A healthy social life? Sociality and health indicators in wild red-fronted lemurs

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“Here's to the crazy ones.

The misfits.

The rebels.

The troublemakers.

The round pegs in the square holes.

The ones who see things differently. [...]

You can quote them, disagree with them, glorify or vilify them.

About the only thing you can't do is ignore them.

Because they change things.

They invent. They imagine. They heal.

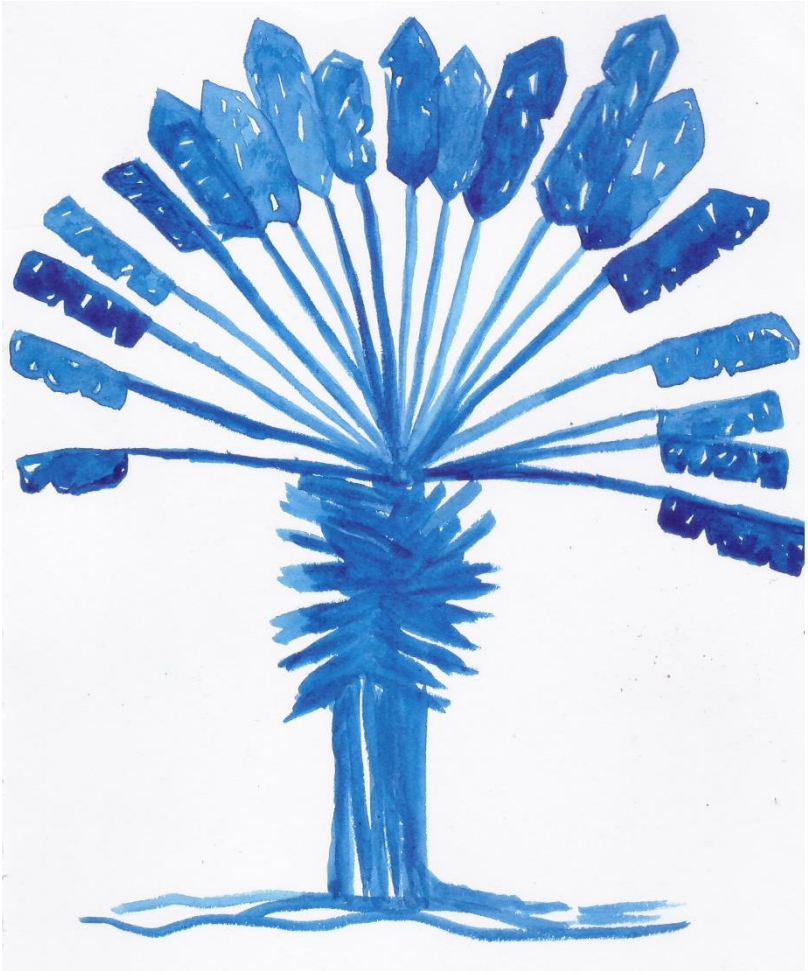
They explore. They create. They inspire. [...]

Because the people who are crazy enough to think they can change the world, are the ones who do.”

— *Rob Siltanen*

Content

General Introduction



Always laugh when you can. It is cheap medicine.

—Lord Byron

Sociality (i.e. life in permanent groups: Alexander, 1974; Kappeler & van Schaik, 2002; Krause & Ruxton, 2002) comes in many forms, with social species (and sometimes populations or groups) differing in their social organisation (e.g. group size, composition and cohesion), mating system (e.g. polygamy, polyandry, monogamy) and social structure (social relationships between individuals) (Clutton-Brock, 2016; Hinde, 1976; Kappeler & van Schaik, 2002; Silk & Kappeler, 2017).The transition from a solitary lifestyle to permanent group living is considered one of the major transitions in evolution (Clutton-Brock, 2009; Szathmary & Maynard-Smith, 1995). To understand the evolution of sociality, many researchers have investigated the factors favouring this transition and the costs and benefits of sociality at an individual level (Krause & Ruxton, 2002).

In the last 40 years, an increasing number of studies from medicine, neurosciences and psychology demonstrated links between many aspects of sociality, health and fitness in humans and laboratory animals (Eisenberger & Cole, 2012; Holt-Lunstad, 2018; Holt-Lunstad et al., 2010). Some studies highlighted strong detrimental effects of social isolation with effects comparable in magnitude to those of diabetes, obesity and heavy alcohol consumption (Holt-Lunstad et al., 2010) In contrast, multiple studies, in a range of primate species including humans, highlighted powerful benefits of strong or diverse relationships on longevity, reproductive success and susceptibility to communicable and non-communicable diseases (Cohen et al., 1997; Holt-Lunstad et al., 2010; Schülke et al., 2010; Silk et al., 2003).

However, most of these findings derived from captive studies or the medical sciences, whereas few studies took place in natural conditions under which the mechanisms driving these effects evolved. Furthermore, results appear sometimes equivocal or species-specific and the directionality of the sociality-health-fitness relationship remain unclear. As a consequence, our understanding of the mechanisms underlying this link and how it evolved in different animal societies is still limited (Nunn et al., 2015; Silk, 2007). Finally, despite an increased interest from a variety of scientific fields, ranging from evolutionary ecology to sociology and medicine, there are methodological, logistic and conceptual obstacles still to overcome to fully understand how sociality, health, and fitness are related.

In this chapter, I will outline the current knowledge and findings on the sociality-health-fitness link. Then I will shortly explain how I aimed to answer some of the remaining open questions during my PhD, focusing on the relationships between sociality and susceptibility to disease, parasite exposure and physiological responses.

What is health, what is sickness and how to measure it in wildlife?

Defining health is quite complex. One common adage for health, attributed to the Roman poet Juvenal says “a sound mind in a sound body”. Since 1946, the World Health Organization (WHO) defined health as "a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity” (WHO 1946, page 1315), adding a social dimension to this definition. While being very comprehensive by including the mental and social domains on top of the physical one, this definition has since then been criticised many times (Breslow, 1972; Callahan, 1973; M. Huber et al., 2011; J. S. Larson, 1996). First, the absoluteness of the term "complete" would lead to almost all living entities being considered unhealthy. Then, defining and measuring completeness of well-being would prove inherently difficult. Finally, this definition does not take into account the individual resilience and ability to cope with physical, mental and social challenges. Thus, this definition was revised many times. Considering health, not as a state, but as a dynamic process, Huber and colleagues (2011), reformulated the definition of health as "the ability to adapt and self-manage" (Huber et al., 2011, page 3) when facing social, physical, and emotional challenges. Throughout this thesis, I will follow this more recent definition.

Health issues are diverse (GBD 2013 Mortality and Causes of Death Collaborators, 2015). Injuries such as wounds and broken bones are a frequent health issue for humans and other animals (Archie et al., 2014; Baker et al., 1974; Bunnell, 2001; Glass et al., 1988; Horan & Mallonee, 2003; Pollock et al., 2016; Wang et al., 2018). They can induce pain and discomfort, alter behaviour, result in infections and ultimately reduce life quality and lead to death (Anderson & Hamm, 2012; Archie, 2013; Archie et al., 2014; Bunnell, 2001; GBD 2013 Mortality and Causes of Death Collaborators, 2015; Glass et al., 1988; Rojas et al., 2002; Wang et al., 2018; Wilson, 1992). Malnutrition also contributes to health issues as it deteriorates multiple body functions and is associated with a high mortality risk, especially in children (Bhutta et al., 2017; GBD 2013 Mortality

and Causes of Death Collaborators, 2015). Finally, diseases are the most common health issue.

In humans, it is estimated that approximately 36 million people die each year from non-communicable disease (World Health Organization, 2011), i.e. disease resulting from organic or systemic failures such as cardiovascular disease, cancer, diabetes and chronic lung disease. Communicable diseases which are caused by macroparasites (e.g. nematodes, ticks, lice) and microparasites (e.g. viruses, bacteria, fungi and protozoan), are even more of an issue because of their potential to spread among individuals. They cause millions of deaths every year (GBD 2013 Mortality and Causes of Death Collaborators, 2015; Kyu et al., 2018).

Macro- and microparasites or parasites and pathogens (hereafter called parasites, (Nunn & Altizer, 2006) are ubiquitous in the wild (Ezenwa et al., 2016; Nunn & Altizer, 2006). They are infectious organisms living in or on another organism (i.e. host), using some of its resources for their own benefits and to the detriment of their host (Combes, 2001). While there is an extensive knowledge of the parasite of humans and their domestic animal, the disease they cause and their effects on health and fitness in wildlife are much less known.

This lack of knowledge can be partly explained by the difficulties and challenges associated with measuring health or disease in wild animals. Yet, from a conservation standpoint, understanding the transmission and consequences of detrimental infections is crucial. This led to a magnitude of studies on potentially deadly diseases : *Chytridiomycosis* in amphibians (Rowley & Alford, 2007; Scheele et al., 2014); white-nose syndrome in bats (Langwig et al., 2012); transmissible facial tumours in tasmanian devils (McCallum et al., 2009); *Pasteurella multocida* type B in Saiga antelopes (Fereidouni et al., 2019); Anthrax and Ebola in great apes (Leendertz et al., 2006); Tuberculosis in buffalo (Ezenwa et al., 2010).

In contrast, the effects of non-lethal infections, such as ectoparasites and gastrointestinal parasites infections, which tend to be chronic, are more subtle, more difficult to detect and often underestimated (Krief et al., 2008), particularly when researchers are restricted to non-invasive sampling. Still, non-lethal infections can be energetically costly to their host, for example due to the resources used by the parasites for growing and reproduction and by the host for mounting and maintaining an immune

response (Bonneaud et al., 2003a; Colditz, 2008). Gastro-intestinal parasites can have long-term negative consequences on host health and fitness (Pedersen & Greives, 2008), including deteriorated body condition (Sánchez et al., 2018), increased susceptibility to other parasites (Ezenwa et al., 2006; Müller-Klein et al., 2019) and increased interbirth intervals (Akinyi et al., 2019).

Since social life increases the risk of parasite transmission and because of their detrimental effects on their hosts, parasites can act as a selective force for social evolution (Freeland, 1976). This impact of parasites on fitness and ultimately evolution led to an increase in research on parasite infections determinants (Altizer et al., 2003; Benavides et al., 2012; Habig et al., 2019; Hawley et al., 2011; Poirotte et al., 2016; VanderWaal & Ezenwa, 2016) and their fitness consequences (Akinyi et al., 2019; Chapman et al., 2009; Ghai et al., 2015).

Hosts' behavioural changes in reaction to infections in wild or semi-wild populations remains a largely unexplored topic (Ghai et al., 2015; Hetem et al., 2008; Krief et al., 2005; Müller-Klein et al., 2019; Weary et al., 2009). This paucity could be attributed to reduced activity and social interest in sick individuals, which may differ between individuals, context and parasites (Hennessy et al., 2014), but would make behavioural observations and sample collection more difficult. Indeed, as a result of infection, hosts can display a group of behavioural changes called "sickness behaviour" that are triggered by pro-inflammatory cytokines (Aubert, 1999; Dantzer, 2009). These changes in behaviour aid in fighting the infection (Johnson, 2002; Robert Poulin, 1995), reduce predator exposure (Hart, 1988) and, in social animals, reduce disease spread to the group (Shakhar & Shakhar, 2015). Sickness behaviour is characterised by a reduction in social interest, appetite and activity levels and sometimes accompanied by fever (Aubert, 1999; Dantzer & Kelley, 2007; Hart, 1988; Johnson, 2002). Sickness behaviour have been described in many taxa (insects (Sullivan et al., 2016), birds (Marais et al., 2013; Owen-Ashley & Wingfield, 2006), rodents (Aubert et al., 1997), ungulates (Hetem et al., 2008) but mostly for acute infections with pathogens in laboratory animals, as they can be provoked by lipopolysaccharide (LPS) injections

Diagnostics of health status in wild population remains a challenge. While deadly diseases make observation easier and allow for sampling from multiple tissues when finding dead individuals (Leendertz et al., 2006), repeated observation of sick individuals

can prove difficult. A major limitation of wildlife research is the number and type of samples and parameters one can measure. Capturing individuals - including anaesthesia, handling and taking samples - can not only be detrimental to the animals and raises ethics concerns (Behringer & Deschner, 2017; Cunningham et al., 2015; Fedigan, 2010), but it can also prove difficult with some species.

In the last two decades, multiple alternatives to blood tests have been developed. The improvement of genetics techniques made the detection of bacteria and viruses from faecal samples possible (Köndgen et al., 2010) but this kind of analysis remains costly and rather exploratory as most wildlife microparasites are not described yet. However, because of their potential social transmission (MacIntosh et al., 2012; Rimbach et al., 2015), their negative effect on their host condition and health (Pedersen & Greives, 2008; Sánchez et al., 2018) and the ease to monitor their presence non-invasively in faeces (Gillespie, 2006), gastro-intestinal parasites are routinely studied to determine infection determinants and consequences in wildlife.

Additionally, several techniques exist to measure health and physiology non-invasively (Behringer & Deschner, 2017; Wikelski & Cooke, 2006). In particular, endocrinology is a leading field with techniques allowing non-invasive measurements of physiological markers of e.g. stress, nutritional balance, dominance and competition, bond formation or maintenance (see Behringer & Deschner, 2017 for a review). Other ways to assess an individual general health condition can be via visual cues (wounds: Archie, 2013; running noses Boesch, 2008; fur condition Berg et al., 2009; Borg et al., 2014), behavioural changes such as lethargy (Patrono & Leendertz, 2019), non-invasive temperature measurements (Chaise et al., 2019; Löhrich et al., 2018) and body mass recordings (J. Altmann & Alberts, 1987; MacLeod et al., 2013) to get a general overview of an individual's health condition.

While a large range of tests of immune status and immune response are available using blood (e.g. antibodies, inflammatory markers, cytokines), it is more challenging to deal with non-invasive samples because the techniques were traditionally designed for blood samples (Behringer & Deschner, 2019; Dibakou et al., 2019; Gesquiere et al., 2020; Higham et al., 2015, 2020; Müller et al., 2017). However, it seems necessary to develop non-invasive markers of immunity and collect these jointly with infection status and

behavioural data (Müller-Klein et al., 2019) to identify the causes and consequences of chronic non-lethal diseases in the wild.

Costs of sociality on health and fitness

Increased risk of disease transmission is one of the major costs of group-living (Anderson & May, 1979; Freeland, 1976; Kappeler et al., 2015; Loehle, 1995). Transmission consists of two distinct processes, both of which can be impacted by social interactions: exposure to infectious pathogen stages (behavioural mechanism) and susceptibility to the respective pathogen (physiological mechanism). Exposure to parasites increases in social species in comparison to solitary ones because conspecifics act as transmission pathways and as parasite reservoir, especially due to their agglomeration in space and time. In particular, the increased density of potential hosts, use of same territory (for example for foraging, drinking and resting), as well as social contact (e.g. mating, grooming, playing) have been identified as determinants of increased parasite exposure (Altizer et al., 2003; Nunn et al., 2011). These differences do not only exist between social and solitary species, but between and within groups of the same social species (MacIntosh et al., 2012; Rimbach et al., 2015; Springer et al., 2016). Intraspecific variations in group size and density have been associated with variation in infection risk and parasite richness (Altizer et al., 2003; MacIntosh et al., 2012; Patterson & Ruckstuhl, 2013; Vitone et al., 2004). Similarly, social interaction patterns can vary among group members and thus influence parasite transmission. In particular, factors such as the number of social or mating partners (Paciência et al., 2019; Wren et al., 2016), proximity with group members and time spent in affiliative behaviours (Friant et al., 2016; González-Hernández et al., 2014; Hernandez & Sukhdeo, 1995; MacIntosh et al., 2012) have all been positively associated with parasite transmission in primates and rodents. In particular, social network position plays an important role for parasite spread, with more central individuals contributing more to transmission than peripheral individuals as they can connect subgroups through the network (Griffin & Nunn, 2011; Hamede et al., 2009; MacIntosh et al., 2012; Rimbach et al., 2015; Springer et al., 2016).

Infection risk differs according to the parasite transmission mode, which is why sociality can play different roles in parasite exposure depending on the parasite species.

Most parasites with direct life cycles are transmitted environmentally (Altizer et al., 2003; Anderson, 2000; Bethony et al., 2006; Hawley et al., 2011), via contact with infectious stages or by ingestion. Close proximity and social contact such as grooming increase the spread of directly transmitted parasites such as viruses, bacteria and some gastro-intestinal nematodes and protozoans (Anderson, 2000; Bethony et al., 2006; Craft et al., 2011; Fenner et al., 2015; Godfrey et al., 2009; Hernandez & Sukhdeo, 1995; Konrad et al., 2012; MacIntosh et al., 2012; Mossong et al., 2008; Rimbach et al., 2015; Springer et al., 2016; Vicente et al., 2007). Aggressive behaviour can also contribute to parasite exposure. For example, the transmission of the Tasmanian devil tumour disease is associated with aggressive behaviour (Hamede et al., 2013) and tuberculosis transmission in meerkats is associated with grooming given but also aggression received (Drewe, 2009). However, whether social behaviours contribute to disease transmission is context and pathogen dependent, as demonstrated by the reduction of ectoparasites by grooming in non-human primates (Duboscq et al., 2016) and decreased transmission of fungal infections in ants (Cremer et al., 2007; Konrad et al., 2012).

Under certain circumstances, social environment can lead to detrimental health effects for individuals via increased disease susceptibility to disease (communicable or not). Agonistic interaction between dominant individuals and their subordinates, which are typical of group living, can provoke elevations of steroid hormones (e.g. testosterone and glucocorticoids), as can social instability (Abbott et al., 2003a; Capitanio & Cole, 2015; Sapolsky, 2005a). In return, prolonged elevation of steroid levels can result in reduced immunocompetence, muscle breakdown, osteoporosis, deteriorated memory and vigilance abilities and decreased reproductive functions (Sheldon Cohen et al., 2012; Koolhaas et al., 2011; McEwen, 2012). Finally, groups usually comprise more genetically closely related individuals (e.g. usually females in non-human primates, all colony members in eusocial insects). Since relatives tend to have similar genotypes, this makes close kin more susceptible to similar diseases due to their immunogenetic similarities (Benton et al., 2016; Shykoff & Schmid-Hempel, 1991; Wilson-Rich et al., 2009).

In addition, environmental factors such as temperature and rainfall (Benavides et al., 2012; Gillespie et al., 2010; Nunn & Altizer, 2006) and individual-related factors such as age (Izhar & Ben-Ami, 2015; Lo et al., 1998; Nunn & Altizer, 2006; Pacala & Dobson,

1988), sex (Escobedo et al., 2010; Klein, 2004; Morales-Montor et al., 2004; Nunn & Altizer, 2006), reproductive status (Klein, 2004), or hormone levels (Nunn & Altizer, 2006; Roberts et al., 2004; Sapolsky, 2005b) can influence exposure and susceptibility to disease, making the picture quite complex. Longitudinal and comprehensive studies on parasite infections are rare, some focusing on social factors, some on environmental factors and other on individual related factors (Grear et al., 2013; Pebsworth et al., 2012), but almost never all three together. In my thesis, I aim to assess the relative importance of all three components of parasite infections.

The previous paragraphs highlighted the importance of social factors on both exposure and susceptibility to parasites, which in return can impact individual health and ultimately fitness (French et al., 2009; Graham et al., 2010). Thus, why would species evolve to live in social groups, with such costs to bear?

Social strategies to mitigate costs of sociality on health:

Animals evolved different strategies to mediate costs of sociality on health and especially increased infectious disease risk. In social insects, a set of collective cooperative strategies and called social immunity have developed to lower disease transmission risk (Cremer et al., 2007; Fefferman et al., 2007; Schmid-Hempel, 2017; Traniello et al., 2002; Walker & Hughes, 2009). Social insects are particularly vulnerable to infectious disease because of their close proximity in their nests, their high level of interaction and the high relatedness between all individuals (Cremer et al., 2007; Schmid-Hempel, 2017). These strategies are based on control and elimination of parasites at the colony-level. Ants have been observed isolating heavily infected individuals (Cremer et al., 2007), removing corpses from the nest (Diez et al., 2014), reorganising their social network into smaller subgroups to limit parasite transmission (Stroeymeyt et al., 2018) and grooming mildly infected individuals as a way to remove parasite but also transfer immunity to the groomer (Konrad et al. 2012). Interestingly, social insects involved in social immunity do not bear the immunity-reproduction trade-off because only the queen reproduces (Cremer et al., 2007; Schmid-Hempel, 2003) but by participating in social immunity they increase their inclusive fitness. Thus, studies of social insect's social immunity are a great avenue of research to understand the evolution of social strategies to limit parasite transmission (Schmid-Hempel, 2003).

A set of strategies called parasite avoidance behaviours is also used by social animals to mediate increased risk of parasite transmission (Rivas et al., 2014; Sarabian et al., 2018). This consists of a set of behavioural responses to reduce the risk of infection e.g. avoiding parasites and signs of their presence in conspecifics, heterospecifics, habitat, food and drinks (Curtis, 2014; Curtis & Biran, 2001; Hart, 1994; Hart, 2011; Sarabian et al., 2018). Animals from different taxa avoid infected conspecifics, for example, mandrills (*Mandrillus sphinx*) groomed less group members with a higher diversity of oro-faecally transmitted protozoans (Poirotte et al., 2017)and Carribean spiny lobsters (*Panulirus argus*) avoided sharing shelters with lobsters infected with a lethal virus (Behringer et al., 2006)). Another adaptive behaviour is to avoid parasite rich-environments: ungulates and marsupials avoid grazing on faecally contaminated patches (Coulson et al., 2018) and redfronted lemurs avoid faecally contaminated water sources (Amoroso et al., 2019).

Finally, to mediate or avoid costs of sociality related to parasite transmission, some animals use self-medication, a set of behaviours used by a host to prevent or reduce the negative effects of parasites and other causes of disease (Huffman, 1997, 2003). These behaviours include dietary selection (e.g. Krief et al., 2005), ingestion of non-nutritional substances for a therapeutic purpose (e.g. Huffman, 1997) and anointment of a substance to the body (e.g. Birkinshaw, 1999; Gasco et al., 2016; Morrogh-Bernard, 2008; Weldon, 2004). For example, chimpanzees (*Pan troglodytes*) are known to use plants containing secondary compounds with therapeutic effects against gastro-intestinal nematode infection (Huffman, 1997, 2015). Anointment of various plant or animal parts as protection against ectoparasites has been observed in a wide range of taxa (several primates species: (Huffman, 2015; Laska et al., 2007; Lynch-Alfaro et al., 2012; Zito et al., 2003); several bird species: (Morozov, 2015; Potter, 1970; Sazima, 2009; Wenny, 1998), and many other species e.g. European hedgehogs (*Erinaceus europaeus*, D'Havé et al., 2005), grey squirrels (*Sciurus carolinensis,* Hauser, 1964) and giant pandas (*Ailuropoda melanoleuca,* Charlton et al., 2020).

Multiples mechanisms exist to avoid or mitigate costs of sociality on health. Additionally, some studies showed that sociality can provide benefits on health and fitness.

Benefits of sociality on health and fitness

While sociality generates costs due to increased disease transmission, there is also evidence for benefits of sociality on health and fitness in humans and other animals (Holt-Lunstad, 2018; Holt-Lunstad et al., 2010; House et al., 1988; Silk et al., 2003, 2009, 2010), potentially buffering against costs of sociality. These benefits are essentially based on social integration and dyadic affiliative relationships. When stable over long periods and specific to a few social partners, dyadic affiliative relationships have been referred to as social bonds (Silk, 2002). Maintaining social bonds can be costly in terms of time and energy invested (Dunbar, 2009). However, the existence of social bonds and the fact that individuals compete over valuable partners (Haunhorst et al., 2020; Mielke et al., 2019; Palombit et al., 2001) highlight their importance.

In humans, meta-analysis pinpointed the adaptive effects of sociality and especially social integration and social bonds on health and fitness Holt-Lunstad et al., 2010; House et al., 1988). Social integration and strong social bonds predict lower mortality, with effect sizes comparable to known predictors of mortality risk, like smoking or heavy alcohol consumption (Holt-Lunstad et al., 2010). Individuals with more diverse relationships have increased longevity and are less susceptible to communicable and non-communicable disease (Cohen et al., 1997; Sheldon Cohen & Janicki-Deverts, 2009; Holt-Lunstad et al., 2010; Miller et al., 2011). In non-human animals as well, correlational studies pointed out long-term effects, similar to those found in humans. In many species, there is a positive relationship between females' strong social bonds and reproductive success, longevity and infant survival (baboons: Silk et al., 2003, 2009, 2010; horses: Cameron et al., 2009; bottlenose dolphins: Frère et al., 2010; humpbacked whales: Ramp et al., 2010). Males can also improve their reproductive success via sociality. For example, in macaques, coalitionary support increases reproductive success (Berghänel et al., 2011; Schülke et al., 2010; Young et al., 2013).

Furthermore, studies on isolation and stress showed that social integration and social support impact both behaviour and physiology and are vital for social animals including humans. In non-human vertebrates, models of loneliness in captivity resulted in changes in activity patterns, deteriorated physiological states and increased mortality rates (Cacioppo et al., 2015; Hawkley & Capitanio, 2015). Similarly, in humans, low socio-

economic status and stress affect health negatively (Thoits, 2010; Umberson et al., 2014) and these effects can last across an individual's complete life course (Taylor, 2010; Umberson et al., 2010). For example, stress and lack of social support are associated with depression (Stafford et al., 2011), early onset and faster progression of several types of cancer (Antoni et al., 2006), and a decrease of life expectancy by up to 50% (Holt-Lunstad et al., 2010).

While many studies highlighted the importance of the quality of social bonds, others showed the importance of their quantity, opening a debate (Silk et al. 2018). In particular, number of social partners improved thermoregulation and survival in Barbary macaques (McFarland & Majolo, 2013) and vervet monkeys (*Chlorocebus pygerythrus*) (McFarland et al., 2015). Additionally, high number of social partners also increased infant survival in chacma baboons (McFarland et al., 2017). Thus, some researchers suggested that the integration of an individual within its social network rather than the quality or quantity of dyadic relationships is of importance for the sociality-health relationship (Brent, 2015; Brent et al., 2013; Cheney et al., 2016; Ellis et al., 2017; Ellis et al., 2019; Stanton & Mann, 2012). For example, in an experimental infection with respiratory viruses, socially well integrated individuals had a lower infection risk (humans: Cohen et al., 1991, 2003, 2015; long tailed macaques (*Macaca fascicularis*): Cohen et al., 1997). However, despite the many studies pointing out costs and benefits of sociality on health in humans and other animals, the proximate mechanisms mediating these positive or negative effects and their evolutionary foundation remain largely unexplored (Hawkley & Capitanio, 2015; Kappeler et al., 2015; Nunn & Altizer, 2006; Nunn et al., 2015; Ostner & Schülke, 2018). Among the potential mechanisms mediating these effects, physiological stress received a lot of attention and seems pivotal in the sociality-health relationship.

The role of physiological stress

Stressors are aversive and often unpredictable stimuli that destabilise an individual's homeostasis and cause a physiological stress response (Madliger & Love, 2014; McEwen, 2007, 2014; Sapolsky et al., 2000). Two main physiological systems are involved in the stress response: the sympathetic nervous system and the neuroendocrine system. Initially, the hypothalamus activates the sympathetic nervous system within seconds or

even milliseconds (Sapolsky et al., 2000) and norepinephrine and epinephrine are secreted, eliciting an increase in heart rate, blood pressure and respiration. Within minutes to hours of this initial response, the neuroendocrine system is activated (Sapolsky, 1992; Sapolsky et al., 2000), with a rise in hypothalamic–pituitary–adrenal (HPA) axis activity (Mendoza et al., 2000; Sapolsky et al., 2000) leading to increased secretion of glucocorticoid (GC) hormones. Then, within minutes to days, elevations of GC levels cause reallocation of energy and resources to systems directly linked to survival (e.g. immune function and cognition), while others are inhibited (e.g. digestion and reproductive function) (McEwen, 2007; Sapolsky et al., 2000). Hence, under normal conditions, acute GC elevation is beneficial, as it allows individuals to cope with short-term challenges and to return to homeostasis (Charmandari et al., 2005; Sapolsky et al., 2000). However, multiple stressors accumulating over time, or becoming chronic or unavoidable, can result in chronically elevated GC levels (Edes & Crews, 2017; McEwen, 1998).

Whether chronically elevated GC levels are adaptive or detrimental is still debated. On the one hand, no study has found detrimental effects of chronically elevated GC levels on individual fitness in natural populations (Beehner & Bergman, 2017; Boonstra, 2013). On the other hand, a large number of biomedical studies and studies on captive animals have shown detrimental effects of chronically elevated GC levels or dysfunctions of the regulatory mechanisms of GCs on individual health and fitness by reducing investment in reproduction and immune function (Dhabhar et al., 1995; Elenkov & Chrousos, 1999; Korte et al., 2005; McEwen, 1998, 2014; Romero et al., 2009), a concept called allostatic load (Edes & Crews, 2017; McEwen, 1998). If chronically elevated or when regulatory mechanisms are impaired, high GC levels can affect the immune system by dysregulating inflammation processes (Cohen et al., 2012), modifying production and activation of cells involved in the immune system (Dhabhar et al., 1995; Elenkov, 2004; Romero & Butler, 2007) and provoking a shift towards cell mediated T-helper type 2 (Th2) immune responses (Elenkov & Chrousos, 1999). Finally, long-lasting GC levels elevations can lead to immunosuppression (Beldomenico & Begon, 2016; Sapolsky et al., 2000) and negatively affect survival (Hufschmid et al., 2014; Muehlenbein, 2006; Stowe et al., 2001).

From studies in human and non-human primates, there is evidence that social stress can have profound negative effects on health. In humans, social isolation is associated with higher levels of perceived stress and deteriorated physiological functioning (Cacioppo & Hawkley, 2003) and with higher HPA axis activity and inflammatory signalling (Hawkley et al., 2012; Hennessy et al., 2014). Higher stress levels or perceived social stress were also associated with altered transcription patterns of immune genes (Cole et al., 2007) and metabolic, cardiovascular disease and mental illnesses in humans (Caserta et al., 2008; Glaser & Kiecolt-glaser, 2009; Hawkley & Capitanio, 2015; Holt-Lunstad et al., 2010; Kiecolt-Glaser et al., 2010). Similarly, in non-human animals, social stress and elevated GC levels are associated with lower cognitive performances, adverse health outcomes and lower longevity (Blas et al., 2007; Bonier et al., 2009; Buchanan et al., 2008; Cavigelli et al., 2009).

Additionally, in non-human animals, many studies found a strong relationship between health, fitness and social status (Archie et al., 2012; Cavigelli & Caruso, 2015; Cavigelli & Chaudhry, 2012; Marescot et al., 2018; Sapolsky, 2005b). In particular, dominance rank is associated with variations in GC levels with differences between dominants and subordinates depending on the social system (Abbott et al., 2003b; Gesquiere et al., 2011; Ostner et al., 2008; Sapolsky, 2005b; Schoof & Jack, 2013). Social instability (i.e. periods of rank change or male immigration) generally lead to increased GC levels (Engh et al., 2006; Sapolsky, 2005b; Wittig et al., 2008). Ultimately, chronic social stress can lead to increased disease susceptibility (Capitanio et al., 1998; Dhabhar, 2009; Glaser & Kiecolt-Glaser, 2005).

In contrast, affiliative interactions, strong bonds and social support can increase an individual's ability to cope with challenging situations, and buffer against the adverse effects of stress (Cheney & Seyfarth, 2009; Sheldon Cohen & Wills, 1985; Kikusui et al., 2006; Kiyokawa & Hennessy, 2018; Taylor et al., 2010). In particular, in baboons, correlational studies showed that while social stress has negative effects on body condition and fitness outcomes, bonding can mitigate these costs (Crockford et al., 2008; Silk et al., 2003, 2009; Wittig et al., 2008). This effect has been referred to as social buffering (Sheldon Cohen & Wills, 1985) and could explain the health benefits of social relationships (Berkman & Syme, 1979; Holt-Lunstad et al., 2010; House et al., 1988) for

both acute and chronic stressors (Abbott et al., 2003b; Cheney & Seyfarth, 2009; Sapolsky, 2002).

Social buffering can occur if an individual' is well integrated within its social network (i.e. structural support: Sheldon Cohen & Janicki-Deverts, 2009; Kikusui et al., 2006; Kiyokawa, 2018), and also by the mere presence of- or interaction with a conspecific during stressful times (i.e. direct support: Ishii et al., 2016; Kiyokawa, 2018). While structural support influences the early response to a stressor, direct support influences the readjustment from a stressor (Kiyokawa, 2018; Kiyokawa & Hennessy, 2018). As a consequence, social buffering can lower the perceived severity of a stressor and GC levels in well integrated individuals, leading to beneficial health outcomes.

Social buffering relies on multiple physiological mechanisms influencing susceptibility to communicable and non-communicable diseases. For example, in humans it was linked with lower cardiovascular reactivity (Uchino & Garvey, 1997), lower inflammation levels (Cacioppo & Hawkley 2003; Kiecolt-Glaser et al. 2010) and lower sympathetic nervous system activation (Cacioppo & Hawkley, 2003; Eisenberger & Cole, 2012; Inagaki & Eisenberger, 2016). In wildlife, positive social interactions have been correlated with increased endorphin levels (Keverne et al., 1989), oxytocin release (Crockford et al., 2013) and reduced HPA axis activation (Aureli & Yates, 2010; Shutt et al., 2007). In non-human primates, strongly bonded individuals had lower GC levels when facing stressful events (Barbary macaques: Young et al., 2014; chimpanzees: Wittig et al., 2016; baboons: Cheney & Seyfarth, 2009).

Studies in non-human primates pinpoint an interplay between behaviour and physiology. For example, strongly bonded chimpanzees had lower GC levels but higher oxytocin levels when facing stressful events (Crockford et al., 2013; Samuni et al., 2017). Oxytocin can downregulate HPA axis activity (Kikusui et al., 2006; Li et al., 2017) but also facilitate social relationships (Witt et al., 1992). Additionally, following social stressors, baboons who adapted their grooming behaviour at the onset of HPA axis activation had lower GC levels or returned faster to baseline level (Engh et al., 2006a; Wittig et al., 2008).

Consequently, social stress can have an important impact on health and fitness. On the one hand, there is evidence that social support and integration can provide an immediate beneficial effect on well-being and a protective effect from adverse effects of social stressors (Cohen & Wills, 1985). On the other hand, sociality can bring stress-related costs such as immunomodulation (Sapolsky, 2005b). Moreover, sociality does not always counter the effects of social stress with some findings pointing out potential species-specific or context specific benefits of sociality. For example, in blue monkeys, inconsistent strong bonds decreased survival while consistent strong bonds increased survival (Thompson & Cords, 2018) and marmots with higher partner numbers had lower survival over winter (Blumstein et al., 2018), suggesting a potential negative effect of social stress overriding other benefits.

However, to date, only few studies have simultaneously investigated effects of sociality and stress on health and individual fitness in the wild (Akinyi et al., 2019; Habig et al., 2019; Wascher et al., 2018). This is why mechanisms and causality of the sociality-health relationship remain poorly understood (Beehner & Bergman, 2017; Peter M Kappeler et al., 2015; Julia Ostner & Schülke, 2018), and it is necessary to widen the range of species examined under natural conditions.

Why focus on primates and especially redfronted lemurs?

Non-human primates are excellent models for investigating the relationship between sociality, stress, and health (Beehner & Bergman, 2017; Peter M Kappeler et al., 2015). First of all, most primate species live in groups which can have complex social organisations and exhibit a large diversity of social behaviour (Peter M. Kappeler & van Schaik, 2002; Mitani et al., 2012; Van Schaik & Kappeler, 1997). Primate populations are studied all around the globe in long-term field studies (Peter M. Kappeler & Watts, 2012; Riley & Bezanson, 2018), with data available on their demography and life-histories and the possibility to observe one or more groups over extended periods.

Primate physiology is relatively well described thanks to the similar immune and endocrine systems in vertebrates (Boehm, 2012; Coe, 1983; Martin, 2009) and the use of non-human primates in biomedical research. Primates also host diverse parasites and pathogens, some of which are already described and used as health indicators (Blersch et al., 2019; Erkenswick et al., 2019; Gillespie, 2006; Irwin & Raharison, 2009; Poirotte

et al., 2016). Non-invasive assessment of glucocorticoid metabolites, general body condition and parasite infection status are commonly used in primate studies as health indicators (Gillespie, 2006; Sánchez et al., 2018; Sheriff et al., 2011) and to assess the ability of wild populations to cope with environmental challenges (Chapman et al., 2007; Romero & Wikelski, 2001). Furthermore, the development of techniques for monitoring immune function and inflammation non-invasively in non-human primates is a blooming field (Gesquiere et al., 2020; Higham et al., 2015, 2020). Finally, because of their close relatedness with humans, findings from non-human primate studies can be reused for human health policies and evolutionary pathways can be reconstructed.

Within the primate order, lemurs have received less attention concerning their social relationships and health status. However, some characteristics of the sociality-health relationship might be evolutionary old. Being phylogenetically less close to humans and anthropoid primates such as macaques, baboons and chimpanzee, lemur studies could provide a better understanding of the evolution of the sociality-health relationship and its state in their last common ancestor (Fichtel & Kappeler, 2010). In this sense, lemurs are interesting models as their last common ancestor with primates outside of Madagascar lived in the Eocene (Karanth et al., 2005; Tattersall, 2007). Furthermore, Lemurs have diverse social organisations (Cossio et al., 1993; Alison Jolly, 1966; Peter M. Kappeler & Fichtel, 2015), this variety allowing potential comparative studies. Group living lemurs (as opposed to solitary ones or pair living ones) live in groups on average smaller than in haplorrhines (Peter M. Kappeler & Heymann, 1996) and group living lemurs are characterised by female dominance, with the exception of some species having no clear linear hierarchy (Ostner & Kappeler, 1999; Pereira & McGlynn, 1997; Wright, 1999). Finally, lemurs host a broad diversity of parasites (Alexander et al., 2016; Bublitz et al., 2015; Clough, 2010; Irwin & Raharison, 2009; Zohdy et al., 2015), which can be monitored as health indicators.

Redfronted lemurs (*Eulemur rufifrons*) are particularly of interest to investigate the sociality-health relationship due to their social organisation. I studied a wild population of redfronted lemurs in Kirindy forest, western Madagascar for a longitudinal period of 18 months from May 2015 to October 2016. They are cathemeral, quadrupedal, small-bodied primates (35-48 cm without the tail and 1.2-2.5kg) from the *Lemuridae* family.

This semi-arboreal species uses the ground for walking, foraging and sometimes resting. They live in small multimale-multifemale group with on average 8-9 members and females are philopatric (Mittermeier et al., 2008). All individuals have been habituated to behavioural observations, individually marked and regularly studied since 1996 (Peter M. Kappeler & Fichtel, 2012a). Demography, life history traits, and maternal relatedness of all individuals are known. This population consisted of 42 individuals (1 to 23 years old) from five groups (group size range 3-16).

In contrast to most lemur species, females are not dominant over males and do not form a clear linear hierarchy among them. Instead, there seems to be one central male per group, dominating other males and monopolising most of all mating, with no linear hierarchy between subordinate males (Kappeler & Port, 2008; Ostner & Kappeler, 1999; Pereira & McGlynn, 1997). Additionally, redfronted lemurs show high levels of social tolerance (Fichtel et al., 2018; Pereira et al., 1990) and group cohesion (Pyritz et al., 2011; Sperber et al., 2019). They breed seasonally, mating occurring usually in the middle of the dry season (May/June) and birth at the end of the dry season (September/October) (Julia Ostner et al., 2002, 2008). Aggression rates are most of the time low but frequent periods of social instability can occur all year long with typically two predictable periods of social instability during the mating and the birth season (Peter M. Kappeler & Fichtel, 2012b; Peter M. Kappeler & Port, 2008). During instability periods, females can be evicted by other females group members and males can take over groups.

Additionally, this species' diverse gastro-intestinal parasite community has been already described (Clough, 2010) and the use of toothcomb instead of fingers for grooming is an interesting characteristic of lemurs that could favour transmission of parasite with direct life cycles (Clough, 2010). Previous findings reported a negative correlation between fGCm levels and nematode infection, which is consistent with a polarized Th2 response (Clough et al., 2010). Assays for the non-invasive assessment of faecal metabolites of testosterone and glucocorticoids have already been validated and showed large variability (Julia Ostner et al., 2002, 2008). So far, neither social status nor aggression rates could explain stress levels in terms of fGCm output in male redfronted

lemurs, suggesting that more subtle aspects of social relationships might play a role (Julia Ostner et al., 2002, 2008).

All these characteristics and, in particular, the combination of a socially relatively tolerant species with frequent social instability make this species particularly suited for a study of the relationship between the sociality and health in the wild.

Objectives and structure of the thesis

The main aim of my Ph.D. project is to identify the proximate mechanisms mediating the link between sociality and health - in general and in wild redfronted lemur. In particular, I want to examine the relative importance of social factors on the transmission of and susceptibility to parasites and determine the relative importance of physiological stress (here faecal GC metabolite, fGCm) variations as a mediator of these effects. To do so, I investigate correlations among various social factors (group size, number of social partners, rate of affiliative and agonistic behaviours), susceptibility factors and health indicators (e.g. fGCm, faecal testosterone metabolites (fTESTm), body mass variation as a proxy of body condition, faecal C-reactive protein (CRP), an acute phase protein and marker of inflammation (Mark B. Pepys & Hirschfield, 2003) and gastro-intestinal parasite infections). The central hypothesis is that social life impacts individual health simultaneously via exposure and susceptibility to infectious diseases but with differential effects according to parasite species.

In chapter 1, I investigate patterns of the parasite-glucocorticoids relationship in mammals by conducting a meta-analysis, using two datasets, one from experiments (infections or deworming) and one from observations. My aim is to identify general patterns of this relationship and the main factors affecting it.

In chapter 2, I characterise the parasite community of redfronted lemurs along with two sympatric lemur species the grey mouse lemur (*Microcebus murinus*) and the fat-tailed dwarf lemur (*Cheirogalus medius*). I discuss different measures of parasite diversity plus host specificity.

In chapter 3, I examine determinants of parasite infection with nematodes and protozoan and parasite richness. Then, I investigate physiological and behavioural

correlates of parasite infection, such as activity, feeding, body mass, grooming received and grooming given.

In chapter 4, I report observations of fur-rubbing with millipedes and their subsequent ingestion in redfronted lemurs at the onset of the rainy season as a potential self-medicative behaviour against gastro-intestinal parasites.

In chapter 5, I test the biological validity of non-invasive C-reactive protein (CRP) measurement in faecal samples from two species of lemur: redfronted lemurs and ringtailed lemurs (*Lemur catta*). In particular, I examine faecal CRP metabolites variation in relation to parasite infection and parturition.

In chapter 6, I investigate the determinants of faecal glucocorticoids metabolites (fGCm) variations in redfronted lemurs, and especially the relative importance of sociality in comparison to ecology and reproductive states.

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Chapter 1: Patterns and variation in the mammal parasite–glucocorticoid relationship

With Thomas Merkling and Claudia Fichtel

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Patterns and variation in the mammal parasite–glucocorticoid relationship

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ABSTRACT

Parasites are ubiquitous and can strongly affect their hosts through mechanisms such as behavioural changes, increased energetic costs and/or immunomodulation. When parasites are detrimental to their hosts, they should act as physiological stressors and elicit the release of glucocorticoids. Alternatively, previously elevated glucocorticoid levels could facilitate parasite infection due to neuroimmunomodulation. However, results are equivocal, with studies showing either positive, negative or no relationship between parasite infection and glucocorticoid levels. Since factors such as parasite type, infection severity or host age and sex can influence the parasite–glucocorticoid relationship, we review the main mechanisms driving this relationship. We then perform a phylogenetic meta-analysis of 110 records from 65 studies in mammalian hosts from experimental and observational studies to quantify the general direction of this relationship and to identify ecological and methodological drivers of the observed variability. Our review produced equivocal results concerning the direction of the relationship, but there was stronger support for a positive relationship, although causality remained unclear. Mechanisms such as host manipulation for parasite survival, host response to infection, cumulative effects of multiple stressors, and neuro-immunomodulatory effects of glucocorticoids could explain the positive relationship. Our meta-analysis results revealed an overall positive relationship between glucocorticoids and parasitism among both experimental and observational studies. Because all experimental studies included were parasite manipulations, we conclude that parasites caused in general an increase in glucocorticoid levels. To obtain a better understanding of the directionality of this link, experimental manipulation of glucocorticoid levels is now required to assess the causal effects of high glucocorticoid levels on parasite infection. Neither parasite type, the method used to assess parasite infection nor phylogeny influenced the relationship, and there was no evidence for publication bias. Future studies should attempt to be as comprehensive as possible, including moderators potentially influencing the parasite–glucocorticoid relationship. We particularly emphasise the importance of testing hosts of a broad age range, concomitantly measuring sex hormone levels or at least reproductive status, and for observational studies, also considering food availability, host body condition and social stressors to obtain a better understanding of the parasite–glucocorticoid relationship.

Key words: host–parasite interaction, immune system, pathogen, stress, susceptibility, resistance, disease ecology.

CONTENTS

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Chapter 2: Parasite community overlap in three sympatric lemur species indicates potential for cross-species transmissions

With Josue Rakotoniaina, Anni Hämäläinen and Claudia Fichtel

Abstract

The study of infectious disease transmission is of great importance for managing and conserving endangered species. It is also important for human health by helping to better understand infection patterns, defence mechanisms and, most importantly, to prevent zoonoses. Increased anthropogenic pressure and from this following habitat overlap further favour opportunities of parasite transmission between humans, their domestic animals (livestock, pets) and wild animals. Moreover, since many primates live in sympatry and are most often phylogenetically closely related, the risk of sharing parasites increases. Thus, sympatric species could potentially act as infectious disease reservoir for each other and have an indirect effect on health and fitness of other species. Many lemur species live in sympatry, display versatile gut parasite communities, and are conservation targets because of global warming, poaching and deforestation. However, reports on parasite communities of sympatric lemur species are rare and little is known on parasite cross-transmission or temporal changes in these communities. Here, we investigated the parasite communities of three sympatric lemur species, the grey mouse lemur, fat-tailed dwarf lemurs and redfronted lemurs, in a forest with different degrees of human disturbance in Western Madagascar. Parasite communities were diverse in all three lemur hosts and with the discovery of six new but rare morphotypes, parasite richness was higher than previously described. Because almost half of the of the parasite morphotypes were shared among two or three lemur species and some of them were also found in livestock or humans, further research into the possibility of gut parasite cross-transmission among lemurs and possibly with humans or livestock is indicated.

Keywords: helminths, nematodes, host-parasite interaction, cross-sharing, primate, host specificity, parasite community similarity

Introduction

Parasites are ubiquitous in the wild (Nunn & Altizer, 2006). They have multiple costs for their hosts (e.g. energetics: Magnanou et al., 2006; immuno-modulation: Helmby, 2009; endocrine modulation: Defolie et al., 2020; host survival and reproduction: Nunn & Altizer, 2006; Nunn et al., 2004) and can ultimately act as strong evolutionary forces on their hosts (Nunn & Altizer, 2006). With increasing anthropogenic pressure on wildlife and the consequential increase in interaction between wildlife and humans and their livestock, the risk of parasite cross-transmission and zoonotic diseases is rising (N. J. Clark et al., 2018; Cooper, Kamilar, et al., 2012; W. Li, Feng, & Santin, 2019). Thus, the understanding of parasite dynamics is of great importance for managing and conserving endangered species (Ehlers et al., 2020; Gillespie et al., 2008). Furthermore, a better understanding of infections and defence mechanisms could help prevent zoonoses and potential threats to humans (N. J. Clark et al., 2018; Cooper, Kamilar, et al., 2012; W. Li, Feng, & Santin, 2019).

Many studies focused on hosts extrinsic (season, latitude) and intrinsic (behaviour, physiology, genetics) factors influencing susceptibility and transmission related to a single host-parasite association or a single hosts-parasite community association. Most parasites occur rather in multispecies community than alone (Knowles et al., 2013; Krasnov et al., 2006; Nunn & Altizer, 2006). Parasite community studies are particularly interesting because of the potential interactions between parasite species, where inhibiting and catalysing effects can occur. For example, the energetical costs for the host can increase independently of parasite abundance due to exploitation of different resources by different parasite species and the need for different immune responses against them (Krasnov et al., 2006; Nunn & Altizer, 2006; Poulin & Mouillot, 2004). From the parasite point of view, while living in multispecies communities can have costs: e.g. resource competition between species occupying the same niche; this can also have multiple benefits: e.g. decreased immune defence in hosts or even tolerance due to the energetic demand, leading to a better parasite fitness (Knowles et al., 2013; Krasnov et al., 2006; Nunn & Altizer, 2006; Poulin & Mouillot, 2004).

Some parasite species can exploit many hosts species (generalists), while others infect only one or a few (specialists) (Altizer et al., 2003; Park et al., 2018). Because both

processes at the intra and inter-species level can influence parasite transmission, there is a need for a better understanding of parasite-host relationships at the metacommunity level. In particular, for gastro-intestinal parasites transmitted orofecally, parasite infective stages emitted in faeces can accumulate in the external environment and remain available for different host species, thus, increasing exposure without direct contact between hosts. Moreover, with the intensification of anthropogenic pressure, the increased space sharing between different species of wild animals (e.g. ground and tree use, water sources and food patches) but also between wild animals, humans and their domestic animals raises transmission risk of new parasite species (N. J. Clark et al., 2018; Cooper, Kamilar, et al., 2012; W. Li, Feng, & Santin, 2019).

The typical sympatric life of multiple primate species in the tropics and their close phylogenetic relatedness increases the opportunities of parasite sharing. Since sympatric species usually have similar ecology and use of similar/shared resources, the probability to encounter infective parasite stages is enhanced. In addition, similar immunology and physiology making sympatric primate species susceptible to similar parasites (Davies & Pedersen, 2008; Freeland, 1983). Thus, sympatry should increase the transmission rate of generalist parasites. Since sympatric hosts can potentially act as infectious disease reservoir and transmission hosts for each other, it increases their infection risk and ultimately all hosts species health and fitness. From the parasite point of view, being more generalist could be of importance when facing changes in habitat and resources. Generalist parasites are expected to survive better to the extinction of a host species and to be more easily able to invade new habitats and spread following introduction to new areas than specialist parasites (Robert Poulin et al., 2006).

Despite the recognized complexity of host-parasite community interactions, reports on parasite communities of sympatric species are quite rare (Dewit et al., 1991; Ehlers et al., 2020; V. O. Ezenwa, 2003; Kouassi et al., 2015; Landsoud-Soukate et al., 1995; Obanda et al., 2019; Springer & Kappeler, 2016). As such, little is known about parasite host specificity and indices of diversity and similarity at the community and metacommunity level. This is in part due to research commonly focusing on a single host species, leading to a knowledge bias in favour of documenting the parasites of certain

host species and not others. Some host species are more prone to be studied because of accessibility and economic interests e.g. pets and farm animals or iconic status e.g. anthropoid primates. Therefore, the increased sampling effort brings a better knowledge of their parasites (Vitone et al., 2004). To date, it is known that 77% of livestock's parasites (Cleaveland et al., 2001), 90% of domestic carnivores' parasites (Cleaveland et al., 2001) and almost 50% of anthropoid primates' parasitic helminth species are generalists (Pedersen et al., 2005). Thus, it is important to study generalist parasites and their communities, but also integrating parasite and host dynamics within the appropriate ecological context. This will prove important for species conservation, biodiversity preservation and contributing to zoonotic outbreaks prevention or reduction (McCallum & Dobson, 1995, 2002; Smith et al., 2018; Smith et al., 2006).

In this context, lemurs are of great interest. Lemurs are endemic to Madagascar (Mittermeier et al., 2008), many lemur species live in sympatry and they have a versatile parasite fauna (Irwin & Raharison, 2009; Raharivololona & Ganzhorn, 2009). They are conservation targets because of global warming, poaching and deforestation. As a consequence of increasing anthropogenic pressure, parasite transmission risk between wildlife, humans and their domestic animals has increased (Bublitz et al., 2015; Ehlers et al., 2020; Schwitzer et al., 2014; Zohdy et al., 2015). Changes in parasite community composition with potential parasite host-switches are thus expected in lemur communities. In particular, in Kirindy forest, western Madagascar, four areas with increasing levels of human disturbance are monitored, and host up to eight species of lemur living in sympatry. The parasite status of six of these lemurs have been investigated in multiple studies (Clough, 2010; Hämäläinen et al., 2015; Rakotoniaina et al., 2016; Schwensow et al., 2010; Springer & Kappeler, 2016) and showed that these lemurs share parasites species to some extent (Springer & Kappeler, 2016).

In this study, we describe the parasite community in three sympatric lemur species from the same field site: grey mouse lemur (*Microcebus murinus*), fat-tailed dwarf lemur (*Cheirogalus medius*) and redfronted lemur (*Eulemur rufifrons*). Regulars samples were collected from these three species during longitudinal studies, allowing us to perform this analysis. Our goal was to characterize parasite prevalence and diversity within host species but also diversity and similarity between host species. We additionally assessed

parasite host specificity for all shared parasites. The three focal host species belong to two distinct families (redfronted lemurs: *Lemuridae*; grey mouse lemur and fat-tailed dwarf lemur: *Cherogaleidae*). This difference of phylogenetic relatedness may impact parasite community similarity, as closely related hosts tend to share more parasite species than distant hosts due to coevolution or inheritance from common ancestor (Nunn et al., 2004). Moreover, these three species differ in their ecology and social organisation. Redfronted lemurs are cathemeral, frugivorous and semi-terrestrial primates. By contrast, grey mouse lemurs and fat-tailed dwarf lemurs are nocturnal, omnivorous and arboreal. While red-fronted lemurs live in permanent small multimale-multifemale groups (Julia Ostner & Kappeler, 1999), the two *Cherogaleidae* species exhibit different forms of sociality: fat-tailed dwarf lemurs are pair-living (Fietz, 1999), whereas gray mouse lemurs exhibit a facultative sociality: they are solitary foragers during the night but some individuals, mainly females, sleep in groups of two to ten individuals in sleeping tree holes during the day (Eberle & Kappeler, 2006).

These contrasted socio-ecological traits could influence parasite exposure and susceptibility, depending on parasites' life history traits. For instance, semi-terrestrial hosts should be more exposed than arboreal hosts to gastro-intestinal parasites exhibiting free-living infective stage in the soil, such as most nematode species (Nunn & Altizer, 2006). Diet should also shape the parasite community harboured by a host population. In particular, omnivorous hosts should be more exposed than frugivorous species to cestode parasites with 'complex life-cycle', as they are transmitted exclusively through the ingestion of a parasitized intermediate host from another species. Finally, differences in sociality could explain differences in parasite prevalence and richness. Parasite transmission is thought to be a major cost of social life because social hosts are more exposed to parasites infective stages but also because social stress can modulate susceptibility (Nunn & Altizer, 2006). Based on previous parasitological surveys of these lemur species (Clough, 2010; Hämäläinen et al., 2015; Irwin & Raharison, 2009; Rahariv ololona & Ganzhorn, 2009; Rakotoniaina et al., 2016; Springer & Kappeler, 2016), and according to their phylogenetic, ecological and social differences, we expect (1) different parasite communities in the three host species, but a higher similarity of parasite communities between the two phylogenetically close *Cherogaleidae* species

than between the *Cherogaleidae* species and the redfronted lemurs; (2) strong differences in parasite prevalence between the two *Cherogaleidae* species and the redfronted lemurs when they are common hosts for a parasite morphotype; and (3) parasite host specificity to be low because of the close phylogeny of our three host species.

Material and methods

Study site:

The study site is a long-term field site of the German Primate Center situated in Kirindy forest, a dry deciduous forest about 60 km north-east of Morondava in western Madagascar (Peter M. Kappeler & Fichtel, 2012a). The local climate is characterized by strong seasonal changes with a hot rainy season from November to March and a cool dry season from April to October (Peter M. Kappeler & Fichtel, 2012a). The 60-ha study site is situated within a forestry concession operated by the Centre National de Formation, d'Études et de Recherche en Environnement et Foresterie. We selected four sites, locally known as N5, CS7, Savanna (SV) and Kirindy Village (KV), because of their increasing levels of human disturbance. Two sites have been used as part of the long-term study for behavioural research since 1993 (N5 and CS7), they belong to the core area of the forest concession and have the lowest human disturbance levels. N5 is exclusively used for research but CS7 is also regularly frequented by small groups of tourists. The SV area is at the eastern border between the core forest area and a natural savannah. It is occasionally frequented by the local human population because the forest constitutes a potential source of food and firewood and is thus considered to have higher human impact than N5 and CS7. The KV study area is a forest fragment located close to Kirindy village and crossed by a path that is used daily by locals because it connects neighbouring villages. It is the site most subjected to human incursion because of its use as a source of food and fire and construction wood. The characteristics of the four study sites are summarized in Rakotoniaina et al. 2016. All samples from redfronted lemurs have been collected in CS7, while samples from grey mouse lemurs and fat-tailed dwarf lemurs came from the four study areas (see Supplementary table 1 for more details).

Sample collection and storage:

All data used in this study comes from other projects: samples from redfronted lemurs samples (736 samples from 42 individuals) were collected from May 2015 to November 2016, and samples from grey mouse lemurs (2333 samples from 879 individuals) and fat tailed dwarf lemurs (197 samples from 140 individuals) were collected from March 2010 to June 2014. The three species have been sampled rather equally across seasons and years, except the fat-tailed dwarf lemur because of torpor during the wet season (Dausmann et al., 2004).

For red-fronted lemurs, we used data collected from 42 individuals over a long-term study including five habituated social groups. All individuals were marked with a unique combination of collars and pendants (Kappeler & Fichtel, 2012), and all individuals older than one year old were regularly observed. Faecal samples were collected whenever a known individual was seen defecating within the five minutes following defecation. To avoid any sample contamination, only parts that did not touch the soil were collected. Each faecal sample was immediately homogenised after removal of big seeds and fruit parts and a small portion (1-2g) was transferred in pre-aliquoted tubes containing 15mL of 10% formalin. The samples were stored at room temperature until parasitological analyses were performed.

For the grey mouse lemurs and fat-tailed dwarf lemurs, we used data collected as part of a long-term project. Most individuals of both species' populations have been regularly captured using Sherman life traps baited with banana during regular monthly trapping sessions. At first capture, individuals were marked with a subcutaneous transponder (Trovan EURO ID, Germany), sexed and aged. In addition, faecal samples were collected during handling or from the cleaned traps. One to four pellets of fresh faeces were weighed, homogenized and stored in 10% formalin tubes at room temperature until microscopic observation.

Sample analysis:

Samples were analysed at the Institut Pasteur, Madagascar and the German Primate Center, Germany. All samples were processed using a modified formalin-ethyl acetate sedimentation technique following a protocol described previously by Clough

(2010). To reduce bias, all samples were processed and observed under the microscope in a random order, without regard to the host identity or collection period. We morphologically identified parasites eggs and cyst to the closest genus, in accordance with previously described identification criteria based on size, shape and color (Clough, 2010; Irwin & Raharison, 2009; Rahariviololona & Ganzhorn, 2009). Since, identifying gastro-intestinal parasite at the species level with microscopy is quite uncertain, we only assigned morphotypes at the genus level, potentially underestimating the actual number of species harboured in our three lemur populations.

Estimating prevalence, diversity, host specificity and parasite community similarity:

For each host species and each parasite morphotype, we calculated parasite prevalence as the percentage of individuals infected with this specific parasite morphotype. We chose to calculate parasite prevalence at the individual level to take into account repeated sampling and the variation in the number of repeated sampling occasions of individuals among species, thus, minimising sampling bias. We calculated parasite morphotype diversity, multiple indices to describe parasite morphotype diversity when taking into account abundance, within and between host species and an index of host specificity as indicated below.

At the intra-community level, we calculated:

- Alpha diversity (Whittaker, 1972) or species richness S at the morphotype level, which is the total number of distinct parasites morphotypes an individual harboured.
- Two diversity indexes taking into account the abundance of parasite species:
  - The Shannon index, H (Kouassi et al., 2015; Shannon, 1948). H is calculated as follows: it is the negative sum of each morphotype prevalence multiplied by the logarithm of its prevalence. This index accounts for both species' richness and its evenness. The more unequal the abundances between morphotypes, the smaller the Shannon index is. This index is strongly influenced by species richness and by rare species.
  - The inverse Simpson index, D (Simpson, 1949). D is the inverse of the sum of squared prevalence for all morphotypes in the host species. This index is used to

estimate dominance of some species in a community, it does not account for species richness. It starts at 1 (low diversity of one species) with the maximum being S, the maximum number of species in this host. This index gives more weight to evenness and species which are more common in the parasite community.

- The Rhode's host specificity index, S_i (Rohde & Rohde, 2008) which determines the degree of specificity of a parasite species in a set of hosts. In brief, it is calculated by scaling the prevalence of each parasite morphotype in a given host species to the prevalence of the same morphotype in the host species in which it was most common. We computed this index for each morphotype that occurred in at least two of the three lemur species in our study. We calculated it as follows: S_i is the sum of individuals from one host species infected with a specific morphotype divided by the number of individuals sampled from in this host species multiplied by the rank of this host species based on the prevalence of infection (species with highest prevalence for this morphotype has rank = 1), and repeated for all host species. This is then divided by the sum of prevalence for this morphotype in each host species. This index takes into account the uneven distribution of parasites across different hosts; thus, it is less affected by accidental or ephemeral occurrences of parasite mophotype. The higher the value of this index, the higher the host specificity. Values range between 0 and 1, with 1 meaning the parasite infests only one host.

At the inter-community level, we considered:

- Beta diversity (Whittaker, 1972), which represents the diversity between the three sympatric host species. It is calculated as follow $\beta\text{Diversity} = \text{gamma diversity} - (\text{mean alpha diversity of all the habitats considered})$. It ranges from 0 (low diversity) to N (high diversity), with N being the number of compared habitats (here host species).
- Gamma diversity which is a measure of the overall diversity for the N different habitats within a region (Whittaker, 1972), here it is the total diversity of parasite morphotypes within the three lemur species considered in this study.
- the Jaccard coefficient of community similarity (Jaccard, 1912) which measures the similarity between communities from different habitats and is calculated as follow

$CC_j = \frac{number\ of\ morphotypes\ common\ to\ both\ communities}{total\ number\ of\ morphotypes\ present\ in\ the\ two\ communities}$. This index varies between 0 and 1, the closer the value is to 1, the more the communities have in common. Complete community overlap is equal to 1; complete community dissimilarity is equal to 0.

Results

Parasite morphotypes and their prevalence in the three lemur species

We found a total of 21 morphotypes heterogeneously distributed across the three lemur species (Table 1). In red-fronted lemurs, 61.36% of all samples were positive for at least one parasite morphotype. We distinguished 11 different morphotypes of gastro-intestinal (GI) parasites (seven nematodes, one cestode, one trematode and two protozoa, see Table 1). Clough (2010) previously described two morphotypes of *Callistoura* in this population, however we found it quite difficult and unreliable to distinguish the two morphotypes with microscopy only, so we decided to lump all together under *Callistoura sp.* morphotype. Nine of these morphotypes were already described in the same population of red-fronted lemurs (Clough, 2010), the most prevalent morphotypes being *Calistoura sp.* and *Balantidium sp.* (respectively 34.97 and 33.88% of samples infected, Table 1). Additionally, we observed for the first time in red-fronted lemurs *Oxyuridae sp.* and *Subulura sp.* (see Figure 1A and 1B), but in a few samples only. These two nematode morphotypes were previously described in grey mouse lemurs at the same field site and in the South of Madagascar (Irwin & Raharison, 2009; Rakotoniaina et al., 2016; Springer & Kappeler, 2016).

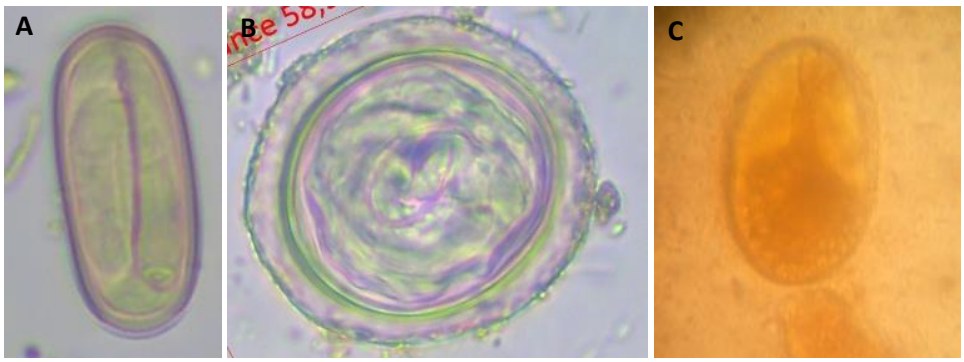

Figure 1: pictures of A. *Oxyuridae sp.*; B. *Subulura sp.* found in redfronted lemurs; and C. *Fasciola sp.* found in mouse lemurs.

Table 1: Parasite prevalence in percentage of individuals infected for each lemur species and host specificity index. Host specificity index was calculated only for morphotypes that were found in more than one host species. We determined the likely parasite transmission mode based on literature for each morphotype. Numbers in brackets following prevalence represent the total number of samples positive for the specific parasite. ✓ indicates morphotypes recorded for the first time in lemur, ✦ indicates morphotypes recorded for the first time in this lemur species.

Parasite morphotype (transmission mode)	*redfronted lemurs (*n individuals=42, n samples=735)	*grey mouse lemurs* (n individuals=879, n samples=2333)	*fat-tailed dwarf lemurs* (n individuals=140, n samples=197)	Total species	Host specificity index
	Prevalence in % infected individuals (n infected individuals)				
Nematoda					
Subulura sp. (indirect)	9.52 (4) ª	49.60 (436)	32.14 (45)	3	0.75
Trichuris sp. (direct)	11.90 (5)	20.82 (183)	12.14 (17)	3	0.69
Ascaris sp. (direct)	--	7.96 (70)	9.29 (13)	2	0.73
Strongylida sp. (direct)	--	12.51 (110)	5.71 (8)	2	0.84
Oxyuridae sp. (direct)	9.52 (4) ª	9.56 (84)	5.71 (8)	3	0.65
Lemuricola sp. (direct)	59.52 (25)	1.02 (9)	1.43(2) ª	3	0.98
Callistoura sp. (direct)	85.71 (36)	--	--	1	--
Oesophagostomum sp. (direct)	--	1.82 (16)	--	1	--
Capillaria sp. (indirect)	--	0.46 (4)	--	1	--
Trichostrongylidae sp. (direct)	38.10 (16)	--	--	1	--
Strongyloididae sp. (direct)	7.14 (3)	--	--	1	--
Cestoda					
Hymenolepsis sp. (indirect)	--	46.08 (405)	31.43 (44)	2	0.80
Anoplocephalidae sp. (indirect)	16.67 (7)	--	--	1	--
Trematoda					
Megatonimus sp. (indirect)	--	2.16 (19)	2.14 (3)	2	0.75
Opisthorchis sp. (indirect)	--	0.57 (5)	0.71 (1) ª	2	0.72
Fasciola sp. (indirect)	--	0.34 (3) ~	--	1	--
Schisostoma sp. (indirect)	--	0.11 (1) ~	--	1	--
Dicrocoeliidae sp. (indirect)	14.29 (6)	--	--	1	--
Protozoa					
Coccidia sp. (direct)	--	3.30 (29)	10.71 (15)	2	0.88
Entamoeba sp .(direct)	66.67 (28)	--	--	1	--
Balantidium sp. (direct)	79.57 (33)	--	--	1	--
Total prevalence	92.86	70.65	55.71		
Parasite morphotype/sample: mean (range)	0.90 (0-4)	0.93 (0-6)	0.81 (0-6)		
Parasite morphotype/individual: mean (range)	3.98 (0-8)	1.56 (0-7)	1.11 (0-6)		

In grey mouse lemurs, 58.17% of samples were positive for at least one parasite. We distinguished 14 different morphotypes of GI parasites (eight nematodes, one cestode, four trematodes and one protozoa, Table 1), with twelve of them already described in this population (Rakotoniaina et al., 2016; Springer & Kappeler, 2016).

Subulura sp. and *Hymenolepsis sp.* were the most prevalent in the grey mouse lemur (respectively 35.02 and 30.60% of the samples, Table 1) but we did find, in a low number of samples, two trematodes: *Fasciola sp.* and *Schisostoma sp.* (Fig. 1C) which were never described in this host species or in lemur before but in livestock in Madagascar (Daynes, 1966; Ribot & Coulanges, 1982).

In fat-tailed dwarf lemurs, 46.60% of samples were positive for at least one parasite. We distinguished 10 different morphotypes of GI parasites (six nematodes, one cestode, two trematodes and one protozoa, Table 1). Eight of them have already been described in this population (Rakotoniaina et al., 2016; Springer & Kappeler, 2016). *Subulura sp.* and *Hymenolepsis sp.* were also the most prevalent in the fat-tailed dwarf lemur (23.86 and 23.35% of the samples, Table 1), but we found a few samples containing one nematode, *Lemuricola sp.* (Fig. 2A), and one trematode *Opisthorchis sp*. morphotypes (Fig. 2B) which were previously described in grey mouse lemurs for both morphotypes and in red-fronted lemurs for *Lemuricola sp.*, but not in fat-tailed dwarf lemurs (Rakotoniaina et al., 2016; Springer & Kappeler, 2016).

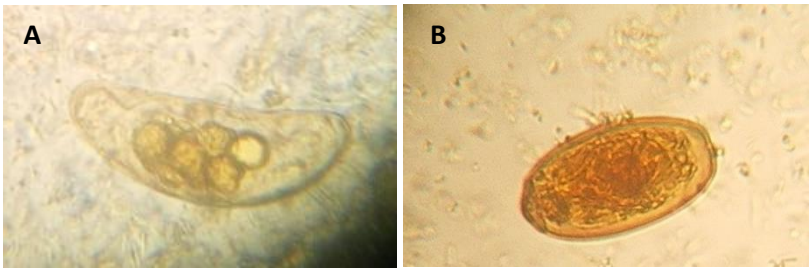

Figure 2: pictures of A. *Lemuricola sp.* and B. *Opisthorchis sp.* found in fat-tailed dwarf lemurs faecal samples.

Parasite diversity and diversity evenness in each host species

Alpha diversity (or parasite morphotype richness) was similar for all host species, it was slightly higher in grey mouse lemur (14 morphotypes) than in red-fronted lemurs (11) and fat-tailed dwarf lemurs (10). The Shannon index was higher in grey mouse lemur than in fat-tailed dwarf lemurs and it was higher in redfronted lemurs than in grey mouse lemurs. The inverse Simpson index was smaller in redfronted lemur than in grey mouse lemurs and it was smaller in grey mouse lemurs than in the fat-tailed dwarf lemur (Table 2). Both index of diversity thus indicated that when taking into account parasite

morphotypes relative abundance, parasite diversity was higher in the fat-tailed dwarf lemur than in the other two lemur species. This reveals a more even distribution of parasite morphotypes in the fat-tailed dwarf lemur than in the other two host species, which had parasite communities dominated by *Callistoura sp., Entamoeba coli* and *Ballantidium sp.* in the redfronted lemurs and *Subulura sp.* and *Hymenolepsis sp.* in the grey mouse lemur.

Table 2: Diversity indices for each host species.

Diversity index	*redfronted lemurs*	grey mouse lemurs	*fat-tailed dwarf lemurs*
Alpha diversity, S	11	14	10
Shannon index, H	2.73	2.11	1.95
Inverse Simpson index, D	0.42	1.87	4.10

Parasite diversity between host species, parasite host specificity and parasite communities' similarity

Table 3: parasite diversity between host species and parasite communities' similarity

Inter-communities' diversity indices	***Eulemur rufifrons* - Microcebus Murinus**	***Microcebus murinus* - *Cheirogaleus medius***	***Cheirogaleus medius* - *Eulemur rufifrons***
Morphotypes present in both communities	21	14	21
Morphotypes in common	4	10	4
Jaccard coefficient of community similarity	0.19	0.71	0.19

The parasite metacommunity was diverse, with a gamma diversity consisting of 21 different morphotypes from four parasite families across all three host species. Nematodes represented the most diverse family in our dataset with 11 morphotypes identified in all faecal samples, while there were five trematodes, two cestodes and three protozoan morphotypes (See table 1 for more details). Beta diversity, however, was 1.8 for the three communities, suggesting a medium diversity between communities. Indeed, seven genera were uniquely found in redfronted lemurs (alpha diversity = 11), four in grey mouse lemurs (alpha diversity = 14), but none in fat-tailed dwarf lemurs (alpha diversity = 10) who shared all its parasites with grey mouse lemurs

(Table 3). Redfronted lemurs also shared the same four morphotypes with both grey mouse lemurs and fat-tailed dwarf lemurs. There were both very prevalent and very rare parasites among the morphotypes found in only one host species and those shared by two or all three host species. Two nematodes, one cestode, two trematodes and one protozoan were shared by two host species. Notably, four nematodes were shared by all three host species (Figure 3). The Jaccard coefficient of community similarity (Table 3) revealed a very strong similarity between the grey mouse lemurs and fat-tailed dwarf lemurs' communities (J = 0.71), while the redfronted lemur community was quite dissimilar to the two other lemur species (J = 0.19). The Rhode's host specificity index, which takes into account the abundance of parasites in all the sampled host species, showed values ranging from 0.65 to 0.98 (Table 1), indicating rather strong host specificity in all morphotypes and especially in *Hymenolepsis sp., Strongylidae sp., Lemuricola sp.* and *Coccidia sp.*

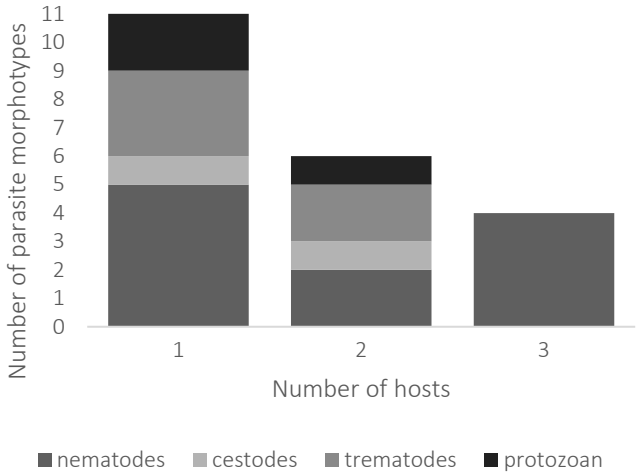

Figure 3: Number of parasite morphotypes present in one, two of three lemur host species at Kirindy field site. The grey shades differentiate between nematodes (11 morphotypes in total), cestodes (2 morphotypes), trematodes (5 morphotypes) and protozoan (3 morphotypes).

Discussion

In this study, we compared parasite communities of three sympatric lemur species. All lemur species were infected with a diverse gastro-intestinal parasite community but parasite diversity as well as parasite prevalence differed between host species, suggesting differences in transmission risk. Rhode's host specificity index indicated that most shared parasite morphotypes had some level of host generalism, while two shared

morphotypes (*Lemuricola sp.* and *Coccidia sp.*) seemed very host specific. Furthermore, with the discovery of new morphotypes in these lemurs, parasite diversity was higher in these three lemur species than described before at the same field site.

When comparing diversity between host species, alpha diversity was higher in the grey mouse lemur compared to the other two species. The unbalanced sample size between the three species (2333 samples from the grey mouse lemurs, 197 from the fat-tailed dwarf lemurs and 736 from the redfronted lemurs), could partly explain this. However, this explanation cannot explain the rather similar alpha diversity between the fat-tailed dwarf lemurs and the redfronted lemur despite large sampling differences. Surprisingly, the Shannon and Simpson diversity indices, which take into account abundance of morphotypes indicated a higher diversity in the fat-tailed dwarf lemurs than in the other two lemur species. As the grey mouse lemurs and the fat-tailed dwarf lemurs have rather similar ecology and social organisation, and are genetically closely related, all these factors influencing greatly parasite transmission, we expected more similarities between these two species. However, it is also known that these two species differ in their life history and abilities to adapt to habitat disturbances (Rakotoniaina et al., 2016). This could lead to differences in immune investment and explain the observed differences in parasite diversity evenness.

Differences in sample size (sampling frequency and samples per individual) and seasonal or yearly variations in environmental conditions can also contribute to variation of parasite diversity (Aivelo et al., 2015; Hokan et al., 2018; Raharivololona & Ganzhorn, 2009). Seasonal effects could be especially important for parasite prevalence at our field site due to strong seasonality with a hot and wet season favourable for nematodes, and a less favourable cold and dry season. In our dataset, we controlled for seasonal variation by sampling during both seasons across two to four years for all three lemur species but some previous studies of parasite in these lemur populations where restrained to short temporal periods: the dry season for redfronted lemurs (Clough, 2010) and the rainy season for grey mouse lemurs and fat-tailed dwarf lemurs (Schwensow et al., 2010). Nevertheless, parasite prevalence and richness differed from parasites studies of the same population a decade earlier (Clough, 2010; Schwensow et al., 2010). The increase in parasite diversity in this study in comparison to previous

studies in the same populations can easily be explained by an increase in the number of samples collected and also in the number of individuals sampled from. Indeed, an increased number of individuals sampled increases the probability to collect rare parasite species (Dallas et al., 2017; Robert Poulin et al., 2006) because parasite distribution in a population is highly heterogenous and many parasite species occurring at low prevalence are therefore likely to be missed (Robert Poulin et al., 2006). This was also evidenced by our discovery of several previously unobserved parasite morphotypes in these relatively well-studied host species. However, the increase in parasite diversity in our study is rather anecdotic as it involves parasites found in only a few samples.

Coevolution of parasites with their host as well as loss of parasites during host evolution are important in determining the parasite communities of individual host species as well as host communities (Robert Poulin, 2004; Robert Poulin et al., 2006). Through different processes of host-parasite coevolution, certain parasites are specialised to infecting specific hosts, while generalist parasite can infect a broader range of host species. Generally, however, parasites tend to infect host species that are phylogenetically similar because they share traits (e.g., immunologic, behavioural, or ecological) that make them susceptible to the same parasites while more distantly related hosts share fewer parasite species because they share fewer such traits (Davies & Pedersen, 2008; Freeland, 1983; Kaesler et al., 2017; Krasnov et al., 2006). We observed more parasite sharing between the three lemur species than described before (Schwensow et al., 2010; Springer & Kappeler, 2016), with nearly half (47.61%, 10/21) of the parasite morphotypes shared by at least two host species. Additionally, nearly a fifth (19.04%, 4/21) of them were shared by all three studied host species. As expected, parasites communities were more similar between the two very closely related nocturnal species (the grey mouse lemur and the fat-tailed dwarf lemur), than between each of them and the more distantly related redfronted lemurs. The overall close relatedness of the sympatric species of lemurs living in Kirindy likely increases the probability of parasite transmission. In consequence, this could increase parasite diversity of each of the host species in this metacommunity (Combes, 2001).

Rhode's host specificity index indicated a very high level of host specificity for half of the shared morphotypes. Parasite transmission mode should determine to a

large extent whether the parasite will be highly specialized or not. For instance, a high level of specialisation is expected for directly transmitted parasites which require social contact or high proximity between hosts while parasites transmitted via intermediate hosts, food or the environment should be much less specific (Pedersen et al., 2005). In our study however, host specificity index did not differ between transmission mode, with high specificity for parasites with intermediate hosts and less specificity for directly transmitted parasites and vice-versa. (see Table1). Other factors may constrain host-switching in sympatric species and promote high levels of host specificity. First, high host specificity is favoured on stable, predictable resources (Desdevises et al., 2002; Robert Poulin et al., 2006; Sasal et al., 1999) and these three lemur species share several ecological traits (long lived mammals, with low reproduction rates and low population densities) making them a stable and predictable resource base for their parasites. Then, differences in host physical environment, ecology, physiology and social organisation may also result in differences in the exposure and susceptibility to parasites (Nunn & Altizer, 2006). The three lemur species included in our study, have different habitat use (semi arboreal for the red-fronted lemurs versus strictly arboreal for the two *Cheirogalidae* species) and activity rhythm (cathemeral for the red-fronted lemurs versus nocturnal for the two *Cheirogalidae* species), which limits the opportunities for transmission of parasite with direct life cycles among different species, thus limiting the potential colonisation of different lemurs species by different parasites. Finally, the limitations of microscopic parasite identification could explain some of these host specificity indices; where some of the shared morphotypes might, in fact, represent multiple cryptic species specialised for their particular host ecology, physiology and social system.

In this study, we also documented six morphotypes in hosts they have not previously been observed in, two in each of the three host species. Interestingly, the new morphotypes in red-fronted lemurs (*Subulura sp.* and *Oxyuridae sp.*) and fat-tailed dwarf lemurs (*Lemuricola sp.* and *Opistorchis sp.*) were all found in grey mouse lemur samples (and reported previously from grey mouse lemurs at other field sites (Irwin & Raharison, 2009). This finding suggests parasite sharing between the sympatric lemurs. In the grey mouse lemur samples, two morphotypes, *Fasciola sp.* and *Schisostoma sp.*,

were never described in lemur species before but have been found in livestock (definitive hosts) in Madagascar (Daynes, 1966; Ribot & Coulanges, 1982). These parasites have a complex life cycle: eggs are shed in their definitive host faeces; they first live in an intermediate host and then as free-living stages they are transmitted through the ingestion of vegetation (for *Fasciola sp.*) or contact with water (for *Schisostoma sp.*) containing free-living stages. Thus, increased anthropogenic pressure in the area could favour a potential transmission from livestock who are typical definitive hosts for these two morphotypes. Potential contamination of fecal samples from the environment is an unlikely explanation for the observation of rare or novel morphotypes in samples, because we applied various precautionary measures to minimize the risk of contamination (e.g. cleaning traps between all captures of the nocturnal lemurs; selecting samples that did not come into contact with soil for the cathemeral lemurs). Occasional transmissions of rare parasites might also originate from other host species (other lemurs or diverse other taxa such as rats, tenrecs, banded mongoose and fossa) that were not followed in this study but that contribute substantially to the parasite network in the ecosystem.

Studying multi-host communities and their parasites is of importance because sympatric species can act in parasite maintenance and transmission to other species (N. J. Clark et al., 2018; Cooper, Griffin, et al., 2012; W. Li, Feng, & Santin, 2019; Wells & Clark, 2019). However, the potential threat these parasites pose to the lemurs at Kirindy forest are unknown. Some of the parasites found in this study (e.g. *Oesophagostomum sp., Trichuris sp, Capillaria so., Schisostoma sp.*) could have veterinary importance because of strong pathologies caused by parasites of the same genera (Beck & Beverley-burton, 1968; Cheever, 1985; Dobson, 1967; Horak & Clark, 1966; Saad et al., 1980; Smith & Stevenson, 1970; Ziem et al., 2006).

The close phylogenetic relationship between human and nonhuman primates is a risk factor for parasite cross-sharing (Davies & Pedersen, 2008; Freeland, 1983) and potentially zoonotic disease (Dewit et al., 1991). Humans and their domestic animals can act as reservoir, and increased use of natural habitat for human activities (agriculture, pastoralism, wood and minerals extraction, habitat) increase the possibility of contact between domestic and wild animals, thus increasing parasite sharing and zoonosis risk

(Ehlers et al., 2020; Obanda et al., 2019; Teichroeb et al., 2009). Such contacts are becoming more and more common under increasing anthropogenic pressure on natural areas (including our study area, (Rakotoniaina et al., 2016). To our knowledge, no study focused on the zoonotic potential of gastro-intestinal parasites of wild lemurs, but evidence of pathogen exchange between human and wild lemur have been shown for diarrhea-associated viruses and bacteria (Bublitz et al., 2015; Zohdy et al., 2015). Given the high prevalence of gastro-intestinal parasites in lemurs and extreme anthropogenic pressure, the potential for lemur gastrointestinal parasites to act as pathogenic and zoonotic agents in humans – or the reverse – should be assessed.

Because of the difficulty of identifying helminths to species level with microscopy (see e.g. (Gasser et al., 2006; Polderman & Blotkamp, 1995), molecular analysis in combination with a coprological survey seems crucial to conclude if there is parasite transmission between sympatric wild species and between human and wildlife and to determine if humans are local reservoirs (de Gruijter et al., 2005; Ehlers et al., 2020; Gasser et al., 2006; Ghai et al., 2014). Parasites we regrouped under the same morphotype might represent completely different species (that may not even have been described yet). Thus, we recommend future studies to combine coprological parasite identification with molecular identification (Gogarten et al., 2020) to identify distinct taxa that cannot be distinguished by microscopy, and sampling multiple potential host species including humans and species associated with them to uncover the true extent of parasite sharing among host species. We also recommend to systematically collect dead lemurs and small mammals when found, to screen for helminth adults in the liver and the guts, as they are easier to distinguish than eggs and cysts and for additional genetic testing.

To conclude, we showed that the parasite metacommunity from three different lemur host species is dynamic but structured. While almost half of the parasite morphotypes were shared in three sympatric species, half had high levels of host specificity within this host species assembly. Furthermore, there were two distinct parasite communities, one more specific to the two more closely related species (the grey mouse lemur and fat-tailed dwarf lemur) and another one more specific to redfronted lemurs. Parasite metacommunity diversity was higher than previously

described, with six new parasite morphotypes in species they were not described in. This indicates potential cross sharing among lemur species and/or with other potential host species in the ecosystem, possibly even including local human or livestock populations. There could be interspecific effects on health if one host acts as a reservoir for a parasite that has stronger effects on another host species. Future studies should aim to assess risks for zoonotic transmission of gastrointestinal parasites between nonhuman primates, domestic animals and humans. Studying ecological networks of parasite infections seems thus a meaningful research avenue to understand dynamics in community structures, which is of importance for one-health and conservation.

Authors contribution: C.D. conceived the study with input from other authors. C.D., J.R. and A.H. collected data and conducted lab work with the help of Eva Pechouskova and field and lab assistants. C.D. performed all analyses, designed figures and tables and drafted the manuscript. All authors provided comments and revised the manuscript.

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Ethical standards: All research reported in this study complied the laws of Madagascar and Germany and the protocols were approved by the Malagasy Ministère de l'Environnement et des Eaux et Forêts and the Centre National de Formation, d'Etudes et de Recherche en Environnement et Foresterie (CNFEREF) of Morondava. The study adhered to guidelines provided by the Association for the Study of Animal Behaviour (ASAB) and the Animal Behavior Society (ABS).

Competing interests: The authors declare that they have no competing interests.

Data accessibility: Raw data on samples, identity of the host and identification of parasites morphotypes are available upon request.

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Chapter 3: Drivers of parasite infection and their short-term consequences in a wild lemur population

With Cémence Poirotte, Michael Heistermann and Claudia Fichtel

Abstract

Pathogens and parasite are ubiquitous in the wild and most individuals are simultaneously infected with several parasite species. While they can have benign consequences individually, these different parasite species may facilitate other infections and have cumulative impact. Thus, understanding the factors determining the number of parasites species carried by an individual and the consequences of these infections is of importance to elucidate the impact of parasitism on host ecology and evolution. In group living animals, many factors can increase parasite transmission at the individual-, group- and at population-level. Because gastro-intestinal parasites are mostly transmitted oro-faecally, close proximity, social contact, and especially grooming in primates are thought to increase transmission by increasing exposure. Social behaviour can also influence susceptibility to infection: competition and dominance may influence an individual's testosterone and/or glucocorticoids levels, which in turn can have immunosuppressive effects. In this study, we investigated the relative importance of individual-, group- and population-level drivers of parasite infection and their potential costs on body condition, activity levels and sociality in 42 wild red-fronted lemurs (*Eulemur rufifrons*) from Kirindy forest, Madagascar during 18 consecutive months. We found essentially an influence of factors at the population- and individual levels on parasite infection. Furthermore, determinants of infection differed between nematodes and protozoan, highlighting the importance of parasite lifecycle for their transmission. Finally, there was no evident short-term costs of parasitism (i.e. reduced body condition, activity levels and sociality), suggesting other mitigation mechanisms or a good tolerance of parasite infections in this lemur species.

Keywords: primates, helminth, nematodes, glucocorticoids, testosterone, sociality, season

Introduction

Wild animals are exposed to and infected with a large array of gastro-intestinal parasites throughout their lives (Altizer et al., 2003). However, such host–parasite systems are characterized by a tremendous heterogeneity of parasite distribution among hosts, with a small number of hosts harbouring the majority of parasites (Poulin,

2007; Shaw & Dobson, 1995). Although many endoparasites generate only moderate clinical symptoms (Ghai et al., 2015; Krief et al., 2005, 2008), they may nevertheless alter host fitness by constantly competing for food resources, damaging host tissues or by leading the host to invest in energetically demanding immune defenses at the expense of growth, reproduction and survival (Akinyi et al., 2019; Chapman et al., 2006; Hillegass et al., 2010; Pedersen & Greives, 2008; Sánchez et al., 2018; Sher et al., 2003). Additionally, co-infection is rather the norm than the exception, infection with some parasite species potentially increasing host susceptibility to other parasites, with sometimes cumulative impact of different parasite species acerbating deleterious effects on host health (Beldomenico et al., 2008; Cox, 2001; A. L. Graham, 2008; Marcogliese & Pietrock, 2011; Vaumourin et al., 2015). Therefore, gastro-intestinal parasites exert significant selective pressures that can impact host population dynamic (Smith et al., 2006; Turner et al., 2014) and shape host physiology and behavioural ecology (Budischak et al., 2012, 2018; Chapman et al., 2016; Defolie et al., 2020; Ezenwa et al., 2016; Ezenwa et al., 2010; Ghai et al., 2015; Müller-Klein et al., 2019).

Exacerbated anthropogenic pressure on wildlife increases the risk of parasite cross-transmission between species, exposing humans and their livestock to the growing threat of infection with novel and potentially harmful pathogens (Clark et al., 2018; Cooper et al., 2012; Li et al., 2019). Unravelling the mechanisms governing parasite distribution within host population is therefore a central objective in ecology to understand evolutionary processes driving animal life history traits and improve our ability to predict disease outcome (Jolles et al., 2008; Pedersen & Fenton, 2015). Ultimately, the identification of individuals at particular risk of infection could also be valuable to manage population of endangered species (Gillespie et al., 2008; McCallum & Dobson, 1995; Smith et al., 2018) and prevent the apparition and spread of zoonosis (Clark et al., 2018; Cooper et al., 2012; Ehlers et al., 2020; Jones et al., 2008; Li et al., 2019).

Parasite infection happens if hosts are exposed to infectious parasite stages (through contaminated soil, food and water, or social contact with contagious conspecifics) and susceptible to parasite infection (for example because of variations in host body condition and immunity). In group living animals, many factors can influence

parasite exposure or susceptibility at three main scales: the population-, group- and individual-level (see Table 1 for an overview from wild animals). At the population level, environmental factors such as increased temperature and rainfall are known to favour parasite development and/or survival in the environment, increasing exposure to parasites for the whole population (Benavides et al., 2012; Gillespie et al., 2010; Nunn & Altizer, 2006). At the group level, group size is expected to favour infection with infectious agents (Côté & Poulin, 1995; Patterson & Ruckstuhl, 2013). Indeed, individuals living in larger groups could face higher parasite exposure due to proximity with multiple partners or the important accumulation of parasites shed by numerous hosts in the environment. Additionally, individuals from larger groups could suffer from increased susceptibility to infection as a consequence of higher social competition (Altizer et al., 2003; Peter M Kappeler et al., 2015; Markham & Gesquiere, 2017; Patterson & Ruckstuhl, 2013). Habitat use is another factor operating at the group level that might contribute to heterogeneity in parasite distribution between groups. In particular, some meta-analyses and empirical studies show that home range size and daily travel distance are positively associated with transmission risk because of increased exposure to parasites in the environment (Brockmeyer et al., 2015; Nunn & Altizer, 2006; Vitone et al., 2004). Alternatively, the use of small areas could increase re-infection because of parasite accumulation in the habitat (Hausfater & Meade, 1982; Nunn & Altizer, 2006) Finally, at the individual level, a broad range of factors can influence parasite infection including host's sex (Klein, 2004; Nunn & Altizer, 2006; Roberts et al., 2004), age (Hudson & Dobson, 1997; Izhar & Ben-Ami, 2015; Nunn & Altizer, 2006), body condition (Irvine et al., 2006; Nunn & Altizer, 2006; Sánchez et al., 2018), reproductive status (S. L. Klein, 2004), dominance rank (Irvine et al., 2006; Nunn & Altizer, 2006; Sapolsky, 2005), hormone profiles (Defolie et al., 2020; R. M. Sapolsky, 2005b) or social relationships and integration (Altizer et al., 2003; Friant et al., 2016; MacIntosh et al., 2012; Rimbach et al., 2015) because they increase exposure and/or susceptibility to parasite.

In particular, although parasite transmission events are undoubtedly linked to animal behaviours, individual variation in behaviours has often been neglected in studies on the sources of heterogeneity in parasite richness, prevalence and load among hosts. For instance, transmission of oro-fecally gastro-intestinal parasites might occur

during social contacts and especially grooming in primates. Highly social hosts exchanging frequent social interactions are therefore expected to be more exposed to these parasites than less integrated individuals (Altizer et al., 2003; Friant et al., 2016; MacIntosh et al., 2012; Rimbach et al., 2015). Agonistic behaviours may also contribute to parasite exposure and susceptibility. For instance, aggressive Tasmanian devils seem particularly exposed to the devil facial tumour disease because they might bite the tumour of infected submissive conspecifics (Hamede et al., 2013). In meerkats, tuberculosis transmission increase with aggression received (J. A. Drewe, 2009), possibly because of the immunosuppressive effect of stress hormones such as cortisol and testosterone (R. M. Sapolsky, 2005b).

In general, immune-endocrine interactions are an under-estimated aspect of the causes influencing gastro-intestinal parasite transmission. However, both androgens and corticoids can cause immunomodulation favouring infection with helminths. Steroid hormone profile might differ between individuals depending on their dominance rank and the species social style, and could be influenced by i.e., reproductive status, mating opportunities, or food competition (Capitanio et al., 1998; R. M. Sapolsky, 2005b). This neuroendocrine effects on parasite susceptibility is therefore expected to account for variation in gastro-intestinal parasitism among hosts. Accordingly, some empirical studies showed that prolonged exposition to testosterone and glucocorticoids (GC) can lead to increased parasitism (S. Cohen et al., 1997; Defolie et al., 2020; Goymann & Wingfield, 2004; Habig & Archie, 2015; Muehlenbein, 2006; R. M. Sapolsky, 2005b).

While host behaviour can have profound impact on parasite transmission, parasite infection also typically affects animal behaviour. First, as a result of parasite infections, hosts can exhibit a suite of behavioural changes known as “sickness behaviour” and characterised by a reduced appetite and decreased activity levels including social activities (A Aubert, 1999; Ghai et al., 2015; Benjamin L. Hart, 1988; Johnson, 2002). In consequence, it could also bring costs such as decreased opportunities to mate (Owen-Ashley & Wingfield, 2006)These behavioural shifts triggered by pro-inflammatory cytokines (A Aubert, 1999; Dantzer, 2009), probably help fighting the infection (Johnson, 2002; Poulin, 1995) but may also incur as indirect costs to the hosts such as decreased food intake, which might exacerbate loss weight usually

associated to gastro-intestinal parasite infection (Sánchez et al., 2018; St Juliana et al., 2014). Contrastingly, some studies showed that individuals increase their feeding time to compensate the energetic cost of parasite infections (Budischak et al., 2018; Coop & Kyriazakis, 1999; Tripet & Richner, 1997; Voutilainen et al., 2008).

Moreover, even though the relationship between animal behaviour and parasitism has been usually studied in the perspective of passive diffusion processes, individuals can also modify their behaviour according to other individuals' infection status (MacIntosh et al., 2012; Müller-Klein et al., 2019; Rimbach et al., 2015; Wren et al., 2016). A few studies have indeed shown that wild animals avoid social interactions with infected conspecifics imposing an additional social cost to infected animals. For instance, mandrills avoid grooming groupmates infected with oro-faecally transmitted gastro-intestinal protozoan (Poirotte et al., 2017). While this defence strategy might efficiently decrease the risk of parasite transmission, it probably comes with the cost of depriving individuals from valuable social bonds. Due to the strong social positive impact of social bonds on individual's fitness (L. J. N. Brent, 2015; S. Ellis et al., 2017; Holt-Lunstad et al., 2010; McFarland et al., 2017; Joan B. Silk et al., 2003), it seems therefore crucial to measure social deprivation of infected hosts, as consequence of self-exclusion or active social avoidance.

Despite its importance, long-term and comprehensive studies evaluating the relative roles of host-, group- and population related factors on parasite transmission in wild animals are still rare, especially in wild population (Benavides et al., 2012; Habig et al., 2019; Poirotte et al., 2017). Moreover, previous results suggest host specific or hosts-parasite specific relationships between diverse factors and infection risk (Habig et al., 2019), calling for more studies to deepen our understanding of the drivers of heterogeneity in wild populations parasite distribution. While parasite affecting humans and livestock have been intensively studied, allowing to gain valuable knowledge on their genetic, ecology and epidemiology (host range, resources used, life cycle, transmission mode, etc.), many wildlife parasites, including non-human primates, are not yet even described and behavioural consequences on their hosts remain largely understudied.

In this study, we investigated during 18 consecutive months the factors influencing parasite infection in five groups of wild redfronted lemurs (*Eulemur rufifrons*) inhabiting the Kirindy forest in Madagascar, comprising in total 42 individuals. Redfronted lemurs are semi-arboreal primates living in relatively small but cohesive multimale-multifemale groups (Julia Ostner & Kappeler, 1999; Pyritz et al., 2011; Sperber et al., 2019) with on average 10 group members. The study groups inhabit a dry deciduous forest subjected to strong seasonally contrasted climatic conditions, with the alternance of a warm and wet season (from April to October) and an extremely dry but colder season (from November to March) (Peter M. Kappeler & Fichtel, 2012a). They are host to a large parasite community of eleven morphotypes (chapter 2). Seven nematodes and two protozoan, directly transmissible between hosts (i.e., they do not require any intermediate hosts to complete their cycle) and one trematode and one cestode, not transmissible between hosts (i.e., they need an intermediate host from another species to complete their cycle) (Clough et al., 2010; Irwin & Raharison, 2009). Redfronted lemurs are particularly suited to study relationships between animal behaviour or immune-endocrine effects with patterns of parasite distribution because they exhibit particular behavioural and physiological traits. First, as all other lemur species, they groom with a tooth comb, a behaviour thought to favour parasite transmission (Clough 2010). Second, in lemur species and contrarily to most anthropoid primates, both sexes excrete testosterone at similar levels outside of the mating season (Drea, 2007). Furthermore, in redfronted lemurs, testosterone and glucocorticoids levels vary over the annual cycle, with typical increase during the mating season (Julia Ostner et al., 2002, 2008).

We focused on three different parasite proxy, evaluated for each individual each month: (1) the global infection status, classifying each host as either infected (if at least one parasite was retrieved from faecal samples collected from this host the studied month) or non-infected; (2) parasite richness, corresponding to the mean number of parasite species recovered from all faecal samples collected from one host on month; and (3) the specific nematode and protozoan infection status, classifying each host as either infected or not by nematode and protozoan. Infection with diverse parasites species might be influenced similarly by some common factors, justifying the study of

global infection status and parasite richness. For instance, a factor inducing high host susceptibility might increase the chances of transmission with any pathogens. Moreover, parasite richness is considered as a robust metric to evaluate the ability of hosts to struggle against multiple parasite infections. However, nematodes and protozoan exhibit different life-history traits and their patterns of infection within a population might thus be controlled by distinct factors, leading us to further investigate separately host nematode and protozoan infection status. Indeed, nematodes exhibit a long life-cycle: they are usually in a non-infected stage when excreted in faeces and require a period of maturation of few days to two-three weeks in their environment, depending on climatic condition (Neveu-Lemaire, 1952). Hosts are typically infected through contact with contaminated substrate, by ingestion of infective larvae. By contrast, protozoan have a short life-cycle: they are directly excreted into infective stages (Neveu-Lemaire, 1952), that could be attached to the fur of infected individuals during body contact or grooming with conspecifics or when using their environment (MacIntosh et al., 2012; Poirotte et al., 2016).

We evaluated the relative importance of several population, group and individual drivers of each parasite proxy. We focused on factors influencing parasite transmission at three different levels. For population-level factors, we considered ecological season and to have a more detailed evaluation of variation due to seasonality, we considered cumulative rainfall over the previous month (mm). For group-level factors we considered group size and monthly percentage of time spent on the ground (calculated at the group level). For individual-level factors, we considered age, sex, monthly mean faecal GC metabolites (fGCm), monthly mean faecal testosterone metabolites (fTESTm) and monthly rate of time in contact with another individual (min/h), including all time grooming or receiving grooming, huddling and sitting in contact.

At the population level, we predicted that the wet season and monthly cumulative rainfall would be positively associated with all three parasite proxies, as it favours survival of nematode parasites in the environment (Hausfater & Meade, 1982; Nunn & Altizer, 2006). At the group level, we predicted that group size and the percentage of time the group spent on the ground would be positively correlated with

all three parasite proxies as it was found in other species before (Vanessa O Ezenwa et al., 2016; Habig et al., 2019; Patterson & Ruckstuhl, 2013; Vitone et al., 2004). At the individual level, we predicted that sex will not influence any of the parasite proxies. Indeed, this difference usually lies in differences in body size and/or testosterone levels (S. L. Klein, 2004; C. Nunn & Altizer, 2006), which do not exist in redfronted lemurs outside of the mating season (Julia Ostner et al., 2002, 2008). We further predicted a positive correlation between each parasite proxy with age because of parasite accumulation overtime and potentially imuno-senescence (Gillespie et al., 2010; Habig et al., 2019; Hayward et al., 2009; Nunn & Altizer, 2006). We also expected each parasite proxy to increase with faecal glucocorticoids metabolites levels (fGCm) and faecal testosterone metabolites levels (fTESTOm) because of the immunosuppressive effect of steroid hormones (Clough et al., 2010; Defolie et al., 2020; Muehlenbein & Watts, 2010; R. M. Sapolsky, 2005b). Finally, due to the contrasted life history traits between nematodes and protozoan (Neveu-Lemaire, 1952), we expected the individual tendency to exchange affiliative behaviours (i.e., grooming and physical contact) to influence particularly or exclusively patterns of protozoan distribution among hosts. Since parasites can be transmitted via grooming or body contact, we predicted that more social individuals are more likely to be infected with protozoan only because of their direct transmission pathway via grooming and body contact (MacIntosh et al., 2012; Poirotte et al., 2016).

In addition to investigating the processes generating patterns of parasite distribution, we evaluated some consequences of parasite infections, with a focus on behavioral consequences. In detail, we measured if body mass and time individuals allocated for resting, feeding, and grooming (given and received) changed with one of the parasite proxies. First, we expected body mass to decrease with parasite richness, due to the presence of multiple parasite morphotypes diverting resources from the hosts, and the increased energetic demand required to mount an immune response (Bonneaud et al., 2003b; Colditz, 2008). As a compensatory mechanism, we further expected that infected individuals (general infection status) would spend more time resting and feeding than non-infected one, to compensate energetical loss due to parasitism (Ghai et al., 2015; Müller-Klein et al., 2019). Finally, because redfronted

lemurs avoid drinking in faecally contaminated waterholes, a probable behavioural strategy to avoid infection with gastro-intestinal parasites (Amoroso et al., 2019), we formulated the hypothesis that individuals would exhibit social avoidance of infected individuals. We therefore expected infected individuals to receive less grooming than non-infected individuals because animals should avoid grooming infected groupmates (especially because the use of their tooth comb should increase the risk of transmission), and/or because infected individuals could re-direct the time usually spent socializing to rest and feed.

Table 1: Potential determinants of parasite infections with hypotheses and evidence from field studies

Factor	Level	Influence	Mechanism	Hypotheses	Evidence from field studies
Rainfall	Population	(+)	Exposure	Moist environment favours parasite development, replication and survival (Nunn & Altizer, 2006)	Chacma baboons (Benavides et al., 2012); eastern chimpanzees (Gillespie et al., 2010) ;
		(-)	Exposure	Wash-out effect of heavy rains (Hausfater & Meade, 1982)	Mandrills (Poirotte et al., 2016)
Temperature	Population	(+)	Exposure	Moist environment favours parasite development, replication and survival (Nunn & Altizer, 2006)	Chacma baboons (Benavides et al., 2012); red-legged partridge (Calvete et al., 2003);
		(-)	Exposure	Desiccation of parasite free-living stages (Hausfater & Meade, 1982)	Mandrills (Poirotte et al., 2016); Amboseli baboons (Habig et al., 2019)
Home range size	Group	(+)	Exposure	Parasite encounter probability increases in a larger home range (Nunn & Altizer, 2006)	Carnivores (Lindenfors et al., 2007)
Home range size	Group	(-)	Exposure	Parasite encounter probability increases in a more intensively used home range (Nunn & Altizer, 2006)	Mammals (Bordes et al., 2009)
Daily travel distance	Group	(+)	Exposure	Parasite encounter probability increases in a more intensively used home range (Nunn & Altizer, 2006)	Primates (Nunn & Dokey, 2006); California meadow mice (Mohr & Stumpf, 1964)
Group size	Group	(+)	Exposure	Parasite encounter probability increases with group density (Côté & Poulin, 1995; Vitone et al., 2004)	Mammals (Patterson & Ruckstuhl, 2013)
Age	Individual	(+)	Exposure	Accumulation of parasites over time (Nunn & Altizer, 2006)	Eastern chimpanzees (Gillespie et al., 2010); White-faced capuchins (Parr et al., 2013)
		(+)	Susceptibility to infection	Immunoscenesencence	Amboseli baboons (Habig et al., 2019); St Kilda Soay sheep (Hayward et al., 2009)
		(-)	Susceptibility to infection	Reinforcement of immune response with age	Artiodactyles (Vanessa O. Ezenwa et al., 2006); grey mouse lemurs (Hämäläinen et al., 2015)
		not linear with an optimal age	Exposure & Susceptibility to infection	At first, accumulation of parasites over time, then reduction of susceptibility with the development of an adapted immune response (Hudson & Dobson, 1997)	Chacma baboons (Benavides et al., 2012)

Table1 (continued)

Factor	Level	Influence	Mechanism	Hypotheses	Evidence from field studies
Sex	Individual	(+) ♂	Exposure & Susceptibility to infection	Increased exposure in dimorphic species because of an increased food intake in ♂ (Nunn & Altizer, 2006); increased susceptibility due to immunosuppressive effects of steroid hormones (S. L. Klein, 2004; Roberts et al., 2004)	Rodents (Krasnov et al., 2005); mouse lemurs (Hämäläinen et al., 2015)
		(+) ♀	Susceptibility to infection	Increased susceptibility in ♀ during gestation and around birth due to immunosuppressive hormones (S. L. Klein, 2004)	Japanese macaques (MacIntosh et al., 2010); Mandrills (Poirotte et al., 2016)
Physical condition/body mass	Individual	(+)	Exposure	Individuals in better condition might eat more and explore more, increasing exposure (Nunn & Altizer, 2006)	Males grey mouse lemurs (Hämäläinen et al., 2015)
		(-)	Susceptibility to infection	Better ability to resist infection for individuals in good condition (Irvine et al., 2006)	Rabbits (Lello et al., 2005); females grey mouse lemurs (Hämäläinen et al., 2015)
Dominance rank	Individual	(+)	Exposure & Susceptibility to infection	Increased exposure in dominants because of an increased food intake (Nunn & Altizer, 2006); Higher susceptibility in dominants due to immunosuppressive effects of steroids (R. M. Sapolsky, 2005b)	Yellow baboons (Hausfater & Meade, 1982); Japanese macaques (MacIntosh et al., 2010)
		(-)	Susceptibility to infection	Higher susceptibility in subordinates in stable rank societies because of immunosuppressive effects of steroids or worst body condition (Irvine et al., 2006; Sapolsky, 2005)	
Glucocorticoids levels	Individual	(+)	Susceptibility to infection	Immunosuppressive effects of glucocorticoids (R. M. Sapolsky, 2005b)	Mammals (Defolie et al., 2020); redfronted lemurs (Clough et al., 2010)
		(-)		Immuno-enhancing effect of glucocorticoids	Amboseli baboons (Habig et al., 2019)
Testosterone levels	Individual	(+)	Susceptibility to infection	Immunosuppressive effects of steroids (R. M. Sapolsky, 2005b)	Chimpanzees (Muehlenbein & Watts, 2010); Redfronted lemurs (Clough et al., 2010)
		(-)		Immuno-enhancing effect of steroids	Redfronted lemurs (Clough et al., 2010)
Affiliative behaviour	Individual	(+)	Exposure	By increasing social contact, individuals increase their exposure and thus probability of infection (Altizer et al., 2003; Ezenwa et al., 2016)	Amboseli baboons (Habig et al., 2019)
		(-)	Exposure & Susceptibility to infection	Resistance, allogrooming and anti-parasitic behaviours could reduce exposure and susceptibility in more social individuals (Ezenwa et al., 2016)	

Materials and methods

Study site and population

We studied a population of redfronted lemurs (*Eulemur rufifrons*) from May 2015 to October 2016 at the research station of the German Primate Center (DPZ) in a 60ha study area in Kirindy Forest, western Madagascar. The study area is a dry deciduous forest, subjected to pronounced seasonality due to a dry season from March to October and a wet season from October to February (Peter M. Kappeler & Fichtel, 2012a). The study population comprised 42 individuals living in five adjacent groups, ranging from 4 to 15 individuals. This species is cathemeral, semi-arboreal and frugivorous (Donati et al., 1999; Mittermeier et al., 2008). Redfronted lemurs are seasonal breeders with a short mating season usually in May/June and birth of single infants in September/October (Julia Ostner et al., 2002). All individuals have been well habituated to the presence of human and are marked with unique combinations of nylon collars and pendants for individual recognition, for more than 20 years (Peter M. Kappeler & Fichtel, 2012a). One adult female per group was equipped with a VHF-radio-collar to facilitate group location. Individual information about sex and age was available because of long-term monitoring of the population. For individuals who had immigrated into the population, age was estimated at first capture of these individuals using tooth wear and sexual maturity.

Behavioural observations

We observed repeatedly each adult individual by conducting focal animal sampling (Jeanne Altmann, 1974) for a duration of one hour. In addition, we observed juveniles over five months old as much as possible. We continuously protocolled all behaviour between 7 a.m. to 11 a.m. and 1 p.m. to 4 p.m. We regrouped all recorded socio-positive interactions (allogrooming, huddling, body contact) in one variable named “body contact” and expressed as minutes per hour of observation. A time-budget analysis (in percent of all time observed) was calculated for each individual by categorizing each behaviour into one of the following five categories: (i) social positive behaviour (allogrooming, huddling and body contact), (ii) moving (walking, running or climbing), (iii) foraging and feeding behaviour, (iv) resting behaviour (sitting, lying down) and (v) all other behaviours.

We defined six different reproductive periods for females: “mating”, “early gestation”, “late gestation”, “early lactation” and “late lactation” and “rest”. Considering a gestation length of 121 days (Julia Ostner & Heistermann, 2003), we defined “mating” for each group as the period of two weeks before and following the estimated average date of conception for the females of the group, based on their average date giving birth. “Early gestation” was defined as the first half of gestation time and took place between “mating” and “late gestation”. Females were in “late gestation” during the second half of gestation period and lasted from the end of “early gestation” to the beginning of “early lactation”, ending when all females in the group gave birth. “Early lactation” covered the first four weeks after giving birth and “late lactation”. “Late lactation” was the time between “early lactation” and “rest” period and lasted 3.5 months. Finally, the period called “weaned” was the period of the year with weaned juveniles and before the following “mating” period. All adult females gave birth in 2015 and 2016. A schematic overview of reproductive periods is given in figure 1.

Figure 1: Schematic presentation of the six reproductive periods considered during our study

January	February	March	April	May	June	July	August	September	October	November	December
Late lactation		Weaned		Mating	Early gestation			Late gest.	Early lact.	Late lactation	

Body mass

Twice a month and for each focal group, a veterinary balance was set on the forest floor to record individual body mass changes.

Faecal sample collection

For parasite and hormone analyses,732 faecal samples from the 42 study animals (15 adult males, 14 adult females, 3 juveniles in 2015 and 18 adult males, 14 adult females, 9 juveniles in 2016) were collected. To limit bias due to temporal variation in parasite and hormone excretion (V. Behringer & Deschner, 2017), samples were systematically collected between 07 am and 11 am, immediately after defecation. After collection, samples were divided in aliquots to run several analyses. One to two grams of faeces were placed in 15ml polypropylene tubes pre-aliquoted with 5ml of 10% neutral-buffered formalin for parasite

analyses, while 0.5 g of faeces went to 15ml polypropylene tubes containing 5ml of 90% ethanol for hormone analyses. Then the tubes were labeled and wrapped with parafilm and brought back to the camp within 3 hours for storage or extraction. Parasites and hormones samples were always collected between 7 a.m. and 10 a.m. to account for a potential circadian effect on parasite egg shedding or hormone levels (Martinaud et al., 2009; Sousa & Ziegler, 1998; Villanúa et al., 2006).

Hormone extraction and analysis

Samples for hormone analysis were extracted at the camp within 4 h of sample collection adapting a protocol described by Ziegler and Wittwer (2005) and modified by Shutt et al. (2012). A small amount of c.a.0.5g of faeces from the homogenised aliquot was weighed into an extraction tube and mixed with 2 ml of 90% ethanol. For logistic reasons, the fecal suspensions were left to stand for 5–12 h, then vortexed for 2 min. Samples were finally centrifuged using a manually operated centrifuge (Hettich GmbH & Co. KG Tuttlingen, Germany) for 2 min (Shutt et al., 2012). The supernatant was poured into a 2 ml polypropylene tube and sealed with parafilm. Samples were stored at the field site in a dark container a t ambient temperature for a maximum of six months and returned to the laboratory in November 2015, April 2016 and November 2016. Upon arrival at our German laboratory, samples were stored at -20°C until hormone analysis. The remaining faecal matter was dried in a solar oven until dry faecal weight was constant, mass to obtain an estimate of the water content of the feces (Shutt et al., 2012; Ziegler & Wittwer, 2005). The hormone extracts were used for measurements of faecal metabolites of immunoreactive testosterone (thereafter fTESTm) and 5b reduced cortisol (3a,11-oxo-CM, thereafter fGCm) using microtitreplate enzymeimmunoassays (EIA). Methods have previously been described and validated for redfronted lemurs (Julia Ostner et al., 2002, 2008). Sensitivity of both assays at 90% binding was 0.3 pg/50µl (androgen) and 1.0 pg/50µl (glucocorticoid). Intra- and interassay coefficients of variation (CV) for androgens of high- and low-value quality controls (QCs) were 6.0% and 8.1% and 8.7% and 12.6% for androgens across both years. Intra- and interassay CV values for glucocorticoid measurements were 6.8% and 8.8% (high) and 7.9% and 13.0%, respectively. All hormone values are expressed as mass per gram dry faecal weight (ng/g). For statistical analyses, we calculated an individual monthly mean from individual sample value.

Parasite analysis

Parasite samples were kept at the camp in the dark, at ambient temperature. They were stored at the field site for a maximum of six months and returned to the laboratory in November 2015, April 2016 and November 2016, were they were kept in the dark, at ambient temperature. For parasite analysis, we followed the methods used by Clough (2010) on the same population of red-fronted lemurs. Faecal samples were processed using a modified version of the formalin-ethyl-acetate sedimentation technique described by Ash and Orihel (1988). Approximately 5 ml of homogenized faecal material was strained into centrifuge tubes and 10% of formalin was added until the total volume reached 10 ml. Then we added 3 ml ethyl-acetate and shook the tube vigorously for 30 s and centrifuged it for 10 min on 2,200 rpm. We then removed the top layer of fat before pouring off the supernatant consisting of ethyl-acetate, formalin, and debris from the centrifuge tube. The remaining sediment was used for subsequent analyses. Details of the methods as well as on the identification of parasite species can be found in Clough (2010). Wet mounts of each sample were prepared with 20 mg of sediment and one drop of Lugol's solution on a microscope slide. One slide was systematically scanned for each sample, looking for helminth eggs and larvae as well as protozoan cysts and trophozoites.

Measurements of parasite infection

We decided to focus only on qualitative parasite measures because all gastro-intestinal parasites have intermittent and unpredictable egg shedding, raising some doubts about the reasonable use of faecal egg counts as a measure of infection intensity (R. M. Anderson & Schad, 1985; Gillespie, 2006). Given the low parasite occurrence of most morphotypes (see Table 3), data for all parasite infections were pooled to consider infection status with any kind of parasites (presence or absence of any parasite infection) for statistical analyses. Our measurements of parasitism include: 1. The individual monthly 'general infection status', classifying each host each month as either infected (if at least one parasite morphotype was retrieved from faecal samples collected from this host the studied month) or non-infected; 2. The individual monthly parasite richness, calculated by summing the number of different

morphotype found in all faeces collected from one individual over a month; 3. The individual monthly 'nematode infection status' and 'protozoan infection status', classifying each host as either infected or not by nematode and protozoan.

Rainfall

Daily rainfall data was measured by a weather station located at the field camp. Cumulative rainfall was calculated for each month of our study period. For each monthly parasite proxy, we considered rainfall data from the previous month, to account for environmental conditions when the parasites might have been in the environment before transmission.

Statistical analyses

In a first set of Generalized Linear Mixed Models ('GLMM'; Baayen et al., 2008), we investigated the potential determinants influencing parasite richness (M.I, & M.II, N = 302 for both, both fit with a Poisson error structure and a logit link function), and the general, nematode and protozoan infection status (*resp.* M.III, M.IV, M.V & M.IV, N = 302 for all, all fit with binomial error structure and logit link function) of the 42 individuals from the five groups (see supplementary Table 1 for a list of all models). In model M.I investigating parasite richness, we included the ecological season as the population explanatory variable, while in model M.II also investigating parasite richness, we instead considered monthly cumulative rainfalls lagged by a month. In model M.III investigating the general infection status, we included the ecological season as the population explanatory variable, while in model M.IV also investigating the general infection status, we instead considered monthly cumulative rainfalls lagged by a month. In Models M.V & M. VI, we decided to consider only monthly cumulative rainfall as the population explanatory variable because it was a more detailed variable than the binary variable "ecological season". We otherwise included for all of these models the same individual explanatory variables, i.e., host's age, sex, monthly rate of time spent in body contact (min/h), and monthly mean fGCm and fTESTm, and the same group explanatory variables, i.e., group size and monthly percentage of time spent on the ground. In all models from this first analytical part, the year was initially included as a covariate, before

being removed for model simplification as it never impacted the response variable. Furthermore, in all these models, we accounted for non-independence of repeated measurements by including individual identity (ID) as a random effect.

In a second set of models (see supplementary Table 1 for a list of all models), we investigated the potential physiological and behavioral consequences of parasite infection. In particular, we conducted a Linear Mixed Model ('LMM', Baayen et al., 2008) to examine potential consequences of parasite richness on body condition of 13 males (M.VII, N = 72), as we expected a stronger negative effect of parasite infection on body mass with the accumulation of different morphotypes. This model was conducted on males only to control for weight gain during pregnancy and loss during lactation and included the following control explanatory variables: fruit abundance, host's age and monthly mean fGCm and fTESTm. Using data collected on the 42 individuals, we finally ran four additional GLMM to examine potential consequences of general infection status on activity budget (% of time spent resting or feeding) and rates (min/h) of grooming given to- or grooming received from- other individuals (*resp.* M.VIII, M.IX, M.X and M.XI, N = 319 for all, all fit with a beta error structure and logit link function). In these models, we controlled for the effect of host's age and sex. We further included as control explanatory variables the monthly fruit abundance in model M.VIII as a it is known to influence time spent feeding and the reproductive periods in model M.IX to M.XI as changes in social relationships might happen between reproductive periods. Because this species is a seasonal breeder with mating and gestation during the dry season, ecological season and reproductive periods are highly correlated and we could not include both in the models. As previously, all models from this second analytical part included individual identity as a random effect to account for the nonindependence of measurements.

All models conducted in this study were applied using R software (version 3.6.2; R Core Team, 2018). Models I to VI were fit using the *lme4* package (Bates et al., 2015) and models VII to X using the *glmmTMB* package. For all models, the non-categorical covariates were z-transformed (to reach a mean of zero and a standard deviation of one) to achieve easier interpretable models and facilitate model convergence (Schielzeth, 2010).

After fitting each model, we controlled the assumptions based on their error structure. We checked for normally distributed and homogenous residuals for the LMM by visually inspecting a qqplot and the residuals plotted against fitted values, and for all GLMMs by using

a function kindly provided by Roger Mundry controlling for overdispersion. We further controlled the normal distribution of the random factor in all models using the same function. Stability of all models was verified using another function provided by Roger Mundry, which excluded data points one by one and compared the derived coefficients. We finally verified the absence of collinearity issues by deriving variance inflation factor using the function *vif* of the R-Package *car* (Fox & Weisberg, 2011) applied to a standard linear model excluding the random effects. For all models, we preliminarily verified that the full models significantly differed from the corresponding null models using likelihood ratio tests before investigating single-predictor effects to reduce the risks of Type I errors (R function ANOVA with argument test set to "Chisq") (A. J. Dobson, 2002; Forstmeier & Schielzeth, 2011). All full-null model comparisons were significant and these results are reported in supplementary Table 1. P-values of each fixed factors were then derived using likelihood ratio tests based on Maximum Likelihood (rather than Restricted Maximum Likelihood; Bolker, 2008) comparing each full model with its respective reduced models (i.e., the model without the considered factor; Barr et al., 2013; R function *drop 1*). Confidence intervals were derived using parametric bootstrapping with an adjusted function provided by Roger Mundry and based on the function *bootMer* from the *lme4* package, with 1000 parametric bootstraps and bootstrapping over the random effects. Finally, for all models from the first analytical part with parasite richness or infection determinants as response variable, we assessed potential autocorrelation by comparing the distribution of the residuals and the effect sizes, SE and p-values of our models with similar Generalized Additive Mixed Models (GAMM) using the package GAMM4 (Version 0.2-5). There were no differences, suggesting no autocorrelation issues.

Results

Table 2: Parasite prevalence in the redfronted lemurs of Kirindy forest

Parasite morphotype	Prevalence in % of individual infected (n=735 samples, 42 individuals)
Nematoda	
Subulura sp.	9.52
Trichuris sp.	11.90
Oxyuridae sp.	9.52
Lemuricola sp.	59.52
Callistoura sp.	85.71
Trichostrongylidae sp.	38.10
Strongyloididae sp.	7.14
Cestoda	
Anoplocephalidae sp.	16.67
Trematoda	
Dicrocoeliidae sp.	14.29
Protozoan	
Entamoeba sp.	66.67
Balantidium sp.	78.57

We found a diverse parasite community with a total of eleven morphotypes of GI parasites in faeces of red-fronted lemurs from Kirindy forest, Madagascar including seven nematodes, one cestode, one trematode and two protozoans (Table 2). The nematodes included *Lemuricola vauceli, Subulura sp., Oxyuridae sp.,* one morphotype of *Callistoura sp., Trichuris sp.,* one trichostrongylid-type and one strongyloid morphotype. We also found one anoplocephalid cestode and a dicrocoelid trematode as well as two protozoan parasites being likely *Entamoeba coli* and *Ballantidium coli*. See chapter 2 for more details on parasite morphotypes and their identification.

Determinants of parasite richness

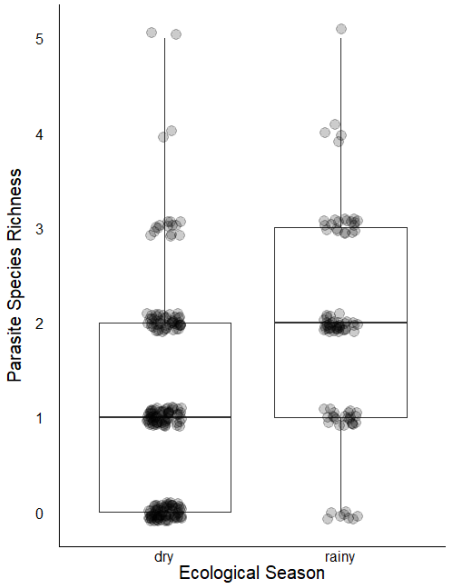

Figure 2: Model I: Parasite richness (y axis) is higher during the wet season than during the dry season (x-axis). Each datapoint is represented by a sheer grey dot. Each box indicates median, upper, and lower quartiles. Whiskers indicate ± 1.5 interquartile ranges.

In model I, we found that seasonality significantly influenced parasite richness: individuals harbored more morphotypes in the rainy compared to the dry season (M.I, Table 4, fig.2). None of the group factors influenced parasite richness (M.I, Table 4). Among individual factors, fGCm showed a tendency to decrease with increasing parasite richness, but all other individual variables did not influence parasite richness (M.I, Table 4). The second model showed that variation in parasite richness was positively correlated with monthly cumulative rainfall (M.II, fig. 3A). In line with the first model, parasite richness was further negatively correlated with fGCm levels in model II (M.II, fig. 3B). Similar to the first model, neither ground use, group size, sex, age, time in body contact and fTESTm were linked to variations in PSR (M.II, Table 4).

Table 4: Results of the model with parasite richness as a predictor

Model	Predictor	Explanatory variable	Estimate	SE	Lower CI	Upper CI	Df	Chi²	p
M.I	**Parasite richness**	(Intercept)	0.13	0.09	-0.07	0.27	[c]	[c]	[c]
		sex (male)[a]	-0.14	0.11	-0.40	0.05	1	1.527	0.217
		age[b]	0.08	0.05	-0.04	0.17	1	2.522	0.112
		group size[b]	-0.10	0.05	-0.21	0.01	1	3.383	0.066
		time in body contact[b]	0.06	0.05	-0.01	0.16	1	1.386	0.239
		fGCm[b]	-0.16	0.08	-0.31	0.01	1	3.606	0.058
		fTESTm[b]	-0.11	0.09	-0.33	0.01	1	1.918	0.166
		season (rainy)[a]	0.46	0.11	0.24	0.71	1	16.43	**<0.001**
		ground use[b]	-0.08	0.05	-0.20	0.03	1	2.489	0.115
M.II	**Parasite richness**	(Intercept)	0.27	0.08	0.07	0.40	[c]	[c]	[c]
		sex (male)[a]	-0.13	0.11	-0.32	0.09	1	1.49	0.22
		age[b]	0.08	0.05	-0.01	0.18	1	2.55	0.11
		group size[b]	-0.10	0.05	-0.21	0.00	1	3.56	0.06
		time in body contact[b]	0.07	0.05	-0.03	0.18	1	1.96	0.16
		fGCm[b]	-0.18	0.08	-0.30	-0.04	1	4.87	**0.03**
		fTESTm[b]	-0.07	0.08	-0.26	0.05	1	0.89	0.35
		rainfall month[b]	0.19	0.05	0.10	0.28	1	14.12	**<0.001**
		ground use[b]	-0.05	0.05	-0.15	0.06	1	0.72	0.40

[a] Reference category being female for sex and dry season for the ecological season
[b] z-transformed
[c] Not shown as not having a meaningful interpretation. For intercepts, p-values would refer to estimated PSR, when all covariates are at zero.
Chi² values are results of a likelihood ratio test comparing the full model with a reduced model lacking the respective term

Determinants of parasite infection

Models III and IV revealed that general infection status was positively correlated with rainy season (M.III) or monthly cumulative rainfall (M.IV) and negatively correlated with fGCm levels(both models) but there was no other significant correlation (M.III & M.IV, Table 5), in line with models I & II. Model V showed that age was correlated with nematode infection status, with older individuals being more infected with nematodes than younger individuals

(M.V, Table 6, fig. 4). Neither sex, time in body contact, fGCm, fTESTm, ground use, group size, nor rainfall were significantly correlated with nematode infections (M.V, Table 6). In model VI, we found that protozoan infection status was positively correlated with monthly cumulative rainfall and negatively correlated with fGCm levels (M.VI, Table 6), similarly to model II and IV. In addition, there were effects of time spent in body contact and group size. Individuals were more likely to be infected with protozoans when they spent more time in body contact and lived in smaller groups (M.VI, Table 6, fig. 5 A, B, C and D). However, age, fTESTm and ground use were not correlated with protozoan infection status (M.VI, Table 6).

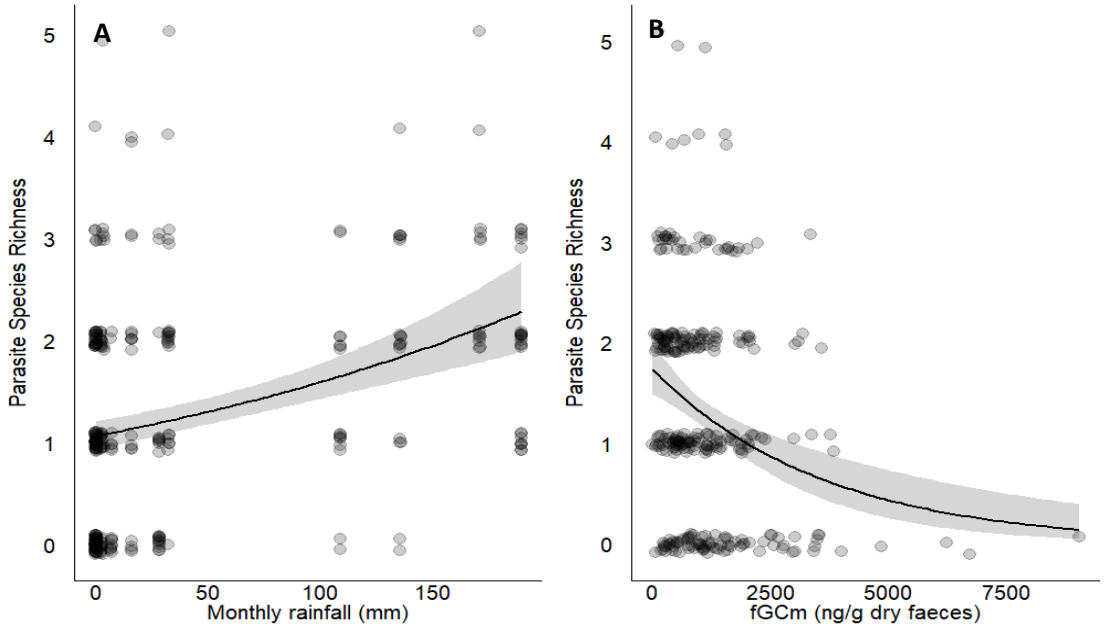

Figure 3: Model II: Parasite richness (y-axis): (A) increases with increasing rainfall (x-axis) and (B) decreases with increasing fGCm (x-axis). Datapoints are represented by sheer grey dots. Plain line represents the model's predicted value, shaded area represents the 95% confidence interval.

Table 5: Results of the models with general infection status as a predictor

Model	Predictor	Explanatory variable	Estimate	SE	Lower CI	Upper CI	Df	Chi²	p
M.III	**General infection status**	(Intercept)	0.95	0.236	0.55	1.59	[c]	[c]	[c]
		sex (male)[a]	-0.477	0.302	-1.12	0.15	1	2.53	0.11
		Age[b]	0.173	0.167	-0.15	0.55	1	1.11	0.29
		group size[b]	-0.275	0.157	-0.53	0.06	1	3.01	0.08
		time in body contact[b]	0.186	0.165	-0.01	0.52	1	1.34	0.25
		fGCm[b]	-0.478	0.194	-0.87	-0.10	1	6.54	**0.01**
		fTESTm[b]	-0.226	0.182	-0.75	0.01	1	1.84	0.18
		season (rainy)[a]	1.377	0.422	0.71	2.51	1	12.75	**<0.001**
		ground use[b]	-0.229	0.166	-0.57	0.06	1	1.87	0.17
M.IV	**General infection status**	(Intercept)	1.46	0.24	1.07	2.05	[c]	[c]	[c]
		sex (male)[a]	-0.49	0.3	-1.06	0.12	1	2.61	0.11
		Age[b]	0.16	0.17	-0.13	0.49	1	0.95	0.33
		group size[b]	-0.3	0.16	-0.66	0.03	1	3.52	0.06
		time in body contact[b]	0.2	0.17	-0.06	0.55	1	1.58	0.21
		fGCm[b]	-0.5	0.19	-0.95	-0.13	1	7.65	**0.01**
		fTESTm[b]	-0.17	0.16	-0.67	0.09	1	1.18	0.28
		rainfall month[b]	0.91	0.26	0.54	1.61	1	18.27	**<0.001**
		ground use[b]	-0.17	0.17	-0.46	0.12	1	1.01	0.32

[a] Reference category being female for sex and dry season for the ecological season
[b] z-transformed
[c] Not shown as not having a meaningful interpretation. For intercepts, p-values would refer to estimated PSR, when all covariates are at zero.
Chi² values are results of a likelihood ratio test comparing the full model with a reduced model lacking the respective term

Table 6: Results of the models with infection status with nematodes or protozoans as a predictor

Model	Predictor	Explanatory variable	Estimate	SE	Lower CI	Upper CI	Df	Chi²	p
M.V	**Nematode infection status**	(Intercept)	0.49	0.18	0.18	0.91	[c]	[c]	[c]
		sex (male)[a]	-0.26	0.26	-0.71	0.21	1	1	0.32
		Age[b]	0.32	0.14	0.12	0.65	1	5.44	**0.02**
		group size[b]	0.01	0.13	-0.26	0.22	1	0	0.96
		time in body contact[b]	0.03	0.13	-0.22	0.29	1	0.05	0.82
		fGCm[b]	-0.26	0.16	-0.59	0.03	1	2.72	0.1
		fTESTm[b]	-0.16	0.15	-0.58	0.1	1	1.3	0.25
		rainfall month[b]	0.11	0.14	-0.16	0.41	1	0.65	0.42
		ground use[b]	0	0.14	-0.21	0.2	1	0	0.98
M.VI	**Protozoan infection status**	(Intercept)	0.11	0.21	-0.4	0.51	[c]	[c]	[c]
		sex (male)[a]	-0.46	0.3	-1.03	0.14	1	2.36	0.13
		Age[b]	0.22	0.15	-0.1	0.54	1	2.01	0.16
		group size[b]	-0.51	0.16	-0.8	-0.22	1	10.32	**<0.001**
		time in body contact[b]	0.31	0.15	0.01	0.66	1	4.48	**0.03**
		fGCm[b]	-0.59	0.23	-1.08	-0.2	1	7.35	**0.01**
		fTESTm[b]	-0.18	0.22	-0.75	0.12	1	0.75	0.39
		rainfall month[b]	1.16	0.21	0.86	1.74	1	46.43	**<0.001**
		ground use[b]	-0.27	0.15	-0.57	0.05	1	2.96	0.09

[a] Manually dummy-coded with the reference category being female
[b] z-transformed
[c] Not shown as not having a meaningful interpretation. For intercepts, p-values would refer to estimated Infection status, when all covariates are at zero.
Chi² values are results of a likelihood ratio test comparing the full model with a reduced model lacking the respective term

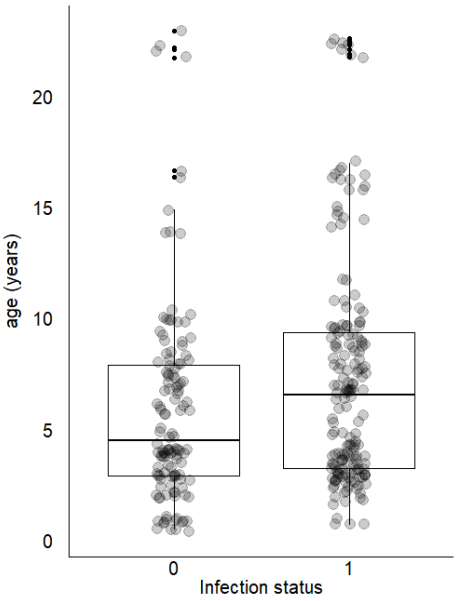

Figure 4: Model IV: Individuals infected with nematodes (x-axis) are older (y-axis) than non-infected individuals. Datapoints are represented by sheer grey dots. Each box indicates median, upper, and lower quartiles. Whiskers indicate ± 1.5 interquartile ranges and outliers are plotted as solid black dots outside of the box and whiskers'area.

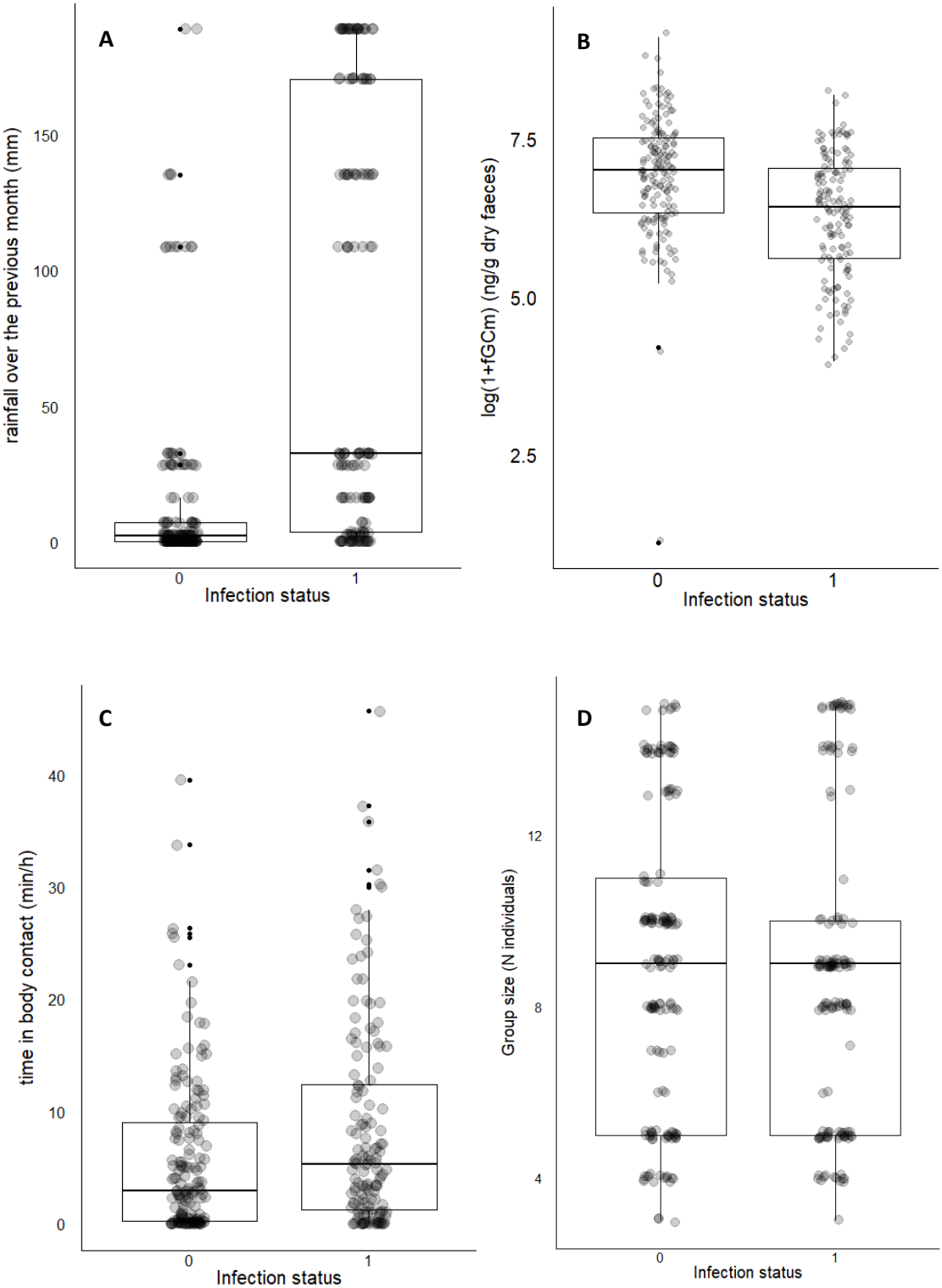

Figure 5: Model V: Individuals are more infected with protozoans (y-axis) when (A) rainfall (x-axis) is high; (B) fGCm (x-axis) are low; (C) they spend more time in body contact with other individuals (x-axis); and (D) they live in smaller groups (x-axis). Each datapoint is represented by a sheer grey dot. Boxes area indicates median, upper, and lower quartiles. Whiskers indicate ± 1.5 interquartile ranges and outliers, when they exist, are plotted as solid black dots outside of the box and whiskers'area.

Consequences of parasite infections

Model VII revealed that body mass was positively correlated with parasite richness (Fig. 5) and age but none of the other predictors included in our model (M.VII, Table 7, fig. 6A).

Model VIII showed that percentage of resting time was positively correlated with cumulative rainfall but negatively correlated with infection status, but none of the other covariates was significantly correlated with resting time (M.VII, Table 7, fig 6B).

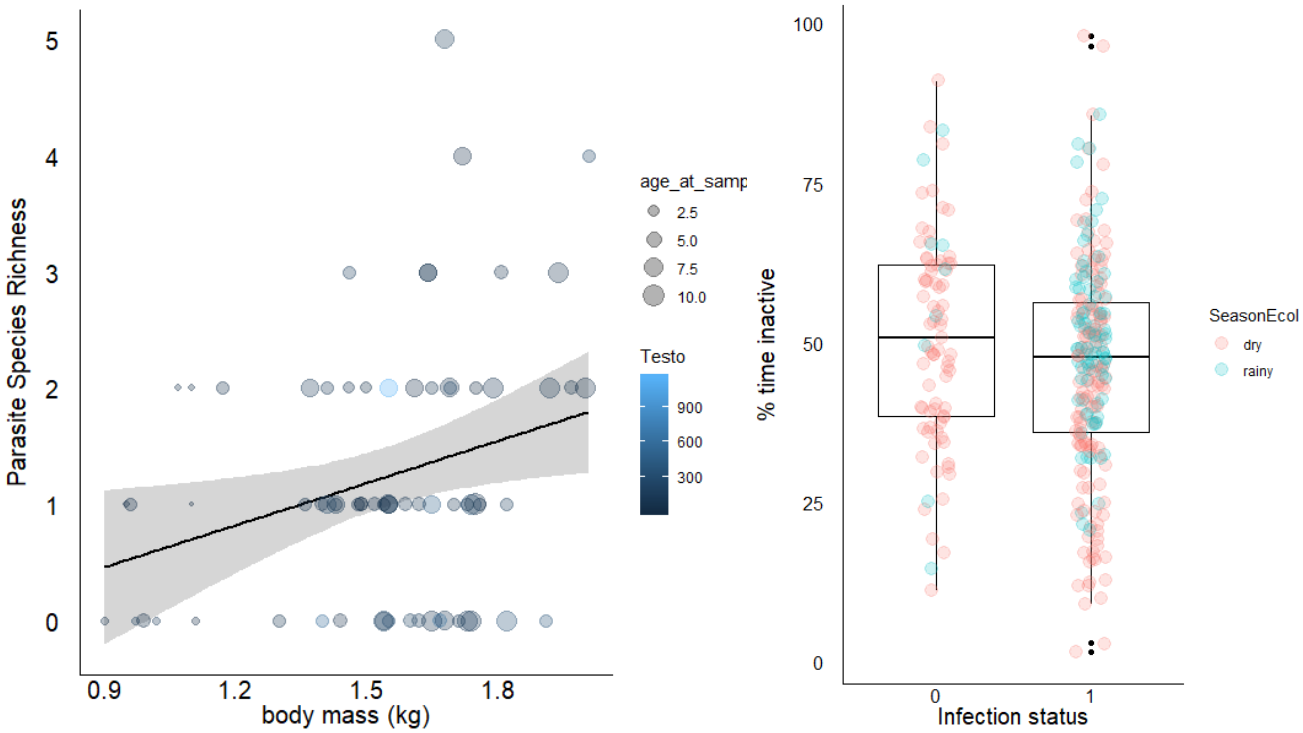

Figure 6: (A) Model VII. Body mass (x-axis) is positively correlated with parasite richness (y-axis). Datapoints are represented by sheer blue dots, the shade deepening with testosterone levels. Dot size indicates age of the individual for each sampling month. The plain line represents the model's predicted value, shaded area represents the 95% confidence interval. (B) Percentage of resting time is negatively correlated with infection. Datapoints are represented by sheer dots. Datapoints from the dry season are in red, the ones from the the rainy season are in blue. Each box indicates median, upper, and lower quartiles. Whiskers indicate ± 1.5 interquartile ranges and outliers, when they exist, are plotted as solid black dots outside of the box and whiskers'area.

Table 7: Results of the models VII, VIII and IX with body mass, or percentage of resting or feeding as predictor

Model	Predictor	Explanatory variable	Estimate	SE	Lower CI	Upper CI	Df	Chi²	p
M.VII	**body mass (kg)**	(Intercept)	1.50	0.08	1.36	1.65	[c]	[c]	[c]
		Age[b]	0.20	0.10	0.02	0.40	1	2.98	0.08
		fGCm[b]	-0.03	0.02	-0.06	0.01	1	1.76	0.19
		fTESTm[b]	0.03	0.02	-0.01	0.07	1	2.59	0.11
		Fruit abundance [b]	0.04	0.02	0.01	0.07	1	3.94	**0.047**
		PSR	0.04	0.01	0.02	0.06	1	13.01	**<0.001**
M.VIII	**% resting time**	(Intercept)	-0.03	0.10	-0.23	0.16	[c]	[c]	[c]
		Age[b]	0.26	0.09	0.26	0.10	1.00	7.00	**0.01**
		fGCm[b]	-0.03	0.02	0.03	-0.08	1.00	2.54	0.11
		fTESTm[b]	0.02	0.02	0.02	-0.01	1.00	1.32	0.25
		PSR	0.05	0.01	0.05	0.03	1.00	17.51	**<0.001**
		fGCm[b]	-0.12	0.05	-0.21	-0.03	1.00	6.96	**<0.001**
		Infection status (infected) [a]	0.09	0.10	0.11	0.28	1.00	0.76	0.38
M.IX	**% feeding time**	(Intercept)	-0.42	0.10	-0.62	-0.22	[c]	[c]	[c]
		Sex (male)[a]	0.02	0.09	-0.16	0.21	0.07	1.00	0.80
		Age[b]	-0.07	0.05	-0.16	0.02	1.99	1.00	0.16
		Fruit abundance [b]	0.10	0.05	0.00	0.19	4.13	1.00	**0.04**
		fGCm[b]	-0.02	0.05	-0.11	0.07	0.23	1.00	0.63
		Infection status (infected) [a]	0.08	0.10	-0.12	0.28	0.61	1.00	0.43

[a] Manually dummy-coded with the reference category being female for sex, weaned for reproductive periods and 0 (no infection) for infection status
[b] z-transformed
[c] Not shown as not having a meaningful interpretation. For intercepts, p-values would refer to estimated Infection status, when all covariates are at zero.
Chi² values are results of a likelihood ratio test comparing the full model with a reduced model lacking the respective term

Model IX showed that feeding time was only correlated with reproductive periods, and none of the other variables, including infection status were correlated (M. IX, Table 7).

Table 7: Results of the models X and XI with grooming given or received as a predictor

Model	Predictor	Explanatory variable	Estimate	SE	Lower CI	Upper CI	Df	Chi²	p
M.X	**grooming received**	(Intercept)	-2.56	0.12	-2.79	-2.34	[c]	[c]	[c]
		Sex (male)[a]	0.05	0.08	-0.10	0.20	1.00	0.43	0.51
		Age[b]	-0.01	0.04	-0.08	0.07	1.00	0.04	0.84
		Grooming given[b]	0.24	0.03	0.19	0.29	1.00	69.65	**<0.001**
		Group size[b]	-0.06	0.04	-0.13	0.01	1.00	2.42	0.12
		Reproductive periods (weaned) [a]					5.00	1.45	0.09
		Mating	0.07	0.12	-0.17	0.32	[c]	[c]	[c]
		Early gestation	-0.01	0.10	-0.20	0.19	[c]	[c]	[c]
		Late gestation	-0.05	0.14	-0.32	0.22	[c]	[c]	[c]
		Early lactation	0.08	0.13	-0.18	0.34	[c]	[c]	[c]
		Late lactation	0.00	0.10	-0.20	0.19	[c]	[c]	[c]
		Infection status (infected) [a]	0.03	0.07	-0.10	0.16	1.00	0.16	0.69
M.XI	**grooming given**	(Intercept)	-2.33	0.09	-2.51	-2.15	[c]	[c]	[c]
		Sex (male)[a]	-0.10	0.05	-0.20	0.00	1.00	3.68	0.06
		Age[b]	0.06	0.02	0.01	0.10	1.00	5.07	**0.02**
		Grooming received[b]	0.27	0.02	0.23	0.30	1.00	141.35	**<0.001**
		Group size[b]	0.02	0.03	0.03	0.08	1.00	0.84	0.36
		Reproductive periods (weaned) [a]				5.00	58.50	**<0.001**	
		Mating	-0.17	0.11	-0.38	0.04	[c]	[c]	[c]
		Early gestation	-0.37	0.08	-0.53	-0.20	[c]	[c]	[c]
		Late gestation	-0.37	0.12	-0.62	-0.13	[c]	[c]	[c]
		Early lactation	0.23	0.10	0.02	0.43	[c]	[c]	[c]
		Late lactation	-0.11	0.08	-0.27	0.06	[c]	[c]	[c]
		Infection status (infected) [a]	0.02	0.06	-0.09	0.14	1.00	0.17	0.68

[a] Manually dummy-coded with the reference category being female for sex, weaned for reproductive periods and 0 (no infection) for infection status
[b] z-transformed
[c] Not shown as not having a meaningful interpretation. For intercepts, p-values would refer to estimated Infection status, when all covariates are at zero.
Chi² values are results of a likelihood ratio test comparing the full model with a reduced model lacking the respective term

Model X revealed that grooming received was positively correlated with grooming given but none of the other covariates including infection status (M.X, Table 8). Model XI showed that grooming given was positively correlated with age, grooming received and reproductive periods, however, it was not correlated to sex, group size or infection status (M.XI, Table 8).

Discussion

We found a diverse parasite community in redfronted lemurs in Kirindy forest, with 11 parasite morphotypes. From these, only two are protozoan and seven are nematodes and most of them have a direct life cycle with oro-faecal transmission (eight over eleven morphotypes, including six nematodes and two protozoan). A combination of factors at the population-and individual-level are driving parasite richness and infection status in redfronted lemurs, with clear differences between nematodes and protozoan. As a result of parasite infection, redfronted lemur showed little to no behavioural changes and physiological costs seemed inexistent, suggesting compensatory mechanisms to bear costs of infection or a potential parasite tolerance, which needs to be tested with another study.

Drivers of parasite richness and infection status

As predicted, we observed a significant positive effect of population-level variables rainy season and monthly cumulative rainfall on all parasite infection proxies except nematode infection status. This suggests that the humid and warm environment during the rainy season is favourable for the parasites infecting redfronted lemurs. Accordingly, several studies found similar results, showing increased parasite richness or parasite prevalence during wet and warm periods (mandrills: Setchell et al., 2007; chacma baboons: Benavides et al., 2012; eastern chimpanzees: Gillespie et al., 2010. The lack of effect of seasonality when taking into account nematodes infection only is quite surprising as nematode larvae usually mature for a few weeks on the soil (Neveu-Lemaire, 1952) and some nematode larvae may undergo a temporary inhibition of their development also called hypobiosis (Eysker, 1997; Gibbs, 1986; Michel, 1974; Schad, 1977) when external conditions are unfavourable. However, these nematodes species, their transmission pathways and life cycles are not well known.

Differences in maturation time could explain why when we tried to associate rainfall and parasite status with a one-month lag-time, we could not find any correlation.

None of the two group-level factors investigated (group size and percentage of time spent on the ground) had effects on all parasite infection proxies, except group size which was negatively correlated with protozoan infection status. This result is quite surprising as a previous study in the same population found a positive relationship between group size and parasite richness (nematodes and protozoan regrouped) (Clough et al., 2010). This negative relationship between parasitism and group size could be due to infection risk dilution in larger groups due to increased group spread and group clustering with little dispersal among groups, reducing contact with infected individuals (Snaith et al., 2008; K. Wilson et al., 2003). Further analysis of individual's proximity and subgrouping in relation to parasite infection could help to validate this hypothesis in redfronted lemurs. The lack of relationship for parasites transmitted through the environment could be explained by the multiple intergroup encounter (personal observations: Esther Bernaldo de Quirós & Louise Peckre) and shared used of fruit trees and water holes by individuals of different groups (Amoroso et al., 2019), making parasite exposure similar between the different groups and group sizes.

Many individual-level factors influenced parasite richness and parasite status, with noticeable differences between nematodes and protozoan. However, the individual levels of fTESTOm levels showed no effect in all our models, suggesting neither an immunosuppressive effect of steroids (Muehlenbein, 2006; Sapolsky, 2005b) nor an immuno-enhancing effect of testosterone as it was suggested in the same population nine years earlier (Clough, 2010) and in other species (Ezenwa et al., 2012; Prall & Muehlenbein, 2014; Setchell et al., 2010). Furthermore, contrary to our predictions, fGCm was negatively correlated with all parasite infection proxies except nematode infection status. While contrasting with a large body of research showing positive correlations between parasite infections and host elevated GC, this result is in accordance with a few other studies (Baboons: Akinyi et al., 2019, Mammals: Defolie et al., 2020, redfronted lemurs: Clough et al., 2010 ; grey-cheeked mangabeys: Arlet et al., 2015). Furthermore, these findings suggest that in our population, different parasites elicit different responses from the immuno-endocrine system (Ezenwa et al., 2012; Goldstein et al., 2005; Monello et al., 2010). It further suggests that redfronted lemur could tolerate parasite in a certain measure (Budischak et al., 2018; Kutzer & Armitage, 2016; Råberg, 2014)

instead of resisting against parasite infection (process involving immune response) or that these parasites could alter their hosts HPA axis in order to increase their fitness (Defolie et al., 2020). While less frequently reported in parasite studies, tolerance is an adaptive strategy when facing chronic non-lethal infections because it allows the hosts to give up on the cost of fighting the infection. Another alternative explanation of this results could be that because of the two consecutive years with heavy rain and high fruit availability when we collected data, individuals might have had enough resources to invest both in body condition and immune function, buffering against the immunological cost of heightened steroids (Sapolsky, 2005b).

We found a positive correlation of age with nematode infections only and no effect with all other models. Previous studies showed that the relationship between age and parasitism is complex, with mixed results so far (see Table1). Our result might reflect cumulative exposure to nematodes over time (Nunn & Altizer, 2006) or an increase in susceptibility to infection with age, because of immunosenescence (Tarazona et al., 2002), like it was found in St Kilda Soay Sheep (Hayward et al., 2009), who experience increase in parasite burden with aging. However, a similar study in the same red fronted population 10 years earlier did not find this effect (Clough et al., 2010). The difference could lie in sampling differences: while Clough et al. (2010) only included adult individuals from 3 to 14 years old and regrouped them in three age-class groups, we included individuals from 1 to 23 years old with age as a continuous variable. Finally, there was no effect of rate of body contact on PSR, all parasite infection status and nematode infection status but it did influence protozoan infection status, with infected individual spending more time in body contact than non-infected individuals. This result is (partly) in accordance with previous studies in anthropoid primates (Habig et al., 2019; Vitone et al., 2004), showing that sociality influence parasite transmission. Nematodes and protozoan differences in life cycle can explain the different results between our models. Nematodes usually require maturation in their environment before being infective, while protozoan are directly infective after excretion in the faeces and can thus be transmitted via direct contact.

Overall, sociality (here measured as variation in group size and time spent in body contact) only played a little role in determining parasite infections because of its influence only on protozoan infections in redfronted lemurs. This contrasts with previous findings in anthropoid primates (e.g. Habig et al., 2019; Müller-Klein et al., 2019; Nunn et al., 2003) where

PSR, prevalence and intensity of infection increased with host sociality. Indeed, PSR, prevalence and intensity should increase with sociality for directly transmitted parasite because higher individual density due to larger group size and close proximity or contact among host individuals increases exposure to directly transmitted parasites (Altizer et al., 2003; Anderson & May, 1979; Freeland, 1976; Nunn et al., 2003). Our result is even more surprising, when considering that lemur groom with their tooth comb. This behaviour should be more costly in terms of parasite exposure than grooming with the hands like anthropoid primates do, because of the ingestion of parasite. However, redfronted lemurs, contrary to macaques and baboons spend little time in active social contact. We observed active social contact such as grooming in average 2% of all observation time contrary to in average 10 to 15% in baboons, macaques and chimpanzees (Gust et al., 1993; Shutt et al., 2007; Tennenhouse et al., 2017; Wittig et al., 2008, 2016; Young, Majolo, Heistermann, et al., 2014), exposure risk via contact seemed thus much more limited than in anthropoid primates. Finally, lemur's social organisation in relatively small groups (here range 3-15 individuals) could explain the difference between our study and previous studies with anthropoid primates which compared groups of multiple dozens of individuals. Indeed, in very small groups, such as in redfronted lemurs, all individuals can be connected to each other, at least indirectly, decreasing the influence of direct connectedness.

Individual differences existed and should be explored further: some individuals had consistently low PSR and other high PSR and individual differed in the parasite morphotypes they hosted. These differences could reflect differences in susceptibility, with some individuals having better immune function (Clough et al., 2011), better body condition (Sánchez et al., 2018) or differences in exposure via personality, with higher exposure in bolder and more exploratory individuals (Barber & Dingemanse, 2010; Dunn et al., 2011; Klemme & Karvonen, 2016; Koprivnikar et al., 2012). Because of these individual differences, it would be interesting to see what happens when individuals change group either because of male emigration around maturity or when male take-over a group or when female evictions happen because of frequent periods of social instability (Peter M. Kappeler & Fichtel, 2012a).Finally, we want to emphasize that the hypotheses discussed above are not mutually exclusive, and variables affecting parasite species richness and infection status are likely to covary.

Consequences of parasitism

A recent meta-analysis (Sánchez et al., 2018) linked parasite infections to lower body condition, including body mass. Indeed, parasite infections should increase energetic expenditure and as predicted by life history theory, there should be energetical trade-offs between maintenance, reproduction and immune function. However, and contrary to our expectations, body mass was positively correlated with PSR, and this effect was independent of testosterone levels but not age. Similar results were found in females grey mouse lemurs at the same field site (Hämäläinen et al., 2015) and explained as "an increased investment in self-maintenance, including parasite resistance with increasing resources or improved condition". Males redfronted lemurs in good body condition may be able to use energy to cope with parasite infections on top of investing in reproduction and maintenance. Alternatively, our results could just reflect that with age, individuals grow larger and thus have a higher body mass while they simultaneously accumulate parasites (Nunn & Altizer, 2006).

In studies about "sickness behaviour", infected individuals exhibited reduced social interest, appetite and activity levels (Ghai et al., 2015; Hart, 1988; Hetem et al., 2008; Krief et al., 2005; Marais et al., 2013; Müller-Klein et al., 2019; Owen-Ashley & Wingfield, 2006; Sullivan et al., 2016). These cytokine-induced behavioural changes are thought to help a sick individual to conserve energy for infection fighting. However, in our study, redfronted lemurs infected with parasites spent the same amount of time feeding and socialising and less time resting than non-infected individuals. Thus, redfronted lemurs did not exhibit "sickness behaviour", maybe because these parasites did not trigger enough their host immune reaction and especially pro-inflammatory cytokines secretion to cause sickness behaviour. However, in the same population and using the same parasite samples, we found a correlation between infection status and an inflammation marker (Chapter 5), contradicting this hypothesis.

Redfronted lemurs do not seem to bear short-term costs of parasite infection. First, at the energetical level, the costs seem low because infected individuals do not increase feeding compared to non-infected individuals to compensate energetical cost of hosting parasites. Second, at the social level, there also seem to be no short-term cost because infected individuals did not differ in grooming given or received in comparison to non-infected individuals. This indicates that there is no reduction of social interest when infected contrarily to other species (red colobus monkeys: Ghai et al., 2015) but also that there is no avoidance

of infected individuals, contrarily to other species and especially in primates (mandrills: Poirotte et al., 2017; macaques: Müller-Klein et al., 2019; vervet monkeys: Chapman et al., 2016). It is highly unlikely that refdronted lemurs would be incapable of detecting infection in others as redfronted lemurs avoided parasitized water holes in the wild and contaminated water during experiments (Amoroso et al., 2019). However, the cost of social avoidance could be too much compared to the cost of parasite infection or redfronted lemur could decide to bear the cost of parasite infection and use tolerance mechanisms as the fGCm levels might indicate. This would need further research to be disentangled.

Concerning inactivity and similarly to our findings, rats infected with Toxoplasma gondii also decrease resting time (Webster, 2007) and studies in human showed that parasites from the *oxyurida*e family (3 out of the 11 morphotypes found in our lemurs) can cause restlessness due to itching sensations (Brady & Wright, 1939; Denhoff & Laufer, 1949; Ragan, 1962). Interestingly, in our population, many individuals presented sit-spots (bald areas on their lower back, see Peckre et al., 2018 for pictures) likely caused by repeated rubbing. Some of these individuals were observed performing anointing combined with the ingestion of millipedes (Peckre et al., 2018). These millipede species contain benzoquinone secretions which may act against gastrointestinal parasite infections, and more specifically *Oxyuridae* nematodes. Furthermore, this unusual behaviour supports the ideas that redfronted lemur can mitigate the costs of parasite infection. Indeed, negative effects of parasites can be masked by antiparasitic behaviour of their hosts. For example, when removing specific plant species from Cliff swallow nests, researcher found that higher mite load had detrimental effects on nestling body condition, while there was no effect if this plant species stayed in the nest (Clark & Mason, 1988).

Concerning long-term costs of parasitism, it would be interesting to assess the cost of parasitism on reproduction success and survival, using longitudinal data. In female mammals, helminth infection could constrain reproduction due to its important and long lasting energetic cost during gestation and lactation (Akinyi et al., 2019; Khokhlova et al., 2002; McFalls et al., 1984). In support, the experimental removal of ecto- and endoparasites sometimes leads to increased reproductive success (Gooderham & Schulte-Hostedde, 2011; Hillegass et al., 2010; Neuhaus, 2003; Patterson & Ruckstuhl, 2013; Raveh et al., 2011, 2015). In primates, previous research found that female geladas (Nguyen et al., 2015) and Amboseli

baboons (Akinyi et al., 2019) exhibited longer interbirth intervals when infected with parasites. In humans, previous research showed (Blackwell et al., 2016) contrasting relationships between helminths and interbirth intervals, with shorter interbirth interval in women infected with roundworm infections and longer interbirth interval in women infected with hookworm infections, showing potentially species-specific costs of parasites. During our study period, all adult females were pregnant and delivered healthy babies on the two consecutive reproductive seasons. This could indicate that parasitism had little energetical cost on female reproduction in redfronted lemurs. Accordingly, a previous study in the same population showed no effect of parasitism on redfronted lemurs males reproductive success (Clough et al., 2010).

Conclusion

We investigated both determinants and costs of parasite infections in redfronted considering determinants at the population-, group- and individual-levels and for mechanisms of susceptibility and exposure. While increased risk of disease transmission is proposed as one of the major costs of sociality in obligatory social animals, sociality seems to play a small role in the redfronted lemurs-parasite relationship with influence only with protozoan parasites. Furthermore, different parasites elicited different responses from the immuno-endocrine system reflected by fGCm. So far, we could not show evidence of costs of parasitism on body condition and sociality: social relationship did not change due to parasite infection, but infected individuals increased their activity patterns, probably due to infection symptoms such as *pruritus*. Here we showed that redfronted lemur differ from anthropoid primates concerning the sociality-parasitism relationship and that this could be due to their particular social organization in rather egalitarian small multimale-multifemale groups.

Supplementary material

Supplementary Table1:

Model	Predictor	Explanatory variables	Observations	Full-null comparison
I	Parasite richness	sex + age + time in body contact + fGCm + fTESTm + ground use + group size + ecological season	N = 302	X^2 = 56.64, df = 8, P<0.001
II	Parasite richness	sex + age + time in body contact + fGCm + fTESTm + ground use + group size + rainfall	N = 302	X^2 = 56.32, df = 8, P<0.001
III	General infection status	sex + age + time in body contact + fGCm + fTESTm + ground use + group size + ecological season	N = 302	X^2 = 53.12, df = 8, P<0.001
IV	General infection status	sex + age + time in body contact + fGCm + fTESTm + ground use + group size + rainfall	N = 302	X^2 = 58.64, df = 8, P<0.001
V	Nematode infection status	sex + age + time in body contact + fGCm + fTESTm + ground use + group size + rainfall	N = 302	X^2 = 19.686, df = 8, P=0.01
VI	Protozoan infection status	sex + age + time in body contact + fGCm + fTESTm + ground use + group size + rainfall	N = 302	X^2 = 51.77, df = 8, P<0.001
VII	Body mass	age + fGCm + fTESTm + fruit abundance + PSR	N = 72	X^2 = 32.41, df = 5, P<0.001
VIII	Resting time	age + sex + rainfall + general infection status	N = 319	X^2 = 9.63, df = 4, P=0.04
IX	Feeding time	age + sex + fruit abundance + general infection status	N = 319	X^2 = 10.69, df = 5, P=0.04
X	Grooming received	age + sex + grooming given + group size + reproductive periods + general infection status	N = 319	X^2 = 97.077, df = 10, P<0.001
XI	Grooming given	age + sex + grooming received + group size + reproductive periods + general infection status	N = 319	X^2 = 624.68, df = 10, P<0.001

Authors contribution: C.F. & C.D. conceived the study. Trained field assistants collected data. C.D. and M.H. conducted lab work with the help of lab assistants. C.D. performed all analyses, designed figures and tables and drafted the manuscript with input from C.P. & C.F. All authors provided comments and revised the manuscript.

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Ethical standards: All samples were collected from habituated wild animals without interfering with their activities. the laws of Madagascar and Germany and the protocols were approved by the Malagasy Ministère de l'Environnement et des Eaux et Forêts and the Centre National de Formation, d'Etudes et de Recherche en Environnement et Foresterie (CNFEREF) of Morondava. The study adhered to guidelines provided by the Association for the Study of Animal Behaviour (ASAB) and the Animal Behavior Society (ABS).

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Data accessibility: Raw data available upon request.

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Chapter 4: Potential self-medication using millipede secretions in red-fronted lemurs: combining anointment and ingestion for a joint action against gastrointestinal parasites?

With Louise Peckre, Peter Kappeler and Claudia Fichtel

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ORIGINAL ARTICLE

Potential self-medication using millipede secretions in red-fronted lemurs: combining anointment and ingestion for a joint action against gastrointestinal parasites?

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Abstract
Self-anointing, referring to the behaviour of rubbing a material object or foreign substance over different parts of the body, has been observed in several vertebrate species, including primates. Several functions, such as detoxifying a rich food source, social communication and protection against ectoparasites, have been proposed to explain this behaviour. Here, we report observations of six wild red-fronted lemurs (*Eulemur rufifrons*) of both sexes and different age classes anointing their perianal-genital areas and tails with chewed millipedes. Several individuals also ingested millipedes after prolonged chewing. In light of the features of the observed interactions with millipedes, and the nature and potential metabolic pathways of the released chemicals, we suggest a potential self-medicative function. Specifically, we propose that anointing combined with the ingestion of millipedes' benzoquinone secretions by red-fronted lemurs may act in a complementary fashion against gastrointestinal parasite infections, and more specifically Oxyuridae nematodes, providing both prophylactic and therapeutic effects.

Keywords Fur-rubbing · Benzoquinones · Diplopods · Nematodes · Oxyuridae

Introduction

The act of rubbing a material, object or foreign substance over different parts of the body is typically referred to as self-anointing (Baker 1996; Jefferson et al. 2014). This behaviour has been described in different taxa, including birds, carnivores and primates, and can be elicited by a wide variety of materials, such as plants, arthropods or human-made products (Campbell 2000; Parkes et al. 2003; Weldon et al. 2006; Laska et al. 2007; Morrogh-Bernard 2008; Lynch Alfaro et al. 2012). Given that self-anointing is likely to be costly in terms of both energy expenditure (rapid and repetitive movements) and risk exposure (decreased vigilance and contact with toxic compounds), it is thought to be associated with important benefits. In this context, several functions have been suggested (Birkinshaw 1999). Detoxification, social communication and self-medication hypotheses are most commonly cited.

First, since anointment usually involves toxic or irritant substances that are partially or totally ingested throughout the process, some authors have suggested a function of detoxifying rich food sources (Birkinshaw 1999; Weldon et al. 2006). Indeed, rubbing should aid in the removal of unpalatable secretions before ingestion. In particular, support from this hypothesis derives from several bird species that feather-rub ants (anting behaviour) (Morozov 2015) and lemurs that fur-rub millipedes (Overdorff 1993; Freed 1995; Birkinshaw 1999; Vasey 2000). A comparable prey-handling behaviour that consists of rolling arthropods on the ground prior to ingestion has also been observed in some carnivores (North American striped skunk *Mephitis mephitis*, meerkat *Suricata suricatta*, banded mongoose *Mungos mungo*), and is thought to disable arthropods' defensive adaptations by removing stinging hairs or depleting reserves of defensive chemicals by stimulating glandular discharge (Doolan and Macdonald 1996; Weldon et al. 2006).

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Chapter 5: Can we measure inflammation non-invasively in lemurs? ELISA measurement of faecal C-reactive protein in two species of lemur shows many limitations

With Simon Faber, Michael Heistermann and Claudia Fichtel

Abstract:

The development and availability of tools for non-invasive measurements of health indicators in wildlife will be crucial to understand life history trade-offs relative to immune function. However, non-invasive measurements of health indicators and diseases is only taking its first steps, with just a few techniques being validated in mammals. The measurement of C-reactive protein (CRP), an acute phase protein involved in response to trauma, infection, or inflammation is a good candidate for wildlife studies as it is not specific to a certain disease. Furthermore, enzyme-immuno-assays for the analysis of CRP in blood of non-anthropoid primates are already available and in one study it has been shown in urine and faecal samples that CRP increases due to tissue trauma. Lemurs are the most endangered group of mammals, and multiple studies report spillover of human pathogens making them even more vulnerable to extinction. Therefore, it is necessary to develop non-invasive methods to monitor health and disease in lemurs. In this study, we tested if CRP could also be measured in faecal samples of two species of lemur, the red-fronted lemur, *Eulemur rufifrons* and the ring-tailed lemur, *Lemur catta*. In particular, we tested if parasite infection, glucocorticoid levels or parturition affected CRP levels in lemurs, as these factors are known to increase CRP levels in humans and other animals. While overall less than 30% of the samples had CRP levels above the assay detection threshold, CRP levels were significantly higher with infection status but not after parturition. Thus, we conclude that the measurement of faecal CRP has limitations in lemurs but discuss potential opportunities for improving the technique, as well as alternative methods for measuring inflammation in lemurs.

Keywords: C-reactive protein, health marker, disease, wildlife, glucocorticoids, parasites, prosimian, primate

Introduction

A well-functioning immune system is important for individual's resistance and defense against pathogens and ultimately its survival (Blackwell et al., 2010). According to life history theory, an organism cannot invest in all demands at the same time, therefore there are trade-offs in energetical investments between growth, reproduction and maintenance (Sheldon & Verhulst, 1996; Stearns, 1989). As the immune system is

particularly energetically costly (Murphy, 2008; Sheldon & Verhulst, 1996), understanding such trade-offs is of major importance to understand the role of immune function in light of different life-history strategies. Since the collection of blood samples for immunological analysis is usually impractical and of ethical concern when studying large-bodied animal species, non-invasive methods to assess health in wildlife are crucial (e.g. Gesquiere et al., 2020; Higham et al., 2020).

Non-invasive sampling has many advantages for wildlife studies as it consists of collecting products usually considered as waste such as urine and faeces (see Behringer & Deschner, 2017 for a review). In contrast to invasive methods, non-invasive methods allow to collect multiple samples over time and, most importantly, they allow sample collection without altering animal behaviour and physiology and without ethical issues (V. Behringer & Deschner, 2017; Cunningham et al., 2015; Fedigan, 2010).

Traditionally, non-invasive assessment of health or immune system activation in the wild was done by visual inspection (coat and fur condition: Berg et al., 2009; Borg et al., 2014; Ezenwa et al., 2009; Jolly, 2009; wound healing: Archie, 2013; Ceballos-Vasquez et al., 2015; Weaver et al., 2009) or assessment of gastro-intestinal parasite load and parasite species diversity (Barbosa & Palacios, 2009; Gillespie, 2006; Howells et al., 2011; Olifiers et al., 2015). However, these techniques show limitations. Visual inspection of animal's health status is vulnerable to observer biases, making comparisons between populations or species challenging. Gastro-intestinal parasites excretion is not linear, thus differences in parasite load in faeces do not always reflect differences in parasite load in the gut (Gillespie, 2006). Additionally, parasite presence does not always reflect poor immune function but can also be a sign of parasite tolerance (Budischak et al., 2018; Defolie et al., 2020; Kutzer & Armitage, 2016; Råberg, 2014). Finally, even though tools for genetic identification of parasites, but also bacteria and viruses in fecal samples have been developed (Gogarten et al., 2020; Köndgen et al., 2010; Livia V. Patrono et al., 2020), they nevertheless do not allow to measure an immune activation due to infections.

For these reasons, other non-invasive measurement of health and disease have received much more attention in the last two decades, with multiple attempts to process and analyse non-invasive samples (saliva, urine or faecal samples) for hormones (see Behringer & Deschner, 2017 for a review) and health markers (see Table 1 for an overview).

In particular, methods have been developed to measure different health parameters such as: energy balance and nutritional status (C peptide of insulin from urine samples; T3, rT3 and T4 from faeces and urine); immune system activation (Neopterin from urine; IgM, IgG and IgA from faeces and saliva); infection and tissue inflammation (suPAR soluble urokinase plasminogen activator receptor from urine; haptoglobin from faeces; CRP from faeces, urine and saliva), (see Table 1 for more details and references). However, these new methods to monitor health non-invasively are essentially designed for anthropoid primates, pets or livestock, leaving a big gap for wildlife studies with other animal species.

C-reactive protein (CRP) would be a useful indicator to assess infections non-invasively. CRP is a widely used non-specific inflammatory biomarker, routinely measured in blood (Ansar & Ghosh, 2013; Black et al., 2004; Du Clos, 2000; Hatherill et al., 1999; Kindmark, 1972; Micallef et al., 2009; M. B. Pepys, 1981). It is an acute-phase protein that is very rapidly secreted by the liver in response to infection, inflammation, and tissue trauma, e.g. inflammation due to gastro-intestinal parasites (Bálint et al., 2014; Blackwell et al., 2010; Stadnyk & Gauldie, 1991), and it decreases just as rapidly with the resolution of the condition (Ansar & Ghosh, 2013; Black et al., 2004; Du Clos, 2000; M. B. Pepys, 1981). CRP is commonly used to determine disease progression, and its increased expression is often associated with long-term chronic health consequences (e.g. cardiovascular disease: Ridker et al., 2000; high blood pressure: Lakoski et al., 2005; Shafi Dar et al., 2010; renal failure: Panichi et al., 2001; type 2 diabetes: Pradhan et al., 2001). CRP levels are usually correlated with glucocorticoid levels (Boss & Neeck, 2000; Cubała & Landowski, 2014; Dugué et al., 1993; White & Fletcher, 1985), parasite infections (e.g. Bálint et al., 2014; Blackwell et al., 2010; Stadnyk & Gauldie, 1991), and they increase with parturition with a peak excretion 24 hours after giving birth in humans (Cicarelli et al., 2005; De Meeus et al., 1998; Kääpä & Koistinen, 1993) and up to a week after parturition in other animals (Eckersall et al., 1993; Kováč et al., 2008; Rota et al., 2019). These characteristics make CRP highly suitable for field studies, where researchers will very rarely be able to monitor symptoms or know the precise infection or disease that the animals are suffering from.

CRP measurement from serum is commonly used in studies of infections and non-transmittable diseases in humans (Ansar & Ghosh, 2013; Black et al., 2004; Du Clos, 2000; Hatherill et al., 1999; M. B. Pepys, 1981), but also in non-human primates (macaques: Hart et

al., 1998; Jinbo et al., 1998; Klingström et al., 2002, pets and livestock: Cray et al., 2013; Eckersall & Bell, 2010; Petersen et al., 2004; Pradeep, 2014; Yamamoto et al., 1993)). To date, only few studies have tested the non-invasive measurement of CRP (see Table 1), despite a real need of non-invasive measurement of inflammation markers for both veterinary medicine and wildlife research. Most studies assessed CRP levels in saliva of domestic animals (Gutiérrez et al., 2009; Jacobsen et al., 2014; Lamy & Mau, 2012; Parra et al., 2005; Smets et al., 2010), but sampling saliva requires the capture or cooperation of the animal and are therefore less practical for wildlife studies. So far, only one study compared CRP measurements in blood, urine and faeces in non-human primates, rhesus macaques (Higham et al., 2015). CRP values in urine or faeces did not correlate with values from blood samples, but CRP clearly increased urine or faeces after tissue trauma from surgery. Hence, further validation on the use of CRP measurements as a health indicator in other animals are required.

A particularly worthwhile group of animals to study immune responses in are lemurs. Lemurs are small bodied primates, endemic to Madagascar (Mittermeier et al., 2008). Lemurs are considered to be the most endangered group of mammals today (Schwitzer et al., 2014), and multiple studies report spillover of human pathogens making them even more vulnerable to extinction. Hence, validations of non-invasive markers to monitor their health status are required. Because of conservation of both immune and neuroendocrine systems in vertebrates (Boehm, 2012; Hillgarth & Wingfield, 1997; Sabra L Klein, 2000; Martin, 2009), they likely excrete similar hormones and proteins as anthropoid primates, allowing the reasonable use of enzyme immuno-essays developed for anthropoid primates. Furthermore, C-reactive protein is remarkably similar in structure and function in mammals (M. B. Pepys et al., 1978). Multiple assays exist to assess hormone levels in lemurs (e.g. Fichtel et al., 2007; Ostner et al., 2002, 2008) and several studies have already shown that lemurs host diverse parasite and pathogen communities (Bublitz et al., 2015; Clough, 2010; Irwin & Raharison, 2009; Springer et al., 2015; Zohdy et al., 2015).

Table 1: Overview of health markers measured in non-invasively collected sample material from pet animals, farm animals and anthropoid primates including humans

Health marker	Tissue sampled	Species	Reference
Neopterin	urine	Human	Ledjeff et al., 2013
Neopterin	urine	Chimpanzee	Wu et al., 2018
Neopterin	urine	Rhesus macaque	Higham et al., 2015
Neopterin	urine	Barbary macaque	Müller et al., 2017
Neopterin	urine	Dog	Duch et al., 1984
Neopterin	saliva	Human	Ledjeff et al., 2013
Neopterin	faeces	Human	Kosek et al., 2013
Neopterin	faeces	Human	Ledjeff et al. 2013
Neopterin	faeces	Rhesus macaque	Higham et al., 2015
Neopterin	faeces	Mandrill	Dibakou et al., 2019
Haptoglobin	faeces	Human	Matsumoto et al., 2001
Haptoglobin	faeces	Rhesus macaque	Higham et al., 2015
C-peptide	urine	Bonobo	Deschner et al., 2008
C-peptide	urine	Orangutan	Emery Thompson & Knott, 2008
C-peptide	urine	Chimpanzee	Emery Thompson et al., 2009, 2010
C-peptide	urine	Rhesus macaque	Higham et al., 2011
T3, rT3, T4	faeces	Multiple species	See Behringer et al. 2017 for a review
T3, rT3, T4	urine	Multiple species	See Behringer et al. 2017 for a review
IgM	saliva	Dog	German et al., 1998
IgG	saliva	Dog	German et al., 1998
IgA	saliva	Sheep	Shaw et al., 2012
IgA	saliva	Dog	German et al., 1998
IgA	faeces	Asian elephants	Edwards et al., 2019
IgA	faeces	Baboons	Gesquiere et al., 2020
IgA	faeces	Chimpanzee	Lantz et al., 2018
suPAR	urine	Rhesus macaque	Higham et al., 2020
CRP	urine	Human	Chuang et al., 2010
CRP	urine	Rhesus macaque	Higham et al., 2015
CRP	faeces	Rhesus macaque	Higham et al., 2015
CRP	saliva	Horse	Jacobsen et al., 2014
CRP	urine	Dog	Smets et al., 2010
CRP	saliva	Dog	Parra et al., 2005
CRP	saliva	Cattle	Lamy & Mau, 2012
CRP	saliva	Pig	Gutiérrez et al., 2009

The overall goal of our study is to verify if it is possible to detect CRP immunologically in faecal samples of two species of lemurs, the red-fronted lemur, *Eulemur rufifrons* and the ring-tailed lemur, *Lemur catta* using the commercial monkey-specific CRP ELISA kit validated for rhesus macaques (Higham et al., 2015) as well as to examine the biological validity of such measurement in these two species. As lemurs in these populations are known to host gastro-intestinal parasites (Clough, 2010) and thus possibly have inflammation, we chose to assess CRP due to its properties as a non-specific biomarker of inflammation and infection compared to other biomarkers such as neopterin. Because redfronted and ringtailed lemurs are small-bodied animals and they excrete very little amount of urine, which is challenging to collect in the wild, only faecal samples could be collected for biomarker analysis.

We first tested the rate of detection of CRP in faecal samples collected from wild and captive animals. We then used the samples in which CRP level was measurable above the assay detection limit to examine whether faecal CRP excretion is related to gastro-intestinal parasite status and CRP levels change in response to parturition. Therefore, we predicted elevated CRP levels in animals excreting gastro-intestinal parasites in their faeces in comparison to animals whose faecal samples were parasite-free and an increase in individual levels of faecal CRP after in comparison to before parturition. In addition, we controlled for a possible effect of sex and age on faecal CRP levels in our study population of wild red-fronted lemurs from which the majority of samples were collected (see Methods).

Methods

Study 1: CRP in relation to gastro-intestinal parasite status

From May 2015 to November 2016, we collected faecal samples from 34 red-fronted lemurs (14 females, 20 males, age 1-23 years), living in five social groups in Kirindy forest, western Madagascar. Being part of a long-term study, all individuals were marked with a unique combination of collars and pendants and well habituated to researchers (Peter M. Kappeler & Fichtel, 2012b). Samples were collected within five minutes following defecation and only faecal material that was uncontaminated with urine or soil was collected. Directly after collection of the faecal sample, we removed big seeds and fruit parts and homogenised the rest using a spatula. For parasite analysis, we placed 1-2g of faeces in 15mL of 10%

formalin in pre-aliquoted tubes and stored it at ambient temperature. For CRP analysis, we collected 1-2g of faeces into a polypropylene tube, kept them in a cooler during collection and stored them within two hours after collection at -20°C until laboratory analysis. In total, we collected 245 samples with matching aliquots for parasite and CRP analysis. Samples were equally distributed between males (124 samples) and females (121 samples, see Table 2). At the time of sample collection, all animals looked healthy and showed no obvious signs of illness.

Study 2: CRP in response to parturition

We collected one faecal sample before and after giving birth from five wild red-fronted lemurs from Kirindy forest, Madagascar, seven captive-housed ring-tailed lemurs from the Wildlife Park Affenwald (Germany, six animals) and the German Primate Center (DPZ, Germany, one animal), see Table 2. Samples were only colected from adult females who looked healthy and showed no obvious signs of illness. The "before birth" samples were collected in the last 3 weeks before females gave birth and the "after birth" samples were collected 1-3 days after parturition had taken place. All samples were collected, processed, and stored as described for study 1.

Laboratory analyses

CRP

Samples were thawed and subsequently extracted following the protocol described in detail by Higham et al. (2015). CRP was measured from the faecal extracts using an ELISA kit for monkey-CRP (Life Diagnostics, Inc., USA, Cat. No.2210-4). The assay was performed according to the manufacturer's instructions, and faecal samples were taken undiluted to assay (Higham et al., 2015). The detection limit of the assay was 1.17 ng/ml. Inter-assay coefficients of variation of a high- and low-concentrated quality control were respectively 10.4% (high) and 10.1% (low). To compensate for the effect of potential differences in faecal samples water content on CRP concentrations, the extracted faecal material of each sample was dried in an oven at 50°C to a constant weight and the sample dry weight was subsequently determined. All CRP concentrations are expressed as nanograms per gram of dried faeces.

Parasite status

All samples were analysed at the Behavioural Ecology and Sociobiology laboratory of the German Primate Center. We used a modified formalin-ethyl acetate sedimentation technique (Ash & Orihel, 1988) following the protocol by Clough (2010). We examined faecal smears under the microscope for morphological identification of parasite eggs and cyst morphotypes to the closest genus, in accordance with previously described identification criteria (Clough, 2010; Irwin & Raharison, 2009). We calculated parasite morphotype richness at the genus level as the total number of distinct egg types in the sample. Additionally, we defined parasite status as a binary variable, parasitised meaning individuals excreting gastro-intestinal parasites in their faeces, non-parasitised meaning individuals whose faeces were parasite-free. See chapter 2 for more details on parasite analysis and parasite morphotypes retrieved.

Glucocorticoids

Fecal samples were extracted at the camp within 4 h of sample collection adapting a protocol described by Ziegler & Wittwer, (2005) and modified by Shutt et al., (2012) as previously described in chapter 2. Faecal glucocorticoids metabolites (fGCm) were measured from the faecal extracts following Ostner et al. (2002, 2008), using a group-specific assay for the measurement of 5ß-reduced cortisol metabolites (3α,11-oxo-CM). Fecal extracts were usually diluted 1:300 in assay buffer (0.04 M PBS, pH 7.2) and 50 µl aliquots were measured by microtiterplate enzymeimmunoassay according to the method described by Palme & Möstl, (1997) and Möstl et al., (2002). Serial dilutions of fecal extracts from samples of different individuals gave displacement curves parallel to that obtained for the standard. Intra- and inter-assay coefficients of variation of a high- and low-concentrated quality control were respectively 6.8% and 8.8% (high) and 7.9% and 13.0%. Sensitivity of the 3α,11-oxo-CM assay at 90% binding was 0.3 pg/50µl. To compensate for the effect of potential differences in faecal samples water content on fGCm concentrations, the extracted faecal material of each sample was dried in an oven at 50°C to a constant weight and the sample dry weight was subsequently determined. All fGCm concentrations are expressed as nanograms per gram of dried faeces.

Statistical analyses

For study 1, we examined the link between CRP concentrations and parasite infection status, parasite morphotype richness, and fecal glucocorticoids levels. We also controlled for sex or age effects on CRP concentrations. Because of the small sample size and high number of zero, we tested each variable separately using nonparametric statistics. Specifically, we used Mann Whitney U tests to examine the effect of sex and parasite status on faecal CRP levels, and Spearman rank correlations for a potential effect of age and parasite morphotype richness on CRP concentrations.

For study 2, we used a Wilcoxon Signed Rank Test to examine whether CRP increased in response to parturition by comparing faecal CRP concentrations in the matched samples collected before and after parturition in our study animals.

Results

Study 1: CRP in relation to gastro-intestinal parasite status and faecal glucocorticoids

Overall, only 64 of the 245 samples collected from the wild red-fronted lemurs had CRP levels above the detection limit of the assay. Thus, the detection rate of CRP was low (26.1%), with no difference between female and male samples (Table 2, U = 8488, p = 0.53). Average faecal CRP concentrations were also not different between the sexes (Table 2, U = 7893, p = 0.71) and there was no significant correlation between age and CRP levels (r = 0, p = 0.96, Figure 1D).

When considering samples that had values below detection limit by giving them the minimum value, i.e. the value of the detection limit, the results did not change, so we excluded these values from further analyses (See supplementary figure 1).

We detected parasites in 61.14% of the samples, with up to five different morphotypes per samples (parasite richness range 0-5).

There was no correlation between faecal CRP levels and parasite richness (r = - 0.04, p = 0.47, Figure 1B) or between CRP and fGCm (r = - 0.10, p = 0.12, Figure 1C). However, CRP

levels were higher in parasitised than non-parasitised individuals (U = 19058, p < 0.001, Figure 1D).

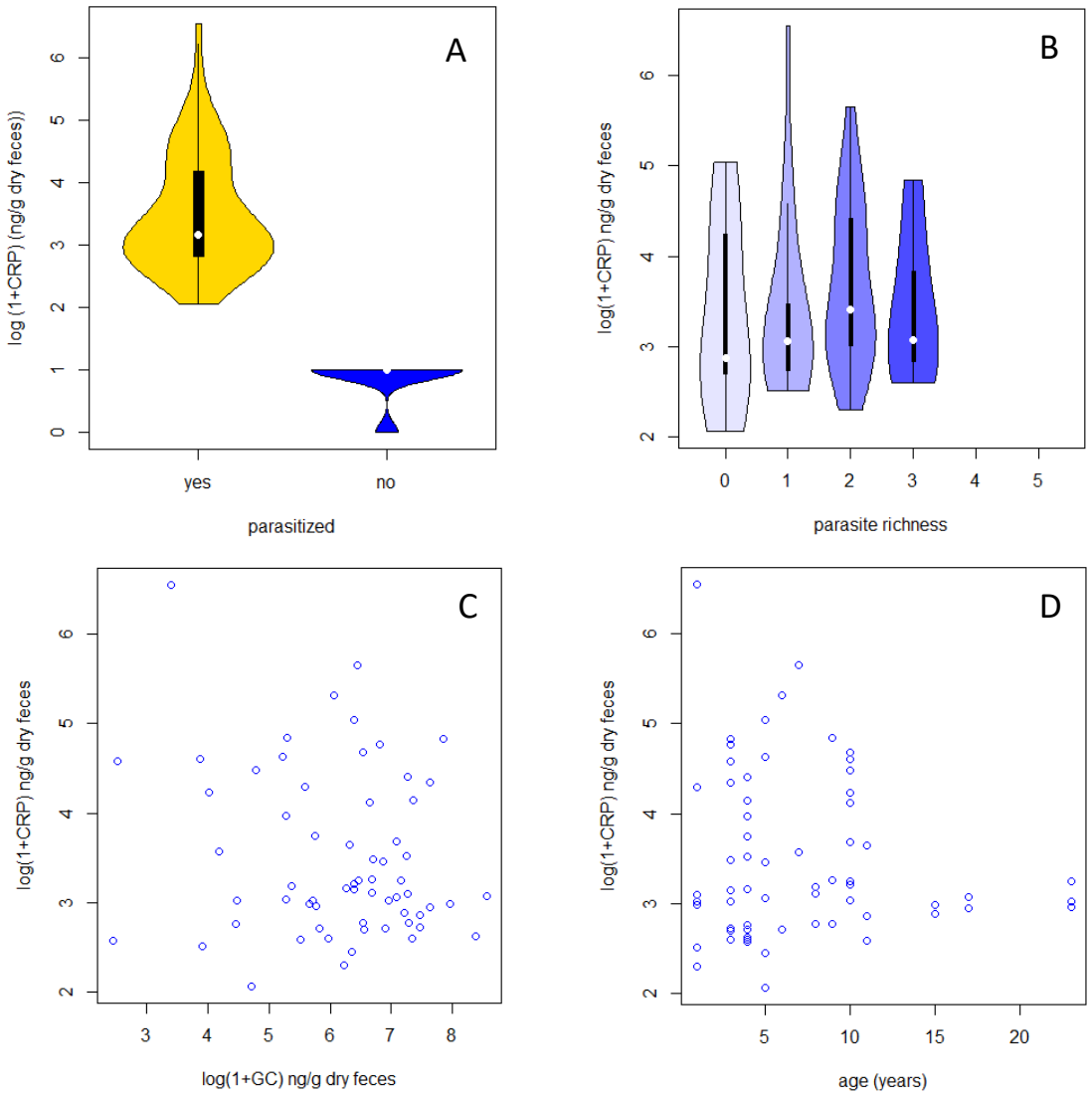


Figure 1: (A) Individuals for which parasites could be detected in their feces (parasite status Yes, x-axis) had higher CRP levels (y-axis) than individuals without parasites. However, CRP levels (y-axis) did not significantly differ in function of (B) parasite morphotype richness (ranging from 0 to 5, y-axis); (C) GC levels (ng/g dry faeces, x-axis) or (D) age (in years, x-axis). GC and CRP levels were log-transformed for visualisation, but not for statistical analyses.

Table 2: Mean CRP values (ng/g dry faeces), sample numbers and detection rate for all samples and for males and females separately.

Sex	N sample	% detected (N)	Mean CRP ng/g dry faeces ± SD
Female	121	25.62% (31)	51.00 ± 55.87
Male	124	26.62% (33)	60.97 ± 123.50
All	245	26.12% (64)	56.14 ± 96.22

Study 2: inflammation due to parturition

Detection rate of faecal CRP before and after parturition in captive and wild female lemurs was also low, albeit slightly higher than in study 1, with 7/24 samples analysed (29.17%) above the detection threshold (Table 3). CRP values were not significantly higher after parturition than before parturition (N = 24, W = 6, p = 0.40, Figure 2).

Table 3: Mean CRP values (ng/g dry faeces) for females ring-tailed lemur (semi-free living) and red-fronted lemur (wild) before and after parturition.

Species	**N sample**	**Living conditons**	**% detected (N)**	**Before parturition** **Mean CRP ± SD** **ng/g dry faeces**	**After parturition** **Mean CRP ± SD** **ng/g dry faeces**
Ring-tailed lemur (n=7)	14	Semi-free ranging	21.43% (3)	0 ± 0	6.84 ± 8.92
Red-fronted lemur (n=5)	10	Wild	40% (4)	7.94 ± 9.51	6.62 ± 8.91
All	24		29.17% (7)	2.55 ± 6.44	6.12 ± 8.35

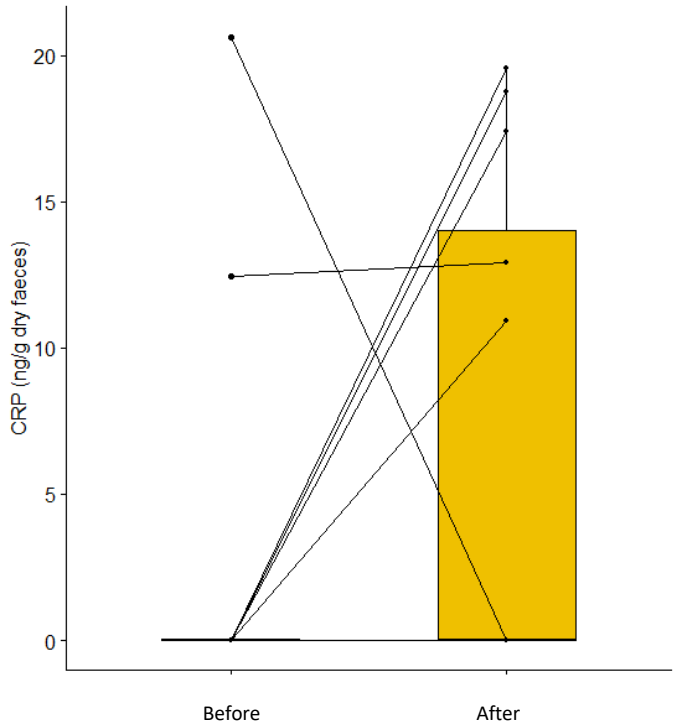


Figure 2: Faecal CRP levels (in ng/g dry faeces, y-axis) did not significantly increase in females after giving birth (x-axis). Each line indicates an individual value before and after parturition, while the boxes indicate mean + standard deviation before and after parturition.

Discussion

The aim of this study was to assess whether CRP can be measured non-invasively in faecal samples of two lemur species, and, if so, whether CRP levels co-vary with gastrointestinal infection and parturition. Our results showed that CRP measurement from lemur feces is at the moment not reliable because of the very low detection rate in samples from both wild and captive lemurs and the inconsistent pattern of CRP levels increase after giving birth. However, CRP levels were positively associated with parasite infection status parasite status in wild redfronted lemurs but they did not correlate with parasite richness. Hence, the given commercial EIA kit we used was, despite the conservative nature of CRP across mammals (M. B. Pepys et al., 1978), probably not suited to assess CRP in faecal samples of lemurs.

Parasite richness did not co-vary with CRP levels but infected individuals exhibited elevated CRP levels. Similarly, in humans, gastro-intestinal parasite infection caused an elevation of CRP because of the inflammation processes of the immune reaction (Bálint et al., 2014; Stadnyk & Gauldie, 1991). Hence, parasitised red-fronted lemurs might have had higher CRP levels than non-paratised individuals, due to a local excretion of CRP in the guts in response to inflammation from the parasite infection. Alternatively, this could also be due to bleeding caused by parasite damage of the intestinal mucosa, leading to a direct transfer of blood CRP in the gut, and, consequently increasing CRP concentration in the faeces.

In general, the detection rate of CRP in faecal samples of the two lemur species was very low and CRP values were, when they reached the detection level, much lower than CRP values found previously in faecal samples of rhesus macaques (Higham et al., 2015).Since CRP is mostly produced and secreted by the liver (Ansar & Ghosh, 2013; Black et al., 2004; Du Clos, 2000; M. B. Pepys, 1981), CRP concentrations in excreta like urine, faeces and saliva are expected to be lower in comparison to CRP levels in blood. Thus, the currently available CRP EIA might generally not be sensitive enough for excreta samples. Therefore, further studies are needed to evaluate whether CRP excretion rates in blood, urine and faeces are generally lower in lemurs than in anthropoid species, and, hence, too low for detection in faecal samples with the given EIA kit.

In principle, when using fecal samples, the gut-passage time of the given species to be considered to take into account the delay in levels measured in feces compared to serum (V.

Behringer & Deschner, 2017). For example, in *Eulemur* the gut-passage time are estimated at c.a. 190 min (Razafindratsima et al., 2014), but fecal glucocorticoid secretions are estimated to be lagged by 24 to 48 hours compared to blood levels (Julia Ostner et al., 2002). Assuming a similar time lag for CRP secretions, the observed CRP variation might reflect variations accumulating over 24 to 48 hours before we took the sample. Since CRP is an acute phase protein, with a fast increase and decrease of its concentrations, accumulation of CRP secretions over 24 to 48 hours may have resulted in low CRP values, and, hence, a lack of detection. This may also explain, that we did not measure heightened faecal CRP levels CRP in samples collected up to three days after giving birth, while in humans, blood CRP increases strongly one to three days after parturition (Cicarelli et al., 2005; De Meeus et al., 1998; Kääpä & Koistinen, 1993).

In our study, we used an EIA commercial kit designed for macaques that was successfully used for CRP detection in fecal samples of rhesus macaques (Higham et al. 2015). In this study, Higham and colleagues found a clear increase of faecal CRP levels with inflammation that we could not replicate in lemurs. Changes of target molecule or their metabolites structure between species may cause binding problems of the EIA kit, explaining the very low detection rate of CRP in lemurs. Most EIA kits are designed for blood samples with intact molecules. Hence, measuring hormones and health markers from faeces and urine can be challenging because of the degradation of the molecule of interest, either by the liver or by bacteria inhabiting the guts (Palme, 2019). The altered form of the target molecule might be too different from the original form or the degradation of different molecules can lead to similar metabolites (e.g. cortisol and testosterone metabolites: Palme 2019). Unfortunately, no information on lemur or macaque CRP structure and their metabolites was available to verify this notion. However, proteins of the CRP family have been conserved with similar molecular architecture and function throughout vertebrate evolution (M. B. Pepys, 1981; M. B. Pepys et al., 1978), suggesting that differences in the initial structure of the molecule are probably small or nonexistent.

Protein structure problems, however, could still arise due to the large diversity of proteins (for example amyloid component) with similar structure in the CRP protein family (Coe, 1983). There is evidence of immunologic cross-reactions among mammalian acute-phase proteins (Gotschlich & Stetson, 1960). These cross-reactions could happen between

the EIA and metabolites of CRP. While hormones in particular, and proteins, more generally, are usually quite similar in mammals, their metabolites are often species-specific (Higham et al., 2010). Cross-reactions and species-specific CRP metabolites could, hence, explain the very low detection rate and the inconsistent pattern of CRP levels we found.

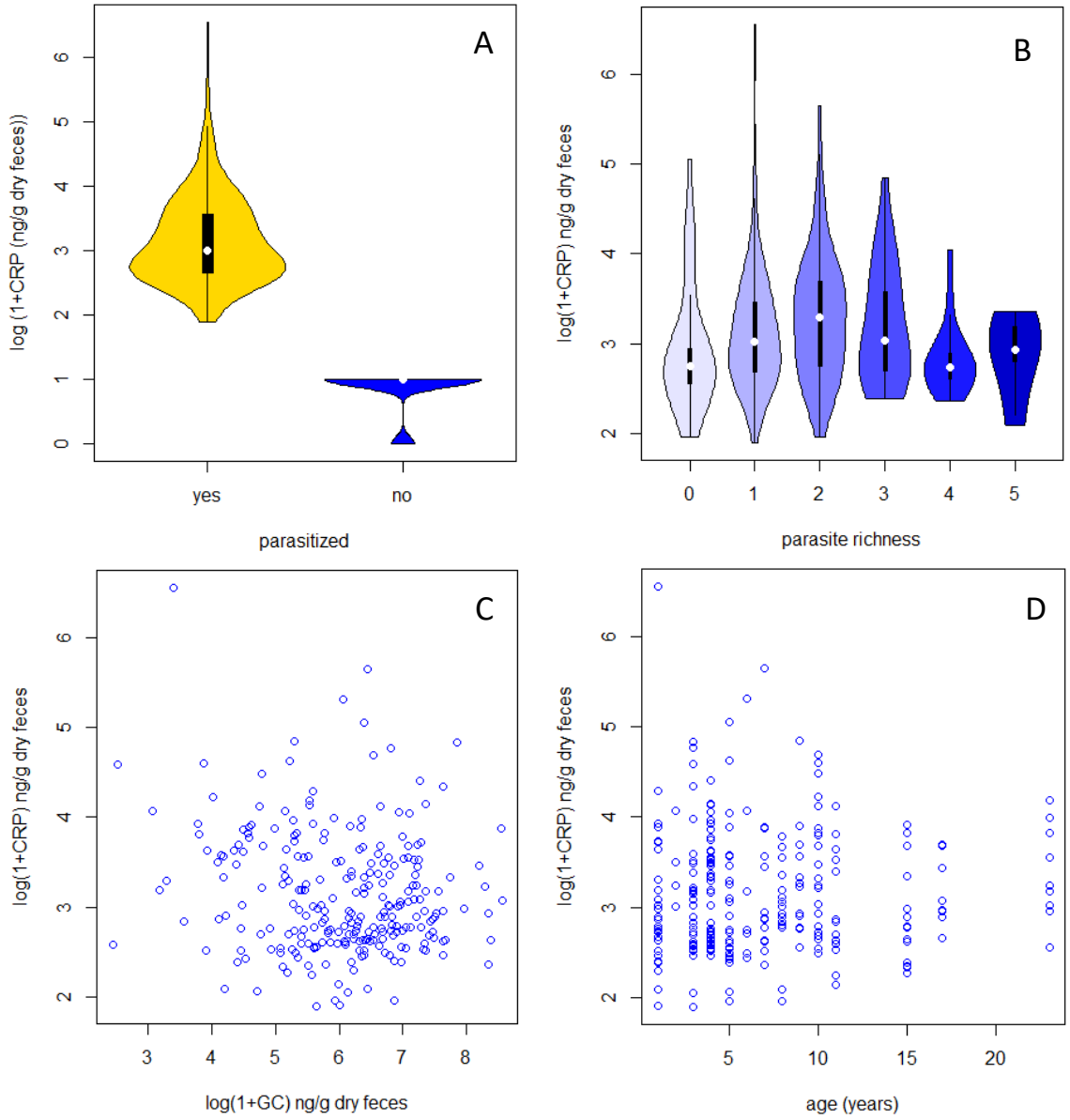
Unfortunately, we cannot consider our study as a successful biological validation for this CRP assay in prosimian faecal samples but this does not mean measuring inflammation non-invasively in prosimians is impossible. Because CRP is secreted by the liver, and also because studies with urinary CRP had better detection rates (humans: Chuang et al., 2010; rhesus macaques: Higham et al., 2015), it might be more appropriate to measure CRP in urine. However, we discarded this option for lemurs because it was difficult to collect the required amount of urine without contamination in semi-arboreal, small bodied animals in the wild.

Other researchers validated saliva measurements of CRP in mammals (dogs: Parra et al., 2005; pigs: Gutiérrez et al., 2009; sheep: Shaw et al., 2012; horses: Lamy & Mau, 2012; humans: Desai & Mathews, 2014; Iyengar et al., 2014), suggesting that saliva samples might be more promising than faecal samples to assess CRP. Saliva samples have, for example, already been collected in semi-free ranging lemurs for a study on androgens, showing that faecal and saliva hormone levels where well correlated (von Engelhard et al., 2000), and suggesting the feasibility of collecting saliva samples for other health markers as well. However, collecting saliva samples in wild animals might be more challenging because animals first have to get used to chew on swabs and release them and it may require some more validation to check if food ingestion before sampling does not affect CRP levels in saliva.

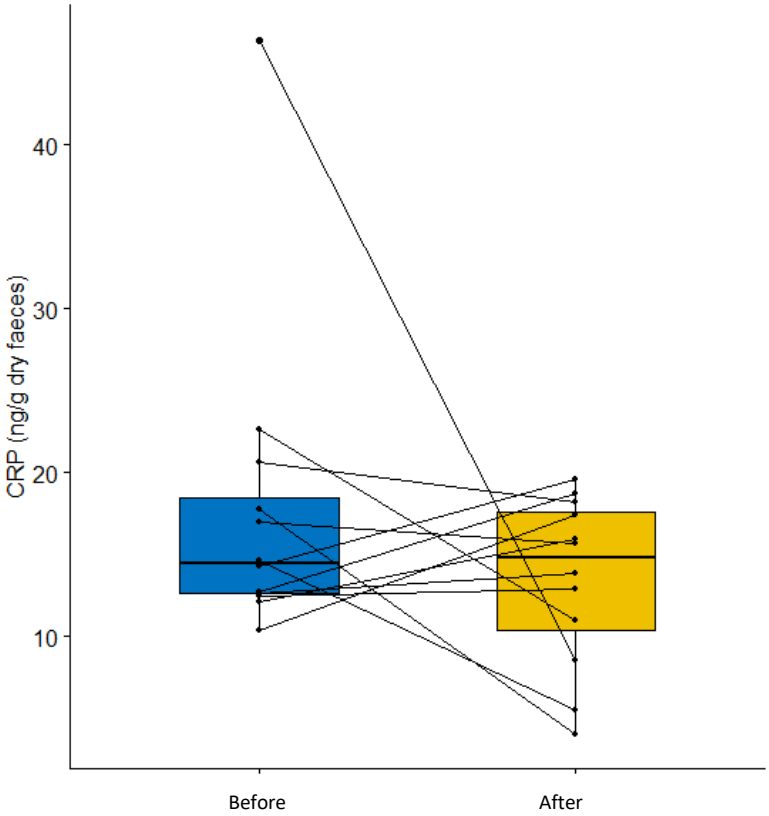
Some markers of local gastrointestinal inflammation designed for human faecal samples, like faecal calprotectin and faecal lactoferrin (Berni Canani et al., 2008; Sherwood, 2012; Sipponen et al., 2010), could also be used in wildlife. In particular, faecal calprotectin has proven more efficient than blood CRP levels (Schoepfer et al., 2013) in the diagnosis of gastrointestinal inflammation and both faecal calprotectin and faecal lactoferrin have high sensitivity, but are not disease specific, opening multiple areas of use in wildlife research.

Conclusion

Despite appearing at first quite practical and useful, our study suggests that measuring CRP in lemur faeces with the available commercial EIA kit for monkeys is not reliable at the moment due to sensitivity issues. Hence, further validation of CRP secretion in other media such as blood, saliva and urine that have lower integration time than faecal samples are required to get a better understanding why the here measured detection rate was so low. In addition, isolation and characterization of the lemur CRP molecules are needed to develop suitable EIA kits for lemurs. An alternative approach to investigate gastro-intestinal inflammation in wild animals might be to measure faecal calprotectin or lactoferrin levels. Finally, with the development of laboratory techniques, methods with higher sensitivity than the traditional ELISA used in this study, such as microfluidic immunosensor chips and lab-on-a-chip devices could make faecal CRP measurement possible in lemurs. However, additional studies are required to confirm CRP structure in lemurs and validate a more sensitive assay or other analytical technique for faecal samples.



Supplementary Figure 1: When taking samples that had values below detection limit into account by giving them the maximum possible value, i.e. the value of the detection limit, the results of Study 1 did not change. (A) Individuals with parasites in their feces (parasite status Yes, x-axis) had higher CRP levels (y-axis) than individuals without parasites. However, CRP levels (y-axis) did not significantly differ in function of (B) parasite morphotype richness (ranging from 0 to 5, y-axis); (C) GC levels (ng/g dry faeces, x-axis) or (D) age (in years, x-axis). To ease visualisation, GC and CRP levels were log-transformed, not for statistical analyses.



Supplementary Figure 2: When taking samples that had values below detection limit into account by giving them the maximum possible value, i.e. the value of the detection limit, the results of Study 2 did not change. Faecal CRP levels (in ng/g dry faeces, y-axis) did not significantly increase in females after giving birth (x-axis). Each line indicates an individual value before and after parturition, while the boxes indicate mean + standard deviation before and after parturition.

Authors contribution: study design CD & CF; data collection: CD & SF; lab-work: CD & MH; statistical analysis and manuscript drafting: CD & SF. All authors provided critical feedback and revised the manuscript.

Ethical standards: All research reported in this study complied with animal care regulations and applicable national laws of Madagascar and was approved by the Ministère de l'Environnement et des Eaux et Fôrets, MINEEF. The study adhered to guidelines provided by the Association for the Study of Animal Behaviour (ASAB), the Animal Behavior Society (ABS) and the International Primatological Society guidelines on the ethical treatment of primates in research. All samples were collected non-invasively, without animal handling.

Competing interests: The authors declare that they have no competing interests.

Data accessibility: data is available upon request.

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Chapter 6: So Happy Together? Ecological, reproductive and social correlates of glucocorticoids in wild redfronted lemurs (*Eulemur Rufifrons*)

With Michael Heistermann and Claudia Fichtel

Abstract

Social organization and social relationships can have a profound impact on the physiological stress response, which in turn plays a pivotal role in mediating the link between sociality and health. On the one hand, sociality can be a source of stress for individuals, as they have to deal with dominance and unpredictable changes in their social network. On the other hand, social support and strong social bonds can reduce the adverse effects of stress and improve an individual´s health and fitness. We investigated ecological and social correlates of physiological stress in redfronted lemurs (Eulemur rufifrons) in Kirindy forest, Western Madagascar. They live in small and cohesive social groups with high level of social tolerance but regular instability periods characterized by male group takeovers and female evictions, upsetting social stability. We observed 42 individuals in five groups over a period of 18 months and collected regular faecal samples for glucocorticoid metabolite (fGCm) analyses. We focused on quality and quantity of social relationships and sex differences on fGCm concentrations. Preliminary results indicate that the dry season is characterized by elevated fGCm levels in all individuals, with higher GC levels when the rainfall is scarce and daily temperature differences are high. Reproductive season also impacted on fGCm, with increased concentrations found in females during gestation and early lactation, and during the mating season and the early lactation period in males. Finally, individual sociality indices were negatively correlated to fGCM in both sexes, supporting an immediate alleviating effect of sociality on stress in this species.

Introduction

Stressors are aversive and often unpredictable stimuli that destabilise an individual's homeostasis and cause a physiological stress response(Madliger & Love, 2014; McEwen, 2007, 2014; Sapolsky et al., 2000). In mammals, one of the primary physiological responses to stress is the increase of adrenal glucocorticoid (GC) secretion via an increase of the Hypothalamic-Pituitary-Adrenal (HPA) axis activity (Cavigelli, 1999; Mendoza et al., 2000; Sapolsky et al., 2000). In the short term (minutes to days), as a result of GC elevation, energy and resources will be reallocated to cope with the stressful situation (Romero et al., 2009; Sapolsky, 2002; Sapolsky et al., 2000). But if the GC elevation is chronic, it can affect many functions such as

reproduction, the immune system and global body condition and eventually the survival of individuals (Bonier et al., 2009; Romero et al., 2009).

Then, within minutes to days, elevations of GC levels cause reallocation of energy and resources to systems directly linked to survival (e.g. immune function and cognition), while others are inhibited (e.g. digestion and reproductive function) (McEwen, 2007; Sapolsky et al., 2000). Hence, under normal conditions, acute GC elevation is beneficial, as it allows individuals to cope with short-term challenges and to return to homeostasis (Charmandari et al., 2005; Sapolsky et al., 2000). However, multiple stressors accumulating over time, or becoming chronic or unavoidable, can result in chronically elevated GC levels (Edes & Crews, 2017; McEwen, 1998).

Whether chronically elevated GC levels are adaptive or detrimental is still debated. On the one hand, no study has found detrimental effects of chronically elevated GC levels on individual fitness in natural populations (Beehner & Bergman, 2017; Boonstra, 2013). On the other hand, a large number of biomedical studies and studies on captive animals have shown detrimental effects of chronically elevated GC levels or dysfunctions of the regulatory mechanisms of GCs on individual health and fitness by reducing investment in reproduction and immune function (Dhabhar et al., 1995; Elenkov & Chrousos, 1999; Korte et al., 2005; McEwen, 1998, 2014; Romero et al., 2009), a concept called allostatic load (Edes & Crews, 2017; McEwen, 1998). If chronically elevated or when regulatory mechanisms are impaired, high GC levels can affect the immune system by dysregulating inflammation processes (Cohen et al., 2012), modifying production and activation of cells involved in the immune system (Dhabhar et al., 1995; Elenkov, 2004; Romero & Butler, 2007) and provoking a shift towards cell mediated T-helper type 2 (Th2) immune responses (Elenkov & Chrousos, 1999). Finally, long-lasting GC levels elevations can lead to immunosuppression (Beldomenico & Begon, 2016; Sapolsky et al., 2000) and negatively affect survival (Hufschmid et al., 2014; Muehlenbein, 2006; Stowe et al., 2001).

From studies in human and non-human primates, there is evidence that social stress can have profound negative effects on health. In humans, social isolation is associated with higher levels of perceived stress and deteriorated physiological functioning (Cacioppo & Hawkley, 2003) and with higher HPA axis activity and inflammatory signalling (Hawkley et al., 2012; Hennessy et al., 2014). Higher stress levels or perceived social stress were also associated with

altered transcription patterns of immune genes (Cole et al., 2007) and metabolic, cardiovascular disease and mental illnesses in humans (Caserta et al., 2008; Glaser & Kiecolt-glaser, 2009; Hawkley & Capitanio, 2015; Holt-Lunstad et al., 2010; Kiecolt-Glaser et al., 2010). Similarly, in non-human animals, social stress and elevated GC levels are associated with lower cognitive performances, adverse health outcomes and lower longevity (Blas et al., 2007; Bonier et al., 2009; Buchanan et al., 2008; Cavigelli et al., 2009).

Additionally, in non-human animals, many studies found a strong relationship between health, fitness and social status (Archie et al., 2012; Cavigelli & Caruso, 2015; Cavigelli & Chaudhry, 2012; Marescot et al., 2018; R. M. Sapolsky, 2005b). In particular, dominance rank is associated with variations in GC levels with differences between dominants and subordinates depending on the social system (Abbott et al., 2003b; Gesquiere et al., 2011; Ostner et al., 2008; Sapolsky, 2005b; Schoof & Jack, 2013). Social instability (i.e. periods of rank change or male immigration) generally lead to increased GC levels (Engh et al., 2006; Sapolsky, 2005b; Wittig et al., 2008). Ultimately, chronic social stress can lead to increased disease susceptibility (Capitanio et al., 1998; Dhabhar, 2009; Glaser & Kiecolt-Glaser, 2005).

In contrast, affiliative interactions, strong bonds and social support can increase an individual's ability to cope with challenging situations, and buffer against the adverse effects of stress (Cheney & Seyfarth, 2009; Sheldon Cohen & Wills, 1985; Kikusui et al., 2006; Kiyokawa & Hennessy, 2018; Taylor et al., 2010). In particular, in baboons, correlational studies showed that while social stress has negative effects on body condition and fitness outcomes, bonding can mitigate these costs (Crockford et al., 2008; Silk et al., 2003, 2009; Wittig et al., 2008). This effect has been referred to as social buffering (Sheldon Cohen & Wills, 1985) and could explain the health benefits of social relationships (Berkman & Syme, 1979; Holt-Lunstad et al., 2010; House et al., 1988) for both acute and chronic stressors (Abbott et al., 2003b; Cheney & Seyfarth, 2009; Sapolsky, 2002).

Consequently, social stress can have an important impact on health and fitness with negative effects via immunomodulation (Sapolsky, 2005b) as well as protective effects via social support and integration (Cohen & Wills, 1985). Moreover, physiological stress can also influence social behaviours (Raulo & Dantzer, 2018). However, to date, only few studies have simultaneously investigated effects of sociality and stress on health and individual fitness in the wild (Akinyi et al., 2019; Habig et al., 2019; Wascher et al., 2018). This is why mechanisms

and causality of the sociality-health relationship remain poorly understood (Beehner & Bergman, 2017; Peter M Kappeler et al., 2015; Julia Ostner & Schülke, 2018), and this is necessary to widen the range of species examined under natural conditions.

In wildlife studies, faecal GC metabolites are regularly used to assess an individual's physiological response to fluctuations in energy allocation, habitat quality or physiological and social constraints (Millspaugh & Washburn, 2004; Palme, 2019; Romero, 2004). For example, differences in GC levels have been correlated with differences in group size and composition, seasonal fluctuations, habitat disturbance, food availability, variation in rates of affiliative and agonistic behaviour, parasite infections, immunocompetence and ultimately reproductive success (Chapman et al., 2006, 2007; Clough, 2010; Gesquiere et al., 2011; Muehlenbein, 2006; Julia Ostner et al., 2008; Schoof & Jack, 2013). However, comprehensive studies including ecological, physiological and social factors are rare and needed (Beehner & Bergman, 2017).

We conducted such a study in redfronted lemurs, a group living lemur species, living in a very seasonal environment characterised by a warm rainy season and a colder dry season, with lower fruit availability. Thus, we expected higher GC levels in the dry season due to cold stress and because fruit are energy rich food items, we expected higher GC levels in the the times of lower fruit availability. This species is breeding only during the dry season, thus we expected higher GC levels in females from mating to the end of lactation with a peak at the end of gestation as it was already found in other mammals (Charpentier et al., 2018; Rudolph et al., 2020). We also expected increased GC levels in males during the mating season due to mate competition (Ostner et al., 2008). We expected parasitised individuals to have lower GC levels than non parasitised individuals due to a TH2 polarised immunity (Clough et al., 2010, Chapter 3). Finally, because our lemur species is known for its frequent periods of social instability with female evictions and male take-overs (Peter M. Kappeler & Fichtel, 2012b), we investigated the effect of social factors like rates of affiliative behaviour, sociality index (Silk et al., 2013) and social instability measured as changes in group composition, as such factors can influence GC levels(Gust et al., 1993; Wittig et al., 2016; Wooddell et al., 2017).

Materials and methods

Study site and population

We studied a population of redfronted lemurs (*Eulemur rufifrons*) from May 2015 to October 2016 at the research station of the German Primate Center (DPZ) in a 60ha study area in Kirindy Forest, western Madagascar. The study area is a dry deciduous forest, subjected to pronounced seasonality due to a dry season from March to October and a wet season from October to February (Peter M. Kappeler & Fichtel, 2012a). The study population comprised 44 individuals living in five adjacent groups, ranging from 4 to 15 individuals. This species is cathemeral, semi-arboreal and frugivorous (Donati et al., 1999; Mittermeier et al., 2008). Redfronted lemurs are seasonal breeders with a short mating season usually in May/June and birth of single infants in September/October (Julia Ostner et al., 2002). All individuals have been well habituated to the presence of human and are marked with unique combinations of nylon collars and pendants for individual recognition, for more than 20 years (Peter M. Kappeler & Fichtel, 2012a). One adult female per group was equipped with a VHF-radio-collar to facilitate group location. Individual information about sex and age was available because of long-term monitoring of the population. For individuals who had immigrated into the population, age was estimated at first capture of these individuals using tooth wear and sexual maturity.

Behavioural observations

We observed repeatedly each adult individual by conducting focal animal sampling (Jeanne Altmann, 1974) for a duration of one hour. In addition, we observed juveniles over five months old as much as possible. We continuously protocolled all behaviour between 7 a.m. to 11 a.m. and 1 p.m. to 4 p.m.

We defined six different reproductive periods: “mating”, “early gestation”, “late gestation”, “early lactation” and “late lactation” and “rest”. Considering a gestation length of 121 days (Julia Ostner & Heistermann, 2003), we defined “mating” for each group as the period of two weeks before and following the estimated average date of conception for the females of the group, based on their average date giving birth. “Early gestation” was defined as the first half of gestation time and took place between “mating” and “late gestation”.

Females were in “late gestation” during the second half of gestation period and lasted from the end of “early gestation” to the beginning of “early lactation”, ending when all females in the group gave birth. “Early lactation” covered the first four weeks after giving birth and “late lactation”. “Late lactation” was the time between “early lactation” and “rest” period and lasted 3.5 months. Finally, the period called “weaned” was the period of the year with weaned juveniles and before the following “mating” period. All adult females gave birth in 2015 and 2016, thus we considered the males as a reference level for the females, to try to distinguish between reproductive and ecological effects. A schematic overview of reproductive periods is given in figure 1.

Figure 1: Schematic presentation of the six reproductive periods considered during our study

January	**February**	**March**	**April**	**May**	**June**	**July**	**August**	**September**	**October**	**November**	**December**
Late lactation		Weaned		Mating	Early gestation			Late gest.	Early lact.	Late lactation	

Adult sex ratios

Adult sex ratios were calculated as the number of adult males divided by the number of adult males and females in the group for each month of the study as there were frequent group changes.

Group size

Group size was calculated each month by adding all individuals (infants, juveniles and adults) present in the group for more than two weeks this month.

Affiliative behaviours

Because affiliative behaviour were very rare, we regrouped all recorded socio-positive interactions (allogrooming, huddling, body contact) over a month in one variable named “all affiliative behaviour” and expressed as minutes per hour of observation. We also included rates of grooming given and received (min per hour).

Number of social partners

We summed up the number of grooming partners each individual had over a month (grooming received and given).

Sociality index

We constructed an individual sociality index (Silk et al., 2013) calculated as the sum of the rate of behavior of interest for the individual divided by the median rate of this behavior for all individuals in the group, all divided by the number of behavior measured. We included grooming received, given and mutual, huddling and time spent in body contact. We calculated this index for each reproductive period during our study period, to account for changes in social relationships due to reproductive states. This index values range from $0 \rightarrow \infty$. High values represent more socially integrated individuals than other members of the group, low values represent less socially integrated individuals than other members of their group.

Agonistic behaviours

Because agonistic behaviour were very rare, we regrouped all agonistic interactions (chasing, biting, slapping, displacing and being displaced) over a month in one variable named "displacements" (as they were essentially displacement events) and expressed as events per hour of observation.

Social instability

Social instability was defined as the two weeks from group composition change, whether an individual disappeared, died or an individual immigrated or emigrated from/to the group.

Food availability, rainfall and temperature

To measure food availability, we conducted monthly phenology transects of 690 trees throughout the study period. We used a semi-quantitative method (Fournier, 1974) in which the abundance of each type of plant part (i.e. leaves, fruit, flowers) was scored, ranging from 0 (absence) to 4 (maximum abundance) (for details, see Koch et al., 2017). We then calculated the monthly average from all the trees for each plant part. Daily rainfall and temperature data were measured by a weather station located at the field camp. Time since the last rainfall was calculated. "Daily temperature differences" is a variable describing average monthly differences between daily lowest and highest temperatures.

Faecal sample collection

733 faecal samples from the 42 study animals (15 adult males, 14 adult females, 3 juveniles in 2015 and 18 adult males, 14 adult females, 9 juveniles in 2016) were collected for parasite and hormone analysis. To limit bias due to temporal variation in parasite and hormone excretion (V. Behringer & Deschner, 2017), samples were systematically collected between 07 am and 11 am, immediately after defecation. After collection, samples were divided in aliquots to run several analyses. One to two grams of faeces were placed in 15ml polypropylene tubes pre-aliquoted with 5ml of 10% neutral-buffered formalin for parasite analyses, while 0.5 g of faeces went to 15ml polypropylene tubes containing 5ml of 90% ethanol for hormone analyses. Then the tubes were labelled and wrapped with parafilm and brought back to the camp within 3 hours for storage or extraction. Parasites and hormones samples were always collected between 7 a.m. and 10 a.m. to account for a potential circadian effect on parasite egg shedding or hormone levels (Martinaud et al., 2009; Sousa & Ziegler, 1998; Villanúa et al., 2006).

Hormone extraction and analysis

Samples for hormone analysis were extracted at the camp within 4 h of sample collection adapting a protocol described by Ziegler and Wittwer (2005) and modified by Shutt et al. (2012). A small amount of c.a.0.5g of faeces from the homogenised aliquot was weighed into an extraction tube and mixed with 2 ml of 90% ethanol. For logistic reasons, the fecal suspensions were left to stand for 5–12 h, then vortexed for 2 min. Samples were finally

centrifuged using a manually operated centrifuge (Hettich GmbH & Co. KG Tuttlingen, Germany) for 2 min (Shutt et al., 2012). The supernatant was poured into a 2 ml polypropylene tube and sealed with parafilm. Samples were stored at the field site in a dark container a t ambient temperature for a maximum of six months and returned to the laboratory in November 2015, April 2016 and November 2016. Upon arrival at our German laboratory, samples were stored at -20°C until hormone analysis. The remaining faecal matter was dried in a solar oven until dry faecal weight was constant, mass to obtain an estimate of the water content of the feces (Shutt et al., 2012; Ziegler & Wittwer, 2005). The hormone extracts were used for measurements of faecal metabolites of immunoreactive 5b reduced cortisol (3a,11-oxo-CM, thereafter fGCm) using microtitreplate enzymeimmunoassays (EIA). Methods have previously been described and validated for redfronted lemurs (Ostner et al., 2008). Sensitivity at 90% binding was 1.0 pg/50µl. Intra- and interassay coefficients of variation were 6.8% and 8.8% (high) and 7.9% and 13.0%, respectively. All hormone values are expressed as mass per gram dry faecal weight (ng/g). For statistical analyses, we used each individual sample value as a data point.

Parasite analysis

Parasite samples were kept at the camp in the dark, at ambient temperature. They were stored at the field site for a maximum of six months and returned to the laboratory in November 2015, April 2016 and November 2016, were they were kept in the dark, at ambient temperature. For parasite analysis, we followed the methods used by Clough (2010) on the same population of red-fronted lemurs. Faecal samples were processed using a modified version of the formalin-ethyl-acetate sedimentation technique described by Ash and Orihel (1988). Approximately 5 ml of homogenized faecal material was strained into centrifuge tubes and 10% of formalin was added until the total volume reached 10 ml. Then we added 3 ml ethyl-acetate and shook the tube vigorously for 30 s and centrifuged it for 10 min on 2,200 rpm. We then removed the top layer of fat before pouring off the supernatant consisting of ethyl-acetate, formalin, and debris from the centrifuge tube. The remaining sediment was used for subsequent analyses. Details of the methods as well as on the identification of parasite species can be found in Clough (2010). Wet mounts of each sample were prepared with 20 mg of sediment and one drop of Lugol's solution on a microscope slide. One slide was

systematically scanned for each sample, looking for helminth eggs and larvae as well as protozoan cysts and trophozoites.

Measurements of parasite infection

We decided to focus only on qualitative parasite measures because all gastro-intestinal parasites have intermittent and unpredictable egg shedding, raising some doubts about the reasonable use of faecal egg counts as a measure of infection intensity (Anderson & Schad, 1985; Gillespie, 2006). Given the low parasite occurrence of most morphotypes (see Chapter 3), data for all parasite infections were pooled to consider infection status with any kind of parasites (presence or absence of any parasite infection) for statistical analyses.

Statistical analyses

Because multiple predictors were correlated (e.g. ecological season and reproductive periods) or the data set was not complete for all predictors, we conducted a set of Linear Mixed Models ('LMM'; Baayen et al., 2008) with gaussian error structure investigating the potential determinants influencing log GC levels. In all these models, we accounted for non-independence of repeated measurements by including individual identity (ID) as a random effect.

In model I and II we investigated the effect of season and seasonal factors respectively. In Model I (N = 731 datapoints, 5 groups, 42 individuals) the predictors were: Season, Year, Adult sex ratio, Group size, Age, and Infection status. In Model II (N = 712 datapoints, 5 groups, 42 individuals), we replaced Season and Year by ecological factors which vary yearly and could explain the effect we found in model I. The predictors were: Temperature difference, Time since last rain, Fruit abundance, Adult sex ratio, Group size, Age and Infection status. In Model III (N = 678 datapoints, 5 groups, 34 individuals), we investigated the effect of reproductive period on GC levels in adults as a proxy of physiological challenges due to reproduction. Both males and females were included, males serving as a comparison of females, because all adult females reproduced during the study period. The predictors were: the interaction of Reproductive periods and Sex, Year, Adult sex ratio, Group size, Age and Infection status. Finally, in Model IV (N = 483 datapoints, 5 groups, 40 individuals), we investigated the effect

of social factors. The predictors were: Season, Displacements, Total affiliative behaviour, Grooming given, Grooming received, Number of grooming partners, Individual sociality index, Social instability, Sex, Age and Year.

All models conducted in this study were applied using R software (version 3.6.2; R Core Team, 2018), using the *lme4* package (Bates et al., 2015). For all models, the non-categorical covariates were z-transformed (to reach a mean of zero and a standard deviation of one) to achieve easier interpretable models and facilitate model convergence (Schielzeth, 2010).

After fitting each model, we controlled the assumptions based on their error structure. We checked for normally distributed and homogenous residuals by visually inspecting a qqplot and the residuals plotted against fitted values. We further controlled the normal distribution of the random factor using the same function. Stability of all models was verified using another function provided by Roger Mundry, which excluded data points one by one and compared the derived coefficients. We finally verified the absence of collinearity issues by deriving variance inflation factor using the function *vif* of the R-Package *car* (Fox & Weisberg, 2011) applied to a standard linear model excluding the random effects.

For all models, we preliminarily verified that the full models significantly differed from the corresponding null models using likelihood ratio tests before investigating single-predictor effects to reduce the risks of Type I errors (R function ANOVA with argument test set to "Chisq") (A. J. Dobson, 2002; Forstmeier & Schielzeth, 2011). All full-null model comparisons were significant and these results are reported in each model Table. P-values of each fixed factors were then derived using likelihood ratio tests based on Maximum Likelihood (rather than Restricted Maximum Likelihood; Bolker, 2008) comparing each full model with its respective reduced models (i.e., the model without the considered factor; Barr et al., 2013; R function *drop 1*). Confidence intervals were derived using parametric bootstrapping with an adjusted function provided by Roger Mundry and based on the function *bootMer* from the *lme4* package, with 1000 parametric bootstraps and bootstrapping over the random effects.

Results

As reported previously in this species (Clough et al., 2010; Julia Ostner et al., 2002, 2008), GC levels were very variable with a minimum recorded at 10.53 and a maximum at

9055.19 ng/g dry faeces. We found a diverse parasite community with a total of eleven morphotypes of GI parasites in faeces of red-fronted lemurs from Kirindy forest, Madagascar. A total of 61.39% of the samples (450/733) were positive with at least one parasite morphotype, see chapter 2 for more details on parasite morphotypes and their identification.

In model I, we found that season, year of data collection, age and infection status had a significant effect on log (GC) (M.I, Table 1, Fig. 2). GC levels were higher in 2015 compared to 2016 and during the dry season than during the rainy season (Fig 2A & 2B). They were also higher in older individuals but they were lower in individuals with gastrointestinal parasites (Fig 2C & 2D). Adult sex ratio did not influence GC levels but there was a tendency (p=0.06) of lower GC levels in smaller groups.

Table 1: Results of model I: a linear mixed model investigating the effect of ecological season, year of data collection, adult sex ratio, group size, age and infection status on log (GC). N = 731 datapoints, 5 groups, 42 individuals, (Df = 6, Chi^2 = 327.59, p <0.001, R^2 = 0.37)

Predictor	Explanatory variable	Estimate	SE	Lower CI	Upper CI	Df	Chi^2	p
log (GC)	Intercept	7.32	0.08	7.18	7.47	[c]	[c]	[c]
	Season (rainy) [a]	-0.77	0.08	-0.91	-0.61	1	92.97	**<0.01**
	Year (2016) [a]	-0.91	0.08	-1.07	-0.76	1	125.58	**<0.01**
	Adult sex ratio [b]	-0.05	0.05	-0.15	0.05	1	0.92	0.34
	Group size [b]	-0.10	0.05	-0.21	0.00	1	3.59	0.06
	Age [b]	0.12	0.05	0.03	0.21	1	6.45	**0.01**
	Infection status (Yes) [a]	-0.19	0.07	-0.32	-0.05	1	6.36	**0.01**

[a] Reference category being dry season for the ecological season, 2015 for the year and not infected for infection status
[b] z-transformed
[c] Not shown as not having a meaningful interpretation. For intercepts, p-values would refer to estimated PSR, when all covariates are at zero.
Chi^2 values are results of a likelihood ratio test comparing the full model with a reduced model lacking the respective term

In model II, we found that the seasonal factors temperature difference and fruit abundance had a significant effect on log (GC), with higher GC levels when average daily temperature difference was high and average fruit abundance index was low, but time since last rain did not influence GC levels directly (M.II, Table 2, Fig.3A & 3B). In accordance with model I, GC levels were higher in older individuals than in younger ones and they were higher in bigger groups than smaller ones (Fig 3C & 3D). Similarly to model II, adult sex ratio did not influence GC levels but infection status did not influence GC levels in this model (Table 2).

Figure 2: Model I. Log (GC) (y axis) were higher (A) in 2015 than in 2016 (x-axis); (B) during the dry than during the rainy season (x-axis); (C) in older than in younger individuals (x-axis); and (D) in non-parasitised than in parasitized individuals (x-axis). Datapoints are represented by sheer dots. In boxplots, each box indicates median, upper, and lower quartiles. Whiskers indicate ± 1.5 interquartile ranges. Outliers are plotted as solid black dots outside of the box and whiskers'area. In scatterplots, the plain line represents the model's predicted value, shaded area represents the 95% confidence interval.

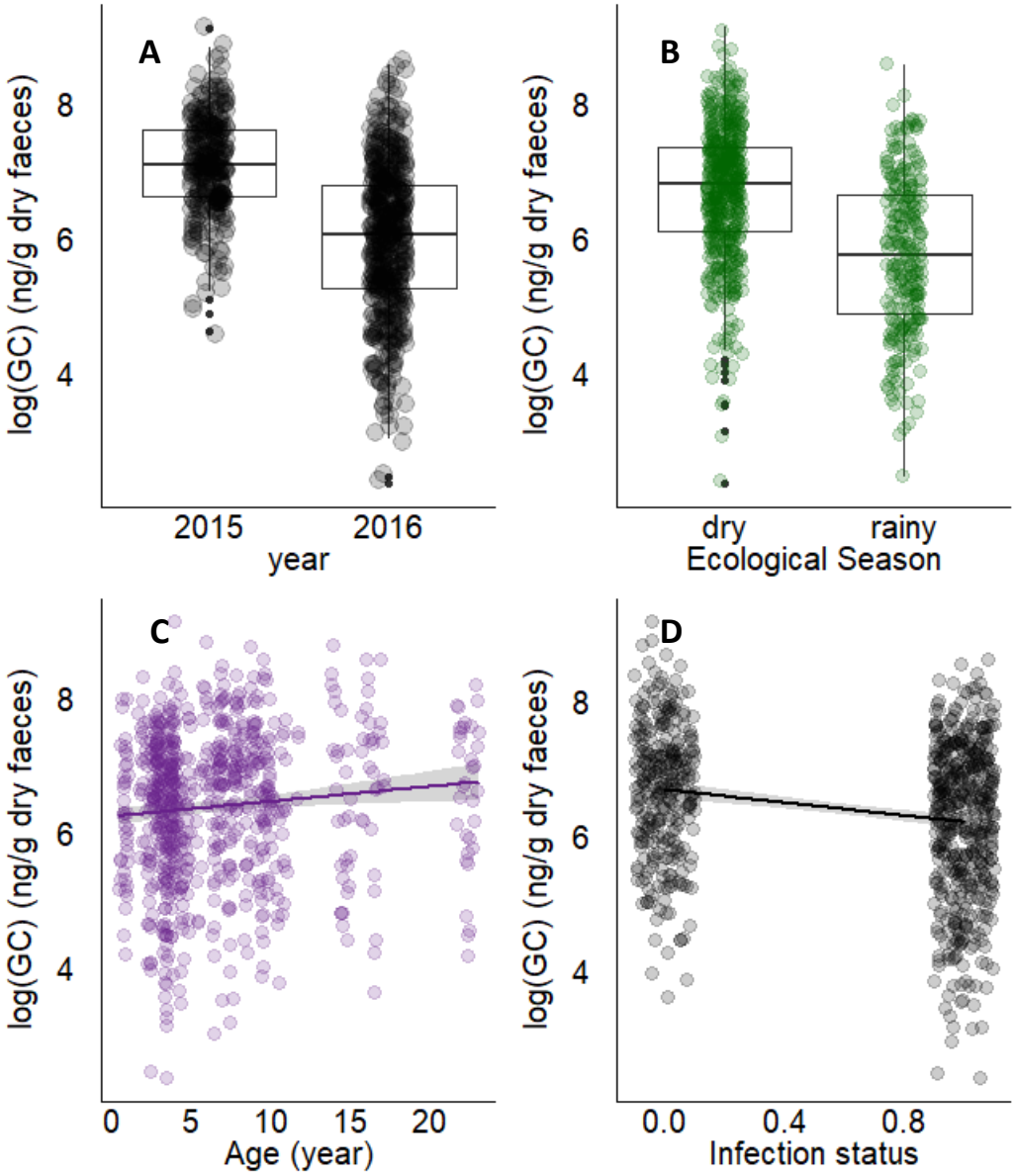


Table 2: Results of model II: a linear mixed model investigating the effect of the ecological factors temperature difference, time since last rain and fruit abundance along with year of data collection, adult sex ratio, group size, age and infection status on log (GC). N = 712 datapoints, 5 groups, 42 individuals, (Df = 7, Chi^2 = 354.12, p <0.001, R^2 = 0.40)

Predictor	Explanatory variable	Estimate	SE	Lower CI	Upper CI	Df	Chi^2	p
log (GC)	Intercept	6.45	0.07	6.71	7.05	[c]	[c]	[c]
	Temperature difference [b]	0.13	0.06	0.31	0.47	1	4.20	**0.04**
	Time since last rain [b]	0.05	0.04	0.00	0.16	1	1.70	0.19
	Fruit abundance [b]	-0.51	0.06	0.03	0.22	1	64.99	**<0.01**
	Adult sex ratio [b]	-0.04	0.05	-0.84	-0.51	1	0.72	0.40
	Group size [b]	-0.14	0.05	-0.23	-0.03	1	6.41	**0.01**
	Age [b]	0.12	0.05	-0.15	0.05	1	5.64	**0.02**
	Infection status (Yes) [a]	-0.10	0.08	-0.27	0.02	1	1.85	0.17

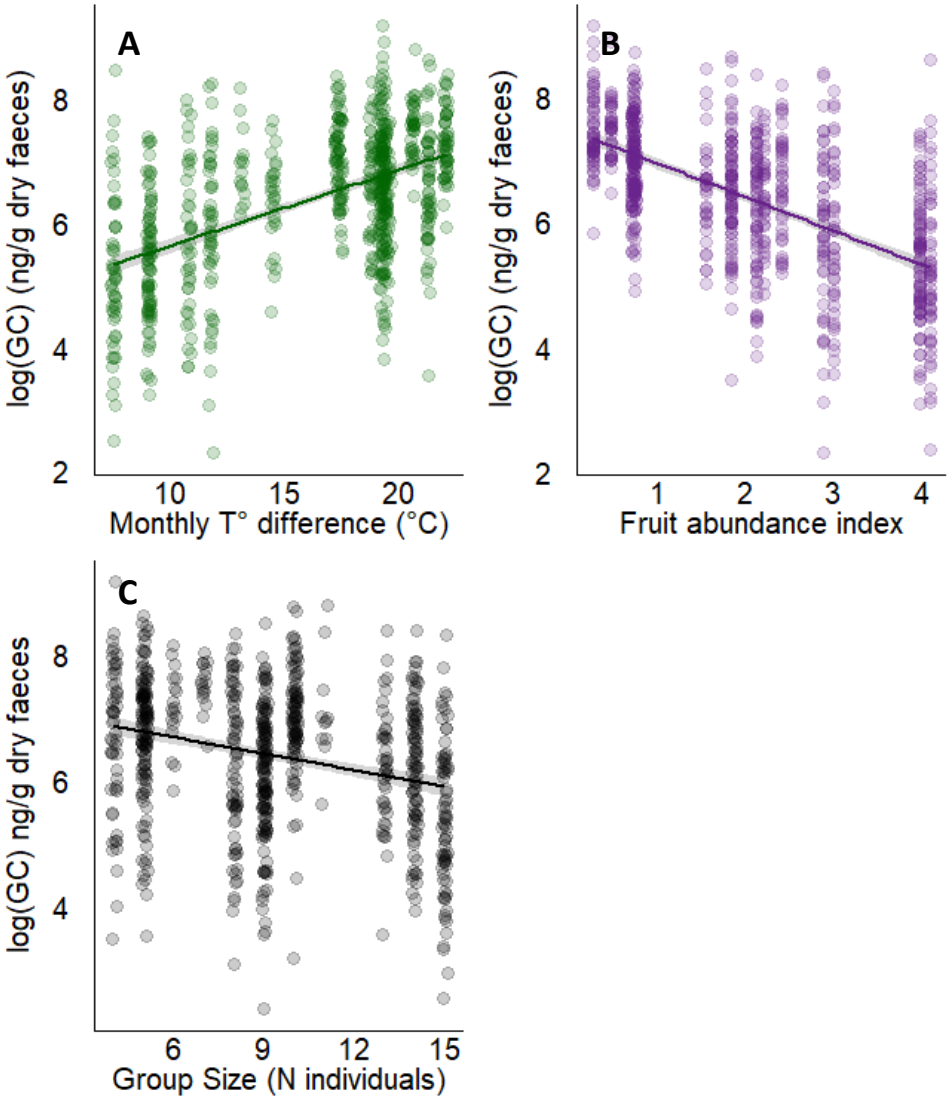
[a] Reference category being not infected for infection status

[b] z-transformed

[c] Not shown as not having a meaningful interpretation. For intercepts, p-values would refer to estimated PSR, when all covariates are at zero.

Chi^2 values are results of a likelihood ratio test comparing the full model with a reduced model lacking the respective term

Figure 3: Model II. Log (GC) (y axis) were higher (A) when temperature difference (x-axis) was large; (B) fruit abundance index (x-axis) was low; and (C) in bigger than in smaller groups (x-axis). Datapoints are represented by sheer dots. The plain line represents the model's predicted value, shaded area represents the 95% confidence interval.



In model III, we found an effect of reproductive period on GC levels that differed according to the individual sex (Table 3, Fig 4): in females GC levels increased from the weaned period (basal levels) to the late gestation period (peak levels), then stayed high until late lactation when they decreased to the basal levels. In males, GC levels increased from weaned period (basal levels) to mating period (peak levels), then they stayed at this level until late lactation, where they decreased to basal levels again. Moreover, in this model, GC levels were higher in 2015 than in 2016 (similarly to model I and II), parasitised individuals had lower GC levels than non-parasitised individuals (similarly to model I) and there was a tendency for older individuals to have higher GC levels than younger ones. Adult sex ratio and group size had no effect on GC levels in this model.

Table 3: Results of model III: a linear mixed model investigating the effect of reproductive periods in function of individual's sex, year of data collection, adult sex ratio, group size, age and infection status on log (GC). N = 678 datapoints, 5 groups, 34 individuals, (Df = 16 , Chi^2 = 333.16, p <0.001, R^2 = 0.39)

Predictor	Explanatory variable	Estimate	SE	Lower CI	Upper CI	Df	Chi^2	p
log (GC)	Intercept	6.48	0.17	6.15	6.84	[c]	[c]	[c]
	Reproductive periods							
	Weaned	-0.67	0.22	-1.07	-0.26	[c]	[c]	[c]
	Mating	0.59	0.20	0.19	0.96	[c]	[c]	[c]
	Early gestation	0.90	0.16	0.58	1.20	[c]	[c]	[c]
	Late gestation	1.60	0.22	1.18	1.99	[c]	[c]	[c]
	Early lactation	1.03	0.24	0.56	1.51	[c]	[c]	[c]
	Late lactation	0.21	0.16	-0.10	0.52	[c]	[c]	[c]
	Sex					[c]	[c]	[c]
	Male	0.35	0.20	-0.05	0.73	[c]	[c]	[c]
	Female	-0.42	0.24	-0.89	-0.02	[c]	[c]	[c]
	Year (2016) [a]	-0.85	0.08	-1.02	-0.69	1	98.09	**<0.01**
	Adult sex ratio [b]	-0.06	0.05	-0.16	0.05	1	1.16	0.28
	Group size [b]	-0.08	0.06	-0.19	0.03	1	2.10	0.15
	Age [b]	0.10	0.05	0.01	0.20	1	3.79	0.05
	Infection status (Yes) [a]	-0.20	0.08	-0.34	-0.05	1	6.65	**0.01**
	Reproductive periods * sex					5	17.64	**<0.01**
	Weaned (male)	-0.08	0.29	-0.68	0.52	[c]	[c]	[c]
	Weaned (female)	0.08	0.29	-0.50	0.68	[c]	[c]	[c]
	Mating (male)	0.08	0.29	-0.49	0.72	[c]	[c]	[c]
	Mating (female)	-0.08	0.29	-0.66	0.53	[c]	[c]	[c]
	Early gestation (male)	-0.54	0.22	-0.96	-0.09	[c]	[c]	[c]
	Early gestation (female)	0.62	0.25	0.14	1.14	[c]	[c]	[c]
	Late gestation (male)	-1.15	0.35	-1.81	-0.46	[c]	[c]	[c]
	Late gestation (female)	1.23	0.37	0.55	1.92	[c]	[c]	[c]
	Early lactation (male)	-0.53	0.31	-1.10	0.12	[c]	[c]	[c]
	Early lactation (female)	0.61	0.34	-0.03	1.22	[c]	[c]	[c]
	Late lactation (male)	-0.50	0.22	-0.93	-0.04	[c]	[c]	[c]
	Late lactation (female)	0.58	0.26	0.07	1.08	[c]	[c]	[c]

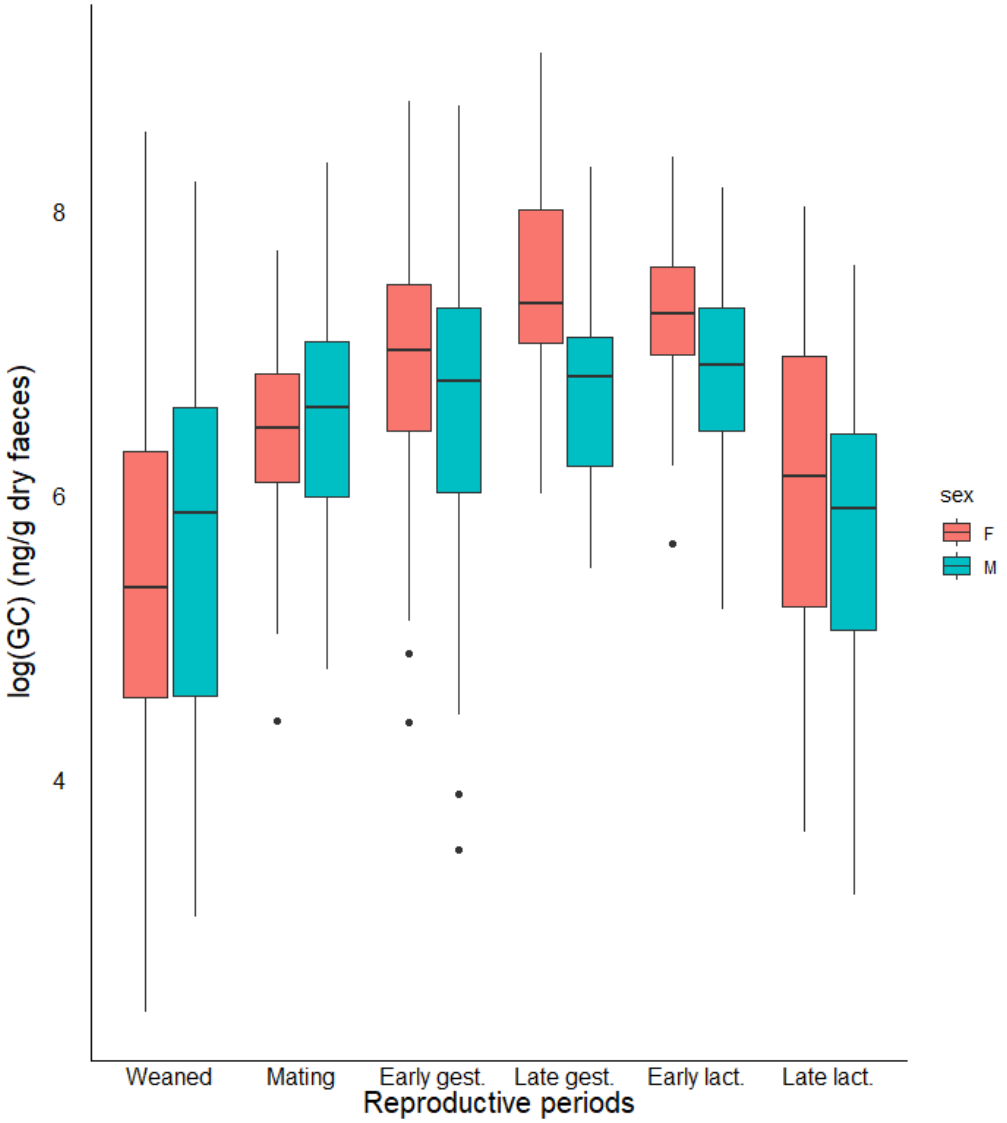
[a] Reference category being not infected for infection status

[b] z-transformed

[c] Not shown as not having a meaningful interpretation. For intercepts, p-values would refer to estimated PSR, when all covariates are at zero.

Chi^2 values are results of a likelihood ratio test comparing the full model with a reduced model lacking the respective term

Figure 4: Model III. Log (GC) (y axis) differed according to the reproductive period (Weaned, Mating, Early gestation, Late gestation, Early lactation, Late lactation: x-axis) and the individual sex (females in red, males in blue). Each box indicates median, upper, and lower quartiles. Whiskers indicate ± 1.5 interquartile ranges. Outliers are plotted as solid black dots outside of the box and whiskers'area.



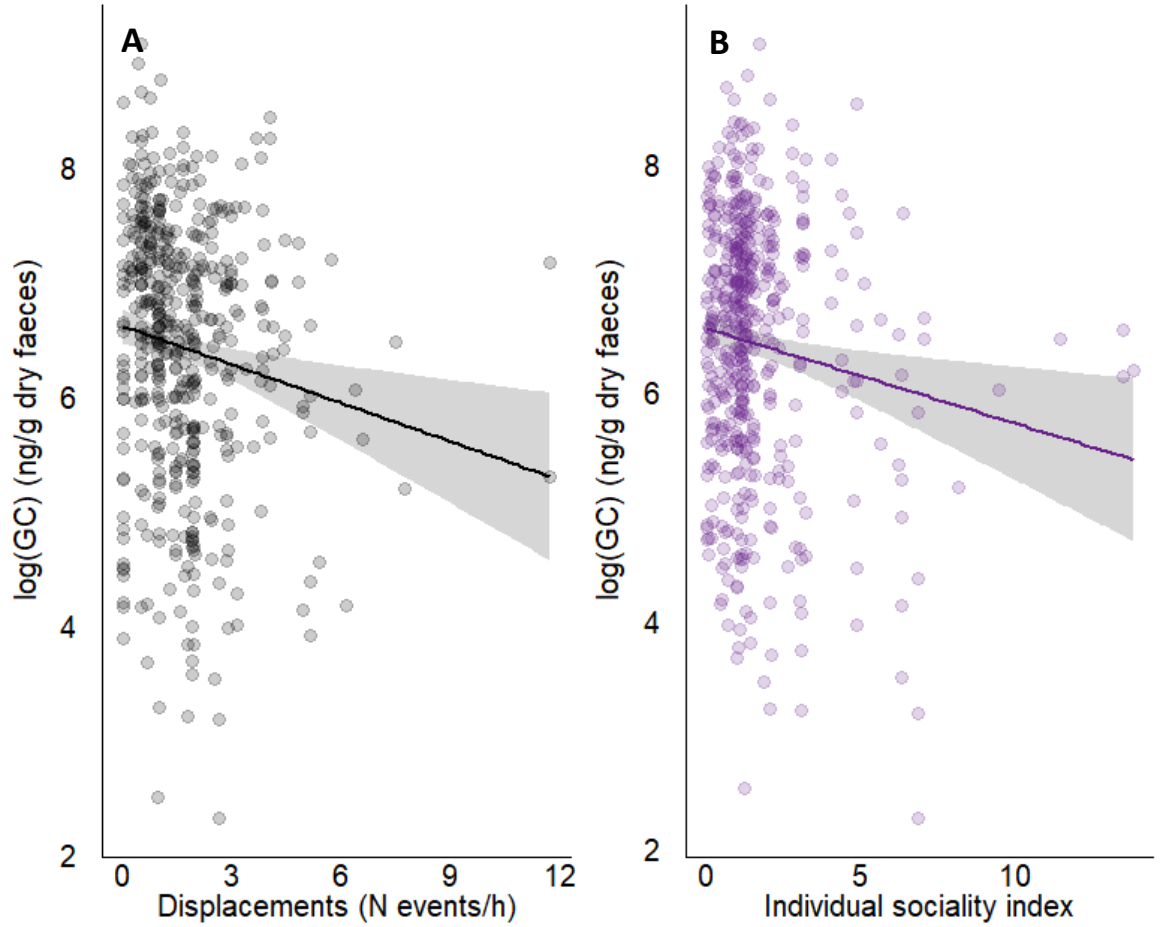
In model IV, we found a negative correlation between log (GC) and (1) of the number of displacements and agonistic behaviours (received and given) and (2) the individual sociality index values (table 4, Fig. 5A & 5B). Similarly to model I and III, GC levels were higher during the dry season and in 2015 compared respectively to the rainy season and the year 2016. None of the other social factors nor age or sex had an effect on GC in this model (Table 4).

Table 4: Results of model IV: a linear mixed model investigating the effect of sociality factors (Number of displacements and other agonistic behaviour per hour, total time in affiliative behaviour per hour, Grooming given per hour, grooming received per hour, Number of grooming partners, individual sociality index, and periods of social instability) plus ecological season, sex, age and year of data collection on log (GC). N = 483 datapoints, 5 groups, 40 individuals, (Df = 11, Chi^2 = 241.97, p <0.001, R^2 = 0.40)

Predictor	Explanatory variable	Estimate	SE	Lower CI	Upper CI	Df	Chi^2	p
log (GC)	Intercept	7.25	0.10	7.06	7.44	[c]	[c]	[c]
	Season (rainy) [a]	-0.86	0.09	-1.03	-0.68	1	76.71	**<0.01**
	Displacements [b]	-0.10	0.05	-0.19	-0.02	1	5.08	**0.02**
	Total affiliative behaviour [b]	0.03	0.05	-0.06	0.13	1	0.43	0.51
	Grooming given [b]	-0.01	0.06	-0.12	0.10	1	0.03	0.88
	Grooming received [b]	0.06	0.06	-0.04	0.18	1	1.21	0.27
	Number of grooming partners [b]	-0.08	0.05	-0.18	0.02	1	2.53	0.11
	Individual sociality index [b]	-0.14	0.05	-0.24	-0.04	1	6.85	**0.01**
	Social instability (Yes) [a]	0.20	0.11	-0.01	0.42	1	3.15	0.08
	Sex (male) [a]	-0.10	0.11	-0.31	0.12	1	0.82	0.37
	Age [b]	0.08	0.05	-0.02	0.19	1	2.43	0.12
	Year (2016) [a]	-0.94	0.09	-1.14	-0.76	1	90.74	**<0.01**

[a] Reference category being dry season for season, no for social instability, female for sex and 2015 for year
[b] z-transformed
[c] Not shown as not having a meaningful interpretation. For intercepts, p-values would refer to estimated PSR, when all covariates are at zero.
Chi^2 values are results of a likelihood ratio test comparing the full model with a reduced model lacking the respective term

Figure 5: Model IV. Log (GC) (y axis) was higher when individuals (A) received and gave less displacements and agonistic behaviours(x-axis) and (B) had lower sociality index values. Datapoints are represented by sheer dots. The plain line represents the model's predicted value, shaded area represents the 95% confidence interval.



Discussion

Our study revealed that a combination of ecological, reproductive and social factors influenced GC levels in redfronted lemurs. As predicted, GC levels were higher in the dry season than the rainy season, because of lower fruit availability and larger daily temperature differences. GC levels were also influenced by the reproductive periods, with differences between the sexes. Males' GC levels increased from the weaned period to the mating season, when they peaked and stayed high until the end of early lactation. Females' GC levels increased from the weaned period to the late gestation period, when they peaked and stayed high until the end of early lactation. Finally, and contrary to our expectations, neither group instability, nor simple measures of sociality like grooming and agonistic behaviour rates explained GC levels, but individual sociality index did, with lower GC levels in individuals with higher sociality index. Altogether, our study shows that energetic demands, caused by environmental or physiological factors, play an important role in redfronted lemurs GC outputs, with sex differences due to differences in life-history strategies. Furthermore, this study shows that social factors seem to have a more subtle influence on redfronted lemur GC patterns.

Seasonality: fruit availability and average daily temperature difference

Seasonality had a strong effect, with higher GC levels in the dry season compared to the rainy season. Animals living in Madagascar, such as lemurs are subjected to strong seasonal changes(Dewar & Richard, 2007; Wright et al., 2005). In particular, in Kirindy forest, we found that during the dry season characterised by large daily temperature differences (cold nights and warm days) but low fruit availability, GC levels were higher than during the rainy season characterised by warmer and wetter weather with high fruit availability.

There was a strong yearly difference, attributed to differences in fruit availability between 2015 and 2016 (supplementary Fig. 1). There was a negative correlation between fruit availability and GC levels. Indeed, one of the main functions of GC is to maintain blood glucose levels (Sapolsky et al., 2000), a very basic but necessary physiological adaptation to short-term food scarcity. This results indicates that reduced food availability cause increased energetic demands and thus, elevate GC levels to maintain homeostasis, as it was shown in other vertebrates (Balestri et al., 2014; Behie & Pavelka, 2013; Bryan et al., 2014; Chapman et al., 2007b; Foley et al., 2001).

In addition, there was an effect of daily temperature differences on redfronted lemurs GC levels. Indeed, to maintain body temperature constant, when exterior temperature vary greatly, would be more costly energetically, which seems to be reflected in redfronted lemurs GC levels, as in many vertebrate taxa (Charpentier et al., 2018; Houser et al., 2011; Huber et al., 2003; Jessop et al., 2016; Jimeno et al., 2018; Rudolph et al., 2020; Sapolsky et al., 2000). Another study on the same population showed that redfronted lemurs modify their behaviour during the dry season to respond to cold stress by increasing inactivity and forming group huddles to benefit from social thermoregulation(Ostner, 2002). These behavioural changes during cold nights in the dry season combined with positive correlations between daily temperature difference and fruit availability with GC levels confirm an increase in energetical demands during the dry season in this species and behavioural and physiological adaptations to face it.

Age

Age was positively correlated with GC concentrations in model I & II and there was a tendency in model III. This effect was found in many other species (Hennessy et al., 2006; Lynch et al., 2002; Rimbach et al., 2013; Rudolph et al., 2020; Tecot et al., 2019), and could reflect the different challenges (physiological or social) individuals face at different life stages (such as puberty, dispersal and group integration, mate competition, senescence), which can affect hormone levels.

Reproductive periods

Males and females redfronted lemurs presented different patterns of GC variation during the different reproductive periods. In males, the annual peak in GC concentrations happened during the mating season, probably because of increased physiological and social challenges during the mating period because of mate competition (Balestri et al., 2014; Fichtel et al., 2007; Girard-Buttoz et al., 2009; Gould et al., 2005; Ostner et al., 2008) and in accordance with the challenge hypothesis(Wingfield et al., 1990). Because redfronted lemurs are seasonal breeders, with the reproductive season happening during the dry season, the extended period of time with heightened GC levels until the early lactation phase suggests that the ecological challenges might contribute to the maintained high GC concentrations.

Finally, it was also suggested, in a study with the same population 15 years earlier, that elevated GC and testosterone levels during the early lactating period might aid in the defence against male take overs and infanticide (Ostner et al., 2008).

In females, GC levels increased during the mating period, to reach a peak at the late gestation period until the early lactation period and decreased at the late lactation period. This result shows the energetic demands of reproduction in mammals (Balestri et al., 2014; Charpentier et al., 2018; Emery Thompson, 2017; Foerster et al., 2012; Maestripieri & Georgiev, 2016; Martínez-Mota et al., 2017). Furthermore, GC levels in females were higher than in males only during gestation and lactation, showing a cumulative effect of reproduction and dry season on GC levels, as gestation, parturition and early lactation happen during the dry season. Thus, gestating and lactating females might face higher energetical burdens than males and non-reproducing females. However, during the two reproductive periods covered by this study, all adult females gave birth to viable babies, so we could not compare reproducing and non-reproducing females.

Sociality

Group size was negatively correlated with GC levels in model II but not in models I and III and adult sex ratios were not correlated to GC levels in any of the models. In redfronted lemurs, groups are relatively small (4-16 during this study) and cohesive (Pyritz et al., 2011; Sperber et al., 2019), with even or slightly male biased adult sex ratios (Julia Ostner & Kappeler, 2004). Furthermore, there is no strict linear hierarchy, high degrees of social tolerance and low levels of aggression (Fichtel et al., 2018; Julia Ostner & Kappeler, 1999; Michael E. Pereira & McGlynn, 1997). The specific social organisation of this species could thus explain the lack of relationship between group size or adult sex ratios and GC levels, especially because of low costs of competition between group members.

Contrary to many studies in anthropoid primates (Gust et al., 1993; Shutt et al., 2007; Wooddell et al., 2017) but in in accordance with numerous lemur studies (Brockman et al., 2009; Fichtel et al., 2007; Ostner et al., 2008; Rudolph et al., 2020), there was no effect of the rates of affiliative behaviour, of grooming given or grooming received on GC concentrations. This result might be due to the short amount of time redfronted lemurs spend socialising (2% of time grooming in my study), in comparison to other social animals such as baboons,

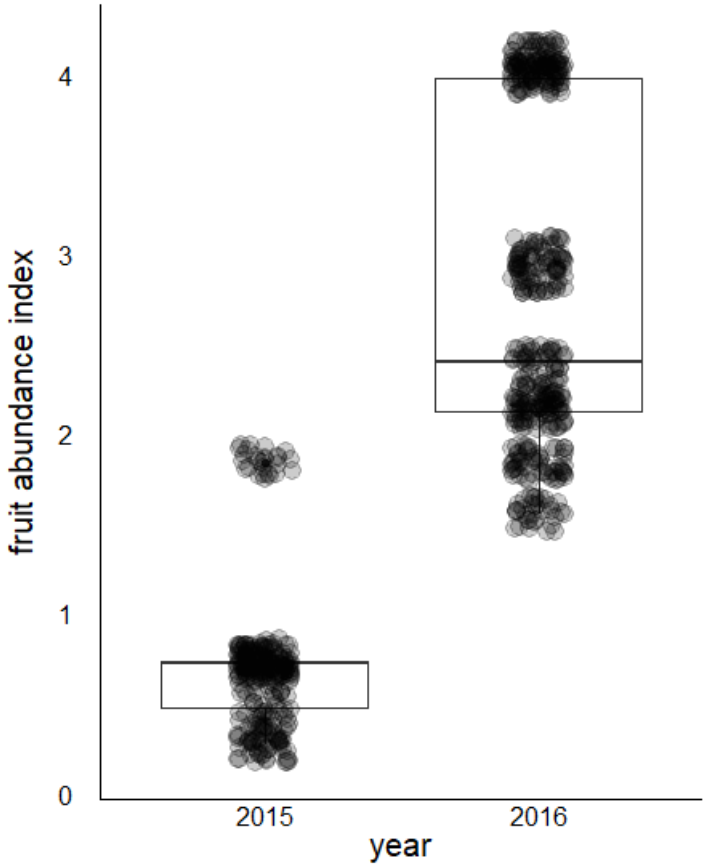
macaques and chimpanzees (10-12% of time grooming (Gust et al., 1993; Shutt et al., 2007; Tennenhouse et al., 2017; Wittig et al., 2008, 2016; Young, Majolo, Heistermann, et al., 2014)).

When taking into acccount a more complex sociality measure, the individual sociality index, we found a positive effect of sociality on GC levels, with lower GC levels in more social individuals (higher sociality index values), similarly to other studies in primates (Girard-Buttoz et al., 2009; Sanders, 2015; Silk et al., 2009; Wittig et al., 2016; Young et al., 2014). Furthermore, individuals receiving and giving low number of displacements and agonistic behaviour per hour had higher GC levels, in contrast with several studies in anthropoid primates (Abbott et al., 2003b; Cavigelli & Caruso, 2015; Maestripieri & Georgiev, 2016; Tennenhouse et al., 2017). Altogether, these results about sociality and GC suggest that, in redfronted lemurs, more subtle factors drive this relationship and could have to do with the species social organisation in small cohesive groups with high level of tolerance but low rates of social interactions, which differ from anthropoid primates.

To conclude, this study showed that in redfronted lemurs, social, reproductive and ecological factors have combined effects on the GC variations. In particular, we could show for the first time in this species a positive effect of sociality on GC levels, not due to the rates of socio-positive behaviour on themselves but to the individual social indices.

Supplementary material

Supplementary figure 1: Fruit abundance (y axis) differed according to the year of data collection (x-axis). Each box indicates median, upper, and lower quartiles. Whiskers indicate ± 1.5 interquartile ranges. Outliers are plotted as solid black dots outside of the box and whiskers'area.



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Summary

In chapter 1, I conducted a literature review and a meta-analysis on the relationship between parasite infections and glucocorticoids (GC) in mammals. I showed a strong support for a positive relationship between parasites and GC throughout the reviewed literature and this relationship was true for both experimental and observational studies in the meta-analysis. Because the experimental dataset consisted of parasite manipulations, I could only conclude on the parasite to increased GC directionality. Collectively, these results suggest that mammal hosts suffer a physiological cost of parasite infection.

In chapter 2, I characterised the parasite community of redfronted lemurs and two sympatric lemur species in Kirindy forest: the grey mouse lemur (*Microcebus murinus*) and the fat-tailed dwarf lemur (*Cheirogalus medius*). I found a diverse parasite community with a total of 21 parasite morphotypes, six shared by two lemur species, four shared by all three species. I discovered six new parasite morphotypes in these populations, two of them usually found in livestock or humans in Madagascar. Additionally, a large proportion of the parasite taxa was shared among two or more lemur species. Thus, my results hint to an increased gut parasite cross-transmission among lemurs and possibly with livestock or humans in the close proximity.

In chapter 3, I investigated the relative importance of individual-, group- and population-level determinants of parasite richness and parasite infection with nematodes and protozoan. Then I examined the potential costs of infections on body condition, activity levels and sociality in wild redfronted lemurs. On an individual level, parasite richness and infection status increased with age and time spent in body contact but decreased with GC levels. On a population level, rainfall during the previous month was positively correlated to parasite richness and infection status. Determinants of infection differed between nematodes and protozoa, highlighting the importance of the parasite's life cycle for their transmission. Finally, there was no evident costs of parasitism on body condition, activity levels and sociality, suggesting other mitigation mechanisms or good parasite tolerance in this species.

In chapter 4, I reported observations of six wild redfronted lemurs of both sexes and different age classes anointing their perianal-genital areas and tails with chewed millipedes and sometimes ingesting it, at the onset of the rainy season. This behaviour could have a potential self-medicative function, because millipedes' benzoquinone secretions may hamper the growth of gastrointestinal parasites. Anointing combined with the ingestion of millipedes may act in a complementary fashion against gastrointestinal parasite infections, providing both prophylactic and therapeutic effects, mediating some costs of parasite transmission due to sociality.

In chapter 5, I tested if C-Reactive Protein (CRP) could be measured non-invasively from faecal samples of two species of lemurs (redfronted lemurs and ringtailed lemurs). As a biological validation of faecal CRP measurement, I tested whether parasite infection, GC levels or parturition affected CRP levels in lemurs, because of the reported elevated CRP levels under these conditions in humans and other animals. CRP levels were above the detection limit of the assay in less than 30% of the samples. There was no correlation between CRP and GC levels or CRP and parturition, but CRP increased with infection status in wild redfronted lemurs. I conclude that faecal CRP has strong limitations as a non-invasive inflammatory marker, discuss improvements of the method and suggest alternatives to measure inflammation in lemur.

In chapter 6, I investigated the determinants of fecal GC metabolites (fGCm) variations in redfronted lemurs. Ecology and reproductive states affected fGCm levels with an increase of fGCm with larger daily temperature fluctuations and lower fruit availability. In males, fGCm elevations seemed to reflect the increased social and physiological demands during the mating season, while elevated levels post mating season were attributable to physiological challenges in the dry season. In females, fGCm variations reflected the physiological cost of reproduction i.e. peak of fGCm levels during the late gestation and early lactation periods, with an amplification of this cost due to the dry season. Sociality had an immediate effect on fGCm levels, with lower fGCm levels in individuals with higher sociality indices, but no effect of social instability. Altogether, my results show that fGCm constitute an appropriate indicator of energetical effects of ecological and reproductive challenges as well as an indicator of immediate consequences of sociality in redfronted lemurs.

Zusammenfassung

In Kapitel 1 führte ich eine Literaturrecherche und eine Metaanalyse zum Zusammenhang zwischen Parasiteninfektionen und Glukokortikoiden (GC) bei Säugetieren durch. Ich habe in der gesamten überprüften Literatur starke Unterstützung für eine positive Beziehung zwischen Parasiten und GC gezeigt, und diese Beziehung galt sowohl für experimentelle als auch für Beobachtungsstudien in der Metaanalyse. Da der experimentelle Datensatz aus Parasitenmanipulationen bestand, konnte ich nur auf eine erhöhte GC-Richtwirkung schließen. Zusammengenommen legen diese Ergebnisse nahe, dass Säugetierwirte unter physiologischen Kosten einer Parasiteninfektion leiden.

In Kapitel 2 habe ich die Parasitengemeinschaft der Rotbarschen und zwei sympatrischen Lemurenarten im Kirindy-Wald charakterisiert: den grauen Mausmaki (Microcebus murinus) und den Fettschwanz-Zwergmaki (Cheirogalus medius). Ich fand eine vielfältige Parasitengemeinschaft mit insgesamt 21 Parasitenmorphotypen, sechs von zwei Lemurenarten, vier von allen drei Arten. In diesen Populationen entdeckte ich sechs neue Parasitenmorphotypen, von denen zwei normalerweise bei Nutztieren oder Menschen in Madagaskar vorkommen. Zusätzlich wurde ein großer Teil der Parasitentaxa auf zwei oder mehr Lemurenarten aufgeteilt. Daher deuten meine Ergebnisse auf eine erhöhte Kreuzübertragung von Darmparasiten zwischen Lemuren und möglicherweise mit Nutztieren oder Menschen in unmittelbarer Nähe hin.

In Kapitel 3 untersuchte ich die relative Bedeutung der Determinanten auf Einzel-, Gruppen- und Populationsebene für den Parasitenreichtum und die Parasiteninfektion mit Nematoden und Protozoen. Dann untersuchte ich die potenziellen Kosten von Infektionen in Bezug auf Körperzustand, Aktivitätsniveau und Sozialität bei wilden Lemuren mit roter Front. Auf individueller Ebene nahmen der Parasitenreichtum und der Infektionsstatus mit dem Alter und der Zeit zu, die im Körperkontakt verbracht wurden, nahmen jedoch mit den GC-Spiegeln ab. Auf Bevölkerungsebene korrelierten die Niederschläge im Vormonat positiv mit dem Parasitenreichtum und dem Infektionsstatus. Die Determinanten der Infektion unterschieden sich zwischen Nematoden und Protozoen, was die Bedeutung des Lebenszyklus des Parasiten für seine Übertragung hervorhob. Schließlich gab es keine offensichtlichen Kosten für Parasitismus in Bezug auf Körperzustand, Aktivitätsniveau und

Sozialität, was auf andere Minderungsmechanismen oder eine gute Parasitentoleranz bei dieser Art hindeutet.

In Kapitel 4 berichtete ich über Beobachtungen von sechs wilden Lemuren beiderlei Geschlechts und verschiedener Altersklassen, die ihre perianal-genitalen Bereiche und Schwänze zu Beginn der Regenzeit mit gekauten Tausendfüßlern salbten und manchmal einnahmen. Dieses Verhalten könnte eine potenzielle selbstmedikamentöse Funktion haben, da die Benzochinon-Sekrete von Tausendfüßlern das Wachstum von Magen-Darm-Parasiten behindern können. Die Salbung in Kombination mit der Einnahme von Tausendfüßlern kann komplementär gegen gastrointestinale Parasiteninfektionen wirken und sowohl prophylaktische als auch therapeutische Wirkungen haben, wodurch einige Kosten der Parasitenübertragung aufgrund der Sozialität entstehen.

In Kapitel 5 habe ich getestet, ob C-reaktives Protein (CRP) nicht-invasiv an Stuhlproben von zwei Lemurenarten (Lemuren mit roter Front und Lemuren mit Ringtail) gemessen werden kann. Als biologische Validierung der CRP-Messung im Stuhl habe ich getestet, ob Parasiteninfektion, GC-Spiegel oder Geburt die CRP-Spiegel bei Lemuren beeinflussen, da unter diesen Bedingungen bei Menschen und anderen Tieren erhöhte CRP-Spiegel gemeldet wurden. Die CRP-Werte lagen in weniger als 30% der Proben über der Nachweisgrenze des Assays. Es gab keine Korrelation zwischen CRP- und GC-Spiegeln oder CRP und Geburt, aber CRP stieg mit dem Infektionsstatus bei wilden Lemuren mit roter Front. Ich komme zu dem Schluss, dass fäkales CRP als nicht-invasiver Entzündungsmarker starke Einschränkungen aufweist, diskutiere Verbesserungen der Methode und schlage Alternativen zur Messung der Entzündung bei Lemuren vor.

In Kapitel 6 untersuchte ich die Determinanten von Variationen der fäkalen GC-Metaboliten (fGCm) bei Lemuren mit roter Front. Ökologie und Fortpflanzungszustände beeinflussten die fGCm-Werte mit einem Anstieg von fGCm mit größeren täglichen Temperaturschwankungen und geringerer Fruchtverfügbarkeit. Bei Männern schienen die fGCm-Erhöhungen die erhöhten sozialen und physiologischen Anforderungen während der Paarungszeit widerzuspiegeln, während erhöhte Werte nach der Paarungszeit auf physiologische Herausforderungen in der Trockenzeit zurückzuführen waren. Bei Frauen spiegelten die fGCm-Variationen die physiologischen Reproduktionskosten wider, d. H. Den

Spitzenwert der fGCm-Spiegel während der späten Schwangerschaft und der frühen Laktationsperioden, wobei diese Kosten aufgrund der Trockenzeit verstärkt wurden. Die Sozialität hatte unmittelbare Auswirkungen auf die fGCm-Werte, wobei die fGCm-Werte bei Personen mit höheren Sozialitätsindizes niedriger waren, die soziale Instabilität jedoch nicht. Insgesamt zeigen meine Ergebnisse, dass fGCm ein geeigneter Indikator für die energetischen Auswirkungen ökologischer und reproduktiver Herausforderungen sowie ein Indikator für die unmittelbaren Folgen der Sozialität bei Lemuren mit roter Front ist.

General Discussion

In this general discussion I will discuss my main results on the sociality-health relationship in a population of wild redfronted lemurs. I will first address traits that might make lemurs special in comparison to other primates concerning the sociality-health relationship. Then, I will discuss parasite transmission modes and life cycle and why it matters for the sociality-health relationship. Next, I will address different immune and behavioural defence strategies against parasites and their costs for their hosts. Finally, I will list my main conclusions and give an outlook for future directions in this field of research.

Sociality and health: are redfronted lemurs so special?

Increased parasite transmission is one of the major costs of sociality (Altizer et al., 2003; Anderson & May, 1979; Freeland, 1976; Kappeler et al., 2015; Loehle, 1995). For instance, larger groups, increased number of social partners or rates of social contacts are associated with higher parasite load, prevalence or richness as shown in diverse taxa such as group-living lizards(Bull et al., 2012), meerkats (Julian A. Drewe, 2010), Tasmanian devils

(Hamede et al., 2009), Belding's ground-squirrels (VanderWaal et al., 2013), wild and domestic ungulates (VanderWaal et al., 2014) and nonhuman primates (Balasubramaniam et al., 2018; Duboscq et al., 2016; MacIntosh et al., 2012; Rimbach et al., 2015; Springer et al., 2016). In accordance with these studies, I found that sociality can have direct effects on health in redfronted lemurs (chapter 3). Sociality facilitated only protozoan parasites, with individuals spending more time in social contact being more infected than others. However, group size had an opposite effect as predicted, with less infections in larger groups. I suggest that infection risk could be diluted in larger groups, due to increased group spread reducing environmental contamination or contact with infected individuals (Snaith et al., 2008).

In various taxa, it was shown that infected individuals suffer numerous costs such as reduced social relationships (Bos et al., 2012; Kennedy et al., 1987; Müller-Klein et al., 2019; Poirotte et al., 2017), poor body condition (see Sánchez et al., 2018 for a meta-analysis), increased resting metabolic rate (Careau et al., 2010), increased interbirth intervals (Akinyi et al., 2019), reduced activity levels (Müller-Klein et al., 2019), and reduced longevity (Soay sheeps: Leivesley et al., 2019; Lynsdale et al., 2017). In wild redfronted lemurs, I did not find evidence of short-term costs of parasitism on body condition, activity patterns or social relationships. Furthermore, I could not show that GC levels can influence susceptibility to parasites with a positive correlation between parasite and GC like in the meta-analysis (chapter 1).On the contrary, in redfronted lemurs, GC levels were negatively correlated with parasitism, replicating findings from 10 years earlier with the same population (Clough et al., 2010).

In anthropoid primates, costs of sociality such as increased GC levels in subordinates (or dominants depending on the species) or during periods of social instability associated with increased GC levels and reduced immune functions have been described (Abbott et al., 2003; Capitanio & Cole, 2015; Sapolsky, 2005). Because of the frequent periods of social instability in redfronted lemurs, I expected to find an indirect effect of sociality on health via GC levels modulation, with social stressors (social instability and displacements) causing an increase of GC, which are known to be immunomodulator hormones (Sapolsky et al., 2000), while social affiliation (body contact, huddling and grooming and number of social partners) would reduce GC levels, indirectly mitigating health costs of sociality. However, I found no effect of social stress or grooming on GC levels but a more complex effect of sociality index

(chapter 6) and contrary to my prediction, I found a negative correlation between GC levels and parasite infections in redfronted lemurs (chapter 3). This lack of association between grooming rates and GC but effect of sociality index suggests that in redfronted lemurs, more subtle aspects of social relationships such as strong social bonds and social network position might affect GC levels and indirectly health. The fact that GC variations happened at predictable periods, such as the mating season and the early lactation period, could also contribute to a faster return to homeostasis and less detrimental effects of increased stressors (Capitanio & Cole, 2015b; Landys et al., 2006; Schultner et al., 2013).

Short- and long-term benefits of sociality on health in primates were reported from species with relatively similar social structure, social organisation and mating systems, i.e. large multimale-multifemale groups with clear linear hierarchy and promiscuous mating. These effects are diverse e.g. physiological stress buffering, reduced susceptibility or transmissibility of infectious agents, improved wound healing, increased survival and infant survival, (E. A. Archie, 2013; Balasubramaniam et al., 2016; Gilbert & Baker, 2011; Joan B. Silk et al., 2003, 2010; Wittig et al., 2016; Young, Majolo, Heistermann, et al., 2014). We did not find social buffering effects per se or effects of grooming rates or number of social partners on health in redfronted lemurs. However, redfronted lemurs differ from these species because they live in relatively small groups with promiscuous mating, high levels of tolerance and frequent periods of social instability, but no clear dominance hierarchy (Fichtel et al., 2018; Kappeler & Fichtel, 2012; Kappeler & Port, 2008; Ostner & Kappeler, 1999; Pereira & McGlynn, 1997). Thus, these differences between species could result from differences in social organisation.

Redfronted lemurs could still profit from other short-term benefits of sociality. For example, in other primate species such as capuchin monkeys, macaques and chimpanzees, individuals with better social relationships had increased access to resources, especially food (Haunhorst et al., 2017; Sabbatini et al., 2012; Tiddi et al., 2011), or protection from predation (Micheletta et al., 2012). In redfronted lemurs, social instability periods characterised by female evictions by other females and male take-over, happen during the mating season and the early lactation period (Kappeler & Fichtel, 2012; Kappeler & Port, 2008; Ostner & Kappeler, 1999). In consequence, maintaining strong social bonds with other group members might be essential to stay in the group and avoid predation. Benefits of

sociality may be different for group members and individuals who are joining or those who are at risk of leaving the group, however, to my knowledge, no study so far attempted to study these differences.

Sociality can have long-term effects on health and ultimately fitness. For instance, despite sometimes causing subclinical effects on the short-term, gastro-intestinal parasites can have cumulative long-term effects, such as decreased life expectancy and reduced reproductive success over a lifetime (Nunn and Altizer 2006). Furthermore, many studies showed an important effect of sociality on survival and infant survival (Stanton & Mann 2012; Silk 2007; Silk et al. 2010; McFarland et al. 2017; Young et al. 2017). Because of the long-term study of redfronted lemurs at Kirindy forest, data on demography, lineage and individual reproduction are collected. These data could contribute to relate measures of parasitism, GC baseline and variations, to longevity and infant survival. In particular, during my study period, all females reproduced but half lost their infant during their first six months of life. Low infant survival could be a critical cost of sociality in redfronted lemurs, and requires further study. Maternal chronic stress and parasite infections could for instance affect infant development in utero and after birth, and be mediators of this cost of sociality on fitness.

Altogether, I showed in my thesis that in redfronted lemurs, costs of sociality on health are only due to increased parasite infections, while I did not find social buffering effects or physiological costs of parasitism. These findings contrast with other findings in non-human primates and humans (Archie, 2013; Balasubramaniam et al., 2016; Gilbert & Baker, 2011; Silk et al., 2003, 2010; Wittig et al., 2016; Young et al., 2014). I suggest that this could be due to either redfronted lemurs social life in small groups with tolerant and relaxed social organisation but a cohesive spatial association (Pyritz et al., 2011; Sperber et al., 2019) or the evolution of a unique combination of traits contrasting gregarious lemur species from gregarious anthropoid primates (thereafter called lemur idiosyncrasies).

Lemur idiosyncrasies are social, demographic, morphological and ecological traits in which lemurs deviate from anthropoid primates. For example, in many gregarious lemur species, females dominate males (Jolly, 1966, 1998; Pereira et al., 1990) and individuals live in pair (Wright, 1999) or in relatively small multimale-multifemale groups with an even adult

sex ratio on average (Kappeler et al., 2009). There is female targeted aggression and direct male and sperm competition (Kappeler & Fichtel, 2012; Kappeler & Port, 2008; Wright, 1999), but no sexual dimorphism in body and canine size (Kappeler, 1990; Kappeler, 1997). Furthermore, many lemur species are cathemeral (Curtis, 2007; Engqvist & Richard, 1991; Kappeler & Erkert, 2003; Tattersall, 1987) and seasonal breeders (Brockman & Van Schaik, 2005; Hrdy & Whitten, 1987) with a high infant mortality and low basal metabolic rates (Harcourt, 2008; Wright, 1999).

Comparative studies between primate species with different social organisations or between different lemur species living in multi-male multi-female groups are required to explore the origin of the contrasting results between anthropoid primates and lemurs. Interestingly, a study in another lemur species, Verreaux sifakas (*Propithecus verreauxi*), in which there is usually one dominant male with or without subordinate males and a harem of females, found no influence of sociality on fGCm (Rudolph et al., 2020). Additionally, in the same population, transmission of bacteria strains was essentially driven by social contact (Springer et al. 2016). In contrast, in ring-tailed lemurs (*Lemur catta*), where females and males form two independent dominance hierarchies, but with females dominating males, Sanders (2015) found effects of sociality on fGCm depending on sex. Males' fGCm levels were correlated with rank and grooming given, whilst in females, fGCm levels were correlated with social bonds quality. Overall, these contrasting results in different lemur species suggest an important role of dominance style in the sociality-GC relationship in lemurs, just as it was found in anthropoid primates for the GC-dominance relationship (Abbott et al., 2003b). Because of their rather unusual dominance style for group living lemurs (no female dominance), redfronted lemurs are an important species to compare the sociality-GC relationship in lemurs.

Parasite transmission mode and sociality

Despite extensive research on infection in laboratory animals, social drivers of parasite transmission in wild or semi-wild populations remain unclear and can be host-parasite association-specific (Akinyi et al. 2019; Hopkins & Nunn 2007; Ghai et al. 2015; Müller-Klein et al 2018; Pedersen et al. 2005; Poirotte et al. 2016). More studies on the relative

contribution of animal behaviour on parasite transmission, in relation to other influences, like host traits and seasonality, is crucial to improve and validate epidemiological models and their predictions. As parasites exhibit a large variety of transmission routes and life cycles, more attention should be paid to these aspects when investigating parasitism in wild populations. In the following paragraphs, I will expand my findings concerning sociality and transmission of gastro-intestinal parasites with what is known about the influence of sociality on other parasite types' transmission mode and what one could expect in redfronted lemurs for other parasite types.

The transmission of directly transmissible gastro-parasites (no need of intermediate hosts), ectoparasites and some viruses and pathogens should be greatly influenced by sociality and in particular proximity and social contact which favours their transmission between individuals (Duboscq et al., 2016; MacIntosh et al., 2012; Nunn & Altizer, 2006; Rimbach et al., 2015). Parasite assessment in my PhD project was not exhaustive as, due to practical and ethical issues, I focused on gastro-intestinal parasites which are usually transmitted orofaecally (direct transmission) or via an intermediate host eaten by the secondary host (indirect transmission) (Nunn & Altizer, 2006).

Among gastro-intestinal parasites, only protozoan infections, but not nematode infections, were determined by sociality in redfronted lemurs, with more parasite infection in individuals spending more time in social contact and grooming behaviours (chapter 3). This difference seems to be linked to the different life cycles of protozoan and nematodes. Protozoan are directly transmissible parasites with short life cycles and are already infectious in faecal excreta, making social contact risky in terms of transmission. However, most directly transmissible nematodes need a maturation period of two to three weeks after excretion in the environment, and some nematode species require an intermediate host to mature in before being transmissible to their secondary host (MacIntosh et al., 2012; Neveu-Lemaire, 1952; Nunn & Altizer, 2006; Springer & Kappeler, 2016).

Interestingly, redfronted lemurs engage in intergroup encounters with neighbouring groups, sometimes with grooming between individuals of different groups (personal communication Ester Bernaldo de Quirós) and share water sources between multiple groups during the dry season (personal communication Ester Bernaldo de Quirós & Louise Peckre).

Thus, intergroup contact and sharing of the same resources in redfronted lemurs could constitute another route of social transmission of endo- and ecto-parasites. This would explain my findings that all groups harboured the same parasite species and is in accordance with a study on bacterial strain transmission between Verreaux sifakas at the same field site (Springer et al., 2016).

Haemoparasites and ectoparasites were found in other *Eulemur* species in Madagascar (Hokan et al., 2017; Junge, 2007; Junge et al., 2008; Junge & Louis, 2005) and in Verreaux sifakas from the same field site (Springer, 2015; Springer et al., 2015). Thus it seems likely that the redfronted lemur of my study would harbour these kind of parasites, and it is expected that social contact and proximity would increase the transmission of ectoparasite, as it was previously found in other primates (Duboscq et al., 2016). Concerning haemoparasites, because they require vectors to be transmitted (Mooring & Hart, 1992; Nunn & Heymann, 2005), two contrasting hypotheses have been suggested regarding sociality and transmission. Sociality could either reduce infection risk due to an encounter-dilution effect in larger groups or increase infection risk, because larger groups might attract more vectors. In lemurs, however, no effect of group size was found so far (Springer et al., 2015) and to our knowledge, no other social influence has been tested.

Another behaviour of interest for further studies of parasite transmission in lemurs is scent-marking behaviour. Scent marking, i.e. the inspection of scent marks and their remarking, provides an important communication mode in lemur species (delBarco-Trillo et al., 2012; Gould & Overdorff, 2002; Kappeler, 1998; Lewis, 2006; Millhollen, 1986). It has been suggested that scent-marking and especially overmarking could constitute an alternative transmission route for oxyurid nematodes (Irwin & Raharison, 2009) and micro-organisms (Springer et al., 2016) because of the contact between an individual and a substrate potentially contaminated by another individual during a previous marking. Thus, marking over a scent-mark of an infected individual could be risky in terms of parasite transmission and constitute a trade-off between infection risk and communication. However, multiple reports indicate that various mammals species are able to discriminate infection statuses of their partners or group members via smell and that, additionally, infected individuals decrease their scent-marking behaviour (Kavaliers & Colwell, 1995; Mitchell et al., 2017; Poirotte et al., 2017; Zala et al., 2004). These results suggest some

ability to assess infection status of conspecifics based on smell and to accordingly modulate their scent-marking behaviour, limiting parasite transmission.

Finally, I want to bring attention to the importance of considering heterospecifics as parasite reservoirs and transmission facilitators while studying parasite transmission. Traditionally, studies on transmittable diseases focused on one species and one or more of its parasites. However, animals share space and resources with other species. Yet, just a small portion of the parasitology literature looked at sympatric species, showing that they share parasite species and influence other species' parasite infection rate (Aivelo et al., 2018; Ezenwa, 2003; Obanda et al., 2019; Radespiel et al., 2015). In chapter 2, I compared the parasite community of redfronted lemurs and two sympatric lemur species and found high levels of parasite sharing, with 47.6% of the morphotypes shared by at least two of the lemur species and 19% shared by all three species. Furthermore, some of the found parasite species were never described before in lemurs but in livestock or humans, suggesting risks of parasite spillover in these endangered primate species, for example due to contact or increased space sharing with humans or livestock. It is of importance, and worth investigating, as spillover of other infectious agents have been discovered in *Eulemur sp.* and *Microcebus sp.* before (Bublitz et al., 2015; Zohdy et al., 2015) Thus, we need to move away from focusing on single species and look at multi-layer patterns and ecosystems (Lively et al., 2014).

Defence mechanisms against parasite infections and their costs

Considering host defences, the traditional focus has been on immune responses, however multiple non mutually exclusive mechanisms evolved to facilitate avoidance of infections, or mitigation of the infection costs. Among these mechanisms, resistance and tolerance are physiological processes (Anthony et al., 2007; Broche et al., 2017; Kutzer & Armitage, 2016; Råberg, 2014) whilst avoidance and self-medication are exclusively behavioural processes (Huffman, 2003; Sarabian et al., 2018). In the next paragraphs, I will explore all these mechanisms in redfronted lemurs infected with gastro-intestinal parasites.

At first, individuals might avoid potentially contaminated environments, food sources or conspecifics to reduce the risk of exposure to infectious agents (Sarabian et al., 2018). For example, carnivores avoid feeding on other carnivore carcasses because of the high proportion of parasite species they share (Moleón et al., 2017) and in a baboon population with some individuals infected with the sexually transmittable bacteria *Treponema pallidum subsp. pertenue*, non-infected females avoid copulations with infected males (Paciência et al., 2019). In the redfronted lemur from Kirindy forest, the picture seemed more complex when it comes to avoidance strategies. While they avoided drinking from potentially contaminated water sources (Amoroso et al., 2019) and thus seem able to detect infection, in my study, infected individuals were not avoided by conspecifics (chapter 3). I argue that this might be linked to the limited time lemur spend socialising (2% of time grooming in my study), in comparison to other social animals such as baboons, macaques and chimpanzees (10-12% of time grooming (Gust et al., 1993; Shutt et al., 2007; Tennenhouse et al., 2017; Wittig et al., 2008, 2016; Young, Majolo, Heistermann, et al., 2014)). In redfronted lemurs, grooming has been hypothesised to act as a crucial social tool, necessary to strengthen group cohesion and to facilitate integration into social networks (Port et al., 2009), especially during the frequent periods of social instability caused by male takeovers and female evictions (Kappeler & Fichtel, 2012; Port et al., 2010). Reducing these social interactions or modifying social networks can have short and long-term consequences for individuals, for example by reducing "political" support and favouring evictions or by reducing mating opportunities, infant survival, access to food patches, and ultimately survival (Brent, 2015; Ellis et al., 2019; Haunhorst et al., 2017; Holt-Lunstad et al., 2010; Larson et al., 2018; Silk et al., 2003; Tiddi et al., 2011).

Redfronted lemurs might have another way to mitigate social costs of parasitism while simultaneously reducing the social costs of conspecifics' avoidance, by using self-medication with millipedes (chapter 4). Following infection, some animals invest time and energy in self-medication, a set of behaviour aimed to suppress or prevent costs of infection and other illnesses (Huffman, 2003). This set of behaviours ranging from the use of insect-repellent leaf material to build nests, the direct application of substances on fur or feathers and the consumption of soil or specific plant parts is widespread in the animal kingdom from ants to birds and mammals (Huffman, 1997, 2003). For instance, chimpanzees (*Pan*

troglodytes) ingest plants containing secondary compounds (Huffman, 2015), resulting in reduced parasite burden and Verreaux Sifakas increase their consumption of tannin-rich plants as anti-helminthic treatment (Carrai et al., 2003). In chapter 4, I reported the observation of several individuals performing anointment of their anogenital region with millipedes containing anti-helminthic benzoquinones and sometimes followed by their ingestion, at the peak of parasite prevalence. This behaviour could act in a complementary fashion against gastrointestinal parasite infections, providing both prophylactic and therapeutic effects to limit parasite burden. I argue that self-medication could be an adaptive technique in redfronted lemurs to mitigate the costs of parasite infection due to their non-avoidance of infected conspecific.

Host ability to limit parasite burden via the immune system (resistance) (Råberg et al., 2007) constitutes an intensely studied mechanism to deal with infection. Resistance consists in the detection and neutralisation of parasites. Both the innate and adaptive immune system can contribute to resistance to infections (Anthony et al., 2007; Broche et al., 2017). In consequence, a well-functioning immune system is of importance for an individual's resistance against parasites and ultimately its survival. For instance, lizards (*Podarcis gaigeae*) heavily parasitized with haemo-protozoan invest more in physiological immune responses than lizards with lower parasite burdens (Sagonas et al., 2016). Although resistance to infection is crucial for host health, it simultaneously comes with multiple costs. First, tissue damage and inflammation often accompanies destruction and elimination of parasites (Day et al., 2007; Holub et al., 2006; Marx et al., 2003; Patel et al., 2009; Sorci & Faivre, 2009). In redfronted lemurs, I found an association of elevated C-reactive protein (a nonspecific marker of inflammation) and parasite infection (chapter 5), indicating parasite resistance or tissue damage by the parasites or the host immune response to parasite infections (Broche et al., 2017; Day et al., 2007; Råberg et al., 2009). Furthermore, I also found a negative correlation of parasite richness and infection status with fGCm levels (chapter 3), indicating a shift toward a Th2 immune response. High GC levels can shift the immune response towards a Th2 immune response, which targets more the extracellular parasites, instead of a Th1 immune response, which targets more the intracellular parasites (Elenkov, 2004b; Franchimont et al., 2000; Kovalovsky et al., 2000). This GC-mediated immunomodulation could thus favour gastro-intestinal infection.

According to life history theory, an organism cannot invest in many tasks at the same time, therefore there are trade-offs in energetical investments between growth, reproduction and maintenance (Sheldon & Verhulst, 1996; Stearns, 1989). Since the immune system is particularly energetically costly (Murphy, 2008; Sheldon & Verhulst, 1996), individuals might give-up all out resistance as the main defence strategy against parasites. When infection is unavoidable and too costly to fight, tolerance, the adaptation to live with a given parasite by limiting its harmful effects and without modifying its load (Kutzer & Armitage, 2016; Medzhitov et al., 2012; Råberg, 2014; Råberg et al., 2009), can occur. This process is well illustrated with Plasmodium-infected mice, who are able to limit the severity of malaria without reducing the parasite burden (Råberg et al., 2007) and Soay sheep infected with gastro-intestinal parasites, who are able to limit weight loss without reducing the parasite burden (Hayward et al., 2014). I did not monitor parasite load in my thesis; thus, I cannot conclude on tolerance in redfronted lemur. However, the lack of physiological costs (such as weight loss) and behavioural changes (such as increased resting time, appetite loss or reduced social interactions) with increasing parasite richness or with infection status suggest a potential tolerance, but require further testing. A deworming experiment with evaluation of changes in parasite load concomitantly to health markers before and after deworming might represent a good approach.

These four defence mechanisms against parasite infections, i.e. tolerance, resistance, avoidance and self-medication, are not mutually exclusive, and trade-off exists between them. In particular, the use of tolerance or resistance mechanisms seems to depend on parasite-host associations, and in some cases, genes regulating these two mechanisms are negatively correlated, suggesting a trade-off between the two (Medzhitov et al., 2012; Råberg et al., 2007). Avoidance remains most likely the most cost-effective mechanism (Curtis, 2014; Hart, 1990; Rivas et al., 2014), however, there are also trade-offs (e.g. social, energetical, hormonal) between avoidance and resistance or tolerance (Fleischman & Fessler, 2011). As a consequence, individuals cannot always afford to use avoidance behaviour and must deal with the consequences of parasitism or invest in resistance and tolerance mechanisms. For instance, isolated human populations living in pathogen-rich environments, such as the Yanomami and the Tsimane of Amazonia, appear to express higher immune activation in response to immune challenge than do western populations

(Blackwell et al., 2016; Clemente et al., 2015). Redfronted lemurs could face such trade-offs, explaining why despite being able to detect and avoid contaminated water sources (Amoroso et al., 2019), they do not seem to avoid contaminated conspecifics (chapter 3). Presumably, the social costs of avoidance are higher than their benefits in this host-parasite association. Experimental testing in captive groups with the possibility to modify group structure and infection status of some individuals whilst controlling for immune response and parasite burden could allow testing of these potential trade-offs.

Finally, at the very moment I am writing this discussion (March 12th 2020), the outbreak of a respiratory disease caused by a novel coronavirus (SARS-CoV-2) has just been declared into a pandemic disease (Adhanom Ghebreyesus, 2020). This unfortunate outbreak provides a world-sized experimental set-up to test a few hypotheses about sociality and health. With an increase of 13-fold of new cases in more than 100 countries in the last two weeks and a death-rate seven-fold higher than the seasonal flu, the WHO director, Dr Adhanom Ghebreyesus, urges governments and citizens to take appropriate measures to limit transmission, using containment and mitigation measures which are essentially based on limiting social contacts (Adhanom Ghebreyesus, 2020). To a scientist in my field, these measures might appear somewhat similar to processes of parasite avoidance already observed in diverse animal taxa. The next weeks will show if this world-sized disease avoidance experiment is sufficient to slow the spread and negative consequences of SARS-CoV-2. However, in that case, human-specific traits of sociality might also play a big role in the effectiveness of these measures. In particular, country differences in healthcare system, developing status, law enforcement and respect of social rules will probably influence the outcome.

Conclusion and perspectives

Coming back to the definition of health in the introduction, it seems that this lemur population was healthy during my study period according to Hubert and colleagues (M. Huber et al., 2011). Altogether, my Ph.D. projects contributed to the current understanding of the interplay between social behaviour, physiology, infection and health. I showed that in a species with small multimale-multifemale groups with a rather egalitarian social organisation,

sociality seems to have little costs or benefits on health on a short-term scale. Grooming rates did not affect GC levels, but individual sociality index did, whereas ecological and behavioural seasons showed strong influences. On the other hand, protozoan but not nematode infections increased in more social individuals. In general, parasite infections seemed to have no short-term costs on individuals' behaviour or physiology. Furthermore, most physiological parameters showed high interindividual variability, with strong differences between individuals in their baseline and response levels, highlighting the importance of individual-based studies.

My results open new questions regarding the influence of different social organisations on the sociality-health relationship. Indeed, in contrast to the studies in wildlife highlighting benefits of sociality on health and fitness, I did not detect such an effect in redfronted lemurs. Most primatological wildlife studies investigated species living in large multimale-multifemale groups, comprising unrelated males and related females and exhibiting linear hierarchies with one dominant male monopolising breeding slots and resources. Results in lemurs are so far quite diverse, with no effect of sociality on GC but on transmission of bacteria in Verreaux's sifakas, another lemur species with female dominance (Rudolph, 2020; Springer et al., 2016), both effects of agonistic and affiliative behaviour on GC but not on parasite transmission in Ringtailed lemurs (Sanders, 2015), and effects of sociality on both GC and transmission of parasite in redfronted lemurs (this thesis).Therefore, it seems important to investigate more the effect of social organisation and dominance hierarchy on the sociality-health relationship in diverse families and taxa. Furthermore, comparing closely related lemurs with similar ecology but different social organisation (e.g. mongoose lemurs with redfronted lemurs) would allow to tear apart the effect of social organisation and phylogenetic relatedness.

While I investigated the short-term health costs and benefits of sociality in redfronted lemurs, the potential long-term benefits remain unclear. Sociality could influence fitness characteristics in redfronted lemurs just as it was found in other social species (Capitanio et al., 1998; Ebensperger et al., 2011; Kalbitzer et al., 2017; Joan B. Silk et al., 2003). To achieve this, long-term data could be used to investigate how individual variations in time spent socialising and in social relationship quality influence fitness traits such as reproductive success, interbirth interval variation and offspring survival.

The use of long-term data from field sites functioning for decades could help understanding the influence of early-life variations in sociality on physiology, health and fitness and the effect of aging on the immune system and sociality. For instance, studies in baboons showed that mother's social bonds quality has a positive effect on infant survival (Joan B. Silk et al., 2003) and that the permanent presence of the father accelerates reproductive maturation, which is a key female fitness determinant, in young female baboons (Charpentier et al., 2008). Additionally, in macaques, prime-aged females with more family members in their group had better survival than females with less family members, but these effects do not remain in older females (Brent et al., 2017). Thus, it would be interesting to see if these advantages remain through a lifetime and to identify their physiological mechanisms. Concerning aging, multiple studies showed increased inflammation as well as decreased immune response with age as well as more selective social interactions (Almeling et al., 2016; Brent et al., 2017; Hayward et al., 2009; Saito et al., 2003). This is why I advocate in favour of comprehensive studies measuring concomitantly a broad age range of ecological, behavioural and physiological factors over multiple life stages to obtain a better understanding of the sociality-health relationship.

The development and validation of non-invasive health markers for wildlife studies is also an important new avenue of research. Indeed, there is a need for reliable tests that are easily doable with non-invasive samples, to infer health status and immune system activation. In particular, I want to raise attention to the need of investigating a large array of infectious agents by developing methods to study viral and bacterial infections from stool, saliva or urine samples. I also would like to see more studies measuring a diverse set of health and immune markers concomitantly, as it is traditionally done in human and veterinary medicine, to improve the identification of physiological causes and consequences of diverse infections in wildlife.

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"We were together. I forget the rest." Walt Withman

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« Ça doit être ça les vrais amis. Ceux qui restent même quand on part, qu'on retrouve en revenant, une semaine, un mois, un an, cinq ans après. » Agnès Ledig

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"For time cannot stop,
But moments,
Seconds,
A fleeting smile,
A kiss in the sunlight,
Can live forever."

Staci Hart

Curriculum Vitae

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Behavioural Ecology and Sociobiology Unit, German Primate Center

Sociobiology/Anthropology department, University of Göttingen

Sociality and Health in Primate research group www.sohapi.de

Research interests: evolution of sociality, sociality-physiology-health-fitness interactions, parasite community effects, parasite avoidance strategies

Education

PhD, Zoology/Anthropology, University of Göttingen & German Primate Center, Germany. (4 years full time equivalent)	2020
MSc, Zoology, *upper second class honors,* Paris 13 University, France.	2012
BSc, Neurosciences, *upper second class honors,* Marseille University, France.	2010

Research positions

PhD project, supervised by Dr. C. Fichtel, Univ. Göttingen, Germany. *A healthy social life? Sociality, stress and indicators of health in wild red-fronted lemurs (Eulemur rufifrons).*	12/2014 – 06/2020 (4 years FTE)
Field team leader, for the Monkeybar project headed by the London School of Hygiene and Tropical Disease, supervised by Dr. M. Salgado-Lynn, Malaysia. *Malaria transmission from macaques to humans. Influence of macaque behavior, density and distribution. Influence of agriculture and forest type.*	2014 (4 months)
Field assistant, for Dr. S. van Belle & Dr A. Estrada, Univ. Mexico, Mexico. *Social and genetic factors mediating individual participation in howling bouts and group defense in black howler monkeys (Alouatta pigra).*	2013 (3 months)
Master's project, supervised by Pr. Dr. H. Meunier, Univ. Strasbourg, France. *Hand preference in object manipulation and gestural communication in Tufted capuchins (Cebus apella). Effects of the experimenter attentional state on gestural communication. Habituation of a group of Cebus capucinus.*	2011-12 (8 months)
Intern, supervised by Dr. F. Levréro, Univ. St-Etienne, France. *Effects of physical and social environment novelty on a group of captive bonobos (Pan paniscus). Search for vocal signature by sex, kinship, hierarchy.*	2011 (4 months)
Intern, supervised by Dr. E. Nowbahari, Univ. Paris 13, France. R*escue behaviour of the ant Cataglyphis cursor, test of the group effect and social facilitation hypothesis.*	2010 (2 months)
Technical assistant, Paleontological Museum of Marseille, France. *Maintenance and inventory of fossils. Creation of data sheets.*	2008 (3 months)

Teaching

Training and supervision of students for their thesis/lab rotations: 4 master students (2012 Marion & Raphaelle, 2015 Roman, 2016 Julia), 1 bachelor student (2017 Jan)
Practical parasitology (1 bachelor student Jan, 1 assistant Simon)
Behavioural observations: 2 field assistants (2012 Jesrine & Daniel) 4 master students (2012 Marion & Raphaelle, 2015 Roman, 2016 Julia)
Basics of biology (high school substitute teacher: 2*6 months in 2013 & 2014, 174 students in 7 groups, ca. 800h of practical and theoretical classes)

Publications

4. **Defolie C.,** Merkling T., Fichtel C. (2019). Patterns and variations in the mammal parasite-glucocorticoid relationship. *Biological Reviews.* https://doi.org/10.1111/brv.12555
3. Peckre, L. R., **Defolie, C.**, Kappeler, P. M., & Fichtel, C. (2018). Potential self-medication using millipede secretions in red-fronted lemurs: combining anointment and ingestion for a joint action against gastrointestinal parasites? *Primates*, 59(5), 483-494.
2. **Defolie C.,** Malassis R., Serre M., Meunier H. (2015). Tufted capuchins (*Cebus apella*) adapt their behaviour in response to human's attentional states. *Animal cognition*, *18*(3), 747-755.
1. Meunier H., Fagard J., Fizet J., Canteloup C., **Defolie C.**, Vauclair J. (2013). Patterns of hemispheric specialization for a communicative gesture in different primate species. *Developmental psychobiology, 55*(6), 662-671.

Presentations

Talks:

10. **Defolie C.,** Rudolph K., Kappeler P., Fichtel C. 2019. Short-term costs and benefits of sociality on physiology: a lemur perspective. Cognition, Behavior & Evolution Network (CBEN) Conference, Amsterdam, Netherland.
9. **Defolie C.,** Heistermann M., Fichtel C. 2019. Caring is sharing: Determinants of parasite richness at the individual, group and population level in wild red-fronted lemurs (Eulemur rufifrons). 16th Conference of the German Society for Primatology (GfP), Göttingen, Germany.
8. **Defolie C.,** Fichtel C., Heistermann M., Kraus C. 2017. *So happy together? Ecological and social correlates of stress in wild red-fronted lemurs (Eulemur rufifrons)*. International Conference on Behaviour, Physiology and Genetics of Wildlife, Berlin, Germany.
7. **Defolie C.,** Fichtel C., Heistermann M., Kraus C. 2017. *So happy together? Ecological and social correlates of stress in wild red-fronted lemurs (Eulemur rufifrons)*. European Federation of Primatology, Strasbourg, France.
6. **Defolie C.,** Fichtel C., Heistermann M., Kraus C. 2017. *Don't worry, be healthy? Sociality, stress and indicators of health in wild red-fronted lemurs (Eulemur rufifrons).* Behaviour ISAB, Estoril, Portugal.
5. **Defolie C.,** Fichtel C., Kraus C. 2015. *Physiological stress and parasite infections in mammals.* GÖZU meeting, Göttingen, Germany.
4. **Defolie C.,** Malassis R., Serre M., Meunier H. 2013. *Tufted capuchins (Cebus apella) adapt their communicative behaviour to human's attentional states*. European Federation of Primatology, Antwerp, Belgium.
3. Levréro F., Déruti L., Touitou S., **Defolie C.,** Mathevon N. 2013. *Who's calling? Reliability of individual signature in bonobo's calls.* European Federation for Primatology, Antwerp, Belgium.
2. **Defolie C.,** Meunier H. 2012. *Brown tufted capuchins (Cebus apella) adapt their communicative behaviour to human's attentional states.* French Speaking Society for Primatology, Lyon, France.
1. Levréro F., Déruti L., **Defolie C.,** Guéry J-P., Mathevon N. 2012. *Reliability of individual signature in bonobo's calls.* French Speaking Society for Primatology, Lyon, France.

Posters:

6. **Defolie C.,** Heistermann M., Fichtel C. 2018. Caring is sharing: Determinants of parasite richness at the individual, group and population level in wild red-fronted lemurs (Eulemur rufifrons). RTG Understanding Social Relationships Conference, Göttingen, Germany.
5. Peckre L., **Defolie C.**, Kappeler P., Fichtel C. 2018. *Potential self-medication using millipede secretions in red-fronted lemurs.* European Conference on Behavioural Biology, Liverpool, UK.
4. **Defolie C.,** Fichtel C., Kraus C. 2017. *Microparasites, macroparasites and physiological stress: a meta-analysis.* Frontiers in Baboon Research Symposium, Göttingen, Germany.
3. **Defolie C.,** Fichtel C., Heistermann M., Kraus C. 2017. *And they lived happily forever after? Ecological and social correlates of stress in wild red-fronted lemurs*. 15th Conference of the German Society for Primatology (GfP), Zurich, Switzerland.
2. **Defolie C.,** Fichtel C., Kraus C. 2016. *Sharing more than friendship? Social network and gastro-intestinal parasites in wild red-fronted lemurs (Eulemur rufifrons).* Networks in Biology symposium, Göttingen, Germany.
1. **Defolie C.,** Guéry J-P., Levréro F. 2012. *Importance of group cohesion in bonobos (Pan paniscus) facing environmental and social changes*. French Society for the Study of Animal Behaviour, St-Etienne, France.

Honors & Awards

GAUSS Finishing Grant (10 200€), 4 grants attributed per year (Göttingen University, Germany).
Dorothea-Schlözer Mentoring Program for young female researchers, 2016-2017, 20 students per year (Göttingen, Germany).
Jury award (500€) at the 2013 annual contest for students' projects given by the science magazine « La Recherche » (Paris, France) with the project « Ecorvids: helpful corvids to harmful waste ».

Laboratory and Analytical training

Linear models and their application in R (2018), 60h course supervised by Dr. R. Mundry
Animal Social network Analysis:
2016, theory and practical with R supervised by Dr. L. Brent
2015, practical with R supervised by Dr. A. Carter
Genetics:
Parasite and microbiome analyses with Next Generation Sequencing (2019), supervised by Dr. C. Roos
Molecular genetics and epidemiology workshop (2016), supervised by Dr. F. Leendertz
Endocrinology:
Practical endocrinology with ELISA (2017), supervised by Dr. M. Heistermann
Field endocrinology workshop (2016), supervised by Dr. T. Deschner
Microscopic parasite analyses (2016-2017), self-taught with support from Dr. N. Müller-Klein & Dr. C. Poirotte
Analysis of dominance relationships (2018), supervised by Dr. C. Neumann
GPS and radio tracking (2013), supervised by Dr. S. van Belle
Video analysis with The Observer (2012), supervised by Prof. H. Meunier
Vocalizations analysis with Praat & Avisoft (2010), supervised by Dr. F. Levréro

Further qualifications

Professional services:
Symposium organisation (2 symposiums: EFP 2017, Behaviour 2017)
Workshop organisation (4 soft skill workshops for PhD students, 2016 - 2017)
DPZ PhD Colloquium organisation (2015 - 2017)
Journal Club (2015 - present)
Languages: French (native speaker), English (fluent), Spanish (conversational + reading), German (basics), Malay (basics)
Leisure activities: Trekking, travel (4 continents), watercolor, yoga, hula-hooping, historical re-enactment.

Declaration

I hereby declare that I have written this thesis independently and with no other aids or sources than quoted.

_____ Besse sur Issole (FR), 20th of May 2020

Charlotte Defolie