE) suggesting there are other predictors of *Trichostrongylus* sp. intensity not included in the model. The main effects accounted for 8.5% of variance ($R^2_{marginal}$= 0.09, ± 0.06 SE). Table 5.5 shows main effects only. For full model results showing individual level splines, see appendix Table B2.

There was no evidence of sex differences in *Trichostrongylus* sp. egg counts across the year (Figure 5.11)

Table 5.5 Summary statistics of Bayesian mixed-effects model for parasite intensity (EPG) of *Trichostrongylus* sp. CI = credible interval; SD = standard deviation. Smooth-term sds() = spline "wiggliness"(spline variance parameter). Estimates for fixed effects where credible intervals do not cross zero are in bold.

	Effect	Estimate	Est.Error	l-95% CI	u-95% CI	$\widehat{R}$
Fixed effects						
Population-Level Effects	Intercept	1.62	0.24	0.97	2.02	1
	Bi-weekly precip	-0.10	0.12	-0.33	0.13	1
	Minimum temp	-0.08	0.11	-0.30	0.14	1
	Maximum temp	0.01	0.12	-0.23	0.25	1
	NDVI	-0.03	0.17	-0.35	0.33	1
	Sex (ref:M)	-0.03	0.21	-0.47	0.37	1
	Rank	-0.11	0.18	-0.47	0.23	1
	fGCMs	0.10	0.07	-0.04	0.23	1
	Sex*Rank	0.07	0.37	-0.64	0.84	1
	s(Date)	0.65	0.78	-0.92	2.12	1
Random Effects	Troop	0.29	0.33	0.01	1.25	1
	ID	0.14	0.09	0.01	0.36	1
Smooth Terms	sds(date)	0.33	0.25	0.01	0.94	1
	sds(Date-Sex1)	0.37	0.27	0.01	1.02	1
	sds(Date-Sex2)	0.40	0.29	0.02	1.08	1
	sds(Date-Sex3)	0.40	0.30	0.01	1.12	1
Family	hu	0.78	0.02	0.74	0.81	1

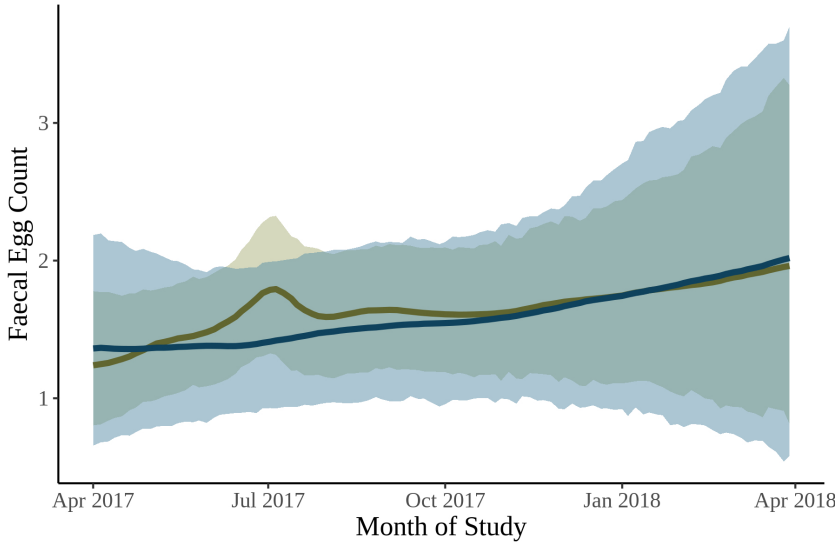

Figure 5.11 Estimate of *Trichostrongylus* sp. in males (blue line) and females (green line) across the study period derived from the fitted Bayesian mixed-effects hurdle model with a Poisson distribution. Upper and lower 95% credible intervals (bands) were derived from the fitted model.

There was no evidence of an interaction between rank and sex, and *Trichostrongylus* sp. intensity (Figure 5.12).

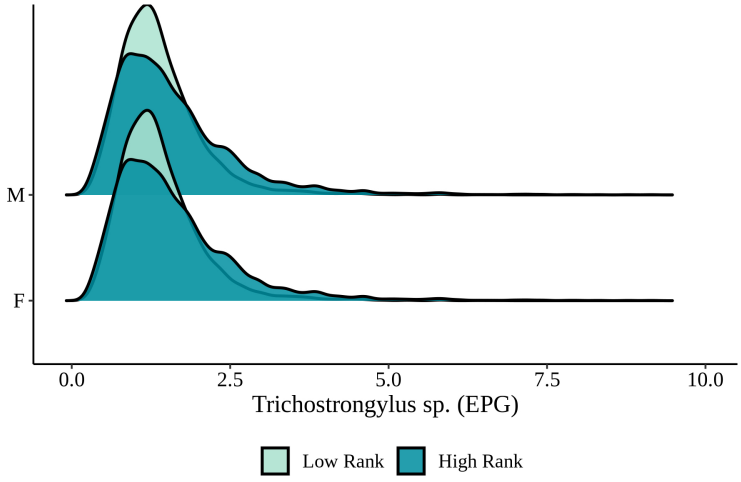

Figure 5.12 Changes in the *Trichostrongylus* sp. intensity (EPG) in males and females when individuals are high ranked and low ranked. Density plots show the range of *Trichostrongylus* sp. intensity (EPG) predicted by the model, with the height of the density curve indicating the probability of *Trichostrongylus* sp. intensity (EPG). The spread of the curve indicates the uncertainty.

There was weak evidence of inter-individual variation in mean *Trichostrongylus* sp. egg count (Figure 5.13, β = 0.14 ± 0.09 SE, l-CI = 0.01, u-CI = 0.36) but no differences were found between troops (Table 5.5, β = 0.29 ± 0.33 SE, l-CI = 0.01, u-CI = 1.25).

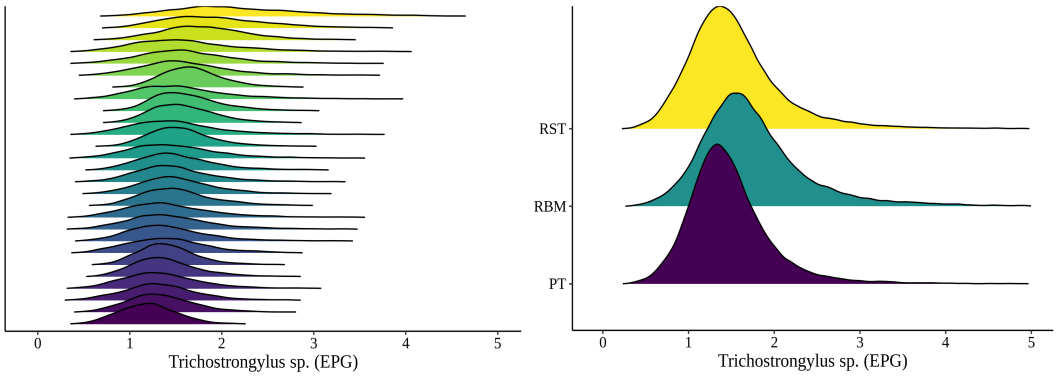

Figure 5.13 Density plots showing mean *Trichostrongylus* sp. intensity (EPG) by ID (left) and troop (right). Density plots show the range of *Trichostrongylus* sp. EPG predicted by the model, with the height of the density curve indicating the probability of *Trichostrongylus* sp. EPG. The spread of the curve indicates the uncertainty.

5.4.4 *Lagged Environmental variables*

There was evidence of a lagged negative relationship between biweekly precipitation (Table 5.6) and minimum daily temperature (Table 5.6) at the approximate time of infection and *Trichostrongylus* sp. prevalence. That is, *Trichostrongylus* sp. was more likely to be present at the time of sampling if precipitation and minimum daily temperature were lower two weeks before. NDVI and maximum daily temperature at the estimated time of infection did not predict the likelihood of *Trichostrongylus* sp. being present (Table 5.6). The full model explained 34.1% of variance ($R^2_{conditional}$= 0.34, ± 0.02 SE) while the main effects accounted for 11.4% of variance ($R^2_{marginal}$= 0.11, ± 0.06 SE).

There were no relationships between lagged environmental variables and parasite richness (Table 5.7). The full model explained 22.2% of variance ($R^2_{conditional}$= 0.22, ± 0.05 SE) while the main effects accounted for 4.6% of variance ($R^2_{marginal}$= 0.05, ± 0.06 SE).

Table 5.6 Summary statistics of Bayesian mixed-effects model for the relationship between lagged environmental variables and *Trichostrongylus* sp. prevalence (presence/absence). CI = credible interval; SD = standard deviation. Smooth-term sds() = spline "wiggliness"(spline variance parameter). Estimates for fixed effects where credible intervals do not cross zero are in bold.

	Effect	**Estimate**	**Est.Error**	**l-95% CI**	**u-95% CI**	$\widehat{R}$
Fixed effects						
Population-Level	Intercept	-2.14	0.76	-3.48	0.03	1.01
	Bi-weekly precip. (lag)	-0.53	0.27	-1.09	-0.03	1
	Maximum temp (lag)	-0.45	0.36	-1.15	0.24	1
	Minimum temp (lag)	-1.74	0.45	-2.62	-0.86	1
	NDVI (lag)	-0.15	0.85	-1.81	1.51	1
	s(Date)	-0.07	1.01	-2.06	1.82	1
Smooth Terms	sds(date)	3.32	1.4	1.38	6.73	1
Random Effects	Troop	0.82	0.9	0.03	3.55	1.01
	ID	1.83	0.4	1.22	2.77	1.01

Table 5.7 Summary statistics of Bayesian mixed-effects model for the relationship between lagged environmental variables and parasite richness. CI = credible interval; SD = standard deviation. Smooth-term sds() = spline "wiggliness"(spline variance parameter). Estimates for fixed effects where credible intervals do not cross zero are in bold.

	Effect	**Estimate**	**Est.Error**	**l-95% CI**	**u-95% CI**	$\widehat{R}$
Fixed effects						
Population-Level	Intercept	-0.74	0.36	-1.34	0.38	1.01
	Bi-weekly precip. (lag)	-0.23	0.16	-0.56	0.08	1
	Maximum temp (lag)	-0.16	0.22	-0.6	0.27	1
	Minimum temp (lag)	-0.39	0.27	-0.91	0.14	1
	NDVI (lag)	0.01	0.48	-0.88	1	1
	s(Date)	-0.03	0.94	-1.9	1.85	1
Smooth Terms	sds(date)	0.74	0.51	0.04	1.91	1.01
Random Effects	Troop	0.46	0.57	0.01	2.31	1.01
	ID	0.74	0.16	0.47	1.11	1
Family	hu	0.02	0.01	0.01	0.03	1

5.5 Discussion

Population-level properties, particularly rainfall and maximum daily temperature, were the strongest predictors of parasite prevalence, diversity and richness in my study population. While there was no evidence of relationships between the measured individual-level characteristics and parasite measures, individual-level variation was a primary predictor of parasite richness, intensity and prevalence. Further, for *Trichostrongylus* sp., there was a relationship between parasite intensity and environmental conditions, particularly rainfall, at the estimated time of infection but no lagged relationship was found for parasite richness.

In the non-human primate literature, host environment is perhaps the most well-documented driver of parasitism (Gillespie et al., 2005; Gillespie & Chapman, 2006; Huffman et al., 2009; Huffman et al., 1997; Mbora & McPeek, 2009; Schwitzer et al., 2010). Here, I found that, at the population level, maximum daily temperature was a strong predictor of *Trichostrongylus* sp. prevalence, with *Trichostrongylus* sp. also being more likely to occur when maximum daily temperatures were higher. This is likely to be due to the sensitivity of larvae to temperature (Andersen et al., 1966): higher temperatures increase larvae abundance and subsequently the likelihood of exposure to larvae in the environment. I also found a negative relationship between ?*Protospirura* sp. intensity and precipitation, where intensity was higher when precipitation was lower. Given that ?*Protospirura* requires an intermediate host, this relationship may be a result of a change in insect consumption when conditions are unfavourable, and points to a need for the identification and study of the intermediate host responsible for transmission.

At the group level, I found no relationship between troop membership and any parasite measures. Variation in parasite measures across social groups would most likely be due to group

size, ranging patterns, home range productivity and in some cases, diet selection. Group size is considered a driver of variation in parasite measures in some species given that intrinsic disease risk and infection rates should increase with group size due to increased proximity and contact rates among individuals and to contaminated substrates (Altizer et al., 2003; Arneberg et al., 1998; Arneberg, 2002; Brown et al., 2001; Freeland et al., 1976; May & Andersen, 1979) but support for this in gastrointestinal helminths is mixed (see: Freeland et al., 1976; Griffin & Nunn, 2012; McGrew, 1989, #53016; Snaith et al., 2008). The lack of any relationship between troop and parasite measures in this population might either support an absence of any linkage between group size and gastrointestinal parasites or, alternatively, could be a result of the similarity in mean troop size at the time of this study (38, 39 and 42 individuals). Given that the parasites under consideration may not be directly transmitted between individuals and the extensive home range overlap across the three troops (Pasternak et al., 2013), it is also possible that group-level exposure to both faecal-oral parasites and parasites with an intermediate host is similar across troops. Finally, given that only a subset of individuals was sampled and juveniles were not included in the analysis, it is possible that inter-troop variation cannot be captured without a full survey of the population, as adults may show age-specific or acquired immunity.

Additionally, at the group-level, I found no relationship between NDVI and parasite measures. Potential links between home range productivity and parasites are two-fold: i) The amount of vegetation present can serve as breeding or sheltering sites for parasites, particularly faecal-oral transmitted and vector-transmitted parasites (Ceccato et al., 2005) or as a surrogate measure of thermal conditions and environmental moisture (Bavia et al., 2001). ii) food availability may affect host body condition and subsequent susceptibility to infection (Benavides et al., 2012). While there is evidence that NDVI influences behaviour, resulting in an increase in

the amount of time spent foraging (Blersch et al., in prep), I do not have data on body condition. Given that the variation in NDVI was relatively low during the study period, it is possible that behavioural changes compensate for lower food availability, resulting in body condition remaining largely unchanged.

At the individual-level, sex, rank, and fGCMs did not predict any parasite measures There was, however, some evidence of sex differences across the year in ?*Protospirura* sp. intensity. Support for links between these host characteristics and parasite measures is mixed across studies and species. My finding of no sex differences for parasite intensity and richness was consistent with two other studies of vervet monkeys (Valenta et al., 2017; Wren et al., 2015), where individual differences explained much of the variance. Females did show a higher degree of variance in ?*Protospirura* sp. intensity across the year with a higher peak in winter/spring (mating season) than males, although this estimate was uncertain. This suggests that females may be more sensitive, and thus susceptible to high levels of infection, when environmental, social or physiological conditions change. However, given the lack of data on other variables such as body condition or age, this should be interpreted with caution.

Rank-related differences in parasite infections vary across primate species, across sexes and according to the parasite measure under consideration. I found no relationship between mean rank and any of the parasite measures included in my study. Similarly, there was no evidence of an interaction between rank and sex. To the best of my knowledge, there are no other studies on vervet monkey gastrointestinal parasites that have considered dominance rank. The lack of relationship between rank and parasite richness is, however, consistent with reports from other primates, including white-face capuchins (Parr et al., 2013), chacma baboons (Benavides et al., 2012), mandrills (Setchell et al., 2007) and ursine colobus (Teichroeb et al., 2009), while the lack

of relationship between prevalence and rank is consistent with a report on red-fronted lemurs (Clough et al., 2010). Similarly, I found no interaction between rank, sex and parasite measures. This, too, is consistent with other primate field studies on parasite richness that investigated a possible interaction (Benavides et al., 2012; Clough et al., 2010; Setchell et al., 2007; Teichroeb et al., 2009).

I found no evidence of a link between fGCM concentration and any parasite measures considered. Although there has been some effort to identify potential links between primate parasites and steroid hormones (Arlet et al., 2015; Chapman et al., 2006; Clough et al., 2010; Foerster et al., 2015; Friant et al., 2016b; Muehlenbein, 2006), the steroid hormone in question varies across studies and, given that glucocorticoids are not "stress hormones" but rather a potential indicator of energetic stress, interpretation of these relationships is difficult. In the primate literature, it is unclear whether the positive relationships between steroid hormones are a result of increased susceptibility to infection due to the immunosuppressive effects of the hormones themselves or whether the parasites induce a stress response (Friant et al., 2016b). Given that I do not know the concentration of steroid hormones required to result in immunosuppressive effects in vervet monkeys, combined with the non-experimental nature of the present study (individuals were not treated, and before and after treatment data are unavailable), the lack of a relationship between fGCMs and parasite infection is difficult to interpret. It is also possible that this lack of relationship is the result of fGCM data collection not being fine-grained enough to detect more short-term increases in fGCMs.

While no individual-level characteristics included in my models directly predicted any of the parasite measures, there was considerable evidence of individual-level variation in ?*Protospirura* sp. intensity, and *Trichostrongylus* sp. prevalence. This suggests that there are

underlying host characteristics that influence parasite infection that were not captured here. These include age (MacIntosh et al., 2010; Monteiro et al., 2007), body condition (Benavides et al., 2012), maternal characteristics (MacIntosh et al., 2010), ranging patterns (Nunn & Dokey, 2006; Nunn et al., 2011) and diet (Rondon et al., 2017; Vitone et al., 2004). Research in other non-human primates has found that the effects of individual behaviour, particularly grooming and proximity measures, supersede the effects of any host individual characteristics on parasite measures (Friant et al., 2016a)—this highlights the need for further investigation into the behavioural correlates of parasitism in the population.

Exploratory analysis showed evidence of a lagged relationship between biweekly precipitation, maximum daily temperature and *Trichostrongylus* sp. Prevalence. *Trichostrongylus* sp. was more likely to be present at the time of sampling if precipitation and minimum daily temperature were lower 2 weeks before. The mechanisms linking gastrointestinal prevalence and temperature are well studied and show species-specific thermal limits for infective stage survival (Pietrock & Marcogliese, 2003). Temperature affects both parasite generation time and/or the production of intermediate stages in their life cycle. Thus, the lower temperature at the time of infection may serve as an indicator of the optimal temperature for development and survival of *Trichostrongylus* sp. larvae in the environment with prevalence being higher as a result of increased exposure to *Trichostrongylus* sp. larvae. However, as with all parasite-host relationships, this lag should be interpreted with caution given that a causal relationship cannot be reliably established. That is, the environmental conditions at the time may not be directly affecting the parasite but rather exerting effects on the host, subsequently increasing susceptibility. Nevertheless, this lagged relationship highlights the need to consider

both present and past environmental conditions when attempting to establish parasite-environment links.

Although I have shown previously that there is temporal variation in egg counts between samples (Blersch et al., 2021; Blersch et al., 2019)— suggesting both that egg count serves as a proxy for infection and that sample collection frequency is sufficient to capture variation in intensity—it should be noted that some authors argue for more frequent sample collection. This is particularly pertinent to species richness measures where sampling effort has been linked to parasite richness measures (Nunn et al., 2003; Walther et al., 1995). Given the overall low frequency of the three genera in our population, sampling every two weeks may not be sufficient to fully capture species richness across time. Further, it has also been proposed that, in wild populations, parasites are not equally distributed with some individuals harbouring a disproportionate number of parasites (Poulin, 2007a). Thus, it is also possible that high-risk individuals were not included in the subset, thus decreasing prevalence and richness measures. Given this, there may be underlying relationships between population-, group and individual level factors, and parasite prevalence and richness, that were not captured here, and more frequent faecal sampling would be needed to investigate this possibility.

Understanding wildlife-parasite dynamics is hugely challenging owing to the large variation in host characteristics (Altizer et al., 2003; Arneberg, 2002; Willis et al., 2015) and behaviour (Nunn & Dokey, 2006), the complexities of social organisation (Altizer et al., 2003), environmental change and anthropogenic disturbances, to name a few (Bradley & Altizer, 2007). As such, establishing and comparing the predictors of parasitism in a wild population is inherently problematic given the multi-scale dynamics of any host-parasite system and the reciprocal nature of any host-parasite relationships (Ezenwa et al., 2016). Nevertheless, these

predictors provide some valuable insight into how host, parasite and environment interact and serve as the primary foundation for research into how parasitism might shape host behaviour.

PART B: GASTROINTESTINAL HELMINTHS AND THEIR BEHAVIOURAL CORRELATES IN VERVET MONKEYS

CHAPTER SIX: SICK AND TIRED: SICKNESS BEHAVIOUR, POLYPARASITISM AND FOOD STRESS IN A GREGARIOUS MAMMAL

A version of this chapter, under the same title, has been submitted as a contribution to the Topical Collection "Sociality and Disease" in the journal Behavioral Ecology and Sociobiology. At the time of first thesis submission, it was in review.

6.1 Abstract

Although sickness behaviour in response to non-lethal parasites has been documented in wild animals, it remains unclear how social and environmental stress might also shape an animal's behavioural response to parasitism, nor do we know whether simultaneous infection with more than one parasite changes the way animals respond. Here, I combine physiological, environmental, behavioural and parasite measures to investigate behavioural responses to infection in wild vervet monkeys living in a semi-arid region of South Africa. I quantified both activity budget and behavioural predictability to investigate the occurrence of sickness behaviour and infection with two non-lethal gastrointestinal parasite genera. Higher parasite load was linked to an increase in the time spent resting. However, the nature of the relationship with other behaviours was contingent on both the parasite genus in question, and parasite species interacted, highlighting the importance of considering co-infection. Overall, food availability was the dominant predictor of behavioural change suggesting that, for monkeys living in a more extreme environment, coping with ecological stress may override the ability to modulate behaviour in response to other physiological stressors. Our findings provide insight into how animals living in harsh environments find ways to cope with parasite infection, avoidance, and transmission.

6.2 Introduction

It has long been established that highly virulent parasites can drive population declines, and may contribute to local extinctions (see: Best et al., 2012; Antonovics 2009; De Castro and Bolker 2005). Although often overlooked, the effects of sub-clinical or non-lethal infections can be costly to host health and fitness, and the consequently on population viability (Bohn et al., 2016). Hosts have evolved several physiological and behavioural responses to cope with the pressures of infection (Lopes 2014) and, while we have some understanding of the physiological immune response to infections in animals, less is known about the behavioural presentation of sickness and its physiological correlates (Dantzer and Kelley 2007).

Sickness behaviour is very broadly defined as a suite of behaviours that occurs in response to infection. This includes lethargy, anorexia, somnolence, and a reduction in grooming (Hart 1988; Dantzer and Kelley 2007). Although originally thought to be simply a by-product of infection, sickness behaviour is increasingly being considered to be part of a highly organised strategy to combat infection by reallocating energy to the immune system and away from non-essential activities (reviewed: Hart 1988; Johnson 2002; Aubert 1999). However, more work is needed to conclusively establish the adaptive nature of sickness behaviour in the wild (reviewed: Poulin 1995). If sickness behaviour is an inherently beneficial strategy to combatting infection, then a trade-off emerges as energetic resources are devoted to fighting infection at the expense of other vital processes, such as growth and reproduction (Lopes 2014). The severity of these costs, and hence the relative benefit of displaying sickness behaviour, depends on ecological context and the value of behaviours that need to be sacrificed. Thus, we should expect to see animals modulating their expression of sickness behaviours when the costs become too high. This is

something particularly pertinent to animals subject to prolonged environmental or social stress given it is likely these animals have an already constrained activity budget and may not be able to express sickness behaviour even if it is beneficial (Cohn and de Sá-Rocha 2006; Moyers et al., 2015).

Sickness behaviour has been extensively documented in captive populations (Weary et al., 2009; Bohn et al., 2016; Lopes et al., 2016; Stockmaier et al., 2020), but we know much less about its occurrence in wild mammals (Krief et al., 2005; Ghai et al., 2015; Hamilton et al., 2020)—most likely due to the challenges associated with long-term environmental and physiological monitoring. Sickness behaviour research in the wild, therefore, has focused almost exclusively on the relationship between parasite infection and behaviour, independent of other stressors. However, the expression of sickness behaviour is more complicated if animals are simultaneously subject to other competing stressors common in natural environments (Cohn and de Sá-Rocha 2006; Moyers et al., 2015), and the expression of sickness behaviour should vary accordingly. Although we have some grasp of the social factors that influence investment in sickness behaviour (for review, see: Lopes 2014), the influence of environmental stressors remains poorly understood. Understanding the interplay between environmental stress and behavioural modification is central to understanding how sickness behaviour may impact long-term fitness in wild populations.

Sickness behaviour research has also been principally concerned with the effects of a single designated parasite or pathogen species on behaviour. Yet, wild animals rarely harbour only a single species, and interactions between parasite species are likely (Bordes and Morand 2011). This interaction can be either synergistic, where the parasite burden of one species magnifies the consequences of another, or antagonistic, where the burden supresses the other's

effects (Graham 2008). At present, we have evidence that polyparasitism predicts infection risk (Telfer et al., 2010), host body condition, and survival (Jolles et al., 2008) in mammals but there is comparatively little research on how multi-parasite infection affects behaviour (see: Huffman and Seifu 1989; Huffman et al., 1997, 1993; Huffman 1997; Alados and Huffman 2000).

While sickness behaviour research generally focuses on activity or time budgets, there are other, more fine-grained, aspects of behaviour that may also be influenced by both physiological and environmental stress, including behavioural predictability and behavioural complexity. Unpredictable behaviour or complex behaviour is thought to be biologically adaptive as it allows organisms to cope with stress or and unpredictable environments (Goldberger 1997; MacIntosh et al., 2011). A decrease in in the complexity of behavioural patterns has been linked to parasite infection in primates and may serve as a proxy measure of health suggesting the behavioural correlates of parasitism stretch beyond activity budget (see: Ghai et al., 2015; Alados and Huffman 2000; MacIntosh et al., 2011). Several measures of behavioural complexity have been used from the frequency of behavioural switching (Ghai et al., 2015) to long-range autocorrelation and fractal analysis (MacIntosh et al., 2011). Current measures used to quantify behavioural predictability and/or structure often require analytical restrictions being placed on the collected data. Typically, analysis is directed at two or three designated behaviours, or at behaviours that have been combined into larger groupings. This is primarily due to the constraints of existing analyses and measures, which often require a single or a binary response variable. For example, MacIntosh et al. (2011) selected foraging and moving, from a broader range of possible behaviours, to assess the consequences of parasite infection in Japanese macaques whereas, to assess the health of chimpanzees, Alados and Huffman (2000) grouped all recorded behaviours into either social or non-social categories. A method of quantification that

allows for the inclusion of more behaviours and/or a non-binary response may provide a broader insight into how animals respond and adapt to environmental changes and where the limits of these changes might lie.

One such measure is entropy rate which provides a way to combine behaviours into a discrete-time sequence of distinct behaviours representing a stationary process in time (Davis et al., 2017). This allows more behaviours to be incorporated to quantify behavioural predictability, which reduces the analytical restrictions of the single or binary-response measures previously mentioned.

Here, I use a comprehensive dataset comprised of detailed physiological (faecal glucocorticoid metabolites), environmental, behavioural and parasite data to assess how these factors interact to shape behavioural responses to infection in a population of a highly social, wild mammal, specifically, the vervet monkey (*Chlorocebus pygerythrus*), in a semi-arid region of South Africa. Previous work in this population has identified complex relationships between behaviour and environmental conditions, with food resources, temperature, rainfall, and standing water availability strongly influencing activity budgets and mortality (McFarland et al., 2014; Young et al., 2019). As in this previous work, I use fGCMs as an index of individual response to environmental stressors (i.e., as a measure of the ability to restore homeostasis), rather than an indicator of an individual animal's stress levels (MacDougall-Shackleton et al., 2019). Given the often harsh environmental conditions in the study area, these monkeys provide an excellent opportunity to determine whether the expression of sickness behaviour occurs in wild animals that are subject to simultaneous external and internal stressors.

I use a combined approach, quantifying both activity budget and behavioural predictability, to investigate the relationships between behaviour and two non-lethal

gastrointestinal parasite genera in the context of food stress. In addition to a more comprehensive dataset, I use a newly developed measure of entropy rate to assess predictability (Vegetabile et al., 2019); this allows a larger range of behaviours to be considered, and is therefore more sensitive than existing analytical techniques. Finally, I consider whether there is an interaction between the two parasite genera studied here, and if co-infection compounds the need to invest in sickness behaviours.

6.3 Methods

6.3.1 *Study Site and Study Species*

We collected behavioural data and faecal samples from August 2017 to April 2018 from three fully habituated groups (PT = Picnic Troop, RBM = River Bend Mob, RST = Riverside Troop) of wild vervet monkeys on Samara Private Game Reserve, South Africa (32°22'S, 24°52'E). These monkeys have been the subject of continuous data collection since 2009. All group members were individually identified based on natural markings, and data for this study were collected from a subset of 27 adult individuals (PT: 4 males, 6 females out of 14 adults; RBM: 2 males, 6 females out of 14 adults; RST: 3 males, 6 females out of 16 adults), selected to be representative of adult demography and to reflect the full range of dominance ranks. The study area comprises semi-arid riverine woodland (Pasternak et al., 2013), with a declining annual average rainfall of 386 mm, and average annual minimum and maximum temperatures of 10ºC and 27ºC respectively. The region experiences periodic droughts that are severe enough to be a primary source of mortality for animals in our study groups (Young et al., 2019).

6.3.2 *Behavioural Data Collection*

Each group was followed for five days each week across the study period, and data were collected for 10 hours each day (McFarland et al., 2015; Young et al., 2019). To assess changes in activity budget, the behaviour of all visible individuals was recorded during 10-min scan sampling blocks (Altmann 1974) conducted every 30 min throughout the day. I selected four, high frequency, mutually exclusive behaviours for analysis: moving, foraging, resting and allo-grooming, either given or received. Notably, I considered foraging to include both manipulation and ingestion of food (for definitions, see: Isbell and Young 1993). It was not possible to record data blind because our study involved sampling individual focal animals in the field, which requires that researchers are able to recognise and follow a specific individual in the context of the social group. However, observers were 'blind' to the parasite loads of the individuals from which data were collected, as all parasite analyses were conducted by RB once data collection in the field was completed.

To investigate changes in behavioural predictability, I conducted 10-min continuous focal sampling (Altmann 1974) twice per week for each of the 27 subjects (N_{total} =1614 focal samples). Randomised focal times were generated for each day. During these focal sampling events, a single individual was followed and a continuous, timed record of its behaviour obtained, using electronic data loggers and proprietary software. The same mutually exclusive behaviours were identified as described above. Owing either to disruptions, such as aggressive encounters between groups, or periods where individuals were out of sight, not all focal samples were exactly 10 minutes long. To account for this, I controlled for focal sample length in our analyses and the final dataset included focal samples where the individual was in sight for a minimum of 7.5 minutes.

Finally, we collected *ad libitum* data on dyadic agonistic interactions among all group members, for which we identified participants and outcomes. Given good visibility at the site I am confident that there was no systematic bias in the likelihood of observing encounters. These agonistic data were used to construct dominance hierarchies (Young et al., 2019). Only decided dyadic agonistic interactions with a clear winner and loser were included in the analysis with the loser being defined as the last individual to show submission during the interaction.

6.3.3 *Dominance Hierarchy*

I divided the study period into four 3-month blocks: July – September 2017, October – December 2017, January – March 2018 and April – June 2018. I used *ad libitum* observations of agonistic interactions to construct hierarchies for each period ($RBM_{Total\ N}$: 963; $RST_{Total\ N}$: 810; $PT_{Total\ N}$: 1135) for all adults in each troop and not only the subset of study subjects. Given male-female co-dominance in this population (Young et al., 2017b), I generated a single matrix that included all decided agonistic interactions regardless of the sex of participants and created a single interdigitated hierarchy.

Dominance ranks in each troop and for each 3-month block were expressed as a standardized David's score using the package 'compete' (Curley 2016). David's scores were standardized to enable direct comparison across groups of different size and interaction rates (Henzi et al., 2013).

6.3.4 *Food availability*

I quantified food availability in each troop's home range by calculating the Normalized Difference Vegetation Index (NDVI) every 16 days (Young et al., 2019) from MODIS data

collected by Earth Observing System (EOS) satellites Terra (EOS AM-1) and Aqua (EOS PM-1). Using Moderate Resolution Imaging Spectroradiometer MOD13Q1 vegetation indices at a 250-meter resolution (Didan 2015), NDVI measures the amount of biomass or chlorophyll activity by calculating the difference between the visible red and near infrared bands divided by their sum. The resultant measure is a range of values between -1 and 1, where negative values indicate an absence of vegetation and positive values approaching 1 indicate larger concentrations of green vegetation (Pettorelli et al., 2005). Given the generalist, largely plant-based nature of vervet diet (Pasternak et al., 2013), the synoptic view of NDVI is a reliable measure of food availability in this species and at this site (Willems et al., 2009; Jarrett et al., 2020).

6.3.5 *Faecal sampling and analysis*

We collected a total of 573 faecal samples (mean = 21/individual, ± 3.1 SD) during the 234 days of the study. Faecal samples were collected twice per month (once during each two-week period) from the 27 subjects. Two corresponding faecal samples, one for parasite analysis and one for faecal glucocorticoid metabolites (fGCM) analysis, were collected from the same defecation event.

6.3.5.1 Parasite analysis

For each sample, approximately 1 g of fresh faeces was weighed in the field immediately after defecation and directly placed into 10% neutral, buffered formalin. Samples were stored in the field lab and transported to the University of Lethbridge, Canada, where faecal flotation and sedimentation techniques were used to identify parasites (Blersch et al., 2019).

I used a modified zinc sulphate flotation to isolate helminth eggs followed by ethyl-acetate sedimentation to isolate potential trematodes that were too heavy to float during ZnSO4 flotation (methods: supplementary S1). For both methods, the entire pellet was examined under the microscope. Parasites were identified to genus level based on egg shape, size, colour, and contents, and all eggs were counted. Representative eggs were photographed.

I recovered 5 parasite genera from faecal samples (Blersch et al., 2019). One parasite could not be identified to genus level, as eggs of *Physaloptera* sp. and *Protospirura* sp. cannot be reliably distinguished based on egg morphology alone. Based on morphological characteristics of the eggs, including their size and the presence of a hyaline substance (Brumpt 1931; Petrželková et al., 2006), I consider it most likely to be *Protospirura* sp. (hereafter referred to as ?*Protospirura* sp.) pending results of ongoing molecular analysis. Preliminary molecular analyses suggest the parasite is a single species. Due to small sample size for three genera (<5% mean annual sample prevalence), namely *Oesophagostomum* sp., *Subulura* sp. and *Ternidens* sp., I selected only ?*Protospirura* sp. and *Trichostrongylus* sp. (>20% mean annual sample prevalence) for these analyses but include other species in the number of genera (parasite richness).

I have established previously that sequential faecal egg count patterns for *Trichostrongylus* sp. and ?*Protospirura* sp. are not stochastic and point to underlying levels of infection in our population (Blersch et al., 2021), and thus use egg counts as a proxy for the extent of helminth infection.

6.3.5.2 Faecal steroid analysis

Samples were collected following the protocol of Young et al. (2017a; 2019). Within 15min of defecation, a 2-5g piece of faecal material was transferred into a plastic vial following

physical homogenization of the full faecal sample. Prior to collection, faecal samples were checked to ensure there was no contamination with urine during excretion or on the substrate where the sample landed. Vials were immediately stored on ice in a thermos flask in the field before transfer to a −20°C freezer at the end of the day. Samples were stored frozen until transport on dry ice to the Endocrine Research Laboratory, University of Pretoria, for analysis.

Samples were lyophilized, pulverized and then sieved to remove seeds and fibrous matter (Young et al., 2017a). The resulting faecal powder (0.10g) was extracted by vortexing for 15min with 80% ethanol in water (3ml) followed by 10 minutes of centrifugation at 1500g. 1.5 ml of the resultant supernatants were transferred into microcentrifuge tubes. Hormone analysis was conducted following the standard procedures of the Endocrine Research Laboratory, University of Pretoria (Ganswindt et al., 2002) using the cortisol enzyme immunoassay (EIA) (Young et al., 2017a). The sensitivity of the EIA used was 0.6 ng/g dry weight (Young et al., 2017a). Inter- and intra-assay coefficients of variation of high- and low-value quality controls were: 4.64–5.96 and 8.13–11.60% respectively. All steroid concentrations are given as ng g^{-1} faecal dry weight.

6.3.6 *Applying entropy rate to the behaviour of free-ranging animals*

6.3.6.1 Simulating data

To determine whether entropy rate can be applied to our observed data, and to get a sense of the sensitivity of the measure, I simulated a dataset that closely matched our observed data. Simulated data allowed me to make specific predictions related to the influence of environmental conditions on behavioural predictability where the outcome is already known. As entropy rate has only been applied narrowly in the field of animal behaviour research, this functioned as a test of whether the entropy rate measure is capable of retrieving the known outcome in simulated

behavioural data comparable to wild vervet monkey behaviour. If the outcome can be successfully retrieved in simulated data, entropy rate can then be reliably applied to explore general relationships between social and environmental factors on behavioural predictability in the wild. Furthermore, simulation provides control over the magnitude of behavioural change in response to environmental change which serves as a coarse measure of the sensitivity of entropy rate to capture changes in behavioural predictability.

I derived the simulation from the prediction that an increase in food availability was associated with a reduction in time spent foraging, and a consequent increase in the time spent engaged in social behaviours. First, I simulated a range of NDVI values between 0.25 and 0.6, which was consistent with our observed data. Then I simulated behavioural sequences across NDVI values, while keeping the sequence length (n = 20 behaviours) associated with the greatest variance, number of focal samples (n = 1553) and number of individuals (n = 27) consistent with our observed behavioural data. Given that our observed dataset extends predominantly through summer, I started with an activity budget similar to the probabilities of behaviours found during the hot-dry period by (Young et al., 2019). I then simulated data such that the time spent foraging decreased with increasing NDVI, using a low (2%), medium (7%), or high (20%) decrease in foraging time between minimum NDVI and maximum NDVI. I then calculated the entropy rate for each generated sequence. This range served as an indicator of how much entropy rate can be expected to vary in relation to the magnitude of behavioural change thus providing a coarse measure of sensitivity. For modelling purposes, I then selected sequences derived from a 7 percent change in foraging time based on previous estimates of seasonal variation in foraging time (Young et al., 2019). These simulated data were used in a Bayesian mixed effects model (brms package Bürkner, 2017; Bürkner, 2018) to test our prediction that an increase in NDVI

would result in a decrease in entropy rate. I used NDVI as our fixed effect and individual ID as our random effect. Other variables, such as troop ID or dominance rank, were not used in this model as our primary interest was whether I could retrieve the known influence of NDVI on entropy rate while aiming to keep the simulation as clear and simple as possible.

6.3.6.2 Entropy rate: Time interval selection

In order to estimate entropy rate, continuous focal samples had to be discretized into coded behavioural sequences. I therefore first determined the sampling time interval that resulted in maximum variance across sequences. This ensured that our measure was sensitive enough to detect small changes in behaviour. I assigned each behaviour a single letter and created coded behavioural sequences by extracting behavior from each focal at 3s, 5s, 10s, 15s, 20s, 30s, 45s, 60s, 90s, 120s and 300s intervals. This generated 11 sets of sequences for each focal that ranged from 2 to 200 consecutive behaviours. I then used the entropy package (Hausser and Strimmer 2014) in R version 3.4.4 (R Core Team, 2018), to calculate the entropy rate, together with the variance and standard deviation (SD) for each sequence for each time interval. A sampling interval of 30 s resulted in maximum variance (Var = 0.157) across sequences and I therefore used sequences from a 30 s sampling interval for further analysis.

6.3.7 *Statistical Analysis*

6.3.7.1 Patterns of co-infection

Egg counts of our two most prevalent parasite genera, ?*Protospirura* sp. and *Trichostrongylus* sp., were used in these analyses. I conducted exploratory analysis to assess whether there was a relationship in parasite intensity between ?*Protospirura* sp. and *Trichostrongylus* sp., using a mixed effects model in a Bayesian framework and specifying a

lognormal distribution. I filtered out samples that were parasite negative (N = 8). ?*Protospirura* sp. intensity, represented as eggs per gram (EPG) was our response variable while *Trichostrongylus* sp. was our fixed effect. I included individual ID nested in troop as our random effect with individual-level random slopes for *Trichostrongylus* sp.

6.3.7.2 Model set 1: The influence of parasite infection and ecology on behaviour

To examine whether infection with ?*Protospirura* sp., *Trichostrongylus* sp. and parasite species richness (the number of genera recovered in each faecal sample) were associated with changes in behaviour, I used scan data (N_{scans}=27,068) to construct a multilevel multinomial behavioural model (Koster and McElreath 2017) with the Rstan package (Stan Development Team 2020). I linked one week of behavioural data (3 days before the faecal sample collection and 4 days after) to each faecal sample for the corresponding individual for both parasite data (Ghai et al., 2015) and fGCM concentrations. I found no qualitative differences in estimates between the reduced and full focal datasets for the variables that could be included (results: supplementary S2).

Multilevel, multinomial behavioural models estimate the likelihood of a given behaviour from a set of categorical behaviours occurring at any given time in relation to a reference behaviour, while controlling for repeated observations from the same individual.

I set behaviour (feeding, resting, grooming given, grooming received, and moving) as our response variable, with moving as our reference variable. Moving was selected, as the reference variable is sensitive to frequency, and moving is a very common behaviour. I included parasite intensity (given as eggs per gram), parasite richness (number of genera), and NDVI as our primary fixed effects. I also controlled for other physiological effects by including fGCMs as a fixed effect, and I also controlled for sex, standardised rank and date. Individual ID and troop

were included as random effects. In addition to summary statistics, I generated predicted probabilities for each behaviour for each predictor variable while holding other coefficients constant. This allowed us to look at changes in all behaviours, including the reference variable. Owing to the use of a reference behaviour (i.e., moving), coefficients of the multinomial model are not straightforward indicators of the effect of a predictor on the probability of performing a given behaviour (Koster and McElreath 2017) thus predicted probabilities are computed to understand the effects of the fixed effects on each behaviour.

6.3.7.3 Model set 2: The influence of parasite infection and ecology on behavioural predictability

I used entropy rate to determine whether parasite infection affects behavioural predictability. Entropy rate quantifies the predictability of the next observation, given the history of observations which occurred before it. Our entropy rate method estimates the distribution of behaviours (the frequency of each) and a transition matrix that describes transitions between behaviours (Vegetabile et al., 2019). An entropy rate of zero would indicate an individual engaged in a single behaviour for the entire observation period whereas an entropy rate of 1 indicates that an individual either engaged in multiple behaviours, switched behaviours frequently or both. As entropy rate has only been applied narrowly in animal behaviour, I began by validating its extension to observational data from wild monkeys, using both simulated and observed data (methods and results: supplementary S3). In order to estimate entropy rate, continuous focal samples were discretized into coded behavioural sequences. I assigned each behaviour a single letter code and created behavioural sequences by extracting behaviour from each focal at 30 second intervals, the optimal time period identified (N=693 faecal sample-

matched sequences). I then used the 'entropy' package (Hausser and Strimmer 2014) in R version 3.5.2 (R Core Team 2018), to calculate the entropy rate.

6.3.7.4 Bayesian mixed-effects model structure

I constructed a mixed effects model with a Gaussian distribution in a Bayesian framework to assess the relationship between parasite intensity and behavioural entropy rate (distribution comparison results: supplementary material S4). Our response variable was behavioural entropy rate and, as with model 1, parasite intensity for ?*Protospirua* sp., *Trichostrongylus* sp., parasite richness and NDVI were included as our primary fixed effects while controlling for fGCM concentration, rank and sex as fixed effects. Given that individuals may be more likely to be active earlier in the morning and resting or grooming during the hottest part of the day, which may affect behavioural predictability, I included a spline on time of day as a fixed effect. Individual ID and troop were included as random effects. As not all focal samples were exactly 10 minutes long, I also controlled for sequence length. I standardised continuous variables (rank, NDVI and sequence length) using one standard deviation (SDs) to allow comparisons of effect sizes across continuous and dichotomous variables. These variables were mean-centred on zero. I ran models with 4 chains and 2000 iterations which allows for a large enough sampling pool to achieve model convergence and conduct posterior sampling (McElreath 2016; Bürkner 2018). I used weakly informative priors (normal(0, 1)) and chain convergence was confirmed by $\hat{R}$ values ≤ 1.01. Model goodness-of-fit was assessed using the "posterior predictive check" (pp_check) function in the "bayesplot" package (Gabry et al., 2019).

6.3.7.5 Posterior plots

Given the nature of the statistical models used, as well as the inclusion of variable interactions, direct interpretation of model estimates is not straightforward. To aid in

interpretation, I generated whole model predictions using the fitted() function from the brms package. These predictions were then used to construct posterior density plots. Importantly, egg count was entered into our models as a continuous variable, but they are categorised as “high” and “low” for visualization purposes in the plots. This was to aid in interpretation only, and no assumptions are made as to the biological significance of what is designated as “high” or “low”.

6.4 Results

6.4.1 *Applying entropy rate to the behaviour of free-ranging animals*

6.4.1.1 Time interval selection

A sampling interval of 30 s resulted in maximum variance (Var = 0.157) across sequences (Figure 6.1) and I therefore used sequences from a 30 s sampling interval for further analysis. Using a 30 second sampling interval, mean entropy rate in our population was 0.76 (±0.40 SD).

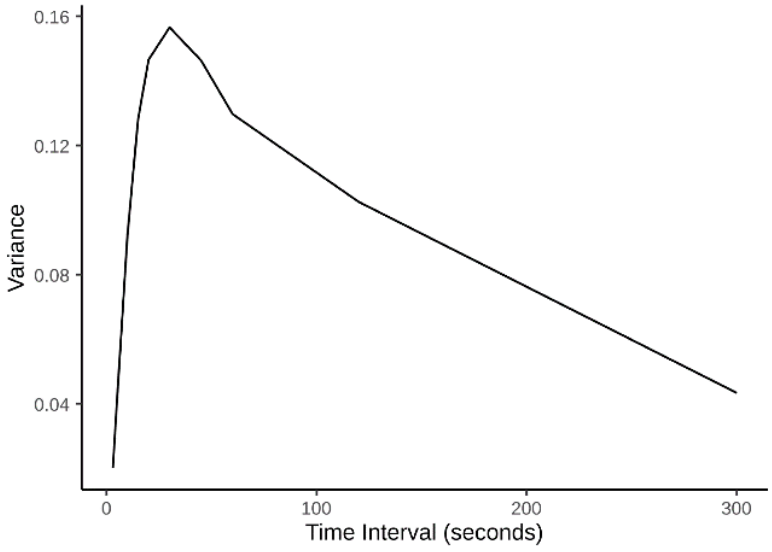

Figure 6.1 Variance in entropy rate for discretized coded behavioural sequences constructed using each time interval. Maximum variance at 30 second sampling time interval

6.4.1.1 Simulated Data and Sensitivity

Based on simulated data, I found that behaviour became more predictable as NDVI increased and the proportion of time spent foraging decreased (Figure 6.2). This indicates that entropy rate successfully captures changes in behavioural predictability in data of similar structure to our observed data. Regarding sensitivity, when considering the magnitude of behavioural change required to detect a change in entropy rate, simulation showed that a 2% decrease in foraging between minimum and maximum NDVI does not result in a reliable change in entropy rate while I may expect a change in entropy rate of approximately 0.3 with a 19% decrease in foraging and increase in social interactions

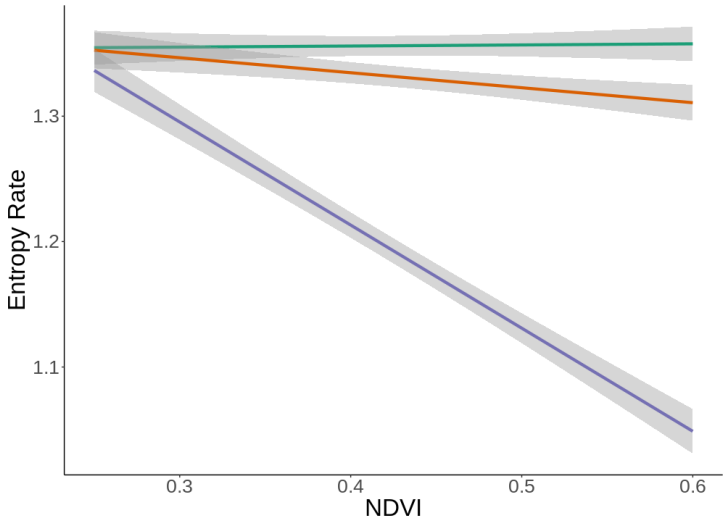

Figure 6.2 Plot of simulated data showing the resultant change in entropy rate as foraging decreases while NDVI increases. Data were simulated with 2% decrease in foraging (green), 9% decrease in foraging (orange) and 19% decrease in foraging (purple). Bands show upper and lower 95% credible intervals

6.4.2 *Patterns of infection and co-infection*

?Protospirura sp. had a mean annual sample prevalence of 98.74 % (±1.74 SD) and host group prevalence of 99.33% (±1.51 SD) with only 8/573 samples negative for all parasites.

Trichostrongylus sp. had a mean annual sample prevalence of 22.04% (± 17.56 SD) and host group prevalence of 25.69% (±17.53 SD). Thus, all samples that were positive for *Trichostrongylus* sp., were also ?*Protospirura* sp. positive.

For ?*Protospirura* sp., annual minimum and maximum egg counts from positive samples (ps) were 2 eggs per gram (EPG) and 5841 EPG respectively ($mean_{ps}$ = 752.22, $median_{ps}$ = 425.75, ±861.33 SD) while for *Trichostrongylus* sp., egg counts ranged from 2 to 47 EPG ($mean_{ps}$ = 6.5, $median_{ps}$ = 5.28, ±5.29SD).

I found no evidence of a population-level relationship between ?*Protospirura* sp. infection intensity and *Trichostrongylus* sp. infection intensity (Estimate = 0.39, Estimate error = 0.63, lower 95% credible interval = -0.98, upper 95% credible interval = 1.56).

I found some evidence of inter-individual differences in random slopes for co-infection patterns of parasite intensity (Figure 6.3). For some individuals, infection intensity of ?*Protospirura* sp. was high when *Trichostrongylus* sp. was absent or intensity is low. However, when *Trichostrongylus* sp. infection intensity was higher, ?*Protospirura* infection intensity was also high for some individuals. This pattern is stronger for some individuals than others. Note that estimate uncertainty is high for some individuals due to smaller individual-level sample size and this result should be interpreted with caution. Full model results are provided in the appendix Table C3 and figure including credible intervals provided in appendix figure C3.

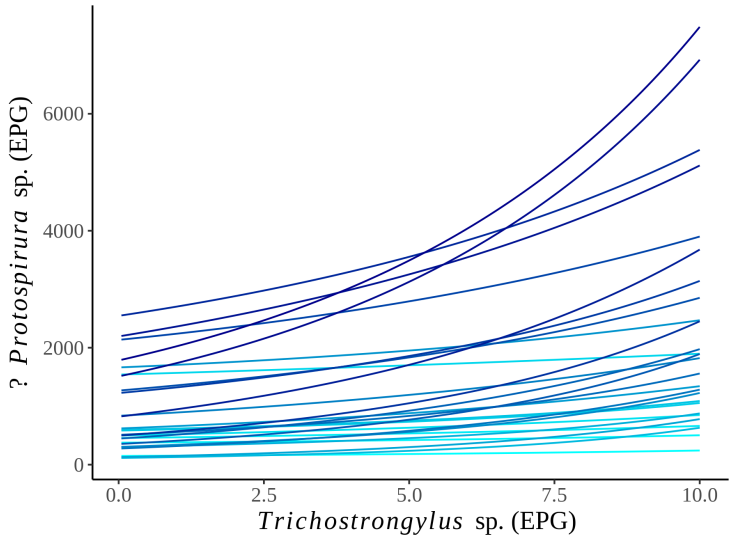

Figure 6.3 Individual-level estimates of faecal egg count (eggs per gram, EPG) of ?*Protospirura* sp. as a function of *Trichostrongylus* sp. faecal egg count derived from the fitted Bayesian mixed-effects model. Dark blue lines show individuals with a strong positive relationship between *Trichostrongylus* sp. and ?*Protospirura* sp. Upper and lower 95% credible intervals excluded for clarity

6.4.3 *Model set 1: Influence of parasite infection and ecology on behaviour*

6.4.3.1 Fixed effects

I found evidence of parasite-induced lethargy (i.e., increased resting time) and anorexia (i.e. reduced feeding time) as ?*Protospirura* sp. egg count increased (Figure 6.4a). The probability of resting increased by 8.7% (l-CI = 2.2, u-CI =14.9) when egg counts were highest. This was predominantly traded off against moving, which showed a 7.4% decrease (l-CI = 2.9, u-CI =12.2) and there was also a 4.3% decrease (l-CI = 0.16, u-CI =8.3) in the probability of foraging. The probability of both giving and receiving grooming were largely unchanged.

Conversely, I found that an increase in *Trichostrongylus* sp. loads resulted in a 15.4% (l-CI = 6.3, u-CI =24.6) reduction in the probability of resting. There was also an 8.8% (l-CI = 0.2, u-CI = 21.1) increase in the probability of foraging, while the probability of moving remained

largely unchanged (Figure 6.4b). The probability of both giving and receiving grooming increased slightly, by 4.0% (l-CI = -0.8, u-CI = 17.8) and 3.04% (l-CI = -1.6, u-CI = 11.4) respectively when *Trichostrongylus* sp. egg counts were higher; however, credible intervals were wide indicating uncertainty.

An increase in parasite species richness resulted in a slight decrease in the probability of resting (4.2%, l-CI = -1.7, u-CI = 10.4). However, credible intervals were wide and uncertainty high. Parasite richness did not influence the probability of the other behaviours occurring (Figure 6.5a).

Although parasite intensity predicted changes in activity budget, the strongest predictor was change in food availability (Figure 6.5b). When food availability was high, the probability of foraging decreased by 18.4% (l-CI = 12.3, u-CI = 23.8). This was accompanied by a 12.3% (l-CI = 8.1, u-CI = 16.0) increase in the probability of resting and a 10.1% (l-CI = 5.5, u-CI = 14.8) increase in the probability of moving. The probability of grooming given and received decreased slightly by 2.1% (l-CI = 0.09, u-CI = 7.9) and 1.9% (l-CI = 0.6, u-CI = 4.4), respectively. The full model output and summary can be found in the appendix Table C4.

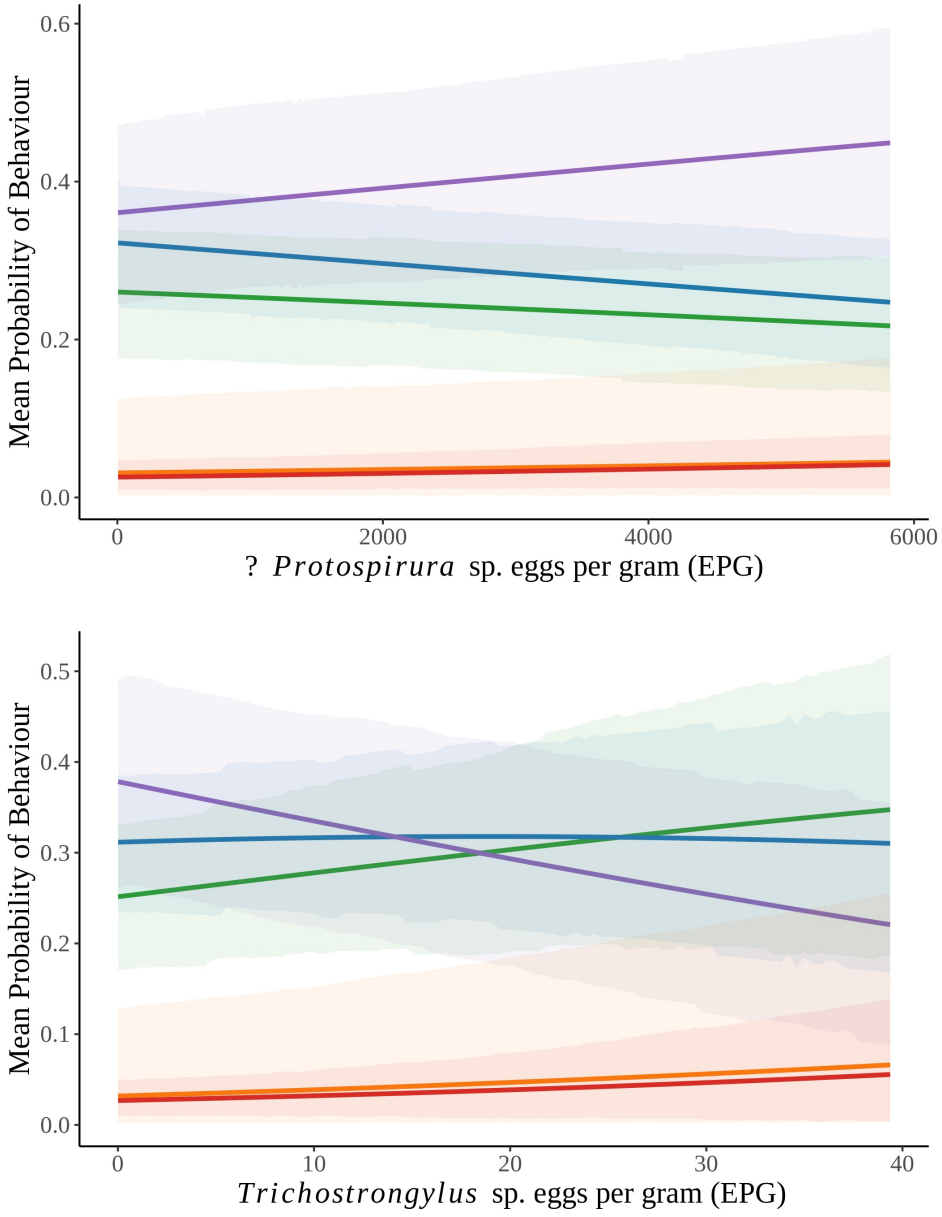

Figure 6.4 The relationships between the probabilities of behaviours being expressed as a function of a) ?Protospirura sp. (EPG) and b) *Trichostrongylus* sp. (EPG). The 5 behaviours are: foraging (green), resting (purple), moving (blue), grooming in (red) and grooming out (orange). Shaded regions show 89% percentile intervals as calculated from the posterior samples

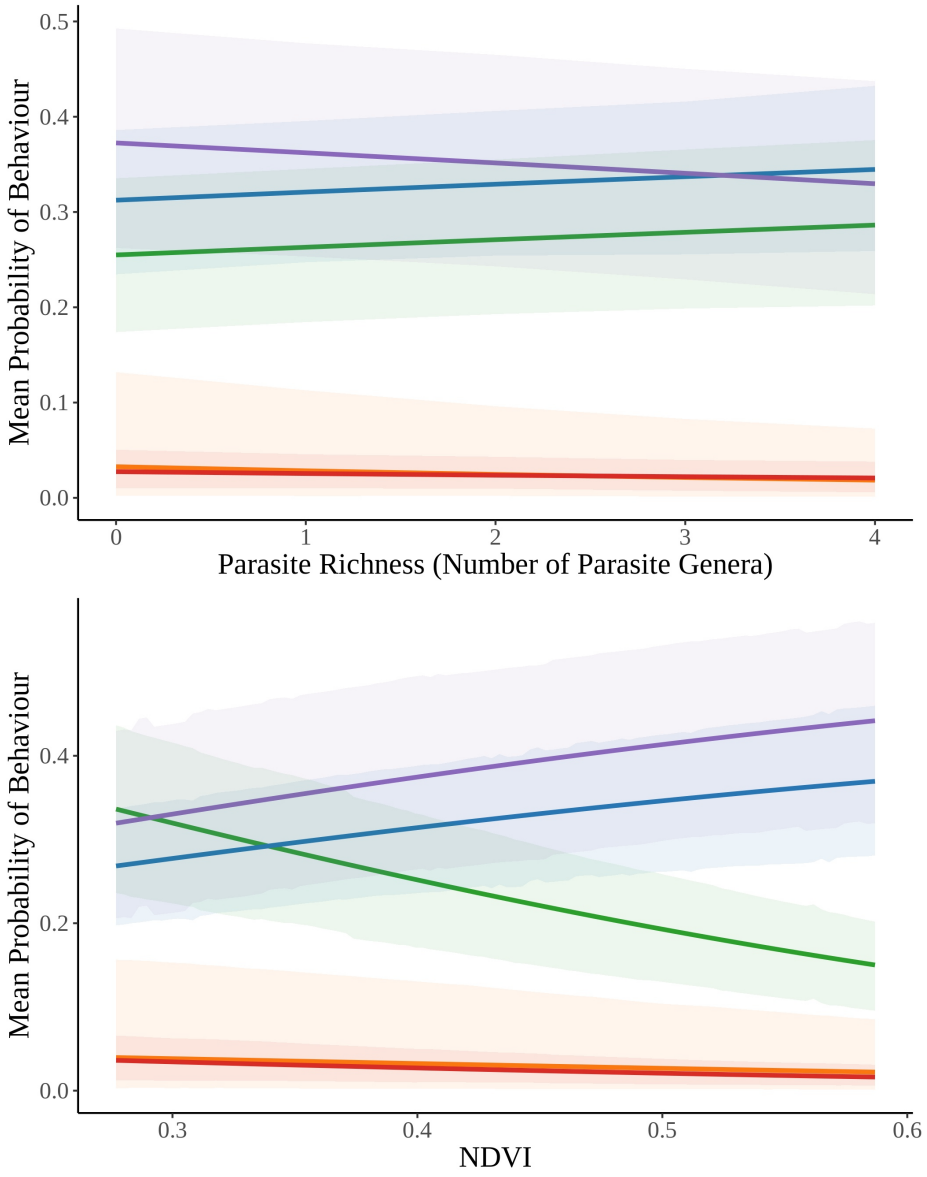

Figure 6.5 The relationships between the probabilities of behaviours being expressed as a function of a) Parasite richness and b) Food availability (NDVI). The 5 behaviours are: foraging (green), resting (purple), moving (blue), grooming in (red) and grooming out (orange). Shaded regions show 89% percentile intervals as calculated from the posterior samples. NDVI = Normalized Difference Vegetation Index

6.4.3.1 The influence of co-infection on behaviour

I found that, when *Trichostrongylus* sp. infection intensity was low (2 EPG), the probability of resting increased, feeding decreased and moving decreased as ?*Protospirura* sp.

egg count increased (Figure 6.6). When *Trichostrongylus* sp. was high (35 EPG), the mean probability of resting was lower overall but still rose with increasing ?*Protospirura* sp. egg count and the probability of foraging decreased further. The probability of moving remained the same.

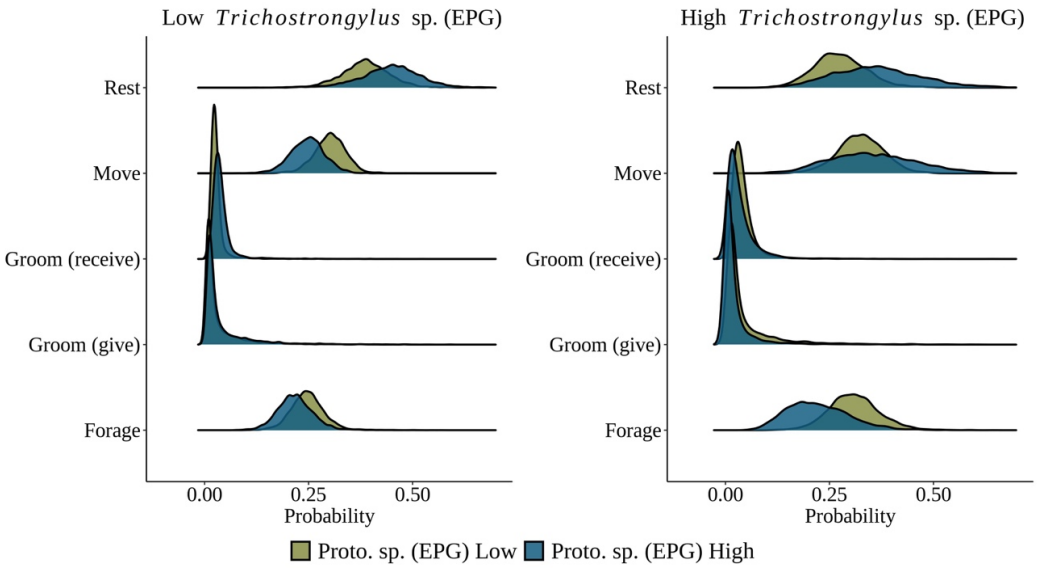

Figure 6.6 Changes in the mean probability of behaviours in response to high ?Protospirura sp. (Proto. sp.) when *Trichostrongylus* sp. intensity (EPG) was low (green) and high (blue). Density plots show probability of behaviours predicted by the model, with the height of the density curve indicating the probability of the predicted behaviour. The spread of the curve indicates the uncertainty

6.4.4 *Model set 2: Influence of parasite infection and food availability on behavioural predictability*

I found evidence of a positive relationship between NDVI and entropy rate (Est. = 0.10, Est. Error = 0.03, l-CI = 0.04, u-CI = 0.16). This indicates that an increase in food availability was associated with a decrease in behavioural predictability (Table 6.1). I found some evidence of a non-linear relationship between entropy rate and time of day (sds Est. = 0.27, Est. Error = 0.23, l-CI = 0.01, u-CI = 0.89) where sds is the spline variance parameter. Behavioural predictability was lowest in the early morning and increased until mid-day (appendix Figure C5).

I found no evidence that ?*Protospirura* sp. and *Trichostrongylus* sp. parasite intensity or parasite richness influenced entropy rate (Table 6.1). Similarly, fGCM concentration, sex, rank and individual ID did not influence behavioural predictability. I found no effect of sequence length on entropy rate, which supports our use of a 6min focal cut off time. The full model only explained 9.2% of variance (R^2 = 0.09, Est. Error = 0.02, l-CI = 0.06, u-CI = 0.13) suggesting there are other underlying drivers of behavioural predictability.

Table 6.1 Summary statistics of generalised additive mixed-effects model examining the influence of parasite infection and social factors on entropy rate. CI = credible interval; SD = standard deviation. Smooth-term sds() = spline "wiggliness"(spline variance parameter). (R^2=0.09, Est.error = 0.02, l-CI = 0.05, u-CI = 0.13)

		Effect	**Estimate**	**Est.Error**	**l-95% CI**	**u-95% CI**	$\widehat{R}$
Fixed effects	Population-level	Intercept	0.81	0.09	0.61	1.01	1
		?*Protospirura* sp. (EPG)	-0.04	0.04	-0.11	0.03	1
		Trichostrongylus sp. (EPG)	-0.06	0.06	-0.17	0.05	1
		Interaction (Proto. sp. .*Trich. sp.)	0.08	0.04	0.01	0.16	1
		Parasite richness (No. of genera)	-0.03	0.04	-0.11	0.05	1
		NDVI	0.1	0.03	0.03	0.16	1
		fGCM concentration	-0.01	0.03	-0.07	0.05	1
		Sex (ref: male)	-0.03	0.04	-0.1	0.05	1
		Rank	0.03	0.04	-0.05	0.1	1
		Sequence length	-0.05	0.03	-0.11	0	1
		Time of day (spline)	-0.32	0.46	-1.24	0.7	1
	Smooth Terms	sds(sTime of day)	0.27	0.23	0.01	0.88	1
Random effects	ID	sd(Intercept)	0.04	0.02	0	0.09	1
	Troop	sd(Intercept)	0.09	0.11	0	0.42	1
Family		sigma	0.38	0.01	0.36	0.4	1

I found some evidence of a small, positive interaction between ?*Protospirura* sp. intensity (EPG) and *Trichostrongylus* sp. intensity. When *Trichostrongylus* sp. was low (2 EPG), entropy rate decreased with increasing ?*Protospirura* sp. intensity (Figure 6.7). Conversely, when

Trichostrongylus sp. egg count was high, entropy rate increased with increasing ?*Protospirura* sp. infection intensity (Figure 6.7).

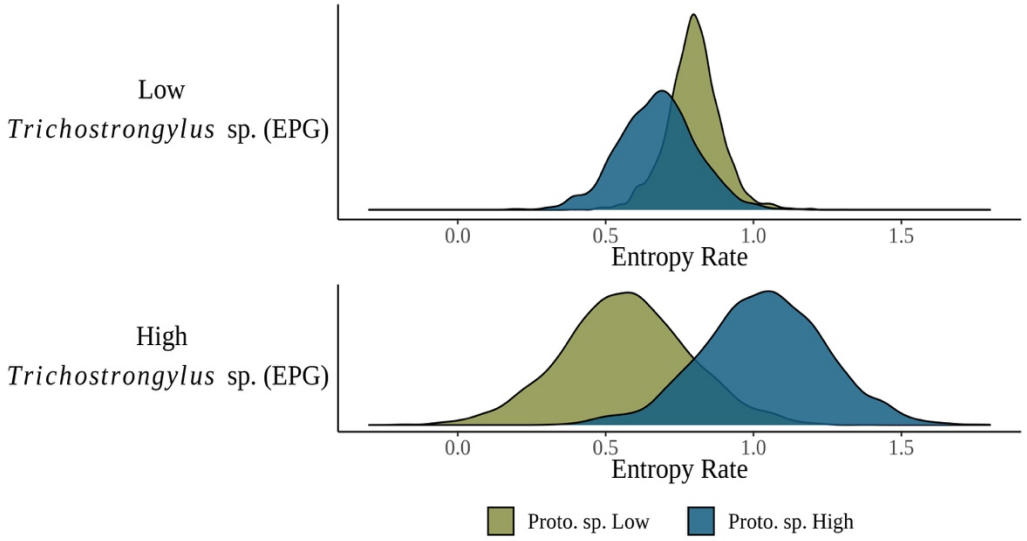

Figure 6.7 Changes in entropy rate in response to high ?Protospirura sp. (Proto. sp.) when *Trichostrongylus* sp. intensity (eggs per gram, EPG) was low and high. Density plots show entropy rate predicted by the model, with the height of the density curve indicating the probability of the predicted entropy rate. The spread of the curve indicates the uncertainty

6.5 Discussion

Our results showed a relationship between parasite intensity and behavioural change, providing evidence for sickness behaviour in vervet monkeys. The nature of this relationship was not straightforward, however: I found that higher parasite loads predicted an increase in time spent resting, but that other behavioural changes were contingent on both the parasite species in question, and their interactions. This highlights the benefit of considering multiple parasite infections when assessing the links between behaviour and infection in wild non-human primates. Although I found evidence for changes in the overall amount of time devoted to particular activities, I found only limited evidence for more fine-grained changes in behavioural predictability (i.e., behavioural entropy rate) in response to increased parasite intensity. Given

that food availability was the best overall predictor of behavioural change, it is likely that, for monkeys living in more extreme environments, coping with ecological stress overrides any fine-scaled ability to modulate behaviour in response to other stressors.

In line with previous work on non-human primates (Huffman et al., 1996; Huffman 1997; Huffman and Caton 2001; Ghai et al., 2015; Friant et al., 2016), I found evidence of sickness behaviour in response to two non-lethal gastrointestinal parasite infections. I found that increases in parasite intensity (EPG) of both ?*Protospirura* sp. and *Trichostrongylus* sp. were linked to changes in activity budget suggesting that these monkeys modify their behaviour in response to high parasite infection load. High ?*Protospirura* sp. parasite intensity resulted in "typical" sickness behaviour—increased resting, and reduced foraging and moving. This is notable as ?*Protospirura* sp. transmission relies on an intermediate arthropod host, so we might expect a positive relationship between foraging and increased parasite load. The inverse relationship in this case provides further support for the idea that what we see here is, indeed, sickness behaviour. It is possible that the change in behaviour is due to other underlying physiological processes that also occur when ?*Protospirura* sp. infection intensity is high. However, I found no relationship between faecal glucocorticoid metabolites (fGCM) concentration and behaviour, suggesting that changes in behaviour may be a result of gastrointestinal parasite infection rather than an indication that individuals are coping with other stressors. Still, it is possible that this lack of relationship may also be a result of fGCM data collection not being fine-grained enough and a failure to detect more short-term increases in fGCMs. This emphasises the value of considering multiple physiological variables in assessing parasite-host relationships.

In the case of *Trichostrongylus* sp. I found a different pattern, where high infection intensity was associated with an increase in the amount of time spent foraging, along with a

decrease in the probability of resting. The implication here is that different gastrointestinal parasites may exert different physiological pressures on the host and the manner in which they successfully cope with different non-lethal infections. For example, nutrition plays a vital role in a host's ability to cope with the negative effects of gastrointestinal parasites (Ezenwa 2004), which could result in the need to forage more when *Trichostrongylus* sp. infection is high. Alternatively, high *Trichostrongylus* sp. parasite intensity may coincide with other environmental or social changes that influence host behaviour or parasite dynamics. I found no relationship between temperature, rainfall, or NDVI and *Trichostrongylus* sp. parasite intensity (Blersch et al., 2021) suggesting that monkeys are not simply foraging more when *Trichostrongylus* sp. is high because food availability is lower. It is also possible that, given the relatively low egg counts of *Trichostrongylus* sp., individuals may not have been harbouring sufficiently high parasite burden to illicit typical sickness behaviour.

I was also able to consider the co-occurrence of the two parasites. I found no strong relationship between *Protospirura* sp. and *Trichostrongylus* sp. faecal egg counts indicating that there is neither a synergistic nor antagonistic relationship between these two parasites, which further suggests there is no direct competition between them (Bordes and Morand 2011). I did find differences in egg counts with ?*Protospirura* sp. egg counts being both higher and more variable than *Trichostrongylus* sp. egg counts. I did find, however, that co-infection with these two nematodes was linked to different activity budget changes. When parasite intensity was high for both species, shifts in behaviour were different from those seen when only a single infection was considered. Specifically, I found that, when *Trichostrongylus* sp. infection intensity was high, monkeys still rested more with increasing ?*Protospirura* sp. egg count (i.e., showed the same pattern as when I considered ?*Protospirura* sp. infection alone), but they also moved more

and decreased foraging further, which contrasts with the findings for ?*Protospirura* sp. alone. While the presence of both infections may also be linked to external environmental or social changes, it lends support to the hypothesis that multiple infections exert differential changes on the wild host (reviewed: Bordes and Morand 2011) and highlights the need to address co-infections when assessing animal health.

Contrary to some previous work on bats (Stockmaier et al., 2018, 2020) and non-human primates (Ghai et al., 2015), I found no marked change in the probability of either giving grooming or receiving grooming for individual infections, and only a small reduction in allogrooming when both ?*Protospirura* sp. and *Trichostrongylus* sp. infection intensity were high. While investment in sickness behaviour may fundamentally beneficial, and suppression of sickness behaviour may be detrimental to host fitness and survival, animals have to weigh the cost of modulating behaviours in response to infection (Lopes 2014). Minimal change in grooming in relation to infection intensity suggests these vervets maintain social relationships in the face of such external pressures. Young et al. (2019), however, found that vervets engaged in fewer social behaviours when environmental conditions were sub-optimal. Given the harsh semi-arid environment, these vervets may be unable to further reduce the amount of time spent grooming in response to parasite infection; that is, they may have already reduced their grooming investment to the extent that any further reductions would incur unsustainable costs with respect to individual social benefits, and/or to group cohesion (Cohn and de Sá-Rocha 2006; Moyers et al., 2015).

While our focus here was solely on time spent grooming, social interaction has been linked to infection susceptibility and transmission in several social species (Otterstatter and Thomson 2007; Drewe 2010; Briard and Ezenwa 2021) including non-human primates (Wren et

al., 2015; Romano et al., 2016). This suggests that, despite the lack of change in the time spent grooming, increased parasite load may result in alternative suppressive strategies, such as changes in the number or identity of grooming partners. However, these strategies may be contingent on the route of parasite transmission which, for ?*Protospirura* specifically, is unlikely to be from direct transmission between individuals. More detailed grooming analysis is required to fully understand whether these vervets do, at least in part, modulate their grooming behaviour in response to infection and the risk that maintaining grooming frequency may incur. Alternatively, the relationship between grooming and parasite infection simply may be less clear given the lower time invested in grooming in comparison to other behaviours.

I also considered whether parasite infection intensity was linked to changes in behavioural structure. Behavioural entropy rate, derived from focal data, was not influenced by individual parasite infections but, when *Trichostrongylus* sp. infection intensity was high, entropy rate increased with increasing ?*Protospirura* sp. egg shedding. Thus, polyparasitism was associated with decreased behavioural predictability, indicating that monkeys engaged in more behaviours, changed behaviours more frequently, or both. This contrasts with studies on non-human primates that found a reduction in behavioural complexity or the rate of behavioural switching when individuals were parasite positive (Ghai et al., 2015) or had impaired health (Alados and Huffman 2000; MacIntosh et al., 2011). Given that detrended fluctuation analysis (Alados and Huffman 2000; MacIntosh et al., 2011) and the rate of behavioural switching (Ghai et al., 2015) measure different aspects of behaviour, direct comparison between previous results and ours is difficult. However, our study shows that polyparasitism may be an important and more realistic consideration in the assessment of behavioural predictability or behaviour

switching, particular given that an unpredictable behaviour is thought to be biologically adaptive (Goldberger 1997; MacIntosh et al., 2011).

Although I found that parasite infections was associated with both activity budgets and behavioural structure, the primary drivers of behavioural change were shifts in food availability; changes in both activity budget and behavioural structure were strongly linked to this. Previous work in our population has identified complex relationships between behaviour and environmental conditions, with food resources, temperature, rainfall, and standing water availability strongly influencing activity budgets and mortality (McFarland et al., 2014; Young et al., 2019). Our findings here augment this previous work, providing the first evidence that food availability also affects behavioural structure: behavioural predictability decreased markedly when food availability was higher. This change likely resulted from a trade-off between a decrease in time spent foraging and an increase in both moving and resting when food availability was high. Changes in aspects of behavioural predictability have been shown to have short- and long-term consequences on fitness and survival. These include the success of predator performance in predator-prey interactions where unpredictable prey are more likely to be predated on by aggressive predators (Chang et al., 2017) and mating success, where consistent does not correlate with mating success (Jennings et al., 2013). However, beyond knowing that behavioural structure can serve as proxy measure of health (Alados and Huffman 2000), the implications for non-human primates are not yet well understood. Here, the use of entropy rate, rather than existing binary approaches, should allow us to identify the consequences of more complex behavioural trade-offs.

Sickness behaviour is increasingly being viewed as an adaptive response to infection (reviewed in Hart 1988; Johnson 2002; Aubert 1999), however relatively little is known about

the consequences of sickness behaviour in social groups. Based on the idea of cytokine-induced sickness behaviour, Hart (1988) proposed that sickness behaviour is an adaptive response to reduce energy consumption when there is a high-energy demand that is necessary to maintain a fever. There was early support for the concept of adaptive behaviour where rats repeatedly chose inactivity over exercise when injected with endotoxin an endotoxin known to produce an immune response which suggested that that they were motivated to rest (Miller 1964). However, while sickness behaviour may aid in coping with infection, there can be corresponding negative consequences. For example, in the same study population, McFarland et al. (2021) found that monkeys who were febrile and exhibiting sickness behaviour were twice as likely to receive aggression and 6 times more likely to be injured than afebrile monkeys. This suggest that, in social groups, sickness behaviour may incur significant fitness costs. More work is required to fully examine how sickness behaviour may influence the long-term fitness of gregarious mammals.

Taken together, our results provide the foundation for further research on both polyparasitism and the more fine-grained influences of non-lethal parasite infections on behaviour. I suggest that considering multiple parasite infections provides a new perspective on how parasitism shapes behaviour and that further investigation in other populations or with other parasite genera could deepen our knowledge of sickness behaviour in the wild. I also highlight the importance of using a detailed, comprehensive dataset when investigating how environment, physiology and parasitism interact to shape behaviour. In sum, our findings provide additional insight into how animals living in a harsh environment, with strong activity budget constraints, may adopt alternative approaches to parasite infection, avoidance, and transmission reduction.

CHAPTER SEVEN: THE RELATIONSHIPS BETWEEN GASTROINTESTINAL PARASITE INFECTION AND SOCIALITY

7.1 Abstract

Social interactions are an integral part of living in a social group, however, these exchanges elevate the risk of parasite and pathogen transmission and are considered to be a major cost of sociality. Social network analysis has emerged as a powerful tool to describe fine-grain interactions in social groups that may be relevant to transmission. Additionally, social network analysis can be used to explain how parasite infection may shape an individual's behaviour and conversely, how conspecifics in the group may react to heavily parasitised individuals. Traditionally, social network analysis was used to model transmission of epidemic viruses in social groups but has since been applied to parasites that have previously been thought to be indirectly transmitted only, either through faecal-oral transmission via substrate contamination or via an intermediate host. Here, I investigate the relationships between social and spatial associations, and parasitism in my study population of vervet monkeys. I investigate whether infection intensity of two parasite genera, ?*Protospirura* sp. and *Trichostrongylus* sp., with differing transmission modes, influences the number of an individual's unique spatial partners and grooming partners. I found that individuals with lower egg counts of ?*Protospirura* sp. have fewer spatial partners, while no links were found between *Trichostrongylus* sp. and parasite richness, and spatial partners. Additionally, and in contrast to previous work on primates, I found no relationships between parasite infection and unique grooming partners. These results highlight the inconsistencies in primate-parasite social interactions stemming from the parasite measure used (intensity versus richness), the parasite genus considered, and the network metric used. Nevertheless, they provide valuable insight into the links between sociality

and parasitism and will serve as a foundation for future work incorporating a bi-directional approach to the investigation of sociality-parasite links.

7.2 Introduction

Group living incurs costs and benefits that are often interlinked. Social living can decrease the risk of predation (Hamilton, 1971), benefit foraging and reproduction (de Ruiter & van Hooff, 1993; Ward & Webster, 2016), offer communal defence when raising young (Ward & Webster, 2016), and accrue physiological benefits, such as better thermoregulation (McFarland et al.,, 2015) and a reduction in physiological stress through affiliative behaviour. However, social living also plays a role in pathogen and parasite transmission, load, prevalence and species richness. These links are complex and, while social grooming can decrease ectoparasite load (Akinyi et al., 2013; Duboscq et al., 2016), social interactions can also increase the risk of exposure and subsequent infection (Rimbach et al., 2015; Romano et al., 2016).

Group size has traditionally been used as a key metric in primate research to understand how social behaviour shapes parasite transmission, where it serves as a proxy for the number of social contacts that occur between individuals (Nunn & Altizer, 2006). However, the links between group size and parasitism are mixed and influenced by other factors, such as transmission mode and the parasite measure used (abundance, prevalence or richness). Given this, group size is not fine-grained enough of a measure to capture adequately the nature of social associations relevant to transmission within a social group (Briard & Ezenwa, 2021). To overcome this, researchers have turned to social network analysis as a means to more accurately assess social interactions in primate groups and how these might influence parasite and pathogen transmission. Social network analysis has been broadly applied in non-human primate research

to describe transmission pathways and their consequences for both ecto-parasites (Duboscq et al., 2016) and endo-parasites (e.g. Rimbach et al., 2015; Rushmore et al., 2013).

In brief, social network analysis provides a framework to visualise the social structure and connectedness of a social group and capture the structural complexity of a population on an individual level (Godfrey, 2013). Networks are visualised as a graph consisting of 'nodes' that are connected via 'edges', where each node represents an individual and each edge represents a link between two individuals. Networks therefore capture a key aspect of group life relevant to parasite transmission: social heterogeneity. In all social groups, individuals may interact preferentially with mates or family members, thus varying the degree to which they interact or change the number and identity of their social partners (Briard & Ezenwa, 2021; Godfrey, 2013). Social network analysis captures such fine-scale variations in both direct and indirect social contacts (i.e., edges that connect two nodes and edges that connect two nodes via intermediary nodes, respectively), and thus provides a clearer picture of group connectedness. In addition, social networks can be constructed from numerous forms of social interaction, such as aggression, grooming and spatial proximity. This provides researchers with the flexibility to select dyadic behaviours relevant to transmission.

One common network relevant to social animals, including primates, is derived from social grooming. In primates, grooming is often highlighted as a means to establish and maintain social bonds and group cohesion (Dunbar, 1991) while also serving hygienic functions (Akinyi et al., 2013; Duboscq et al., 2016). However, social grooming can also facilitate the transmission of some pathogens and parasites. While transmission through grooming may be more obvious for protozoa, viruses and bacteria transmitted through physical contact, there is some evidence that social grooming also facilitates the spread of other parasites. For example, Macintosh et al.

(2012) found that high-ranking female Japanese macaques who occupied more central positions in group networks showed increased infection with *Oesophagostomum aculeatum* (EPG) and *Strongyloides fuelleborni* (probability)—two nematodes considered to be indirectly transmitted by a faecal-oral route. They suggest that rank-mediated physical contact between individuals is also an important mechanism of transmission for these parasite species. This transmission would occur either via faecal contamination of body parts being groomed or infective larvae being unknowingly ingested when grooming another individual. In brown spider monkeys (*Ateles hybridus*), the network measures of node strength (the sum of link weights of a given individual) and "betweenness" centrality (the number of paths that pass through an individual if the shortest paths between all other pairs of individuals are traced) were both correlated with infections by *Strongyloides* sp. and *Trichostrongylus* sp. suggesting that social interactions play a role in the transmission of gastrointestinal parasites (Rimbach et al., 2015). These studies suggest that while network analysis typically may be used to model directly transmitted parasites and pathogens, the potential relevance to gastrointestinal parasite transmission should not be overlooked.

A second, less common form of network used to assess pathways of transmission is one based on spatial association. Broadly, spatial networks describe non-contact associations between individuals (i.e., individual proximity measures) but such networks have also been applied more broadly to include, for example, the extent of home-range overlap between dyads (Godfrey, 2013) and asynchronous refuge sharing (Leu et al., 2010). Depending on the type of association considered, spatial networks provide greater flexibility than contact networks as direct observation of contacts is not required, which enables researchers to use telemetry (Hamilton et al., 2020) to infer spatial proximity events that represent possible transmission routes (Godfrey, 2013), or other proxies for spatial position, such as space sharing and home-

range overlap (Fenner et al., 2011; Leu et al., 2010). In primate research, spatial networks are typically derived from direct observation of individual nearest neighbours. Links between spatial proximity and parasitism are mixed. For example, in brown spider monkeys, Rimbach et al. (2015) there was no evidence for a relationship between spatial proximity and gastrointestinal parasites, whereas, in chimpanzees, Deere et al. (2021), more gregarious individuals—those who spent more time with more individuals in the same space—had higher parasite richness. In a study of red-capped mangabeys that examined the predictors of parasite reinfection following anti-parasitic treatment, more central individuals in proximity networks were at increased risk of helminth, but not protozoan, reinfection Friant et al. (2016a). While proximity networks appear to be related to some parasite measures in primates, this area requires more attention.

There are several measures of network centrality that are commonly used to describe the topology of proximity and contact networks (Perkins et al., 2009). Degree is the most straightforward of these, and represents the number of connections, or edges, an individual possesses. Network degree can be directional and calculated as "in-degree"—the number of links directed toward an individual (e.g., contacts from receiving grooming)—and "out-degree"—those directed away from an individual toward others (e.g. contacts from giving grooming). Degree also can be weighted to describe the relevant importance of these contacts (network strength). In combination, degree measures assess an individual's risk of direct exposure to pathogens/parasites via social conspecifics (Duboscq et al., 2016). More complex measures of centrality, such as "betweenness", "closeness" and eigenvector, can be used to provide information on the relative importance of an individual within the network, thus extending estimations of exposure risk (Perkins et al., 2009). These include measures such as closeness centrality—the fewest number of links needed to reach all individuals in the network from a

given individual—and betweenness centrality—the number of paths that pass through an individual if the shortest paths between all other pairs of individuals are traced (Rimbach et al., 2015).

While social network analysis is frequently used to describe transmission pathways, some recent research suggests that networks may also provide insight into how animals change their behaviour in response to a high parasite burden and, conversely, how their group mates may react to heavily parasitised individuals (Chapman et al., 2016). Thus, networks may represent a series of behavioural responses to infection (how the probability of parasite infection influences behaviour) rather than an index of how transmission is occurring (how behaviour influences parasite infection). For example, Chapman et al. (2016) found that, in vervet monkeys, individuals increased their number of nearest neighbours and had more frequent interactions following deworming, suggesting that group mates were avoiding infected individuals. The importance of considering the bi-directional nature of host behaviour-parasite links has begun to receive more attention recently and is key to understanding the ecological and evolutionary processes that underpin parasitism (Hawley et al., 2021).

Here, I investigate the relationships between social/spatial associations and parasitism in my study population of vervet monkeys. I investigate whether infection intensity of two parasite genera, ?*Protospirura* sp. and *Trichostrongylus* sp., with differing transmission modes, influences the number of an individual's unique spatial partners and grooming partners. As in this previous work, I also include faecal glucocorticoid metabolites (fGCMs) as an index of individual response to environmental stressors (i.e., as a measure of the ability to restore homeostasis), rather than an indicator of an individual animals stress levels (MacDougall-Shackleton et al., 2019). I have shown previously that, when parasite load is high, individuals

show sickness behaviour expressed as an increase in resting and decrease in foraging. However, contrary to other studies, time spent grooming remained unchanged. I build on that finding by considering whether there are more subtle changes in grooming behaviour in response to high parasite load. I also consider whether parasitised individuals are more socially isolated during times of high infection and associate with fewer unique individuals. Given the constraints of associating behavioural data with parasite data and the subsequent sample size, I only consider degree and do not consider other social network measures.

7.3 Methods

7.3.1 *Study Subjects and site*

Data were collected across 12 consecutive months – April 2017 to March 2018 – from three fully habituated groups (PT, RBM, and RST) of wild vervet monkeys at Samara Private Game Reserve, South Africa (32º22'S, 24º52'E). These monkeys have been the subject of continuous data collection since 2009 and are individually identifiable from natural markings. Faecal samples were collected from a subset of 27 adult individuals (PT: 4 males, 6 females from 16 adults; RBM: 2 males, 6 females from 14 adults; RST: 3 males, 6 females from 14 adults), selected to be representative of adult demography and to reflect the full range of dominance ranks. Table 7.1 shows troop demographics across the study period. Behavioural data were collected from all adult and juvenile individuals.

Table 7.1 Mean troop sizes (± SD) across the study periods. Adults are individuals who have reached sexual maturity. Infants were born between August 2017 and February 2018.

Troop	RBM	PT	RST
Adults	14 (1.5)	16 (0.95)	14 (0.6)
Juveniles/yearlings	27 (1.25)	21 (0.43)	23
Infants (born)	2	5	2
Annual mean	42 (2.3)	39 (1.63)	38 (1.4)

The study area is semi-arid riverine woodland, an area characterised by low rainfall, very hot summers and cold winters (McFarland et al., 2015; Pasternak et al., 2013). The area is under escalating risk from climate change (Jury, 2013) and has a declining annual average rainfall of 386 mm, and average minimum and maximum temperatures of 6.1º C and 21.2 º C, respectively (Pasternak *et al.*, 2013).

7.3.2 *Behavioural data collection*

Spatial and grooming data were collected during 30min scan sampling of all troop members, excluding infants, located within a 15-min time window. Grooming data were recorded as either grooming given (out) or received (in) and the identities of grooming partners was recorded. For spatial data, during each scan, the nearest male, female and juvenile individual, as well as their distance to the scan individual, were recorded regardless of the behaviour of the scan subject. Proximity partners were classified as individuals within 3m of the scan subject.

I collected *ad libitum* data on dyadic agonistic interactions, for which I identified participants and outcomes. These agonistic data were used to construct dominance hierarchies. Only decided dyadic agonistic interactions with a clear winner and loser were included in the analysis.

I divided the study period into four 3-month blocks: April – June 2017, July – September 2017, October – December 2017 and January – March 2018. I used *ad libitum* observations of agonistic interactions to construct hierarchies for each period ($RBM_{Total\ N}$: 963; $RST_{Total\ N}$: 810; $PT_{Total\ N}$: 1135) for all adults in each troop and not only the subset of study subjects. Given male-female co-dominance in this population where all males do not out-rank all females (Young et al., 2017b), I generated a single matrix that included all decided agonistic interactions regardless of the sex of participants and created a single interdigitated hierarchy that allows for male-female co-dominance .

Dominance ranks in each troop and for each 3-month block were expressed as a standardized David's score using the package 'compete' (Curley, 2016). David's scores were standardized to enable direct comparison across groups of different size and interaction rates (Henzi et al., 2013).

7.3.3 *Faecal sampling and extraction*

Faecal samples were collected by 4 to 5 observers spread over 3 troops during each of the 234 10h study days. Faecal samples were collected non-invasively twice per month (once during each two-week period) from the 27 subjects. Two corresponding faecal samples, one for parasite analysis and one for faecal glucocorticoid metabolites (fGCM) analysis, were collected from the same defecation event. I analysed a total of 573 faecal samples (mean = 21/individual, ± 3.1 sd).

7.3.3.1 Parasite analysis

For each sample, approximately 1 g of fresh faeces was weighed in the field immediately after defecation and directly placed into 10% neutral, buffered formalin. Samples were stored in

the field lab and transported to the University of Lethbridge, Canada, where faecal flotation and sedimentation techniques were used to identify parasites (Blersch et al., 2019).

A modified zinc sulphate flotation method was used to isolate helminth eggs, whereby an additional washing step was included in the faecal flotation to avoid egg damage, which had been evident in the initial samples that were analysed (Blersch et al., 2019). Briefly, faecal samples suspended in formalin were placed in 15 ml Falcon tubes and centrifuged at 1,389 g for 6 min after which the supernatant was discarded. The Falcon tube was filled with water, mixed with the faecal material, centrifuged at 1,389 g for 6 min, and the supernatant was discarded. The deposit was resuspended in $ZnSO_4$ (specific gravity 1.3), vortexed to mix, and centrifuged at 617 g for 8 min. The supernatant was pipetted into 4x15 ml tubes and combined with water. The pellet that remained after flotation was kept aside for sedimentation. This step reduced the specific gravity of the $ZnSO_4$ after flotation, thus preventing egg damage and allowing the eggs to deposit upon sedimentation. These supernatant-water tubes were centrifuged at 964 g for 6 min. The supernatant was discarded, and the deposits were combined into 1 test tube, which was filled with water and centrifuged at 964 g for 6 min. The supernatant was discarded, and the entire pellet was examined under the microscope.

Ethyl-acetate sedimentation was used to isolate potential trematodes that were too heavy to float during ZnSO4 flotation. Here, the deposit from the flotation was suspended in water, vortexed, and centrifuged at 964 g for 6 min. The supernatant was discarded, and the sample was rewashed. Water was added to the pellet to the 7 ml mark of the centrifuge tube and vortexed. Then, 3 ml of ethyl-acetate was added to the tube, mixed thoroughly, and centrifuged at 1,389 g for 6 min, and the supernatant was then discarded. The entire pellet was examined under the

microscope. For both methods, parasites were identified to genus- level based on egg shape, size, colour, and contents, and all eggs were counted. Representative eggs were photographed.

7.3.3.2 Faecal steroid analysis

Samples were collected following the protocol of Young et al. (2017a; 2019). Within 15min of defecation, a 2-5g piece of faecal material was transferred into a plastic vial following physical homogenization of the full faecal sample. Prior to collection, faecal samples were checked to ensure there was no contamination with urine during excretion or on the substrate where the sample landed. Vials were immediately stored on ice in a thermos flask in the field before transfer to a −20°C freezer at the end of the day. Samples were stored frozen until transport on dry ice to the Endocrine Research Laboratory, University of Pretoria, for analysis.

Samples were lyophilized, pulverized and then sieved to remove seeds and fibrous matter (Young et al., 2017a). The resulting faecal powder (0.10g) was extracted by vortexing for 15min with 80% ethanol in water (3ml) followed by 10 minutes of centrifugation at 1500g. 1.5 ml of the resultant supernatants were transferred into microcentrifuge tubes. Hormone analysis was conducted following the standard procedures of the Endocrine Research Laboratory, University of Pretoria (Ganswindt et al., 2002) using the cortisol enzyme immunoassay (EIA) (Young et al., 2017a). Sensitivity of the EIA was 0.6 ng/g dry weight (Young et al., 2017a). Inter- and intra-assay coefficients of variation of high- and low-value quality controls were: 4.64–5.96 and 8.13–11.60% respectively. All steroid concentrations are given as ng g^{-1} faecal dry weight.

7.3.4 *Statistical Analysis*

All statistical analyses were conducted in a Bayesian framework, using the 'brms' package (Bürkner, 2017, 2018) in R version 3.5.2 (R Core Team, 2018). I constructed

hierarchical generalized additive mixed models to allow for non-linear relationships between explanatory and response variables (Pedersen et al., 2019).

I present summary statistics and posterior density plots ("bayesplot" package Gabry et al., 2019) for posterior means, standard errors and 95% credible intervals (CIs) for the main effects, and for individual variance within the random effects. For the smooth terms, I present summary statistics of the spline variance parameter ("wiggliness") for the global smooth, and male and female smooths. I conducted prior predictive checks (Gabry et al., 2019) for each model and specified weakly informative priors (normal (0, 1)), unless otherwise indicated. I ran models with 4 chains and 2000 iterations, which provided me with a large enough sampling pool to conduct posterior sampling and achieve model convergence (Bürkner, 2018; McElreath, 2016). Chain convergence was confirmed by $\hat{R}$ values ≤ 1.01, and model goodness-of-fit was assessed using the 'posterior predictive check' (pp_check) function from the "bayesplot" package (Gabry et al., 2019). I assessed potential collinearity of fixed and random effects visually using pairs plots which produce univariate histograms and bivariate scatterplots for each parameter (mcmc_pairs function: "bayesplot" package). Collinearity would manifest as narrow bivariate plots, which were not observed between our predictor variables. I used the "bayes_R2" function to generate conditional R^2 values for each model (Gelman et al., 2019). All continuous variables were standardized by subtracting the mean and dividing by two times the standard deviation to facilitate comparisons of the effect sizes across continuous and dichotomous variables (Gelman, 2008). To make full model predictions, I used the "fitted()" function in brms to predict the expected values of the response variable for a fitted model (Bürkner, 2017). This allowed me to interpret model results that are not clear from the parameters estimated by the model. These whole model predictions formed the basis of all plots.

For purposes of plotting and visualisation in the results section, I split egg counts in "high EPG" and "low EPG" based on the minimum and maximum egg counts for each genus. However, it should be noted that these variables are continuous variables in the model and not binary (high versus low).

7.3.4.1 Hierarchical generalized additive mixed effects models: Spatial Partners

To examine whether infection with ?*Protospirura* sp. and *Trichostrongylus* sp. was associated with changes in the number of spatial partners, I used proximity data from scan sampling (N_{Scans} = 28 106) to construct a generalised additive mixed-effects model. I used parasite egg count as a measure of infection intensity for both genera. As I have shown that total faecal egg counts between successive individual samples are correlated within an individual, I have suggested that egg counts of ?*Protospirura* sp. and *Trichostrongylus* sp. may be a reliable indicator of an underlying infection of these parasite genera in these vervet monkeys, rather than reflecting some stochastic process (Blersch et al., 2021; Blersch et al., 2019).

For each individual, I quantified monthly parasite measures and matched these parasite measures to the corresponding behavioural data. For parasite intensity, I summed the total number of eggs recovered in the month for each genus and divided that by the total faecal weight of samples in the month. This gave me a monthly eggs/gram measure for each individual for each month of the study period. Cumulative egg count was used to avoid the possibility of over-estimating parasite intensity if only the highest egg of the month was used. To assess this approach, I repeated analyses using the maximum egg count for the month and found no qualitative differences in the results (appendix D.1). For parasite richness, I counted the number of genera that were recovered in that month (maximum richness), while for fGCMs, I calculated a monthly mean for each individual.

From the behavioural data, I extracted the identities of the nearest female, male and juvenile within 3m for each subject and calculated spatial degree (number of unique spatial partners). This resulted in a single spatial degree measure for each subject for each month. To control for sampling effort, I also calculated the number of times an individual was scanned per month, hereafter referred to as scan count.

I constructed a Bayesian GAMM with a Poisson distribution. The response variable was spatial degree and the fixed effects included were ?*Protospirura* intensity (EPG), *Trichostrongylus* sp. intensity (EPG), parasite richness, fGCMs, rank, sex, date and a spline on scan count. I used a spline for scan count given that the number of potential spatial partners is constrained by group size and is subsequently non-linear. ID and troop were specified as random effects.

Given that the posterior predictive checks of the Poisson model were not optimal, I also ran a negative binomial and a Gaussian model. While Gaussian distributions are not used for count data, I wanted to assess whether there were qualitative differences in model results where the model fit the data better. Additionally, I included observation-level random effects (OLRE) in the negative binomial model. OLREs model the extra-Poisson variation in the response variable by accounting for variation between data points or observations. OLREs have been shown to be effective when modelling overdispersed count data (Harrison, 2014). I then compared all models using the "loo_compare" function. The Gaussian model fit the data best but could not be used given the nature of the data. However, there were no qualitative differences between the results of the Gaussian model and Poisson model suggesting that, despite sub-optimal prior predictive checks, the Poisson model was performing adequately and fit the data

better than the negative binomial; and I therefore proceeded with this model. All model comparisons and results are included in appendices E2 – E6.

7.3.4.2 Hierarchical generalized additive mixed effects models: Grooming Partners

To examine whether infection with ?*Protospirura* sp. and *Trichostrongylus* sp. was associated with changes in the number of grooming partners, I used grooming events from scan sampling. As with spatial partners I calculated monthly parasite and fGCM measures. Given that juveniles form an integral part of the group, they were included in the measures. However, juvenile scan sampling protocols changed during this field season and they could thus only be counted/analysed as grooming partners not as scan subjects, that is, only scan where the adult was the focal monkey were used. This resulted in a total of 9752 grooming events. I calculated the number of unique grooming partners (combined in and out degree) for each week of matched behavioural data. This resulted in a single grooming degree measure for the individual from which the faecal sample was obtained. Given individuals vary in their overall grooming effort, I counted the total number of times the individual was present in grooming dyads for the week, hereafter referred to as dyad count.

I constructed a Bayesian GAMM with a negative binomial distribution. The response variable was grooming degree and the fixed effects included were ?*Protospirura* sp. intensity (EPG), *Trichostrongylus* sp. Intensity (EPG), parasite richness, rank, sex, date and a spline on dyad count. I used a spline on dyad count. Splines allow for the inclusion of non-linear relationships. The number of times an individual can appear in a dyad is constrained by the number of scans conducted and the troop size and a non-linear curve emerges. ID and troop were specified as random effects.

7.4 Results

7.4.1 *Spatial Proximity*

When pairing faecal samples with one month of behavioural data, there was a mean of 183.1 (±59.2 SD) scans recorded per faecal sample and a mean of 32.2 (±5.36 SD) unique spatial partners. I found evidence of a small positive association between ?*Protospirura* sp. intensity and spatial degree (Figure 7.1). When egg counts were lower, individuals had fewer spatial partners (Figure 7.1). I found no effect of date on spatial degree (Table 7.2).

Table 7.2 Summary statistics of Bayesian mixed-effects model for unique spatial partners. CI = credible interval; SD = standard deviation. Smooth-term sds() = spline "wiggliness"(spline variance parameter). Est.Error = Standard error of the estimate. $\widehat{R}$ (Rhat) provides information on convergence. At convergence, Rhat = 1. Estimates for fixed effects where credible intervals do not cross zero are in bold. Trich = *Trichostrongylus* sp. Proto = ?*Protospirura* sp.

	Effect	Estimate	Est.Error	l-95% CI	u-95% CI	$\widehat{R}$
Fixed effects						
Population-Level	Intercept	3.46	0.1	3.25	3.68	1
	Proto (EPG)	**0.06**	**0.02**	**0.01**	**0.1**	**1**
	Trich. (EPG)	-0.04	0.03	-0.09	0.01	1
	Rank	-0.02	0.03	-0.07	0.03	1
	Richness	0	0.03	-0.06	0.06	1
	fGCM	0.01	0.02	-0.03	0.05	1
	Sex (ref:M)	0.01	0.03	-0.04	0.07	1
	Month	-0.03	0.02	-0.07	0.02	1
	s(scan count)	1.27	0.67	-0.07	2.61	1
Smooth Terms	sds(scan count)	0.6	0.28	0.24	1.3	1
Random Effects	sd(Troop)	0.15	0.16	0.02	0.61	1
	sd(ID)	0.01	0.01	0	0.04	1

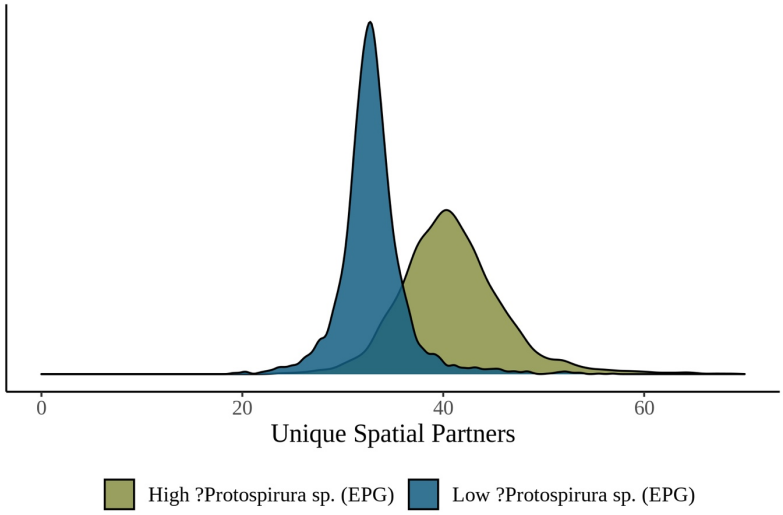

Figure 7.1 Changes in the number of unique spatial partners when ?*Protospirura* sp. egg counts (EPG) are high and low. Density plots show the range of unique spatial partners predicted by the model, with the height of the density curve indicating the probability and the spread of the curve indicating the uncertainty.

I found no evidence of any relationship between spatial degree and *Trichostrongylus* sp. egg counts or parasite richness (Figure 7.2).

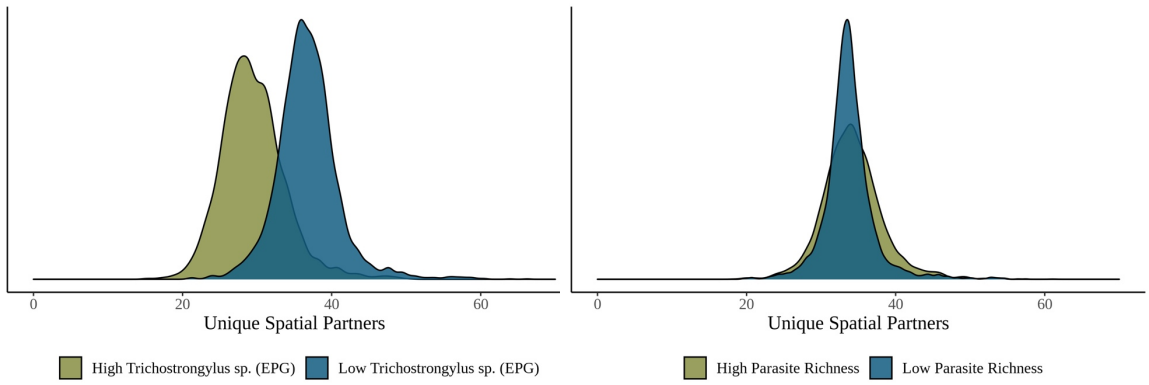

Figure 7.2 Changes in the number of unique spatial partners when *Trichostrongylus* sp. egg counts (EPG) are high and low (left) and when parasite richness is high and low (right). Density plots show the range of unique spatial partners predicted by the model, with the height of the density curve indicating the probability of spatial partners. The spread of the curve indicates the uncertainty.

There was no evidence of a relationship between spatial degree, and rank, sex or fGCMs (Table 7.2) There was some evidence of inter-individual variation in spatial partners in this

population although the effect was small (Table 7.2), as well as an effect of troop with RST having fewer unique spatial partners overall while PT and RBM were the same (Figure 7.3). The full model explained 0.53% of variance (R^2 conditional= 0.54, ± 0.04 SE), with the main effects accounting for 45.29% (R^2 marginal= 0.45, ± 0.06 SE).

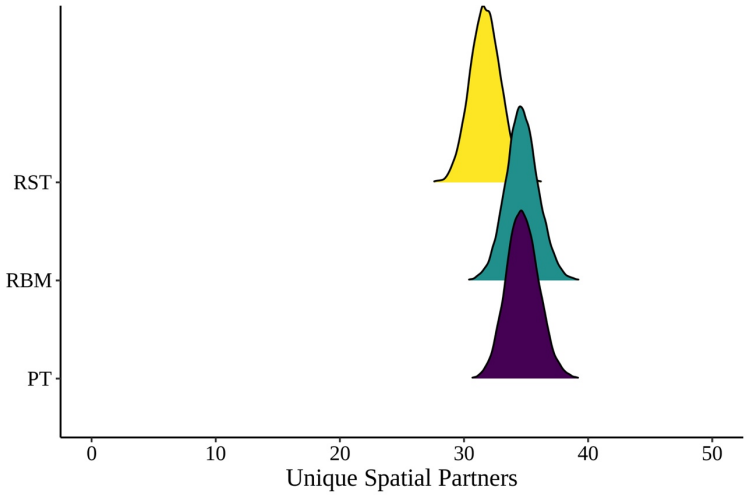

Figure 7.3 Density plots showing mean number of unique spatial partners by troop. Density plots show the range of spatial partners predicted by the model, with the height of the density curve indicating the probability and spread of the curve indicating the uncertainty.

7.4.2 *Grooming*

When pairing faecal samples with one month of behavioural data, there was a mean of 21.7 (±13.6 SD) grooming dyads recorded per faecal sample and a mean of 8.9 (±4.1 SD) unique grooming partners. I found no evidence of a relationship between either parasite genus or grooming degree (Table 7.3, Figure 7.4). I found no effect of date on grooming degree (Table 7.3).

Table 7.3 Summary statistics of Bayesian mixed-effects model for unique grooming partners. CI = credible interval; SD = standard deviation. Smooth-term sds() = spline "wiggliness"(spline variance parameter). Est.Error = Standard error of the estimate. $\widehat{\boldsymbol{R}}$ (Rhat) provides information on convergence. At convergence, Rhat = 1. Trich = *Trichostrongylus* sp. Proto = ?*Protospirura* sp.

	Effect	Estimate	Est.Error	l-95% CI	u-95% CI	$\widehat{\boldsymbol{R}}$
Fixed effects						
Population-Level	Intercept	2.12	0.18	1.73	2.48	1
	Proto (EPG)	0.02	0.05	-0.07	0.11	1
	Trich. (EPG)	-0.01	0.05	-0.11	0.09	1
	Rank	-0.11	0.06	-0.22	0.01	1
	Richness	-0.04	0.05	-0.15	0.06	1
	fGCM	0.02	0.04	-0.06	0.09	1
	Sex (ref:M)	-0.01	0.07	-0.15	0.13	1
	Month	0.02	0.04	-0.06	0.11	1
	s(dyad presence)	1.85	0.86	0.15	3.5	1
Smooth Terms	sds(dyad presence)	1.31	0.43	0.64	2.31	1
Random Effects	sd(Troop)	0.25	0.24	0.04	0.92	1
	sd(ID)	0.07	0.04	0.01	0.15	1.01

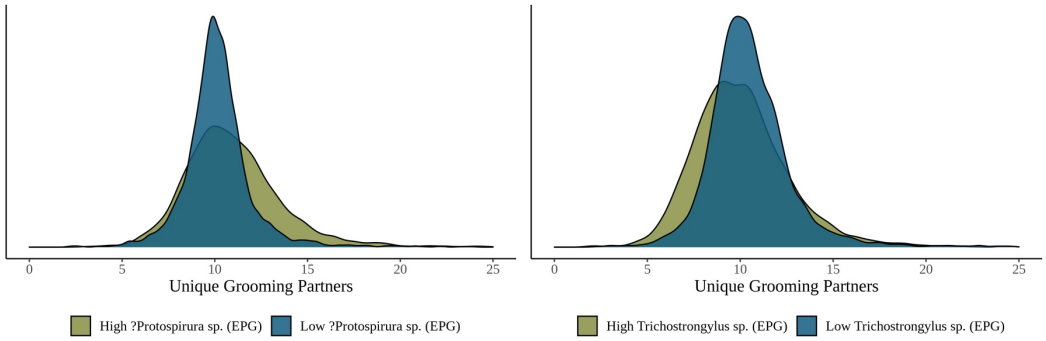

Figure 7.4 Changes in the number of unique grooming partners when ?Protospirura sp. egg counts (EPG) are high and low (left) and when *Trichostrongylus* sp. egg counts (EPG) are high and low (right). Density plots show the range of unique spatial partners predicted by the model, with the height of the density curve indicating the probability of spatial partners. The spread of the curve indicates the uncertainty.

There was also no evidence of a relationship between fGCMs and grooming degree (Figure 7.5). Similarly, there was no evidence of a relationship between rank and sex, and

grooming degree (Table 7.3). The full model explained 0.75% (R^2 conditional= 0.75, ± 0.02 SE), while the main effects accounted for 68.59% of the variance (R^2 marginal= 0.69, ± 0.07 SE).

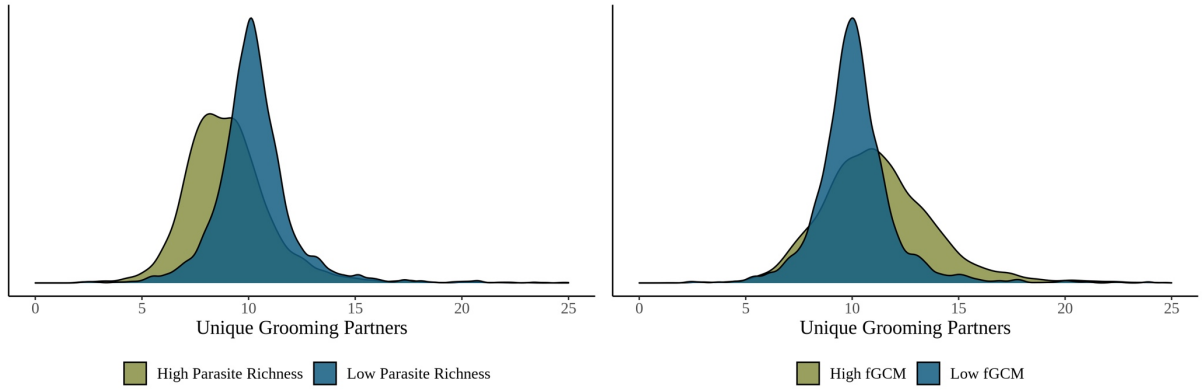

Figure 7.5 Changes in the number of unique grooming partners when parasite richness is high and low (left) and when fGCM concentrations are high and low (right). Density plots show the range of unique spatial partners predicted by the model, with the height of the density curve indicating the probability and the spread of the curve indicating the uncertainty.

There was evidence of inter-individual variation in the number of unique grooming partners (Figure 7.6) as well as inter-troop differences in grooming degree, with RST troop showing a lower overall number of grooming partners (Figure 7.6).

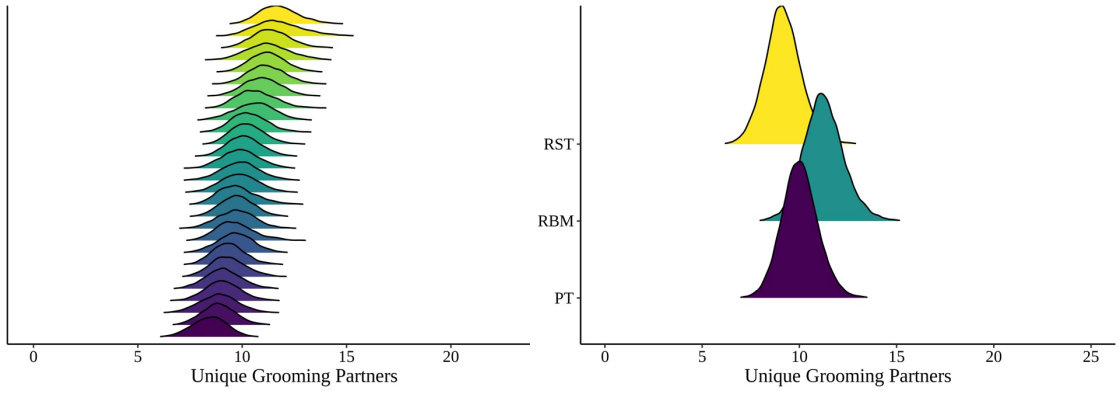

Figure 7.6 Density plots showing mean number of unique grooming partners by ID (left) and troop (right). Density plots show the range of grooming partners predicted by the model, with the height of the density curve indicating the probability and spread of the curve indicating the uncertainty.

7.5 Discussion

Our results show that the relationships between parasite infection and two forms of social association—spatial proximity and grooming—varied according to both parasite genus and measure (richness versus intensity). I found a relationship between parasite infection and the number of unique spatial partners but not the number of unique grooming partners. However, this spatial relationship was contingent on the parasite measure in question. There was a positive association between ?*Protospirura* sp. intensity (eggs per gram) and unique spatial partners, but no relationship between *Trichostrongylus* sp. intensity or parasite richness, and either spatial or grooming partners.

While there is some agreement between our results and those of previous studies, two important points need to be highlighted here. First, links between parasites and network measures are heavily dependent on the parasite genus in question. Second, inconsistency in the primate literature with respect to both the network measures used and direction of relationships found limits both comparison and interpretation of results between study species and populations. These limitations are critical when attempting to generalise results.

Contrary to previous work, I found that when egg counts were lower, individuals had fewer unique spatial partners. (Chapman et al., 2016) reported a reduction in spatial partners when infected, but other studies have shown no relationship between helminth infections and spatial partners Rimbach et al. (2015). However, direct comparison across studies is problematic, given variation in the parasite genera considered and whether the study investigated how parasites influence social networks or how networks influence parasitism. Given the nature of transmission of ?*Protospirura* sp. via an intermediate host, it is unlikely that this relationship is indicative of transmission between individuals, but could point to synchronous exposure to

arthropod vectors while foraging. Alternatively, given that I have also found evidence for sickness behaviour in our population, it is possible that an increase in spatial partners when individuals are sick serves as an additional coping mechanism in response to infection. Given that these vervets are subject to high predation risk from several terrestrial predators, including black-backed jackal (*Lupulella mesomelas*) and caracal (*Caracal caracal*) (Ducheminsky et al., 2014), and that heavy parasite infection can increase the risk of predation (Hudson & Dobson, 1992), increased gregariousness may benefit monkeys coping with high infection intensity.

Parasite richness and *Trichostrongylus* sp. intensity were not linked to spatial partners despite some evidence in primates that *Trichostrongylus* sp. may be directly transmitted between individuals (Rimbach et al., 2015), as well as evidence showing that parasite richness and spatial partners are linked (Deere et al., 2021). From a transmission perspective, given that three out of the five parasite genera are faecal-orally transmitted, sharing space could result in uninfected individuals being exposed to parasites shed by infected individuals (Grear et al., 2013). This is not apparent in our results, but there is a time delay related to the development time of nematode infectious stages in the environment, so it is possible that the time scale used here fails to capture that relationship adequately. Combined, these results highlight inconsistency in parasite-sociality relationships related to both parasite measure (richness versus intensity) and parasite genus.

I also found no relationships between parasite infection and unique grooming partners across all parasite metrics. Grooming behaviour has been linked to parasite infection in other primates (Ghai et al., 2015) and a reduction in grooming has been attributed to sickness behaviour. While I found evidence of sickness behaviour in response to infection, time spent grooming remained unchanged. These results therefore add further support to our suggestion that, given strong environmental constraints, our vervets may be unable to further change their

grooming behaviour in response to parasite infection; that is, their grooming time is already so restricted that any further changes would incur unsustainable social costs (Cohn & de Sá-Rocha, 2006; Moyers et al., 2015).

It is also possible that these vervets do not alter the number of grooming partners in response to the particular parasite genera considered here. Links between infection status and grooming have previously been reported in vervets, however Wren et al. (2016) found that individuals infected with hookworm had more grooming partners than hookworm-negative individuals, although this relationship was not found for the other five parasite genera considered. Similar variation between different parasite genera and grooming metrics has been noted elsewhere (Rimbach et al., 2015). Thus, as with spatial associations, links between grooming partners and parasite infection appear to depend on the parasite genus in question, pointing to a need to be cautious in interpreting both these results and, more importantly, when attempting to generalise across study populations.

It should be noted that while egg shedding of the genera considered may not be a stochastic process in our population, without necropsies, I cannot get an accurate estimate of exact adult worm burden. That is, egg shedding may be high but that is not an absolute measure of the extent of worm burden. Therefore, it is possible that grooming partners do not vary because egg counts may be low enough that there is no impetus or awareness for them to restrict proximity and grooming partners. Experimental work and necropsies would be needed to confirm this.

Inter-individual variation was low with respect to both the number of spatial partners and, to a lesser extent, grooming partners. Given the overall cohesiveness of the group, all individuals will be spatially proximate to all other individuals over time, which may have contributed to low

overall inter-individual variation. However, I did find troop differences in spatial and grooming degree. These differences are likely a function of troop size and the results correspond to troop size, that is, the larger of the three troops had a higher overall mean number of spatial and grooming partners. However, this was not explicitly tested and inter-troop differences in cohesiveness are possible.

In this study, I took a more fine-grained approach to assessing the links between social behaviour and parasitism by using one month of matched behavioural data for each parasite measure. While this provided us with a good view of how parasitism might drive direct social links, I was limited to the use of grooming and spatial degree at the expense of other, more detailed, network measures. Given the complex relationships between primate social networks and parasites described in other studies primates (see: Deere et al., 2021; Duboscq et al., 2016; Rimbach et al., 2015), further work, at a coarse scale, is required to fully describe the relationships between sociality and parasitism in these vervets.

While the links between sociality and parasitism have been investigated widely in non-human primates, establishing causality is problematic, largely given the non-experimental nature of studies. Further, little attention has been paid to the bi-directional relationships of host behaviour-parasite interactions (Hawley et al., 2021). In general, the primate literature focuses on one of two processes: how behaviour shapes parasitism or how parasitism shapes behaviour. That is, considering behaviour before infection and after infection, rather than at the time of infection as is often the case, may provide a clearer picture of the links between sociality and parasitism. However, these relationships are bi-directional, simultaneous and likely occur in feedback loops. This has only recently been addressed with respect to parasite richness and

social networks (see: Poulin & Filion, 2021), but has not been investigated for other parasite measures.

Further, while there are certainly limitations in the generalisability of primate-parasite network analysis results, the use of social network analysis does provide a useful tool to describe the possible mechanisms underlying parasite-sociality relationships in primates. Their versatility allows for a range of parasites to be considered and they can capture different scales of transmission processes. This enables broad applications across a range of systems (Godfrey, 2013). The results presented here therefore offer some preliminary, but nevertheless valuable, insights into links between sociality and parasitism and will serve as the foundation from which to pursue a more bi-directional approach to the question of sociality-parasite links.

CHAPTER EIGHT: GENERAL DISCUSSION

8.1 Primates and their parasites: coping with infection in the wild

Although decades of research on wild non-human primates have strengthened our understanding of their behaviour, ecology, and evolution, we still know relatively little about how they cope with infection (see: Friant et al., 2016b; Ghai et al., 2015; Huffman et al., 1996; Huffman, 1997; Huffman & Caton, 2001). This is largely due to the inherent complexity of natural environments and the competing challenges these pose to wild primates. In captivity, animals are kept in conditions that favour their welfare through carefully controlled conditions, including temperature and light regimes, diet and housing. Wild primates, obviously, are not afforded such luxuries and are exposed to multiple competing stressors, resulting in on-going behavioural trade-offs to maximise fitness in the face of infection or diseases.

Competing stressors pose challenges to both primates and the researchers who study them. Captive studies allow for the isolation of particular variables of interest, through stringent environmental control, thus improving the ability of researchers to infer causal relationships in primate-parasite systems. Further, captive studies more readily allow for the experimental introduction or removal of parasites, which also allows for detailed causal analyses of parasite and pathogen transmission in social groups (captive Barbary macaques: Müller-Klein et al., 2019; semi-free-ranging red-capped mangabeys: Friant et al., 2016a; Friant et al., 2016b). Under wild conditions, however, experimental introduction or removal of parasites is rare (for wild vervet monkeys, see: Chapman et al., 2016) and there are external environmental variables that may or may not be amenable to control (either experimentally or statistically).

Despite these constraints, there has been considerable effort to both measure and control for factors that potentially shape primate-parasite interactions in the wild. These factors operate

across the individual- (e.g. age, sex and social rank), group- (e.g. home-range size and productivity) and population-level (e.g. climatic conditions). This means that, in wild studies, not only are there a large number of variables to consider, but one also has to recognise that these are likely to interact in complex ways. This is evident in the contradictory results of multi-scale studies of the socio-ecological predictors of parasitism in primates, as found in this thesis and across studies of other primates. This points to the need to carefully consider what variables are being measured, the methods that are used, and the analyses conducted as there seems to be no consistency with respect to how external factors shape parasitism within species, across populations or between environments. In what follows, I discuss these issues in the context of my own study, and offer some suggestions for how to extend and strengthen primate parasite research in the future.

8.2 Primate-parasite interactions in extreme environments

Many primate species now live in disturbed and/or fragmented habitats and primate-parasite ecology has shifted to focus on how these conditions shape parasitism (for example, see: Lane et al., 2011; Mbora & McPeek, 2009; Trejo-Macías & Estrada, 2012; Valenta et al., 2017). This research has identified trends such as increased parasite prevalence and richness in fragmented forests or highly disturbed areas, thus informing decisions on what should be considered in primate-parasite research in fragmented or disturbed environments and how results should be interpreted. However, despite a firm grasp on how local, more direct anthropogenic disturbance, such as deforestation or urbanisation, might shape parasitism (reviewed: Bradley & Altizer, 2007), we know very little about the impact of extreme climatic conditions on primate-parasite relationships. There has been some effort to investigate how short-term, extreme weather

events might shape parasitism (e.g. hurricanes: Behie et al., 2014) but more work needs to be done to establish the influences of chronic environmental stress on primate populations, such as those living in semi-arid environments. Decades of primate-parasite research has highlighted the diversity of these relationships and, given this, a next step would be to expand our focus beyond tropical and sub-tropical regions to include areas where competing stressors may change what we know about the potential drivers and consequences of parasitism in the wild.

In particular, primate-parasite interactions in extreme environments offer us a novel set of questions that often cannot be asked under other conditions. These range from how extreme heat or drought impact parasite establishment through to how such conditions influence the capacity of animals to respond to parasite infection. One primary question that can be asked is: what happens, in the face of parasitism, when primate behaviour is constrained, and flexibility is limited?

Food availability plays a large role in primate life. The impacts of food shortages are generally short-term and range from a reduction in body condition to an increase in aggression related to food access (Beehner & McCann, 2008; Thompson, 2017). However, in extreme environments, food shortages are often chronic, and the pressures are long-term. Animals are also subject to additional, often compounding, stressors, including temperature extremes and reduced water availability. These conditions previously have been linked to stress and mortality in our population (Young et al., 2019) and there is evidence to suggest that the vervets have some behavioural flexibility and can tolerate the current environmental variability at the study site (McFarland et al., 2014). However, little is known about whether these vervets have the capacity to further modulate behaviour in response to non-climatic factors, such as parasite infection, and how these competing stressors may interact.

Our study population therefore offered me the ideal opportunity to assess how chronic environmental and physiological stress shape behaviour and to consider how that might influence long-term fitness outcomes. It is perhaps not surprising, but nevertheless important, to note that resource availability often swamped our ability to detect the influence of parasites on behaviour, and apparently limited the animals' ability to respond adaptively. That is, although sickness behaviour potentially is beneficial for highly parasitised individuals in our population, these monkeys simply may not have the capacity to flexibly adjust their time-budgets in the manner needed. Given the controlled conditions in captive studies and the lack of chronic environmental stress in earlier wild studies, this kind of interaction has either not been found or not considered (Alados & Huffman, 2000; Chapman et al., 2016; Ghai et al., 2015; MacIntosh et al., 2011). This seems to have resulted in a perception among researchers that sickness behaviour is both likely to be seen and likely to be adaptive among primates in general. My findings suggest more caution may be needed in this respect.

More specifically, I found that, although there were some behavioural changes in response to infection, there was no reduction in grooming, which is often seen as an energetically expensive activity (Coelho et al., 1976) and likely to be reduced in the face of ecological stressors of various kinds (Ghai et al., 2015; Young et al., 2019). My suggestion, following the finding by Young et al. (2019), was that the vervets' activity budget is already so severely constrained that they lack the flexibility to modify it yet further. This suggests that grooming behaviour may always be under a certain level of stress due to the particular ecological challenges of the semi-arid karoo, and further reductions are not sustainable without leading to (possibly detrimental) shifts in individual social connections and/or group cohesion. It is also possible that other essential behaviours not explicitly considered here, such as copulations or

aggressive interactions, may also be ecologically constrained; a reduction in copulations has been reported in response to infection in primates (Ghai et al., 2015). These variables were not considered in this thesis due to their low frequency in scan samples but they do warrant further investigation, given the potential knock-on effects of reducing those behaviours further in response to infection, such as a subsequent drop in birth rates.

Changes of this nature are given added importance due to a rapidly changing climate. Hot and dry areas are increasingly experiencing periods of severe, frequent drought, further exacerbating the environmental stress to which these monkeys are exposed (Hoffman et al., 2009; Jury, 2013). In other ecotypes, primates are, or soon will be, experiencing a reduction in climatically suitable habitats (Graham et al., 2016), and their survival will depend on their ability to disperse (Schloss et al., 2012). However, in a study of more than 50 primate species, Schloss et al. (2012) concluded that primates exhibit limited dispersion capacity when compared to other mammals, and that climate change will likely outpace the response capacity of many species. Additionally, climate change is likely to shape the intensity and distribution of infectious diseases (Altizer, 2013; Chapman et al., 2005; Lafferty, 2009). Thus, local population survival will likely depend on local flexibility and the more fine-scale ability of primates to cope with the more extreme environmental conditions in which they live.

Given this, understanding flexibility and its limits in primates in response to environmental conditions and physiological pressures in habitats that are already hot and dry provides a useful window into how primates may adapt and survive in what could become climatically unsuitable habitats. While understanding which behaviours are constrained in these environments is a good first step, understanding the potential long-term fitness consequences of these constraints, in conjunction with infection, provides a new avenue of primate-parasite

ecology research in areas where long-term monitoring is possible. There are several avenues for this including attention to the long-term survival of heavily infected individuals based on their past behavioural responses to infection and/or investigating how mothers respond to infection during gestation/lactation and how this affects infant survival. There are some data to suggest that parasite loads differ in relation to reproductive status: female Japanese macaques have been reported to experience a higher parasite intensity during the birth season (MacIntosh et al., 2010). The implications of this, however, are unknown. Projects of this nature require sustained and consistent long-term monitoring and are therefore demanding of time and resources, but such long-term data are crucial if we hope to move primate-parasite ecology toward a deeper understanding of how current and future infectious diseases shape primate survival and reproductive success, and exert effects on fitness.

8.3 Complex modelling for complex problems

In many cases primate-parasite ecologists have lagged behind other ecologists in embracing new analytical and modelling techniques. Given the number of possible variables, and the interactive and non-linear nature of relationships between these variables, primate-parasite ecology would benefit hugely from a shift in both study design and statistical approach. This approach would be two-fold: i) sophisticated modelling to explain current relationships, and ii) modelling to predict future relationships.

With respect to the first point, advances in statistical modelling have allowed researchers to increase their ability to assess the nature of specific relationships in a given study system. Such modelling was previously inaccessible due to the difficulties associated with computing power and the necessary statistical software. Computing power is now more accessible and the

widespread use of opensource software, such as R, has allowed for the more extensive use of existing techniques, and the development and dissemination of new techniques. More sophisticated modelling allows for the variation that is placed under experimental or environmental control in captive studies, to be controlled statistically among wild populations.

One specific example of the benefits of advanced modelling is the inclusion of non-linear variation, a particular challenge in wild environments. Several relationships considered in primate-parasite ecology are likely to be non-linear, often as a result of seasonal variation. That is, although there may be a relationship between the time of year and parasite measures, this is unlikely to be captured if approached using only linear regression; a common modelling approach in primate-parasite ecology (for examples, see: Benavides et al., 2012; MacIntosh et al., 2010; Rondon et al., 2017). This is evident in this thesis where, using hierarchical generalized additive models (Pedersen et al., 2019), I found that annual variation in parasite measures could be captured using a spline and would otherwise only have been captured if season was entered as a categorical variable or, potentially, not at all if a linear approach had been taken. In much primate-parasite research, seasonal variation is typically assessed by dividing data into seasonal blocks (for example, see: MacIntosh et al., 2010; Rondon et al., 2017; Teichroeb et al., 2009), however, parasite measures can vary continuously, peaking, for example, over a period that spans the end of spring and beginning of summer, and which is then unlikely to be captured in a categorical variable despite being valuable information. In this regard, one of the primary benefits of additive models is that they allow us to fit models without making any assumptions about the shape of the curve, and permit the consideration of non-linear annual variation in a continuous manner, without the need to divide into seasonal blocks. This flexibility in modelling is highly beneficial for primate-parasite systems given that we know relatively little

about the underlying predictors of parasitism in wild primates and how they may vary. While clearly helpful to understanding relationships in primate-parasite systems, these models are underused by primate-parasite ecologists despite their wide use in other fields of ecology. This is a point worth emphasizing as these types of models can be applied to any variable where non-linear variation might be expected. This includes, for example, time of day (evident in the entropy rate results presented in this thesis), rank effects and many ecological variables.

Given the current propensity for the use of linear models in primate-parasite ecology, it is certainly possible that these approaches have, at least in part, led to incorrect conclusions and interpretations about the predictors of parasitism in wild primates. By constraining the shape of the curve or taking a categorical approach, we may have missed variation that is critical to fully understanding primate-parasite interactions. Conversely, by constraining models and making assumptions before analysis, we may also be capturing variation that is less meaningful than it appears. Embracing new modelling techniques allows for flexibility but also, critically, could aid in more accurate interpretation of any variation found. However, it should be noted that these types of models are susceptible to overfitting and sufficient caution should be applied when using them (Pedersen et al., 2019). Nevertheless, it is certainly possible that some of the inconsistency found in the predictors of parasitism in primates may not be a function of characteristic variation in these systems but simply may be a by-product of not capturing the variation that exists, due to the particular analytical strategies employed.

With respect to the second point, statistical modelling can also take on a more predictive role either through the use of simulated data or the application of predictive modelling techniques to real-world data. In primate disease ecology, predictive modelling, in conjunction with network analysis, has been successfully applied to simulate the spread of infectious diseases

in social groups (Romano et al., 2016) and assess how sub-grouping might mitigate disease risk in social groups (Griffin & Nunn, 2012). Understanding the role of social interactions in disease transmission has important implications for predicting disease spread from a conservation and management perspective, and provides insight into the trade-offs that may arise as a result of group living.

These types of models have most often focused on how social organisation predicts parasite risk and transmission and often exclude the role of the environment. Agent-based modelling, however, has emerged as a powerful tool to incorporate resource distribution into disease dynamics and predict how landscape changes may affect host and parasite dynamics (Bonnell et al., 2010). Bonnell et al. (2010) used an agent-based model to simulate movement and foraging in red colobus monkeys to infer how landscape changes can affect host and parasite dynamics. They found that the density of resource rich sites and the overall heterogeneity of the landscape are important factors contributing to this spread.

While Bonnell et al. (2010) focused on the impacts of habitat fragmentation on disease dynamics, agent-based models also provide a tool with which to simulate other environmental scenarios. Of particular interest is how different climatic scenarios might predict disease transmission in populations and what consequences this may have on overall health. Constructing these models requires detailed environmental and behavioural data and, given I have access to these data, I plan to combine social and environmental drivers of parasitism and provide predictions on how diseases transmission dynamics in this social primate may change in the face of climate change.

In sum, embracing more sophisticated modelling techniques in primate-parasite ecology will greatly improve both our ability to describe relationships in the study system, whether it be

host-parasite relationships or the influence of the environment on these systems, and to predict future outcomes for a group of mammals that is highly sensitive to environmental change.

8.4 Parasite manipulation for a testable framework

Given the non-invasive nature of much primate-parasite research in the wild, links between behaviour and parasitism are largely correlative. This is most often due to the diverse research conducted at field sites and the disruptive nature of experimental parasite manipulation. However, parasite removal experiments, where animals are given anti-parasitics, provide us with a more testable framework to study the links between parasitism and various aspects of behaviour. Removal experiments are notably less invasive than introducing new parasite infections into a population yet provide avenues to overcome some of the correlative problems faced by primate parasite ecologists.

Removal experiments have been used in other fields to address a range of questions. For example, Hudson and Dobson (1997) treated red grouse (*Lagopus lagopus scoticus*) with Levamisole hydrochloride to test for host-mediated reductions in worm fecundity and establishment. Specifically, whether parasite reinfection rates are regulated by competition for resources or by host-mediated immune response. Parasite removal, in this case, allowed Hudson and Dobson (1997) to quantify three components of parasite transmission (egg production rates, infection rates and establishment of larvae in a new host) that would otherwise not be possible in an observational-only analysis.

Parasite removal experiments have been used in some primate studies although they are rare. For example, Friant et al. (2016b) treated semi-free-ranging red-capped mangabeys with anti-parasitic drugs and assessed subsequent changes in glucocorticoid production and individual

behaviour. Additionally, they used this removal experiment to assess the physiological and behavioural correlates of parasite re-infection (Friant et al. 2016a). To quantify how gastrointestinal parasite infection affects the behaviour of vervet monkeys, Chapman et al. (2016) conducted a removal experiment and assessed changes in activity budget before and after treatment. Additionally, they investigated whether individuals' position in the spatial network were linked to parasitism.

While removal experiments are most often not possible in wild populations where parallel research on other aspects of primatology is occurring, they do provide a relatively non-invasive means to assess parasite-haviour links more empirically. Subsequently, the inclusion of removal experiments in primate-parasite research could provide primate-parasite ecologists with an additional means to study parasite-behaviour links and should be conducted in other populations where possible.

8.5 When generalisability fails: Methodological consistency for a comparative future

One of the primary issues facing primate-parasite ecologists is the inability to generalise results across both primate study species and study areas. Such a state of affairs is a serious impediment to truly comparative work. As Gillespie (2006) has pointed out, divergent methodologies have been used for both sample collection and parasite analysis, hindering longitudinal or comparative work. While this is certainly true, I suggest a similar problem is posed by the manner in which researchers collect behavioural data and the way in which different statistical approaches are used. One clear example of this is the use of social network analysis (SNA) to understand the links between sociality and parasites. Although SNA is certainly a promising tool with great potential for making comparisons across populations and

species, there is limited consistency across studies and, in practical terms, comparison is all but impossible. This lack of consistency can be seen in the variety of parasite measures considered (richness, prevalence and intensity) and the network metrics used (e.g. node strength, closeness centrality or betweenness). While selecting a network measure is certainly question-dependent, there are areas in social network research that could be more standardised. For example, from a methodological standpoint, behavioural data needs to be matched to the period over which parasite data were collected. In my thesis, I used a period of one week, following the approach of Ghai et al. (2015). In the main, however, this issue of scale is an ongoing problem, with a lack of consistency in behaviour-parasite matching across studies where, in many cases, such matching is not even mentioned. Having no indication of the scale at which these processes are occurring severely hampers comparison.

Consistency in analytical techniques is even more important for primate-parasite research, given that the study systems already vary widely, and introducing any additional variation into research makes useful comparison even more difficult. Primate-parasite interactions are diverse, and relationships vary from the population level to individual-level. With so many variables in play, it becomes hard to tease out whether patterns are due to the particular population of animals in a particular environment, the particular parasite community in that particular environment, or the interactions of those particular animals with this particular parasite community in that particular environment. This is evident in my sociality-parasite findings. Contrary to other work, for example, I found no links between grooming and parasite measures. This could be a result of several processes including: i) the parasite genera considered do not exert sufficient pressure on the monkeys to elicit a response ii) the environment constrains the monkey's ability to change behaviour or iii) the relationship is bi-directional with parasites constraining monkey behaviour

and monkey behaviour influencing parasite measures. Although one could say that my results contrast with previous work (see: Chapman et al., 2016; Macintosh et al., 2012; Rimbach et al., 2015; Wren et al., 2016), no direct comparisons can legitimately be made owing to the differences in statistical methodology. The lack of comparative options is a loss to primate-parasite ecology given the breadth of data available on different species, populations and habitats. Having said all this, and despite these challenges, primate-parasite ecology is a burgeoning field that, with some tweaking, has much to offer in the way of solid comparative research and we can surely improve our ability to generalise via a concerted effort to standardise methods and analysis within the field.

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APPENDICES

Appendix A: Supplementary Material for Chapter 4

A.1 Morphological Characteristics Used to Designate Parasite genera

Table A.1 Description and size range of parasite genera identified.

Genus	Size range (µm)	Description
?Protospirura sp.	49 - 58 x 36 - 43	Eggs colourless, tick-shelled, ellipsoidal and surrounded by a hyaline substance
Trichostrongylus sp.	81 - 95 x 38 - 49	Eggs colourless, thin-shelled eggs pointed at either one or both ends. Eggs are non-larvated
Ternidens sp.	74.8 - 94 x 39 - 49	Eggs ellipsoidal, thin shell and non-larvated. Eggs contained numerous blastomeres
Subulura sp.	50 - 56 x 63 - 65	Eggs rounded, relatively thick-shelled and contained coiled larva
Oesophagostomum sp.	51 - 70 x 35- 47	Eggs ovular with rounded extremes, thin shell and non-larvated. Eggs contained numerous blastomeres

A.2 Data Overdispersion Test for Parasite Richness

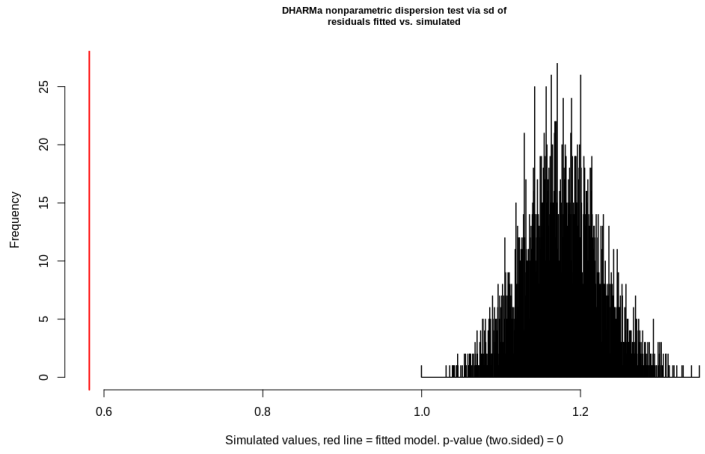

Figure A.2 Results from the DHARMa non-parametric dispersion test showing underdispersion in the parasite richness dataset when modelling a Poisson distribution. ratioObsSim = 0.4945, p-value <- 2.2e-16.

A.3 Parasite Richness: combined strongyle measure

Given potential problems with strongyle egg differentiation, this model was run with *Oesophagostomum* sp. and *Ternidens* sp. combined into one family: strongyle. Owing to low frequency, no substantial differences were found between the two models.

Table A.3 Summary statistics of Bayesian mixed-effects model for parasite richness (number of genera). CI = credible interval; SD = standard deviation

	Effect	Estimate	Est.Error	l-95% CI	u-95% CI	$\widehat{R}$
Fixed effects						
Population-level	Intercept	-0.86	0.30	-1.41	-0.20	1
	Precipitation (bi-weekly)	-0.39	0.21	-0.81	0.01	1
	Minimum Daily Temperature	-0.24	0.19	-0.62	0.15	1
	Maximum Daily Temperature	0.3	0.18	-0.04	0.66	1
	NDVI	-0.39	0.23	-0.83	0.07	1
	Sex (reference: male)	-0.48	0.32	-1.08	0.16	1
	s(Date)	-0.53	0.9	-2.13	1.31	1
Smooth Terms	sds(Date)	0.62	0.44	0.04	1.68	1
Random effects						
ID	sds(ID)	0.64	0.15	0.39	0.98	1
Troop	sds(Troop)	0.31	0.32	0.01	1.20	1
Family						
	hu	0.02	0.01	0.01	0.03	1

A.4 Summary Statistics for GI Model: ?*Protospirura* sp. Parasite Intensity

Table A.4 Summary statistics of Negative binomial Bayesian generalised additive mixed-effects model for parasite intensity (egg counts) of ?*Protospirura* sp. CI = credible interval; SD = standard deviation. Estimates where credible intervals do not cross zero are in bold.

	Effect	Estimate	Est.Error	l-95% CI	u-95% CI	$\widehat{R}$
Fixed effects						
Population-level	Intercept	5.95	0.27	5.41	6.47	1

	Precipitation (bi-weekly)	-0.19	0.07	-0.33	-0.05	1
	Minimum Daily Temperature	-0.06	0.09	-0.24	0.13	1
	Maximum Daily Temperature	0.01	0.08	-0.14	0.17	1
	Sex (reference: male)	0.28	0.36	-0.43	0.97	1
	NDVI	0	0.12	-0.24	0.25	1
	s(Date)	0.86	0.95	-0.99	2.74	1
Smooth Terms	sds(Date)	2.04	0.33	1.4	2.7	1
	sds(Date ID1)	0.48	0.24	0.05	0.99	1
	sds(Date ID2)	0.64	0.18	0.35	1.06	1
	sds(Date ID3)	0.25	0.18	0.01	0.69	1
	sds(Date ID4)	0.7	0.23	0.34	1.24	1
	sds(Date ID5)	0.27	0.23	0.01	0.86	1
	sds(Date ID6)	0.23	0.19	0.01	0.71	1
	sds(Date ID7)	0.55	0.27	0.08	1.09	1
	sds(Date ID8)	0.21	0.17	0.01	0.63	1
	sds(Date ID9)	0.67	0.31	0.14	1.34	1
	sds(Date ID10)	0.7	0.2	0.39	1.14	1
	sds(Date ID11)	0.61	0.23	0.23	1.13	1
	sds(Date ID12)	0.26	0.21	0.01	0.77	1
	sds(Date ID13)	0.66	0.21	0.31	1.14	1
	sds(Date ID14)	0.52	0.26	0.06	1.07	1
	sds(Date ID15)	1.4	0.3	0.84	2.01	1
	sds(Date ID16)	0.23	0.18	0.01	0.71	1
	sds(Date ID17)	0.47	0.22	0.08	0.96	1
	sds(Date ID18)	1.29	0.27	0.8	1.88	1
	sds(Date ID19)	0.37	0.31	0.01	1.12	1
	sds(Date ID20)	0.68	0.21	0.33	1.16	1
	sds(Date ID21)	0.29	0.22	0.01	0.82	1
	sds(Date ID22)	1.12	0.23	0.72	1.62	1
	sds(Date ID23)	0.18	0.15	0.01	0.57	1
	sds(Date ID24)	0.92	0.29	0.37	1.5	1
	sds(Date ID25)	0.31	0.19	0.02	0.77	1
	sds(Date ID26)	0.57	0.23	0.2	1.09	1
	sds(Date ID27)	0.27	0.23	0.01	0.85	1
Random Effects						
ID	sds(ID)	0.93	0.13	0.72	1.21	1
Troop	sds(Troop)	0.25	0.21	0.01	0.8	1
Family						
	Shape	2.3	0.17	1.98	2.64	1

A.5 Summary Statistics for GI Model: ?*Trichostrongylus* sp. Parasite Intensity

Table A.5 Summary statistics of Bayesian mixed-effects generalised additive hurdle model for parasite intensity (egg count) of *Trichostrongylus* sp. CI = credible interval; SD = standard deviation.

	Effect	Estimate	Est.Error	l-95% CI	u-95% CI	$\widehat{R}$
Fixed effects						
Population-level	Intercept	1.63	0.19	1.1	1.92	1.01
	Precipitation (bi-weekly)	-0.1	0.12	-0.33	0.12	1
	Minimum Daily Temperature	-0.08	0.11	-0.29	0.13	1
	Maximum Daily Temperature	0.04	0.12	-0.19	0.27	1
	Sex (reference: male)	-0.01	0.16	-0.34	0.3	1
	NDVI	-0.04	0.16	-0.36	0.29	1
	s(Date)	0.65	0.77	-0.97	2.11	1
Smooth Terms	sds(Date)	0.33	0.24	0.01	0.89	1
	sds(Date ID1)	0.28	0.23	0.01	0.86	1
	sds(Date ID2)	0.39	0.31	0.01	1.13	1
	sds(Date ID3)	0.27	0.23	0.01	0.82	1
	sds(Date ID4)	0.38	0.22	0.04	0.89	1
	sds(Date ID5)	0.3	0.24	0.01	0.89	1
	sds(Date ID6)	0.3	0.24	0.01	0.94	1
	sds(Date ID7)	0.27	0.21	0.01	0.78	1
	sds(Date ID8)	0.35	0.28	0.02	1.02	1
	sds(Date ID9)	0.4	0.29	0.02	1.08	1
	sds(Date ID10)	0.28	0.23	0.01	0.85	1
	sds(Date ID11)	0.4	0.3	0.02	1.13	1
	sds(Date ID12)	0.31	0.25	0.01	0.92	1
	sds(Date ID13)	0.74	0.25	0.32	1.29	1
	sds(Date ID14)	0.4	0.3	0.02	1.09	1
	sds(Date ID15)	0.3	0.22	0.02	0.81	1
	sds(Date ID16)	0.33	0.26	0.01	0.98	1
	sds(Date ID17)	0.4	0.3	0.02	1.14	1
	sds(Date ID18)	0.41	0.29	0.02	1.06	1
	sds(Date ID19)	0.37	0.27	0.02	1.01	1
	sds(Date ID20)	0.18	0.15	0.01	0.55	1
	sds(Date ID21)	0.29	0.24	0.01	0.88	1
	sds(Date ID22)	0.4	0.31	0.01	1.13	1
	sds(Date ID23)	1	0.22	0.62	1.48	1
	sds(Date ID24)	0.37	0.29	0.01	1.07	1

	sds(Date ID25)	0.29	0.21	0.02	0.8	1
	sds(Date ID26)	0.23	0.2	0.01	0.74	1
	sds(Date ID27)	0.4	0.25	0.03	0.97	1
Random effects						
ID	sds(ID)	0.2	0.21	0.01	0.83	1.01
Troop	sds(Troop)	0.11	0.08	0	0.3	1.01
Family						
	Shape	0.78	0.02	0.74	0.81	1

Appendix B: Supplementary Material for Chapter 5

B.1 Summary Statistics for GI Model: Socio-ecological drivers of ?*Protospirura* sp. Parasite Intensity

Table B1 Summary statistics of Bayesian mixed-effects model for parasite intensity (EPG) of ?*Protospirura* sp. CI = credible interval; SD = standard deviation. Smooth-term sds() = spline "wiggliness"(spline variance parameter). Estimates for fixed effects where credible intervals do not cross zero are in bold.

	Effect	Estimate	Estimate Error	l-95% CI	u-95% CI	$\widehat{R}$
Population-Level Effects	Intercept	6.14	0.49	5.18	7.18	1.01
	Bi-weekly precip	-0.19	0.07	-0.34	-0.05	1
	Minimum temp	-0.09	0.09	-0.27	0.09	1
	Maximum temp	0.01	0.08	-0.16	0.18	1
	NDVI	-0.03	0.13	-0.28	0.21	1
	Sex (ref:M)	0.18	0.42	-0.65	1.01	1
	Rank	-0.08	0.32	-0.72	0.55	1
	fGCMs	0	0.07	-0.13	0.14	1
	Sex*Rank	0.37	0.5	-0.61	1.35	1
	s(Date)	1	0.98	-0.94	2.92	1
Random Effects	Troop	0.54	0.65	0.01	2.45	1
	ID	1.02	0.16	0.77	1.38	1
Smooth Terms	sds(date)	2.02	0.34	1.38	2.7	1
	sds(Date ID1)	0.53	0.25	0.06	1.07	1
	sds(Date ID2)	0.64	0.19	0.34	1.08	1
	sds(Date ID3)	0.27	0.18	0.01	0.69	1
	sds(Date ID4)	0.68	0.23	0.32	1.23	1
	sds(Date ID5)	0.28	0.23	0.01	0.86	1
	sds(Date ID6)	0.22	0.18	0.01	0.68	1
	sds(Date ID7)	0.57	0.26	0.07	1.13	1
	sds(Date ID8)	0.2	0.16	0.01	0.61	1
	sds(Date ID9)	0.58	0.28	0.13	1.21	1
	sds(Date ID10)	0.68	0.19	0.37	1.13	1
	sds(Date ID11)	0.55	0.24	0.13	1.1	1
	sds(Date ID12)	0.32	0.22	0.01	0.85	1

	sds(Date ID13)	0.67	0.22	0.31	1.18	1
	sds(Date ID14)	0.56	0.28	0.06	1.16	1
	sds(Date ID15)	1.42	0.31	0.87	2.07	1
	sds(Date ID16)	0.24	0.2	0.01	0.74	1
	sds(Date ID17)	0.4	0.23	0.03	0.88	1
	sds(Date ID18)	1.22	0.26	0.76	1.76	1
	sds(Date ID19)	0.31	0.27	0.01	1.01	1
	sds(Date ID20)	0.69	0.22	0.33	1.18	1
	sds(Date ID21)	0.29	0.22	0.01	0.84	1
	sds(Date ID22)	1.08	0.23	0.69	1.61	1
	sds(Date ID23)	0.19	0.16	0.01	0.6	1
	sds(Date ID24)	1	0.27	0.51	1.57	1
	sds(Date ID25)	0.28	0.17	0.02	0.69	1
	sds(Date ID26)	0.52	0.25	0.08	1.08	1
	sds(Date ID27)	0.29	0.24	0.01	0.89	1
	sds(Date-Sex1)	0.43	0.31	0.02	1.16	1
	sds(Date-Sex2)	0.4	0.3	0.01	1.13	1
	sds(Date-Sex3)	0.4	0.31	0.02	1.15	1
Family- Specific Parameters	shape	2.29	0.17	1.98	2.62	1

B.2 Summary Statistics for GI Model: Socio-ecological drivers of *Trichostrongylus* sp. Parasite Intensity

Table B2 Summary statistics of Bayesian mixed-effects model for parasite intensity (EPG) of *Trichostrongylus* sp. CI = credible interval; SD = standard deviation. Smooth-term sds() = spline "wiggliness"(spline variance parameter). Estimates for fixed effects where credible intervals do not cross zero are in bold.

	Effect	**Estimate**	**Estimate Error**	**l-95% CI**	**u-95% CI**	$\widehat{R}$
Population-Level Effects	Intercept	1.62	0.24	0.97	2.02	1
	Bi-weekly precip	-0.1	0.12	-0.33	0.13	1
	Minimum temp	-0.08	0.11	-0.3	0.14	1
	Maximum temp	0.01	0.12	-0.23	0.25	1
	NDVI	-0.03	0.17	-0.35	0.33	1
	Sex (ref:M)	-0.03	0.21	-0.47	0.37	1

	Rank	-0.11	0.18	-0.47	0.23	1
	fGCMs	0.1	0.07	-0.04	0.23	1
	Sex*Rank	0.07	0.37	-0.64	0.84	1
	s(Date)	0.65	0.78	-0.92	2.12	1
Random Effects	Troop	0.29	0.33	0.01	1.25	1
	ID	0.14	0.09	0.01	0.36	1
Smooth Terms	sds(date)	0.33	0.25	0.01	0.94	1
	sds(Date ID1)	0.28	0.24	0.01	0.88	1
	sds(Date ID2)	0.4	0.3	0.02	1.12	1
	sds(Date ID3)	0.29	0.23	0.01	0.85	1
	sds(Date ID4)	0.38	0.22	0.03	0.88	1
	sds(Date ID5)	0.3	0.24	0.01	0.88	1
	sds(Date ID6)	0.29	0.24	0.01	0.92	1
	sds(Date ID7)	0.29	0.22	0.01	0.82	1
	sds(Date ID8)	0.36	0.26	0.02	0.98	1
	sds(Date ID9)	0.4	0.3	0.01	1.14	1
	sds(Date ID10)	0.28	0.23	0.01	0.86	1
	sds(Date ID11)	0.4	0.31	0.02	1.17	1
	sds(Date ID12)	0.31	0.26	0.01	0.96	1
	sds(Date ID13)	0.67	0.27	0.14	1.23	1
	sds(Date ID14)	0.4	0.3	0.01	1.12	1
	sds(Date ID15)	0.29	0.22	0.01	0.84	1
	sds(Date ID16)	0.34	0.27	0.01	0.99	1
	sds(Date ID17)	0.41	0.3	0.01	1.13	1
	sds(Date ID18)	0.4	0.29	0.02	1.06	1
	sds(Date ID19)	0.37	0.27	0.01	1.01	1
	sds(Date ID20)	0.19	0.16	0.01	0.62	1
	sds(Date ID21)	0.31	0.23	0.01	0.87	1
	sds(Date ID22)	0.4	0.3	0.02	1.11	1
	sds(Date ID23)	0.97	0.23	0.58	1.46	1
	sds(Date ID24)	0.37	0.29	0.01	1.08	1
	sds(Date ID25)	0.29	0.21	0.01	0.78	1
	sds(Date ID26)	0.25	0.2	0.01	0.73	1
	sds(Date ID27)	0.43	0.26	0.03	1.03	1
	sds(Date-Sex1)	0.37	0.27	0.01	1.02	1
	sds(Date-Sex2)	0.4	0.29	0.02	1.08	1
	sds(Date-Sex3)	0.4	0.3	0.01	1.12	1

Family Specific Parameters:	hu	0.78	0.02	0.74	0.81	1

Appendix C: Supplementary Material for Chapter 6

C.1 Dataset reduction validation

Matching faecal sample data to a single week of behavioural data reduced our behavioural data set significantly (%). To assess whether there were any clear quantitative differences between the full and reduced datasets, I ran a generalised additive mixed model (GAMM) using the full focal dataset for comparison. The response variable was entropy rate and the predictor variables were sex, rank, NDVI, sequence length and time of day with ID nested in troop as a random effect.

I found no qualitative differences in estimates between the reduced and full focal datasets for the variables that could be included (Table C1) and I therefore proceeded with the reduced dataset for our entropy rate analysis.

I ran a generalised additive mixed-effects model to assess whether the reduction in our dataset that resulted from matching faecal samples to behavioural influenced results. I found no qualitative differences in these models and proceeded with the reduced dataset.

Table C1 Summary statistics of generalised additive mixed-effects model examining the influence of environmental and social factors on entropy rate (N= 1553). CI = credible interval; SD = standard deviation. Smooth-term sds() = spline "wiggliness"(spline variance parameter). (R^2 0.05, Est.error = 0.01, l-CI = 0.03, u-CI = 0.74)

	Effect	Estimate	Est.Error	l-95% CI	u-95% CI	$\widehat{R}$
Population-level	Intercept	0.76	0.09	0.46	0.95	1.01
	NDVI	0.11	0.02	0.07	0.15	1
	Sex (ref: male)	-0.02	0.03	-0.08	0.03	1.01
	Rank	0.03	0.03	-0.02	0.08	1
	Sequence length	-0.03	0.02	-0.07	0.01	1.01
	Time (spline)	-0.09	0.53	-1.13	0.95	1
Troop	sd(Intercept)	0.11	0.15	0	0.54	1.01
ID	sd(Intercept)	0.02	0.02	0	0.06	1.01

Smooth Terms	sds(time)	0.39	0.23	0.13	0.99	1
Family-specific	sigma	0.39	0.01	0.37	0.4	1.01

C.2 Model comparison results

To account for the large number of zeros in our estimates of entropy rate (N = 59/747), i.e., where individuals engaged in one behaviour for the full observation time, I first fitted a hurdle model with a Gaussian distribution as well as a non-hurdle model with a Gaussian distribution. I then used the widely applicable information criterion (WAIC: Gelman et al., 2014) to compare the hurdle and non-hurdle models by applying the "loo_compare" function from the loo package (Vehtari et al., 2017). Model comparison indicated that while the hurdle model accounted for the zeros, the non-hurdle model produced a better fit ($elpd\_diff_{gaussian} = 0$, $se\_diff_{gaussian} = 0$; $elpd\_diff_{hurdle\text{-}gaussian} = -64.1$, $se\_diff_{hurdle\text{-}gaussian} = 8.5$). Model estimates were also qualitatively similar between models. I therefore proceeded with the non-hurdle model.

Table C2 Summary statistics of generalised additive mixed-effects hurdle model with a Gaussian distribution examining the influence of environmental and social factors on entropy rate. CI = credible interval; SD = standard deviation. Smooth-term sds() = spline "wiggliness"(spline variance parameter)

	Effect	**Estimate**	**Est.Error**	**l-95% CI**	**u-95% CI**	$\widehat{R}$
Population-level	Intercept	0.86	0.08	0.67	1.01	1.01
	NDVI	0.11	0.03	0.06	0.16	1
	Sex (ref: male)	0.02	0.04	-0.05	0.09	1
	Rank	-0.01	0.03	-0.07	0.05	1
	Sequence length	-0.02	0.02	-0.07	0.03	1
	?*Protospirura* sp. EPG	-0.02	0.03	-0.08	0.04	1
	Trichostrongylus sp. EPG	0.01	0.04	-0.07	0.09	1
	Number of species	-0.03	0.04	-0.11	0.04	1
	fGCM concentration	0	0.03	-0.05	0.05	1
	Time (spline)	-0.47	0.39	-1.37	0.26	1
Troop	sd(Intercept)	0.09	0.13	0	0.49	1.01

ID	sd(Intercept)	0.04	0.02	0	0.08	1.01
Smooth Terms	sds(time)	0.18	0.17	0.01	0.6	1.01
Family-specific parameters	hu	-2.32	0.13	-2.59	-2.07	1
	sigma	0.32	0.01	0.3	0.34	1.01

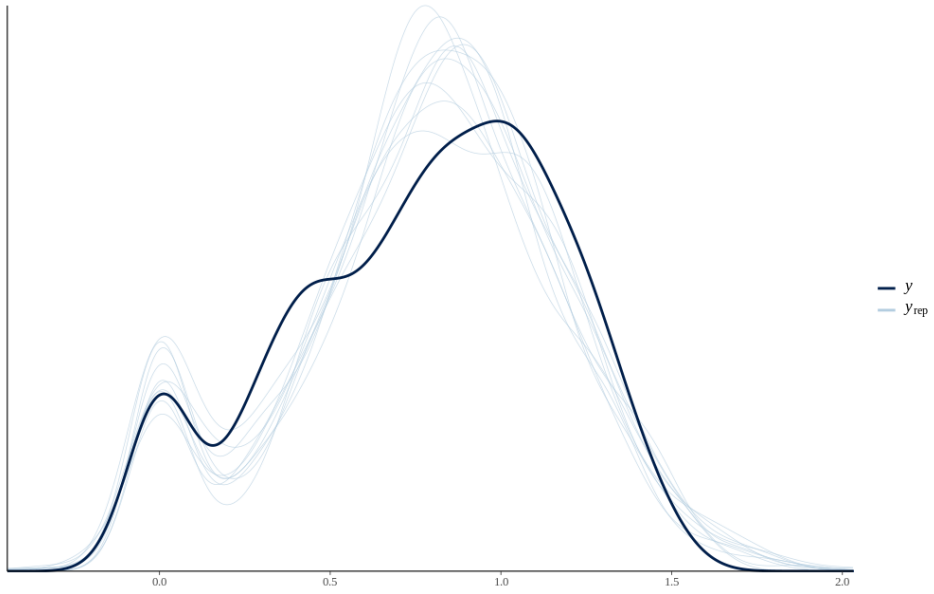

Figure D2 Posterior predictive check for generalised additive mixed-effects hurdle model with a Gaussian distribution comparing the observed outcome variable (y) to simulated datasets (y_{rep}) from the posterior predictive distribution

C.3 Co-infection model results

Full results for the Bayesian mixed effects model examining the relationship between ?*Protospirura* sp. infection and *Trichostrongylus* sp. infection

Table C3 Summary statistics of the mixed-effects model examining the relationship between ?*Protospirura* sp. infection intensity (eggs per gram) and *Trichostrongylus* sp. infection intensity. CI = credible interval; SD = standard deviation. N=565 faecal samples. (R^2=0.61, Est.error = 0.03, l-CI = 0.55, u-CI = 0.65)

		Effect	Estimate	Est. Error	l-95% CI	u-95% CI	$\widehat{R}$
Fixed-effects	**Population-level**	Intercept	2.3	1.13	0.16	4.8	1
		Trichostrongylus sp. (EPG)	0.38	0.64	-1.04	1.56	1
Random effects	**Troop**	sd(Intercept)	2.33	0.64	1.04	3.59	1
		sd(*Trichostrongylus* sp. (EPG))	0.67	0.39	0.09	1.59	1
		cor(Intercept, *Trichostrongylus* sp. (EPG))	0.17	0.51	-0.83	0.94	1
	ID	sd(Intercept)	0.93	0.15	0.68	1.28	1
		sd(*Trichostrongylus* sp. (EPG))	0.3	0.24	0.01	0.9	1
		cor(Intercept, *Trichostrongylus* sp. (EPG))	-0.21	0.48	-0.95	0.82	1

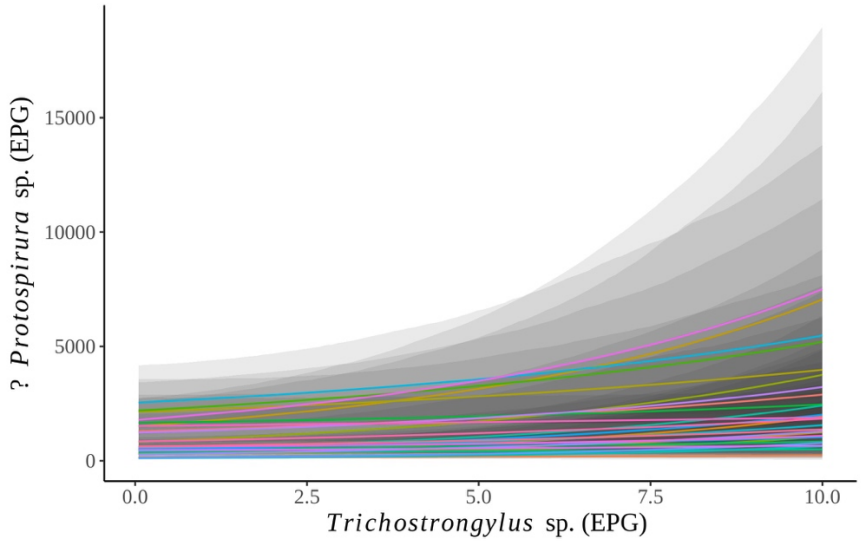

Figure C3 Estimate of faecal egg count (eggs per gram, EPG) of ?*Protospirura* sp. as a function of *Trichostrongylus* sp. faecal egg count derived from the fitted Bayesian mixed-effects model. Upper and lower 95% credible intervals (grey bands) shown

C.4 Multinomial full results

Table C4 Multinomial mixed effects model results of the coefficients of the fixed and random effects. These represent the effects of a one-unit increase in the predictor on the log-odds of exhibiting each behaviour instead of the reference category, conditional on the other parameters. Reference behaviour: moving

	Variable	**Behaviour**	**Mean**	**Standard Error**	**Standard Deviation**	**2.5% CI**	**97.5 % CI**	$\widehat{R}$
Fixed Effects	Intercept	Groom (give)	-2.78	0.06	1.10	-4.28	-0.23	1.02
		Rest	0.15	0.01	0.24	-0.33	0.63	1.00
		Groom (receive)	-2.51	0.02	0.48	-3.11	-1.02	1.01
		Forage	-0.22	0.01	0.20	-0.59	0.15	1.00
	NDVI	Groom (give)	-0.45	0.00	0.08	-0.60	-0.30	1.00
		Rest	0.01	0.00	0.04	-0.07	0.09	1.00

	Groom (receive)	-0.54	0.00	0.08	-0.70	-0.38	1.00
	Forage	-0.54	0.00	0.04	-0.62	-0.46	1.00
?*Protospirura* sp. (EPG)	Groom (give)	0.20	0.00	0.08	0.05	0.34	1.00
	Rest	0.15	0.00	0.04	0.07	0.23	1.00
	Groom (receive)	0.22	0.00	0.08	0.07	0.38	1.00
	Forage	0.02	0.00	0.04	-0.06	0.11	1.00
Trichostrongylus sp. (EPG)	Groom (give)	0.14	0.00	0.09	-0.03	0.17	1.00
	Rest	-0.10	0.00	0.06	-0.21	-0.01	1.00
	Groom (receive)	0.11	0.00	0.11	-0.10	0.21	1.00
	Forage	0.06	0.00	0.05	-0.05	0.12	1.00
Number of Species	Groom (give)	-0.18	0.00	0.07	-0.31	-0.05	1.00
	Rest	-0.06	0.00	0.04	-0.13	0.02	1.00
	Groom (receive)	-0.11	0.00	0.08	-0.26	0.04	1.00
	Forage	0.00	0.00	0.04	-0.07	0.08	1.00
Sex (ref: male)	Groom (give)	1.21	0.01	0.26	0.66	1.69	1.01
	Rest	0.04	0.00	0.11	-0.17	0.26	1.00
	Groom (receive)	0.47	0.00	0.13	0.20	0.71	1.00
	Forage	0.14	0.00	0.08	-0.01	0.30	1.00
Date	Groom (give)	0.19	0.00	0.07	0.05	0.33	1.00
	Rest	0.24	0.00	0.04	0.17	0.32	1.00
	Groom (receive)	0.58	0.00	0.09	0.41	0.75	1.00
	Forage	0.12	0.00	0.04	0.04	0.19	1.00
fGCM	Groom (give)	-0.15	0.00	0.08	-0.30	0.00	1.00
	Rest	0.03	0.00	0.03	-0.03	0.10	1.00
	Groom (receive)	-0.07	0.00	0.07	-0.22	0.06	1.00
	Forage	-0.01	0.00	0.03	-0.07	0.06	1.00
Rank	Groom (give)	-0.40	0.00	0.17	-0.76	-0.08	1.00
	Rest	0.01	0.00	0.09	-0.14	0.19	1.00
	Groom (receive)	0.25	0.00	0.12	0.01	0.46	1.00
	Forage	0.06	0.00	0.07	-0.07	0.20	1.00
Interaction	Groom (give)	-0.17	0.00	0.09	-0.37	0.00	1.00
	Rest	-0.01	0.00	0.05	-0.12	0.09	1.00
	Groom (receive)	-0.11	0.00	0.10	-0.31	0.07	1.00
	Forage	-0.05	0.00	0.05	-0.16	0.05	1.00

Random Effects	Troop	Groom (give)	1.06	0.07	1.11	0.01	3.69	1.02
		Rest	0.16	0.00	0.19	0.00	0.69	1.00
		Groom (receive)	0.28	0.02	0.43	0.00	1.64	1.00
		Forage	0.13	0.01	0.19	0.00	0.61	1.00
	ID	Groom (give)	0.48	0.00	0.10	0.32	0.71	1.00
		Rest	0.22	0.00	0.04	0.15	0.31	1.00
		Groom (receive)	0.19	0.00	0.06	0.08	0.31	1.00
		Forage	0.14	0.00	0.03	0.09	0.21	1.00

C.5 Entropy rate results

Relationships between time, NDVI and entropy rate

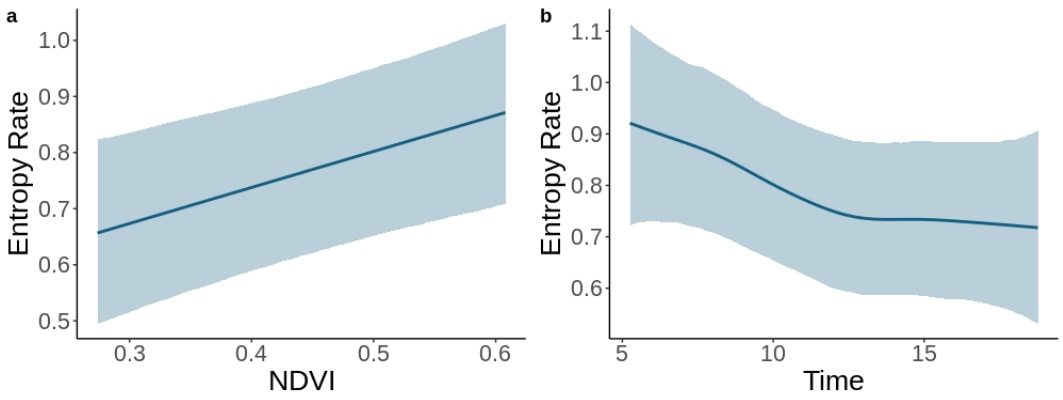

Figure D5 Changes in entropy rate in response to NDVI (a) and time of day (b) derived from the fitted generalised additive mixed effects model. Upper and lower 95% credible intervals (bands) were derived from the fitted model.

Appendix D: Supplementary Material for Chapter 7

D.1 Selection of Monthly Parasite Measure

For each individual, I quantified monthly parasite measures and matched these parasite measures to the corresponding behavioural data. For parasite intensity, I summed the total number of eggs recovered in the month for each genus and divided that by the total faecal weight of samples in the month. This gave me a monthly eggs/gram measure for each individual for each month of the study period. Cumulative egg count was used to avoid the possibility of over-estimating parasite intensity if only the highest egg of the month was used. To assess this approach, I repeated analyses using the maximum egg count for the month. Results were qualitatively similar (Tables E1.1 and E1.2)

Table D1.1 Summary statistics of Bayesian mixed-effects model for unique spatial partners. CI = credible interval; SD = standard deviation. Smooth-term sds() = spline "wiggliness"(spline variance parameter). Est.Error = Standard error of the estimate. $\widehat{R}$ (Rhat) provides information on convergence. At convergence, Rhat = 1. Estimates for fixed effects where credible intervals do not cross zero are in bold. Trich = *Trichostrongylus* sp. Proto = ?*Protospirura* sp.

	Effect	**Estimate**	**Est.Error**	**l-95% CI**	**u-95% CI**	$\widehat{R}$
Fixed effects						
Population-Level	Intercept	3.46	0.13	3.19	3.74	1
	Proto (EPG)	**0.06**	**0.02**	**0.01**	**0.1**	**1**
	Trich. (EPG)	-0.02	0.03	-0.08	0.03	1
	Rank	-0.02	0.03	-0.07	0.03	1
	Richness	-0.03	0.03	-0.08	0.02	1
	fGCM	0.01	0.02	-0.03	0.05	1
	Sex (ref:M)	0.01	0.03	-0.04	0.06	1
	Month	-0.03	0.02	-0.08	0.02	1
	s(scan count)	1.26	0.65	0.01	2.57	1
Smooth Terms	sds(scan count)	0.6	0.27	0.24	1.26	1
Random Effects	sd(Troop)	0.17	0.18	0.03	0.67	1
	sd(ID)	0.01	0.01	0	0.04	1

Table D1.2 Summary statistics of Bayesian mixed-effects model for unique grooming partners. CI = credible interval; SD = standard deviation. Smooth-term sds() = spline "wiggliness"(spline variance parameter). Est.Error = Standard error of the estimate. $\widehat{R}$ (Rhat) provides information on convergence. At convergence, Rhat = 1. Estimates for fixed effects where credible intervals do not cross zero are in bold. Trich = *Trichostrongylus* sp. Proto = ?*Protospirura* sp.

	Effect	**Estimate**	**Est.Error**	**l-95% CI**	**u-95% CI**	$\widehat{R}$
Fixed effects						
Population-Level	Intercept	2.09	0.19	1.67	2.5	1
	Proto (EPG)	0.02	0.05	-0.07	0.11	1
	Trich. (EPG)	-0.01	0.05	-0.11	0.09	1
	Rank	-0.11	0.06	-0.22	0	1
	Richness	-0.02	0.05	-0.13	0.07	1
	fGCM	0.02	0.04	-0.06	0.09	1
	Sex (ref:M)	-0.01	0.07	-0.15	0.13	1
	Month	0.03	0.04	-0.05	0.11	1
	s(dyad presence)	1.95	0.84	0.27	3.56	1
Smooth Terms	sds(dyad presence)	1.27	0.43	0.59	2.22	1
Random Effects	sd(Troop)	0.27	0.25	0.04	1	1
	sd(ID)	0.07	0.04	0.01	0.14	1.01

D.2 Posterior predictive check for the mixed effects model with a Poisson distribution (selected model used)

Due to poor model fitting and resulted posterior predictive checks for the relationships between parasite measures and unique social partners, several models, with different distributions, were fitted to improve model fit. These included the original Poisson distribution as well as Gaussian, negative binomial and negative binomial with observation level random effects. These models were then compared and the qualitative similarities of results assessed.

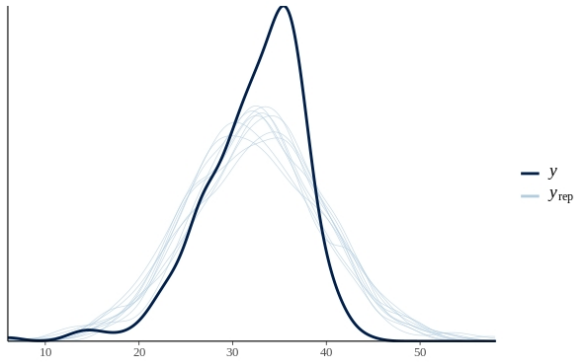

Figure D2 Posterior predictive check for generalised additive mixed-effects model with a Poisson distribution comparing the observed outcome variable (y) to simulated datasets (y_{rep}) from the posterior predictive distribution

D.3 Data Overdispersion Test for Unique social partners

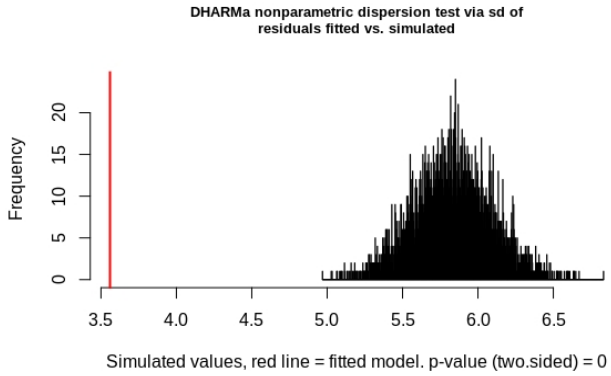

Figure D3 Results from the DHARMa non-parametric dispersion test showing underdispersion in the spatial proximity dataset when modelling a Poisson distribution. ratioObsSim = 0.61059, p-value < 2.2e-16

D.4 Posterior predictive check for the mixed effects model with a Gaussian distribution

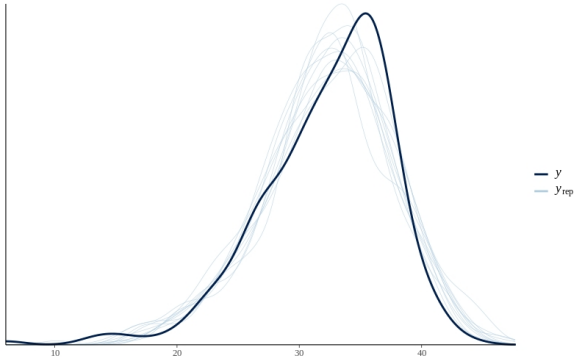

Figure D4 Posterior predictive check for generalised additive mixed-effects model with a Poisson distribution comparing the observed outcome variable (y) to simulated datasets (y_{rep}) from the posterior predictive distribution

Table D4 Summary statistics of generalised additive mixed-effects hurdle model with a Gaussian distribution examining the influence of environmental and social factors on entropy rate. CI = credible interval; SD = standard deviation. Smooth-term sds() = spline "wiggliness"(spline variance parameter)

	Effect	**Estimate**	**Est.Error**	**l-95% CI**	**u-95% CI**	$\widehat{R}$
Fixed effects						
Population-Level	Intercept	32.21	0.98	30.4	34.16	1.01
	Proto. (EPG)	**1.47**	**0.45**	**0.58**	**2.32**	**1**
	Trich. (EPG)	-0.64	0.5	-1.61	0.33	1
	Rank	-0.31	0.51	-1.3	0.69	1
	Richness	-0.07	0.51	-1.04	0.94	1
	fGCM	0.18	0.42	-0.62	1.01	1
	Sex (ref:M)	0.24	0.53	-0.82	1.26	1
	Month	-0.51	0.47	-1.4	0.42	1
	s(scan count)	0.49	1.02	-1.55	2.47	1.01
Smooth Terms	sds(scan count)	3.93	0.54	2.92	5.06	1
Random Effects	sd(Troop)	1.43	0.46	0.69	2.52	1
	sd(ID)	0.71	0.37	0.05	1.46	1.01

D.5 Posterior predictive check for the mixed effects model with a negative binomial distribution

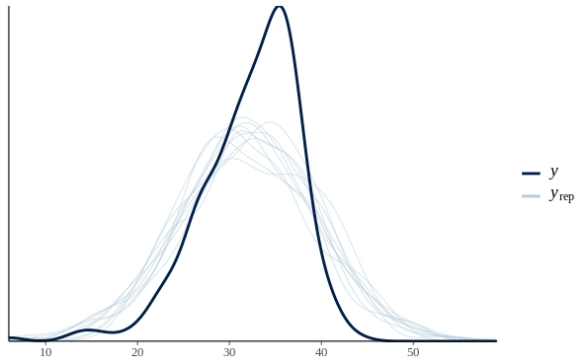

Figure D5 Posterior predictive check for generalised additive mixed-effects model with a negative binomial distribution comparing the observed outcome variable (y) to simulated datasets (y_{rep}) from the posterior predictive distribution

D.6 Posterior predictive check for the mixed effects model with a negative binomial distribution and observation-level random effects (OLREs)

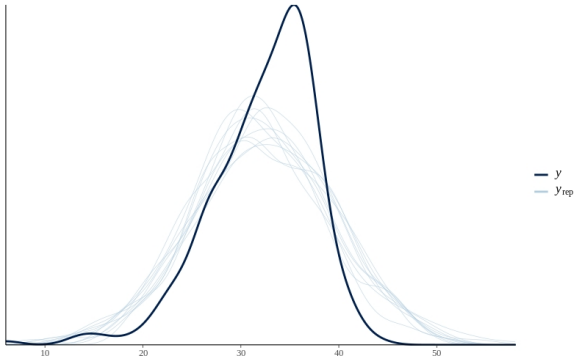

Figure D6 Posterior predictive check for generalised additive mixed-effects model with a negative binomial distribution and OLREs comparing the observed outcome variable (y) to simulated datasets (y_{rep}) from the posterior predictive distribution