Influence of climate, season and anthropogenic changes on the energy budget and stress responses of *Lepilemur edwardsi* (Lepilemuridae; Primates)

Dissertation

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Summary

Seasons cause yearly changes in ambient temperatures and precipitation and therefore strongly influence an animals' resource availability in its habitat. Hence, animals living in seasonal habitats have to adapt their energy expenditure to these annual changes. Some species use heterothermic strategies (i.e., daily torpor or hibernation) to cope with challenging seasons, whereas other species strongly increase their energy expenditure (e.g., for thermoregulation) or use behavioral mechanisms, such as huddling during cold periods or the change of posture or microclimates at high ambient temperatures. The highly seasonal western deciduous dry forests of Madagascar are characterized by a hot and humid wet season and a cooler dry season with almost no precipitation for about six months. These changes are accompanied by fluctuations in resources, with a lower resource availability and also quality during the dry season than during the wet season.

Lepilemur edwardsi, a small nocturnal and folivorous lemur species, living in these forests in north western Madagascar, has to adapt to these seasonal fluctuations of ambient temperature and resources. The protein content of the folivorous diet of *L. edwardsi* decreases during the dry season with increasing leaf age and additionally the high daytime ambient temperatures increase the plant secondary metabolites content of the leaves, resulting in a decrease of the food-quality towards the end of the dry season. We examined the seasonal influences and the influence of sex, sleeping site quality and body condition on the energy budgeting and stress response of *L. edwardsi*. The study took place in the Ankarafantsika National Park at the forest station in Ampijoroa in the wet season and late dry season in 2018 and the early dry season in 2019. We measured the resting metabolism via open-flow respirometry, the stress levels via an analysis of hair cortisol concentrations and the intestinal and ectoparasite prevalence of individuals of *L. edwardsi* during the three sampling periods directly in in the dry forest. To cope with the increased toxicity of its diet, *L. edwardsi* presumably has to increase its resting metabolic rate from the well-resourced wet season to the poor-resourced dry season. We could not detect any regular employment of heterothermy,

however one individual with a low body mass showed a “torpor-like” episode possibly due to the food deprivation during respirometry in the late dry season. The dietary constraints during the dry season are presumably also shown in the higher stress levels and intestinal parasite prevalence of this species, particularly towards the end of the dry season. As individuals also showed a loss in body mass, it seems that *L. edwardsi* might only have a small scope to future climatic changes (e.g., a prolongation of the dry season). Furthermore, reproducing male individuals seem to be particularly stressed by the reproductive season, as these individuals showed a significant increase in stress levels during the mating season (early dry season).

Ultimately, the results of my dissertation contribute to a better understanding of the impact seasonal changes have on arboreal folivorous mammals, such as *L. edwardsi* and may help to predict the response of such species or species living in the same or similar habitats to future climatic changes. Additionally, this data might contribute to the current and future conservation action plan of *L. edwardsi*.

Zusammenfassung

Jahreszeiten führen zu jährlichen Veränderungen der Umgebungstemperaturen und Niederschläge und haben einen starken Einfluss auf die Ressourcenverfügbarkeit von Tieren in ihrem Lebensraum. Daher müssen Tiere, die in saisonalen Lebensräumen leben, ihren Energiehaushalt an diese jahreszeitlichen Veränderungen anpassen. Einige Arten nutzen heterotherme Strategien (z.B. täglicher Torpor oder Winterschlaf), um mit anspruchsvollen Jahreszeiten zurechtzukommen, während andere Arten ihren Energieverbrauch stark erhöhen (z.B. zur Thermoregulation) oder ihr Verhalten ändern, in dem sie sich z.B. zusammenkauern, um während kälteren Jahreszeiten eine Wärmeabgabe an die Umwelt zu vermeiden; oder bei hohen Umgebungstemperaturen ihre Körperhaltung verändern um Wärme an die Umwelt abzugeben. Die stark saisonalen und laubabwerfenden Trockenwälder im Westen Madagaskars sind durch eine heiße und feuchte Regenzeit und eine kühlere Trockenzeit geprägt, in der etwa sechs Monate lang kaum Niederschlag fällt. Diese Veränderungen gehen mit Schwankungen der Ressourcen einher, wobei die Verfügbarkeit und auch die Qualität der Ressourcen in der Trockenzeit geringer ist als in der Regenzeit.

Lepilemur edwardsi, eine kleine nachtaktive und folivore Lemurenart, die in diesen Wäldern im Nordwesten Madagaskars lebt, muss sich an diese jahreszeitlichen Schwankungen der Umgebungstemperatur und der Ressourcen anpassen. Der Proteingehalt der folivoren Nahrung von *L. edwardsi* nimmt während der Trockenzeit mit zunehmendem Alter der Blätter ab, und zusätzlich erhöhen die hohen Tagestemperaturen den Gehalt an pflanzlichen Sekundärstoffen in den Blättern, was zu einer Abnahme der Nahrungsqualität insbesondere gegen Ende der Trockenzeit führt. In meiner Dissertation haben wir die saisonalen Einflüsse und den Einfluss des Geschlechts, der Schlafplatzqualität und der Körperkondition auf den Energiehaushalt und die Stressantwort von *L. edwardsi* untersucht. Die Studie wurde im Ankarafantsika-Nationalpark an der Forschungsstation in Ampijoroa, in der Regenzeit und der späten Trockenzeit im Jahr 2018 und der frühen Trockenzeit im Jahr 2019 durchgeführt. Wir haben den Ruhestoffwechsel mit Hilfe von open-flow-respirometry gemessen, die

Stressantwort durch die Konzentration des Stresshormons Cortisol in den Haaren der Individuen analysiert; und die Darm- und Ektoparasitenprävalenz von *L. edwardsi* während der drei Probenahmezeiträume gemessen. *L. edwardsi* muss vermutlich seinen Ruhestoffwechsel während der ressourcenarmen Trockenzeit massiv erhöhen, im Vergleich zum niedrigen Ruhestoffwechsel während der ressourcenreichen Regenzeit, um mit der erhöhten Toxizität der Nahrung zurecht zu kommen. Wir konnten keine regelmäßige Heterothermie feststellen, allerdings zeigte ein leichteres Individuum eine "torporähnliche" Episode, während der respiratorischen Messungen in der späten Trockenzeit, was möglicherweise auf den Nahrungsentzug währenddessen zurückzuführen ist. Die geringere Ressourcenverfügbarkeit während der Trockenzeit zeigen sich vermutlich auch in den höheren Stresslevel und der höheren Prävalenz von Darmparasiten, insbesondere gegen Ende der Trockenzeit bei *L. edwardsi*. Da die Individuen auch einen Verlust an Körpergewicht aufwiesen, scheint es, dass *L. edwardsi* nur einen geringen Handlungsspielraum für zukünftige klimatische Veränderungen hat. Insbesondere bei einer Verlängerung der Trockenzeiten könnte *L. edwardsi* kaum Möglichkeiten zu weiteren Anpassungen haben. Darüber hinaus scheinen reproduzierende männliche Individuen durch die Fortpflanzungszeit besonders gestresst zu sein, da diese Individuen während der Paarungszeit (in der frühen Trockenzeit) einen signifikanten Anstieg der Stresslevel zeigten.

Letztendlich tragen die Ergebnisse meiner Dissertation zu einem besseren Verständnis der Auswirkungen von jahreszeitlichen Veränderungen auf arboreale, folivore Säugetiere, wie *L. edwardsi*, bei und können helfen, die Reaktion dieser Arten oder von Arten, die im selben oder ähnlichen Lebensräumen leben, auf zukünftige klimatische Veränderungen vorherzusagen. Zusätzlich dazu, könnten diese Daten zum aktuellen und künftigen Artenschutzprogramm von *L. edwardsi* beitragen.

CHAPTER 1:

GENERAL INTRODUCTION

1.1 Introduction

As the earth cycles the sun on a tilted axis, seasons are created by the changing annual cycle of solar radiation. As a result, ecosystems experience seasonal changes in e.g., ambient temperatures, winds, precipitation and daylight-length. In mid-latitudes, this leads to a three-month cycle with four seasons over the course of a year (spring, summer, autumn and winter). Whereas the poles and the tropics are usually dominated by a six-month cycle, with a wet and dry season (Trenberth 1983). Yearly fluctuations of ambient temperatures in the tropics are not as large as in mid-latitudes, however changes in rainfall are often quite pronounced (Richter 2016). Hence, these seasonal changes result in seasonal fluctuations of resource availability, such as food, water and nutrients (Varpe 2017). Furthermore, the resource quality in habitats changes seasonally, which is particularly problematic for folivorous animals (i.e., herbivorous animals that specialize in eating leaves). As many plants change their nutritional (e.g., protein, nitrogen) and fiber content, and their plant secondary metabolites (PSMs) depending on leaf age (Coley 1983), herbivore defense (Mithöfer and Boland 2012) and ambient temperature (Ganzhorn 1995; Dearing 2013). In tropical seasonal deciduous forests the quality and availability of leaves fluctuates throughout the year, with young, protein-rich (high-quality) leaves with low fiber and high water content in the wet season and mature, protein-poor (low-quality) leaves with high fiber and low water content in the dry season (Coley 1983; Coley and Barone 1996; Coley and Kursor 1996). Additionally, PSM-concentrations of leaves change with ambient temperature, with a high PSM-concentration at high ambient temperatures and vice versa (Dearing 2013). Moreover, leaf-quality increases within the day and is highest in the afternoon (highest protein and sugar content, but also tannin concentration)(Ganzhorn and Wright 1994).

Animals living in seasonal habitats have to behaviorally or physiologically adapt to these daily and seasonal abiotic changes, which act as environmental stressors for animals varying in impact, duration and intensity throughout the year (Romero 2004; Spiga et al. 2014). Additional environmental stressors that can affect the hormonal balance and therefore fitness of animals, can be natural disasters, (e.g., droughts and cyclones)(Sapolsky 1986; Fardi et al. 2018), social status (Koren et al. 2008), diseases (Mormède et al. 2007) and parasite infections (Triki et al. 2016), reproductive activities (Brunton et al. 2008), as well as nutritional stress (Chapman et al. 2015). But, especially variations in ambient temperature cause functional constraints, as they have a strong influence on the energy budgeting of animals (Pörtner and Farrell 2008). Particularly on small endothermic mammals, which have an unfavorable surface-area-to-volume ratio and therefore lose and gain heat more rapidly than large endothermic mammals (Speakman 1999, 2001). Nonetheless, species react differently to changes in ambient temperatures. Some species react behaviorally, such as *Phascolarctos cinereus*, the koala, that rests against cooler tree trunks on hot days (e.g., during heat waves) to enhance conductive heat loss (Briscoe et al. 2014); or *Odocoileus virginianus*, the white-tailed deer, that reduces its activity during the mid-latitude winter (Moen 1976). Whereas other endothermic species are able to physiologically adapt their metabolism during periods of resource shortages, extreme weather, or if they are living in an extreme habitat by entering heterothermic states, such as hibernation, prolonged or daily torpor (Geiser and Ruf 1995; Geiser 2004; Heldmaier et al. 2004; Kobbe et al. 2011; Ruf and Geiser 2015). Heterothermy is a physiological state of controlled interruption of normothermia via a reduction of metabolic rate and other bodily functions, that can last many months (hibernation) or a few minutes (daily torpor)(Geiser 2004; Heldmaier et al. 2004; Ruf and Geiser 2015; Reher and Dausmann 2021). Hibernation is mainly a seasonal physiological strategy, consists of a series of torpor bouts and is normally employed for many months during winter (Dausmann et al. 2004; Heldmaier and Neuweiler 2012; Ruf and Geiser 2015). Daily torpor is characterized by short torpor bouts of less than 24h, whereas prolonged torpor can last for a few days (Kobbe et al. 2011; Heldmaier and Neuweiler 2012; Ruf and Geiser 2015). Prolonged torpor enables the individuals to increase their energy savings in comparison to daily torpor and can be used more flexibly than hibernation, which requires intensive preparation and can only be employed by individuals with sufficient fat reserves and a good body condition (Kobbe et al. 2011). Torpor bout duration, frequency and depth varies within species and with environmental conditions (Turner et al. 2012a; Lovegrove et al. 2014; Reher et al. 2018;

Nowack et al. 2020). Interestingly, daily torpor is sometimes even used as a response to extreme weather events, such as storms, wildfires and floods (Willis et al. 2006; Stawski et al. 2015; Nowack et al. 2017; Barak et al. 2019). Generally, heterothermy enables animals to efficiently cope with demanding environmental conditions by reducing their energy expenditure and has additional advantages, such as predator or parasite avoidance or a reduction in evaporative water loss (Geiser and Brigham 2012; Nowack et al. 2017). In arid and / or hot regions, the savings in water that accompany heterothermy, are maybe most important for animals (Schmid and Speakman 2000; Bondarenco et al. 2014). As, the general water turnover and additional water-depleting processes such as respiration, urine production, and defecation are lower or even absent during heterothermy (Levin et al. 2015; Hill et al. 2016).

Additionally, climatic changes often also cause changes in biotic factors, such as parasite prevalence (Patz et al. 2000; Short et al. 2017). The severity of parasitic infestation can vary greatly depending on ambient temperatures (Studer et al. 2010) or precipitation (Turner et al. 2012b); but also with the hosts' diet-quality, as species with a low-quality diet (e.g., folivorous diet) show a higher endoparasite infestation than species with a high-quality diet (e.g., insectivorous diet)(Coop and Holmes 1996; Coop and Kyriazakis 1999; Vitone et al. 2004; Lange et al. 2014). These factors can also be mutually dependent, as the diet-quality usually decreases during dry periods and high ambient temperatures (see above), often resulting in a higher parasite load of individuals (Ezenwa 2004). Contrastingly, ectoparasite prevalence often decreases during dry periods, as these conditions often disturb their development and increase mortality (Moyer et al. 2002). However, intestinal and ectoparasite prevalence of individuals also depends on various other factors, such as an individuals' body condition (Sánchez et al. 2018) or habitat disturbances (Patz et al. 2000). In the Eurasian red squirrel, *Sciurus vulgaris*, for example, individuals living in forest fragments have a higher intestinal parasite prevalence than individuals living in large continuous forests (Santicchia et al. 2015). Hence, the risk of parasite transmission might increase further with environmental changes, such as climatic changes, deforestation and other anthropogenic disturbances (Patz et al. 2000). This is presumably due to the higher host density and the reduced access to higher-quality food in forest fragments; but also due to the increased stress levels of the individuals, which negatively affect the immune response of species (Pruett 2003; Chapman et al. 2005; Santicchia et al. 2015).

A country which is already severely affect by environmental changes as a result of the anthropogenic climate change, as well as deforestation is Madagascar (Harper et al. 2007; Tadross et al. 2008; Hannah et al. 2008). Madagascar is a country with diverse habitats and climates, from the arid spiny forests in the South, to the dry deciduous forests in the West and North; further to the central highlands and the humid rainforests of the East (Goodman and Benstead 2003; Yoder and Nowak 2006). Species living in these regions, have to adapt to the prevailing conditions of their respective habitats and changes within, such as seasonal changes or unpredictable weather events (e.g., cyclones)(Dewar and Richard 2007; Kobbe et al. 2014; Fardi et al. 2018). Particularly the cool dry season (May – September) is energetically challenging for endothermic Malagasy mammals, as they have to increase their energy uptake despite low ambient temperatures, a reduced food-quality and availability and in the dry regions, almost no rainfall for many months (Simmen et al. 2003; Bethge et al. 2017). Malagasy mammals can respond behaviorally, such as by sunbathing (e.g., *Lemur catta*) (Rasamimananana et al. 2006) or social thermoregulation (e.g., *Hapalemur meridionalis*) (Eppley et al. 2017) or they shift their activity patterns and food choices (e.g., *Propithecus verreauxi*)(Norscia et al. 2006). Interestingly, many Malagasy mammals can react to these changes physiologically, by using heterothermic strategies. Either by hibernating to cope with the low resource-availability and low ambient nighttime temperatures during the dry season, such as in *Cheirogaleus medius* (fat-tailed dwarf lemur)(Dausmann et al. 2004); or by using daily torpor to survive the hottest time of the day (e.g., *Macronycteris commersoni*)(Reher et al. 2018; 2021).

Heterothermy in lemurs however, seems to be restricted to the nocturnal lemur family of the Cheirogaleidae, whose members are widespread throughout Madagascar. Almost all species of this family are presumably becoming torpid during the cool, dry season with varying torpor bout durations and depth, depending on the species, their respective habitat and environmental conditions, their sex and several yet unknown parameters (Dausmann and Warnecke 2016). Whereas the dwarf lemurs (*Cheirogaleus* spp.) hibernate for many months during the dry season (Dausmann et al. 2004, 2005); individuals of the nocturnal mouse lemur species (*Microcebus* spp.), regularly enter daily torpor in the cool mornings during the dry season while resting in their shared resting sites, to survive the often unpredictable conditions in their habitats (Schmid 2000; Kobbe et al. 2011; Dausmann and Warnecke 2016). Torpor in *Microcebus* spp. is used as a flexible response to changing environmental conditions and entry into torpor often depends on an individual's body condition (Schmid and Kappeler 1998; Schmid 2000; Kobbe et al. 2011). In addition to the reduction in energy requirements and the decrease in body temperature, water requirements are often extremely reduced during daily torpor, which may be even more important for the *Microcebus* species in their southern dry habitats (Schmid and Speakman 2000).

The sportive lemurs (Lepilemuridae, Gray 1870), are the sister family of the Cheirogaleidae, with a single genus in this family (*Lepilemur* spp.) and approximately 26 different species (Mittermeier et al. 2010). Sportive lemurs are nocturnal clinger and leapers, which rest in tree hollows, tangles of lianas or leaves, or forked branches during the day (Hladik and Charles-Dominique 1971, Charles-Dominique and Hladik 1974, Mittermeier et al. 2010). Individuals eat mainly leaves but also fruits and flowers when in season (i.e., during the wet season). In fact, sportive lemurs are the smallest folivorous primates known (Ganzhorn 1993), with a body mass ranging from 450 – 1200g and a head body length of approximately 25 – 30cm (Mittermeier et al. 2010). Because of their folivorous low-quality diet, they have an elongated cecum and are hindgut fermenters that re-ingest their feces (i.e., cecotrophy)(Hladik and Charles-Dominique 1974; Chivers and Hladik 1980; Hladik et al. 1980; but see also: Russell 1977).

There is not much known about the sociability of sportive lemurs. *Lepilemur ruficaudatus* and *L. edwardsi* however are pair-living and sometimes share their resting sites with their partners (Rasoloharijaona et al. 2003; Zinner et al. 2003). These resting sites are an important resource, as they presumably provide thermal insulation and protection against predators (Rakotomalala et al. 2017; Wulff 2020). Female *L. edwardsi* also share their resting sites with their offspring, which they transport orally and leave on branches while they forage (Rasoloharijaona et al. 2000). Contrastingly, the two sexes of *L. petteri* never use their resting sites at the same time (as *L. leucopus* in: Dröscher and Kappeler 2013). Sportive lemurs are supposed to be monogamous, however there are reports of polygamy in this genus, as males show relatively big testes during the mating period (Warren and Crompton 1997; Zinner et al. 2003; Randrianambinina et al. 2007). In the so far studied sportive lemur species (*L. petteri, L. ruficaudatus, L. sahamalazensis, L. edwardsi, L. mustelinus*), individuals presumably reach sexual maturity in their second year (Zinner et al. 2003; Randrianambinina et al. 2007; Rasoloharijaona et al. 2008; Seiler et al. 2015; Dröscher et al. 2016). Mating takes place at the beginning of the dry season (May / June) and females are pregnant during the poor-resourced dry season. They give birth to one young at the beginning of the wet season (October / November) and the lactation period lasts throughout this well-resourced season. Finally, females ween their offspring shortly before the beginning of the consequent mating season (Zinner et al. 2003; Randrianambinina et al. 2007; Rasoloharijaona et al. 2008; Seiler et al. 2015; Dröscher et al. 2016). Sportive lemurs generally have small home ranges of approximately 1ha, that are defended by both sexes (Thalmann and Ganzhorn 2003; Zinner et al. 2003; Mittermeier et al. 2010). Sportive lemurs inhabit various different habitats, from the humid rainy forests of eastern Madagascar, to the dry spiny forests in the South and species have to adapt to the respective prevailing environmental conditions in their habitats. Particularly, the southern and western dry forests are dominated by a pronounced seasonality with high daily and seasonal fluctuations of ambient temperatures and hence resource availability (Tadross et al. 2008; Waeber et al. 2015). The two sportive lemur species, *L. ruficaudatus* and *L. petteri*, have an extremely low resting metabolism, of about 50% of what would be expected from their body size (Schmid and Ganzhorn 1996; as *L. leucopus* in: Bethge et al. 2017). *L. petteri* even shifts its thermoneutral zone (i.e., range of ambient temperature where general basal metabolic processes suffice to maintain a constant body temperature) from warmer ambient temperatures (29 – 32 °C) in the poor-resourced and cooler dry season, to lower ambient temperatures (25 – 30 °C) in the better-resourced and warmer wet season. This is counter-intuitive and contrary to the seasonal shift of the thermoneutral zone in the nocturnal *Microcebus griseorufus,* which shifts its thermoneutral zone from cooler ambient temperatures in the dry season, to warmer ambient temperatures in the wet season (Kobbe et al. 2014). Whether sportive lemurs also become heterothermic is not fully examined yet, as authors often have difficulties to distinguish between the already extremely low resting and torpid metabolic rates (Schmid and Ganzhorn 1996).

Lepilemur edwardsi, one of the bigger sportive lemurs with a body mass of 800 – 1000g and a head-body length of 26 – 29cm can be found in the dry deciduous forest of the Ankarafantsika Nationalpark in western Madagascar (Fig. 1)(Mittermeier et al. 2010); a region characterized by a pronounced seasonality. Ambient temperatures during the wet, food-abundant season average between 23°C and 36°C and the annual mean precipitation is high (1562.5mm)(Randrianambinina et al. 2007; Klein et al. 2018). Whereas, during the dry season there is no rainfall for about 6 months and the ambient temperatures average between 17°C during the night and 37°C during the day (Randrianambinina et al. 2007). These dry season conditions are challenging, particularly for folivorous species as the quality of their diet decreases with the ongoing dry season. Especially for *L. edwardsi*, as it eats lower-quality leaves (i.e., leaves with a lower protein content), presumably due to niche-partitioning to *Avahi occidentalis*, a sympatrically living woolly lemur species (Ganzhorn 1988; Thalmann 2001). Contrary to *A. occidentalis*, *L. edwardsi* rests in tree hollows during the day whereas *A. occidentalis* rests in open foliage (Rasoloharijaona et al. 2003; Ramanankirahina et al. 2012). These tree hollows provide good protection against predators, but also affect the ectoparasite and intestinal parasite prevalence of *L. edwardsi*, as it shows a higher parasite infestation than *A. occidentalis*. On the other hand, the tree hollows might protect *L. edwardsi* from vector-borne parasites that are transmitted by mosquitos (Hokan et al. 2017, 2018).

Additionally, *L. edwardsi* is also affected by forest fragmentation and additionally poaching (Craul et al. 2009). Outside of the Ankarafantsika Nationalpark Craul *et al.* (2009) only found *L. edwardsi* in one third of the investigated sites with a low genetic diversity in these populations. But even inside the Ankarafantsika Nationalpark *L. edwardsi* was only found in the largest fragments but showed a high genetic diversity there (Craul et al. 2009), which can be advantageous for populations, as the ability of populations to persist in stressful or changing environment is increased with a higher genetic diversity (Hughes et al. 2008). Moreover, *L. edwardsi* is highly vulnerable to hunting which is probably why this species has mainly disappeared from most of the unprotected fragments (García and Goodman 2003; Craul et al. 2009).

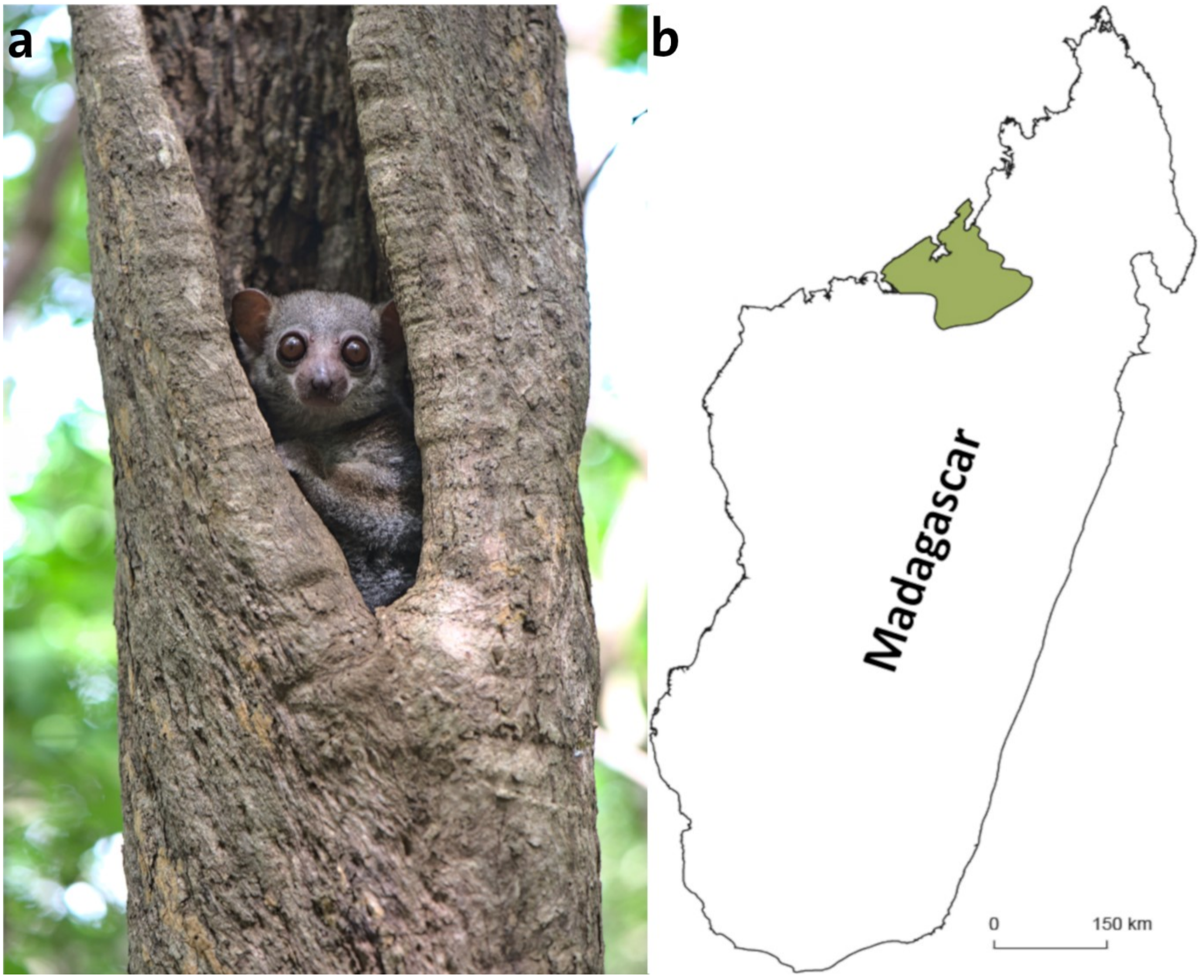

Fig. 1: (a) *Lepilemur edwardsi* resting in a tree hollow during the day. (b) The current distribution of *L. edwardsi* in the dry deciduous forests of northwestern Madagascar.

1.2 Research Questions

Endothermic animals experience daily and seasonal changes in environmental and ecological conditions in almost all habitats. Particularly, fluctuations in ambient temperature have a strong impact on thermoregulation of small endothermic species, as they generally have an unfavorable surface-to-volume ratio (Speakman 1999, 2001; Pörtner and Farrell 2008). Hence, even small changes of these prevailing conditions, particularly with regard to the current climatic changes, can increase the energy requirements and result in additional thermoregulatory challenges for endothermic species. Additionally, species living in dry habitats are often already living at the edge of their thermoregulatory possibilities and may particularly be affected by this (Fuller et al. 2021).

My dissertation investigates the seasonal adaptations in the energy budgeting, stress response and the seasonal changes in the parasite prevalence of the nocturnal, folivorous lemur species, *Lepilemur edwardsi*, in its deciduous dry forest habitat. I chose *L. edwardsi*, because I wanted to examine whether this species shows physiological adaptations to its seasonal habitat, particularly to the lack of rainfall and degrading food-quality during the dry season. *L. edwardsi* is one of the smallest folivorous primates and hence should be adapted to the decreasing conditions during the dry season, such as the hot daytime and low nighttime ambient temperatures, the higher PSM-content and lower protein content in its diet. As *L. edwardsi* mates at the beginning of the dry season, females are pregnant throughout the harshest (i.e., poor-resourced) time of the year, which could make the dry season even more challenging for the females, due to their higher energy demands during pregnancy. To examine the mechanisms *L. edwardsi* uses to adapt to changes in its habitat, we measured the resting metabolism, stress levels and parasite prevalence of this species during three different sampling seasons and directly in its habitat. I put my results in an ecological context and discuss my data by relating it to the seasonal changes and accompanying decreasing food-quality in its habitat. Furthermore, I link my results to the reproductive activity of *L. edwardsi* and discuss the potential future impact of the ongoing climatic change on this species.

Chapter 2 investigates the physiological adaptations of *L. edwardsi* to the abiotic seasonal changes of ambient temperatures and the accompanying food availability and quality. To investigate whether the lack of rainfall and the decreasing resource availability during the dry season has an impact on the energy budgets of *L. edwardsi*, we measured the resting metabolism of this species via respirometry during the wet season, the early dry season and late dry season directly in the field. Furthermore, we measured the skin temperature of the individuals during respirometry and the skin temperature of free-ranging individuals after respirometry. My goal was to test whether *L. edwardsi* also shows the generally low resting metabolism of the Lepilemuridae (resp. *L. petteri* and *L. ruficaudatus*). Specifically, I tested whether the resting metabolism differed between seasons and sexes. Additionally, I aimed to resolve the question whether *L. edwardsi* uses heterothermic strategies to counter unfavorable conditions.

Chapter 3 analyzes if the climatic and nutritional seasonal changes are reflected in the stress response of *L. edwardsi* across seasons. I aimed to investigate whether the stress response of *L. edwardsi* differs among seasons and sexes and if females are generally more stressed, as they are either giving birth (early wet season), lactating (wet season) or are pregnant (early dry season). Furthermore, I tested if the body condition of individuals had an influence on the stress response of *L. edwardsi*, and if individuals with tree hollows, used as resting sites during the day, in larger trees have potentially lower stress levels, as these presumably provide a better protection against predators and thermal insulation. We measured the hair cortisol concentrations during the different seasons and linked the results to the seasonal changes in the habitat and the reproductive activities of *L. edwardsi*.

Chapter 4 investigates whether the elevated metabolic rate (Chapter 2) and stress levels (Chapter 3) during the late dry season are also reflected in the parasite prevalence of *L. edwardsi*. Furthermore, I investigated whether the resting site quality, sex and body condition of the individuals had an influence on the parasite prevalence during the three different sampling periods. We analyzed the intestinal and the ectoparasite prevalence during the three sampling periods and discussed the results in the context of the seasonal environmental changes in the habitat of *L. edwardsi*, particularly the climatic conditions and decreasing food availability and quality.

Seasonal changes in species' environments pose different abiotic and biotic challenges for individuals to which species have to adapt. Ultimately, my dissertation answers the questions how the small nocturnal and folivorous Malagasy lemur species, *L. edwardsi*, copes with the seasonal changes in abiotic factors, such as ambient temperatures and precipitation and the fluctuations in the associated resource availability; but also how these abiotic changes may result in changes of biotic factors, such as the seasonal changes in the parasite prevalence of individuals which is presumably tightly linked to the resource availability in the habitat of *L. edwardsi*.

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CHAPTER 2:

SPORTIVE LEMURS ELEVATE THEIR METABOLIC RATE DURING CHALLENGING SEASONS AND DO NOT ENTER HETEROTHERMY

2.1 Abstract

Animals experience seasonal changes of environmental and ecological conditions in most habitats. Fluctuations in ambient temperature have a strong influence on thermoregulation, particularly on small endothermic mammals. However, different mammalian species cope differently with these changes. Understanding the physiological responses of organisms to different seasons and analyzing the mechanisms that account for intra- and interspecific differences and the ecological consequences of these variations is important to predict species responses to climatic changes. Consequences of climatic changes will be most pronounced in climatically already challenging habitats, such as the dry regions of western Madagascar.

We aimed to identify the seasonal responses and adaptive possibilities in energy budgeting of *Lepilemur edwardsi*, a small primate of this habitat, by measuring metabolic rate (open-flow respiratory) and skin temperature in the field during different seasons. Resting metabolism was generally low, but our study did not detect any signs of regular heterothermic episodes, despite the fact that these are known in other sympatrically living lemurs with a similar lifestyle. Surprisingly, *L. edwardsi* responded by elevating its resting metabolic rate in the poor-resourced dry season, compared to the better-resourced wet season, presumably to master detoxification of their increasingly toxic diet. As body mass decreased over this time, this strategy is obviously not energetically balanced on the long term. This is cause for concern, as it suggests that *L. edwardsi* has a very small leeway to adjust to changing conditions as experienced due to climate change, as dry season are expected to become longer and hotter, straining water budgets and food quality even more. Moreover, our findings highlight the importance of studying physiological parameters directly in the field and under differing climatic conditions.

2.2 Introduction

Endothermic species regulate their body temperature (T_b) mainly endogenously and independently of ambient temperature (T_a). To cope with warm and dry habitats species often express strategies to save water and energy during the hottest and driest periods of the day or the year. For instance, by having a generally low metabolism (Lovegrove 2000, 2003; Swanson et al. 2017), which is sometimes accompanied by tolerance of high daily fluctuations of T_b, as is the case in some rodents and camels from hot deserts (Schmidt-Nielsen et al. 1981; Lovegrove and Heldmaier 1994), and bats roosting in open vegetation at high T_a (Reher and Dausmann 2021). This enables them to reduce their energy requirements and respiratory evaporative water loss (Schmidt-Nielsen et al. 1957). Another response is to shift the thermoneutral zone (TNZ), the range of T_a where basal metabolic rate (BMR) is sufficient to maintain normothermic T_b without any additional energetic costs, from a lower T_a (range in winter) to a higher T_a (range in summer)(Scholander 1955; Heldmaier and Steinlechner 1981). This shift is species-specific and can occur seasonally and regionally and optimizes the zone of minimal energy expenditure to the prevailing conditions (Chamane and Downs 2009; Zhao et al. 2014; Kobbe et al. 2014; Bethge et al. 2017). Adapting to unpredictable changes and fluctuations of environmental conditions can be extremely challenging, making species highly vulnerable to additional anthropogenic changes in their habitats (Pörtner and Farrell 2008; Khaliq et al. 2014; Radchuk et al. 2019). Due to their unfavorable surface-to-volume ratio, small endothermic animals (Kleiber 1947; Hayssen and Lacy 1985), are especially affected by varying and extreme T_a (Pörtner and Farrell 2008). Some small mammals and birds even abandon endothermy and become heterothermic to survive lean seasons, even at comparatively high T_a (McKechnie and Mzilikazi 2011; Dausmann et al. 2012). Heterothermy describes physiological states of active depression of metabolic rate (MR) and thereby a controlled interruption of normothermia and almost all bodily functions, that can last from a few minutes (daily torpor) to many months (hibernation; Geiser, 2004; Heldmaier *et al.*, 2004; Ruf and Geiser, 2014; Reher and Dausmann, 2021). Small mammalian species that remain normothermic throughout the year often show a lower MR in dry and unpredictable habitats in comparison to species in habitats with more reliable resource availability (Speakman 1999; Lovegrove 2000). This is especially true for arboreal folivores that have a generally low metabolism, presumably due to their unfavorable diet that is comparatively low in nutrients and high in fiber and cellulose. Consistent with this low metabolism is the sedentary lifestyle and relatively low muscle mass of arboreal folivores (McNab, 1978a; Cork and Foley, 1991), e.g., in the three-toed sloth (*Bradypus variegatus*, Pauli *et al.*, 2016) or the slow loris (*Loris tardigradus*, Müller *et al.*, 1985).

Indeed, members of the arboreal, tropical and nocturnal Malagasy lemur family Lepilemuridae (e.g., *Lepilemur ruficaudatus* and *L. petteri*) show particularly low mass-specific resting MR (RMR, metabolism during inactivity; Schmid and Ganzhorn, 1996; Bethge *et al.*, 2017, published as *L. leucopus*). The only genus within this family, the sportive lemurs, are able to survive in even the most unpredictable and hottest habitats of Madagascar, from the dry south with its distinctive xerophytic spiny forest to the west with its deciduous dry forests and high degree of seasonality (Mittermeier et al. 2010). Given the body size, lifestyle and diet of this lemur genus, it was previously hypothesized that sportive lemurs might become heterothermic to cope with lean seasons (Ganzhorn 1988; Schmid and Ganzhorn 1996). Moreover, members of a related lemur family, the Cheirogaleidae (*Cheirogaleus spp*. and *Microcebus spp*.) that live sympatrically with *Lepilemur spp*., undergo daily torpor and hibernation regularly as a seasonal response (Dausmann and Warnecke 2016). However, up to now there are no scientifically confirmed cases of heterothemic episodes in the Lepilemuridae. *Lepilemur petteri,* living in the dry south of Madagascar, adapts seasonally by reducing its RMR in the hotter wet season and elevating its resting metabolism in the food constrained and more unpredictable dry season. Overall, this species seems to be more constraint by high T_a, suggesting a small scope to tolerate changing environmental conditions.

Lepilemur edwardsi (800 – 1000g; head-body length ~27cm), sister species of *L. petteri*, lives in less unpredictable, but also challenging conditions in the dry deciduous forests of western Madagascar (Mittermeier et al. 2010), a region characterized by a pronounced seasonality. The wet, food abundant season is characterized by an average T_a between 23°C and 36°C and a high yearly mean precipitation of 1562.5mm

(Randrianambinina et al. 2007; Klein et al. 2018). During the dry season there is no precipitation for about 6 months and animals have to physiologically and behaviorally adapt to the accompanying scarcity of food and water together with slightly lower temperatures (T_a averages around 37°C during the day and 17°C during the night; Randrianambinina *et al.*, 2007). These dry season conditions are assumed to be extremely challenging for *L. edwardsi* due to its folivorous diet (Mittermeier et al. 2010), as the food trees grow young high-quality leaves in the wet season, which decrease their protein content with leaf age and thus the ongoing dry season (Thalmann 2001; Ganzhorn 2002). *L. edwardsi* is pair-living and rests in tree holes during the day (Rasoloharijaona et al. 2003; Mittermeier et al. 2010). These resting sites seem to be an important resource for this species to shelter from climatic extremes, thus making the existing and ongoing habitat destruction due to climatic changes but also due to anthropogenic impacts, especially severe (Rasoloharijaona et al. 2003; Wulff 2020).

In this study, we investigated the physiological adaptations of *L. edwa*rdsi to seasonal changes in climatic conditions and food availability and quality, not least to allow for inferences on flexibility in the face of climate change and anthropogenic disturbances on this highly endangered species (Louis et al. 2020). *L. edwardsi* lives farther north than *L. petteri*, in a different habitat with different climatic conditions, which is why we wanted to examine, if the physiological adaptations found in *L. petteri* are a general trait of sportive lemurs or solely a specific adaptation to their respective habitats. In particular, we wanted to verify whether heterothermic episodes occur in *L. edwardsi*, and if and how resting metabolism is adjusted throughout the season by measuring RMR and skin temperature (T_{skin}) in the field under natural conditions and during different seasons.

2.3 Materials & Methods

Study site

We conducted the study around the Ampijoroa Forest Station within the Ankarafantsika National Park (S 16° 19’, E 46° 48’) in Jardin Botanique A, a 30.6 ha research area with dry deciduous forest in western Madagascar. The region is characterized by a distinct wet season from November to April and a food constraint dry season from May to October. Temperatures are generally high with minimal T_a around 23 °C in the wet and 17 °C in the dry season, and daily maximal T_a around 36 °C and 37 °C, respectively, with almost no precipitation for up to six months (Randrianambinina et al. 2003, 2007).

Animal captures

We captured 27 individuals of *Lepilemur edwardsi* during three sampling periods in 2018 and 2019. We measured the RMR during the wet season to get the baseline RMR, when resources are abundant and T_a is relatively stable. During the early dry season, individuals have to respond to the beginning of the deteriorating environmental conditions and food availability. Whereas, during the late dry season individuals have to cope with the full extent of strongly decreased food availability and the ongoing lack of rainfall. 11 individuals (9 males, 2 females) were captured during the wet season (January – February 2018), 12 individuals (6 males, 6 females) during the early dry season (Mai – July 2019) and 17 individuals (7 males, 10 females) during the late dry season (August – October 2018). We located the individuals in their sleeping sites in the mornings (09:00 – 12:00 h), captured them by hand and anesthetized them with 0.1 ml/kg ketamine hydrochloride (Ketamidor ® 100mg/ml, WDT, Garbsen, Germany). Afterwards, we weighed the individuals to the nearest 1 g, sexed and individually marked them with a subcutaneously injected passive integrated transponder (Trovan, EURO I.D. Usling GmbH, Weilerswist, Germany). Individuals were released after the measurements of RMR at their trapping locations at dusk the next day. Some individuals were used in more than one sampling period. This is accounted for in the statistical models.

Measurements of metabolic rate

Metabolic rate was determined by measuring oxygen consumption using open-flow respirometry in pull mode with a portable oxygen analyzer (OxBox, designed and constructed by T. Ruf & T. Paumann, FIWI, University of Veterinary Medicine Vienna, Austria) with chemo-electric oxygen sensors (Bieler & Lang, Achern, Germany; for more details, see: Dausmann *et al.*, 2009) powered by a 12V (100Ah) battery. The oxygen sensor was calibrated immediately before and after each field trip in the laboratory with calibration gas prepared by a gas-mixing pump (20.95%, 20.32% and 19.90% atmospheric oxygen (O_2); type 2KM300/a; H. Wösthoff Messtechnik GmbH, Bochum, Germany). A closed box served as respiratory chamber (30 cm x 25 cm x 23 cm). It was connected to the oxygen analyzer with airtight tubes (Tygon, Saint-Gobain, Courbevoie, France) and had an air inlet on the opposite side of the box (scheme of the setup: Fig. 1). Measurements started in the mornings (around 09:30 – 12:00 h, depending on capture time) and were stopped after approximately 24h, thus providing continuous measurements over this time. We conducted the measurements directly in the field under naturally fluctuating daily T_a. All animals were constantly monitored to ensure wellbeing and calm behavior. Generally, individuals remained calm during the measurements. In the few cases were animals seemed stressed, the measurements were discontinued and they were immediately returned to their capture sites. Airflow was set at 70 – 100L/h, which has proved appropriate for our set-up in many studies (e.g. Turner *et al.*, 2017), matching the size of the respiratory chamber and adequate wash-out rates, and was constantly monitored and regulated by the flowmeter of the oxygen analyzer (OxBox). The sample air was dried with silica gel and filtered before entering the flowmeter, subsampling and oxygen analysis, and oxygen content was determined once per minute. Reference air (ambient air from the environment next to the respiratory chamber) was also dried with silica gel and filtered before entering the analyzer and analyzed for 5 minutes every hour to account for drift of the oxygen sensors (Fig. 1). We did not scrub the incurrent air into the respiratory chamber of water vapor, as we wanted to measure natural responses to prevailing conditions and avoid physiological stress by being exposed to completely dry air. Volumes were corrected for standard conditions for temperature and pressure, with barometer pressure provided by a nearby weather station and temperature of the airflow measured by the flowmeter of the oxygen analyzer (OxBox). When CO_2 is not scrubbed from the excurrent sample air or measured, as we only had access to silica gel in the field, we can assume a value based on the volume of O_2 ($volO_2$) and substitute the volume of CO_2 by using the respiratory quotient

(RQ) of volO_2 (Lighton 2008). Hence, the rate of oxygen consumption ($\dot{V}O_2$) was calculated as ml $O_2 h^{-1}$ with the equation given by Lighton (2008) specifically for this set-up: $\dot{V}O_2 = \frac{FR_e(F_iO_2 - F'_eO_2)}{[1 - F_iO_2(1 - RQ)]}$, where FR_e is the excurrent flow rate and $(F_iO_2 - F'_eO_2)$ gives the difference in fractional concentration of oxygen entering and exiting the respiratory chamber. When assuming an RQ of 0.85 (oxidation of approximately 50% carbohydrate and 50% fat; see below), the maximum error amounts approximately to 3% (Koteja 1996, Lighton 2008), which is acceptable for our study, as we are interested in patterns and adaptations. Additionally, we scrubbed most of the water from the sample and reference air indicated by $F'eO_2$ (Lighton 2008). The data of the oxygen analyzer were analyzed using ClampFit v10.3.1.4 (Molecular Devices, Sunnyvale, USA); see Dausmann (2009) for details. To calculate mass-specific RMR, we divided RMR by body mass (ml O_2 $h^{-1} g^{-1}$); all RMR data is given as mass-specific RMR.

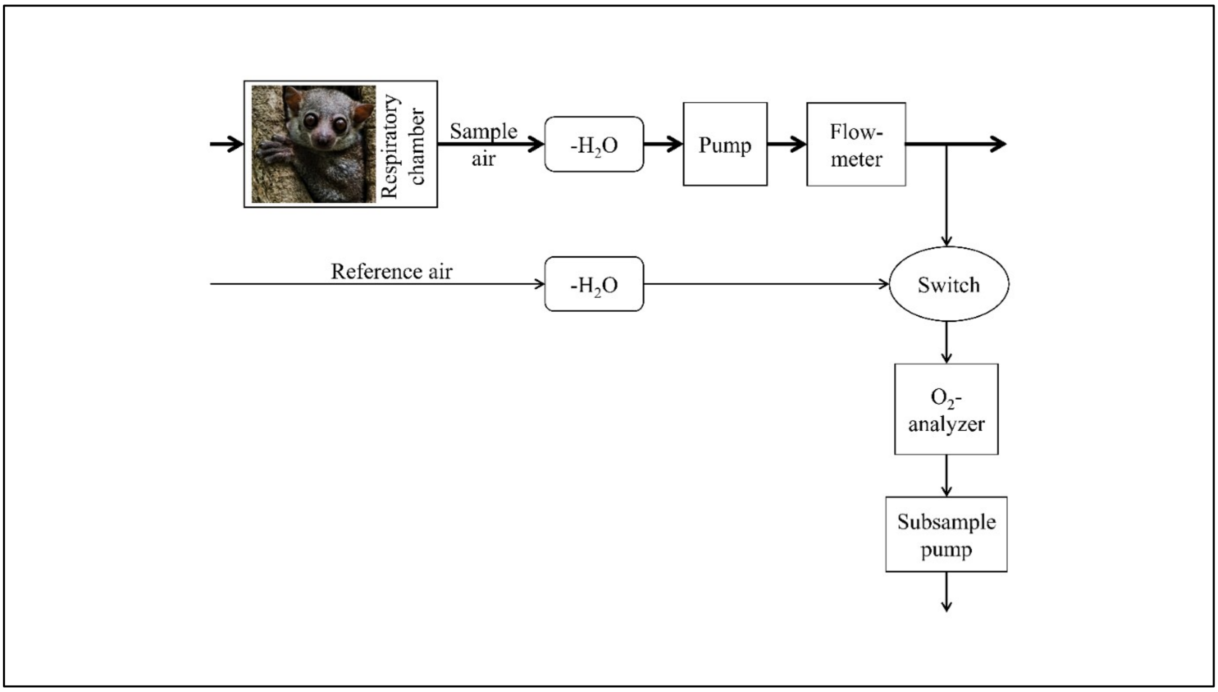

Fig. 1: Setup of the pull-mode O_2-respirometry system using excurrent flow rate measurements. Air is pulled through the respiratory chamber, where the animal is located during the measurements, with a flow rate of 70-10 L h^{-1}. We scrubbed the air of H_20 using silica gel. The dry air is pumped through the flowmeter, and drawn through the oxygen analyzer and subsampler using a smaller pump. The reference air is scrubbed of H_2O using silica gel, before it is drawn through the oxygen analyzer for 5 minutes every hour. (Photo by A. Wulff).

Temperature measurements

Shortly before the RMR measurements we measured the rectal temperature (T_{rectal}) of the individuals as a proxy of core body temperature with a handheld thermometer (resolution: ±0.1°C, MT1P21 flexible, Microlife AG, Widnau, Switzerland). During respirometry, T_{skin} of the animals was recorded to the nearest 0.5°C once per minute using a temperature data logger (custom-made by J. Sannert, Hamburg, Germany) mounted on a collar with the temperature sensor on the inside in direct contact with the skin (4g) Although T_{skin} does not precisely represent core T_b, numerous studies have shown that it gives a valid (and less invasive) proxy to gain insights into general patterns of T_b regulation in small mammals, especially during resting phases, when possible torpor episodes would be expected (Dausmann 2005; Langer and Fietz 2014). Directly before release of the individuals, we equipped the majority of the individuals with a temperature data logger (Thermochron iButton, Maxim Integrated Products, San Jose, USA) glued to the inside of a radio transmitter collar (total weight: 12.5 g; PIP3 longlife tag, Biotrack Ltd., Dorset, UK) to measure longterm T_{skin} (wet season: N = 2; early dry season: N = 7; late dry season: N = 12) every 30 minutes. At the end of the respective sampling period, we recaptured these individuals via radio tracking and recovered the data loggers. The duration of these T_{skin} measurements ranged from 12 to 39 days, depending on the day of respirometry. We defined daily torpor episodes to occur if T_{skin} decreased below 25°C for at least one hour (following the definition of Kobbe *et al.*, 2011), which is clearly below the regular circadian rhythm, with MR decreasing concordantly (if occurring during RMR measurements). We are aware that there might be an influence of environmental heat load on T_{skin}, and therefore chose the threshold for torpor episodes with a corresponding margin.

During respirometry, the temperature in the respiratory chamber ($T_{chamber}$) was recorded simultaneously once per minute with data loggers (Thermochron iButton) placed inside the respiratory chamber. Additional temperature loggers (Thermochron iButton) were placed in- and outside (on the shady side) of tree holes (wet season: N = 13, early dry season: N = 5, late dry season: N = 11) where *L. edwardsi* were known to rest during the day in the dry forest, which recorded temperature in the forest (T_{forest}) and in the treehole ($T_{treehole}$) every 30 minutes.

Data analysis and statistics

Data were analyzed using Cran R (R Core Team 2019) and the packages “plyr” (Wickham 2011), “dplyr” (Wickham et al. 2019), “lubridate” (Grolemund and Wickham 2011), “ggplot2” (Wickham 2016), “mgcViz” (Fasiolo et al. 2020) and “mgcv” (Wood 2017).

We only included the lowest 30% of the continuous daily and nightly RMR data of each individual in the analyses to exclude any phases of short stress, disturbance or activity of the individuals (Bethge et al. 2017). This is a conservative approach to ensure only resting values are considered. To control for autocorrelation, we calculated hourly means per individual per season and assigned these to the average $T_{chamber}$ during this hour. We wanted to examine the influence of different variables on the RMR (ml O_2 h^{-1} g^{-1}) of *L. edwardsi* and model this functional relationship. As the visual data exploration showed a non-linear relationships between the explanatory variables (T_a, month, sex) and the response variable (RMR) we chose a generalized additive mixed model (GAMM) to estimate smooth functional relationships between theses variables (Zuur et al. 2009; Wood 2017). The pACF-plots still showed a slight autocorrelation in the data, thus we controlled for autocorrelation using different approaches: firstly, we included time as a random effect; secondly, we implemented an autoregressive structure (autoregressive model (AR1), autoregressive-moving-average model (ARMA)) in the GAMM and thirdly, we used a combination of both approaches (Appendix, Table 1). We selected the “best model” based on Akaike’s information criterion (AIC) and a visual approach using the gam.check()-function of the package “mgcv” (Zuur *et al.*, 2009; Wood, 2017; Pedersen *et al.*, 2019). We modelled 12 GAMMs with different explanatory variables and random effects and incorporated an autoregressive structure into these models (AR1, ARMA). The GAMM with time as a random effect performed better than the models with the autoregressive structure, showed a lower AIC and explained 64.3% of the deviance. Hence, our final model included smoother for $T_{chamber}$ (cubic regression spline smoother), month as a proxy for season (cyclic cubic spline smoother), a smoother for the interaction of $T_{chamber}$ and month, and sex (two-level categorical variable; Table 1). Since some individuals were measured in multiple seasons, we included ID and time as random effects and used restricted maximum-likelihood (REML) instead of cross-validation criteria for smoothing parameter selection (following the recommendation of Wood, 2011).

Since the data were non-independent (some individuals were measured more than once in different seasons) and showed non-normality (Shapiro-Wilk Test: $p < 0.001$), we applied an unpaired Wilcoxon rank sum test with a Bonferroni correction to test for differences between the body mass and the minimal and maximal T_{skin} of all individuals between the seasons. As the wet season sample size for T_{skin} was relatively small (N = 2), the statistical comparisons between the wet season and the two sampling periods during the dry season, should be interpreted cautiously but nevertheless gives a good impression of the overall seasonal adaptations in thermoregulation of *L. edwardsi*.

We calculated energy expenditure (REE; kJ h^{-1}) and daily resting energy expenditure (DREE; kJ $24h^{-1}$) under field conditions assuming an average RQ of 0.85 (Dausmann *et al.*, 2009) and thus an oxycaloric equivalent of 20.37kJ/L O_2 (Schmidt-Nielsen 1997). To interrelate these data to the environmental conditions, average T_{forest} (natural habitat) for each hour of the day was calculated for each sampling period separately (wet season, early dry season and late dry season), to account for the daily rise and fall in temperature, and the RMR determined from the respiratory measurements during this sampling period for this particular temperature was appointed. For DREE the values for each hour throughout the day were added.

Additionally, we calculated the mean energy expenditure of the lowest 30% of the RMR data for each individual during each season, to compare our results with data from other primates by using the mass-specific BMR scaling equation for primates provided by McNab (2008) (BMR (kJ h^{-1}) = 0.037 x body $mass^{0.792}$). In our field set-up, we did not meet all the requirements for BMR measurements, however we are confident that the lowest RMR-values approximate BMR as individuals were resting during the day and the last food intake had been several hours before.

2.4 Results

The median of the minimal T_{forest} during the wet season amounted to 22.9°C during the night and the median maximal T_{forest} to 29.1°C in the middle of the day. T_{forest} in the early dry season fluctuated between the median minimal T_{forest} of 20.75°C during the night and maximal median T_{forest} of 27.5°C during the day. During the late dry season T_{forest} fluctuated more strongly with a minimal median T_{forest} of 22.5°C, however maximal median T_{forest} was higher with 32.5°C during the daytime (Fig. 2). The mean body mass of males and females did not differ significantly between the sexes in any of the different sampling periods. Mean body mass of both sexes in the wet season amounted to 827 ± 84g (N = 11) and was significantly higher in the early dry season with a mean body mass of 929 ± 104g (N = 12; Wilcoxon rank sum test; wet season vs. early dry season, $p = < 0.001$). Mean body mass in the late dry season was 795 ± 111g (N = 12) and was significantly lower than mean body mass in the wet season (Wilcoxon rank sum test; wet season vs. late dry season, $p = < 0.001$). Furthermore, individuals lost weight during the dry season, as mean body mass in the early dry season was significantly higher than in the late dry season (Wilcoxon rank sum test; early dry season vs. late dry season, $p = < 0.001$). Five females were probably pregnant in the late dry season with a mean body mass of 1049 ± 32g (Table 1).

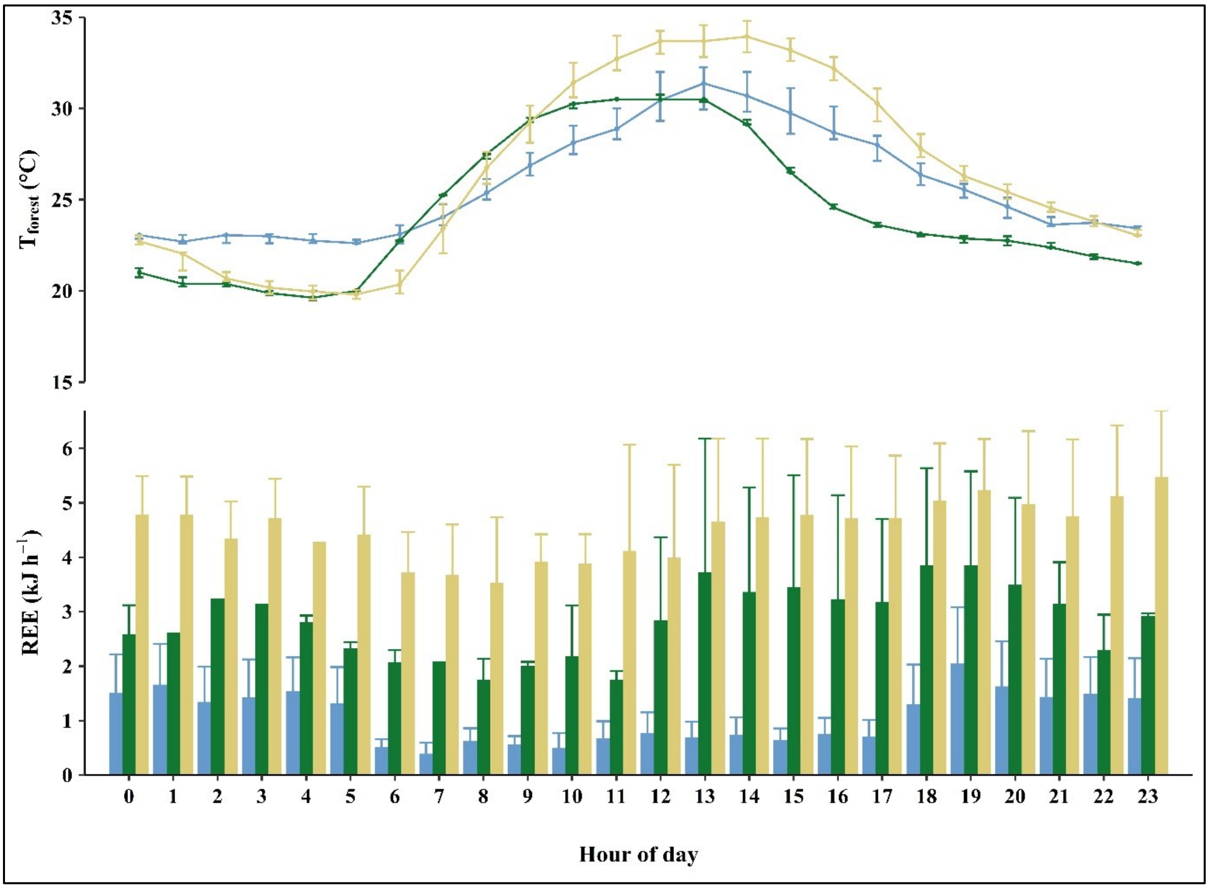

Fig. 2: Resting energy expenditure of *Lepilemur edwardsi* and daily fluctuations of ambient forest temperature during the three sampling periods. Hourly resting energy expenditure (REE) of *Lepilemur edwardsi* (wet season: N = 11, early dry season: N = 12, late dry season: N = 17) and median hourly ambient forest temperature (T_{forest}) in the wet season (blue columns; blue line), the early dry season (green columns; green line) and the late dry season (yellow columns; yellow line) in the dry forest of Ampijoroa. The whiskers of the REE show the standard deviation and the median T_{forest} whiskers show hourly maximal T_{forest} and minimal T_{forest}.

Table 1: Individuals of *Lepilemur edwardsi* measured in the wet season, early dry season and late dry season; sex of the individuals (m = male, f = female); body mass (BM); mean skin temperature (T_{skin}), rectal temperature (T_{rectal}) of the individuals before the metabolic rate measurements and mean resting metabolic rate for each season (RMR). During some of the T_{skin} measurements, the $_{Tskin}$-logger failed, given as NA.

		Wet season 2018					**Early dry season 2019**					**Late dry season 2018**				
Ind.	**Sex**	**BM** (g)	**Mean T_{skin}** (°C)	**T_{rectal}** (°C)	**Mean RMR** (ml O_2 h^{-1} g^{-1})	**Mean RMR** (J^{1} h^{1} g^{-1})	**BM** (g)	**Mean T_{skin}** (°C)	**T_{rectal}** (°C)	**Mean RMR** (ml O_2 h^{-1} g^{-1})	**Mean RMR** (J^{1} h^{1} g-1)	**BM** (g)	**Mean T_{skin}** (°C)	**T_{rectal}** (°C)	**Mean RMR** (ml O_2 h^{-1} g^{-1})	**Mean RMR** (J^{1} h^{1} g^{-1})
1	m	671	36.0±0.7	36.4	0.04±0.02	0.89±0.45						775	37.0±0.2	36.8	0.29±0.03	5.88±0.67
2	m	877	36.4±0.8	36.4	0.08±0.04	1.69±0.88										
3	m	947	35.9±0.3	36.7	0.06±0.03	1.29±0.67	954	36.2±0.6	36.3	0.09±0.02	2.01±0.42					
4	m	798	36.0±0.6	34.5	0.08±0.04	1.65±0.84										
5	f											996	NA	34.9	0.33±0.03	6.70±0.55
6	m	744	36.4±0.3	35.7	0.07±0.03	1.48±0.58										
7	m	836	NA	35.9	0.07±0.03	1.59±0.69						880	NA	37.2	0.28±0.03	5.76±0.63
8	m	910	36.5±0.9	35.0	0.05±0.03	1.04±0.58						952	37.2±0.4	36.8	0.21±0.02	4.19±0.43
9	m	965	35.2±1.1	35.1	0.02±0.01	0.43±0.24						862	NA	35.6	0.28±0.02	4.43±0.39
10	f						946	36.8±0.4	36.4	0.17±0.02	3.61±0.41	1064	37.1±1.1	38.2	0.25±0.07	5.00±1.40
11	m	944	35.9±0.5	35.3	0.08±0.03	1.66±0.57	934	36.5±0.5	35.8	0.15±0.02	3.16±0.44	914	NA	38.3	0.33±0.02	6.62±0.41
12	f	747	36.4±0.4	36.4	0.02±0.01	0.48±0.27	809	35.8±0.7	35.2	0.49±0.02	9.97±0.48					
14	f	864	36.6±0.3	35.9	0.04±0.02	0.89±0.45						911	35.7±0.1	37.1	0.24±0.03	4.88±0.59
15	f											655	32.3±2.9	35.0	0.22±0.08	4.49±1.56
16	f											684	37.3±0.2	37.4	0.22±0.05	4.42±0.92
17	m						943	37.0±0.4	37.1	0.07±0.02	1.40±0.45	887	37.5±0.6	38.0	0.31±0.09	6.41±1.82
18	f											796	35.6±1.4	35.6	0.26±0.05	5.31±0.94
19	f						938	37.0±0.4	35.7	0.20±0.03	3.98±0.70	1047	34.5±1.1	38.4	0.17±0.03	3.55±0.64
20	f											594	36.9±0.5	38.0	0.29±0.02	5.83±0.45
21	f											1084	34.8±0.9	36.4	0.19±0.04	3.82±0.89
22	m											801	35.4±1.6	37.1	0.30±0.06	6.14±1.20
23	f						981	37.3±0.3	NA	0.27±0.02	5.43±0.45	1066	37.1±0.5	35.7	0.26±0.02	5.30±0.35
26	f						1136	NA	35.8	0.10±0.02	1.95±0.40					
28	f						814	NA	35.8	0.14±0.03	2.91±0.60					
31	m						898	37.3±0.4	34.6	0.19±0.01	3.88±0.20					
32	m						858	36.6±0.2	35.8	0.18±0.01	3.74±0.26					
33	m						819	37.8±0.9	35.6	0.20±0.01	4.03±0.30					

Skin temperature of free-ranging *L. edwardsi*

The long-term field T_{skin} measurements of all individuals showed strong fluctuations ranging from 31.1°C to 39.6°C in the wet season (N = 2), from 27.6°C to 41.1°C in the early dry season (N = 7) and from 28.0°C to 40.1°C (N = 12) in the late dry season. During the wet season, individuals reached a maximal mean T_{skin} of 36°C between 12:00 and 17:00 hours when mean T_{forest} was highest (26.6°C – 29.5°C). Minimal mean T_{skin} of 34.4°C was reached at 04:00 hours when mean T_{forest} was 21.6°C during their active phase at night. Mean T_{skin} in the early dry season was highest between 12:00 and 17:00 hours with 36°C at a mean T_{forest} between 23.7°C (17:00 hours) and 29.2°C (12:00 hours); and lowest at 04:00 hours with a minimal mean T_{skin} of 33.8°C and a mean T_{forest} of 18.1°C. In the late dry season individuals had a maximal mean T_{skin} of 37°C between 14:00 and 17:00 hours at a mean T_{forest} between 28.8°C (17:00 hours) and 32.2°C (14:00 hours). During the night *L. edwardsi* showed a minimal mean T_{skin} of 33.6°C between 03:00 and 04:00 hours at a mean T_{forest} of 19°C. Thus, maximal T_{skin} of all individuals during all days of measurement in the wet season did not differ from the maximal T_{skin} in the early dry season, but was significantly lower than in the late dry season (Wilcoxon rank sum test; wet season vs. early dry season, p = 0.46; wet season vs. late dry season, p = 0.009). Whereas maximal T_{skin} during the late dry season was significantly higher than in the early dry season (Wilcoxon rank sum test; late vs. early dry season, $p = < 0.001$). Minimal T_{skin} of all individuals and all days of measurement in the two sampling periods of the dry season did not differ from each other (Wilcoxon rank sum test; late vs. early dry season, p = 0.27). However minimal T_{skin} in the wet season was significantly higher than during both sampling periods of the dry season (Wilcoxon rank sum test; wet season vs. early dry season, $p = < 0.001$; wet season vs. late dry season, p = 0.001) and followed the daily T_a fluctuations. Overall, T_{skin} fluctuated more during the two sampling periods in the dry season, whereas T_{skin} in the wet season did not drop below 31°C (Fig. 3). T_{skin} dropped a few degrees at the beginning of the daily activity period at dusk (around 18:00 hours) with decreasing T_{forest} during all seasons and ranged at lower levels during the night while the individuals were active. This might be due to an increased influence of

T_a on the T_{skin} logger when the animals are not curled up during activity. Nevertheless, as *L. edwardsi* rests in quite narrow tree holes during the day, we expect T_{skin} to approximate T_b during rest. At dawn (around 06:00 hours), T_{skin} increased again continuously with increasing T_{forest} resp. $T_{treehole}$. Particularly, during the late dry season *L. edwardsi* seems to take advantage of the increasing daytime T_{forest} for passive uptake of external heat (resp. $T_{treehole}$; Fig. 3).

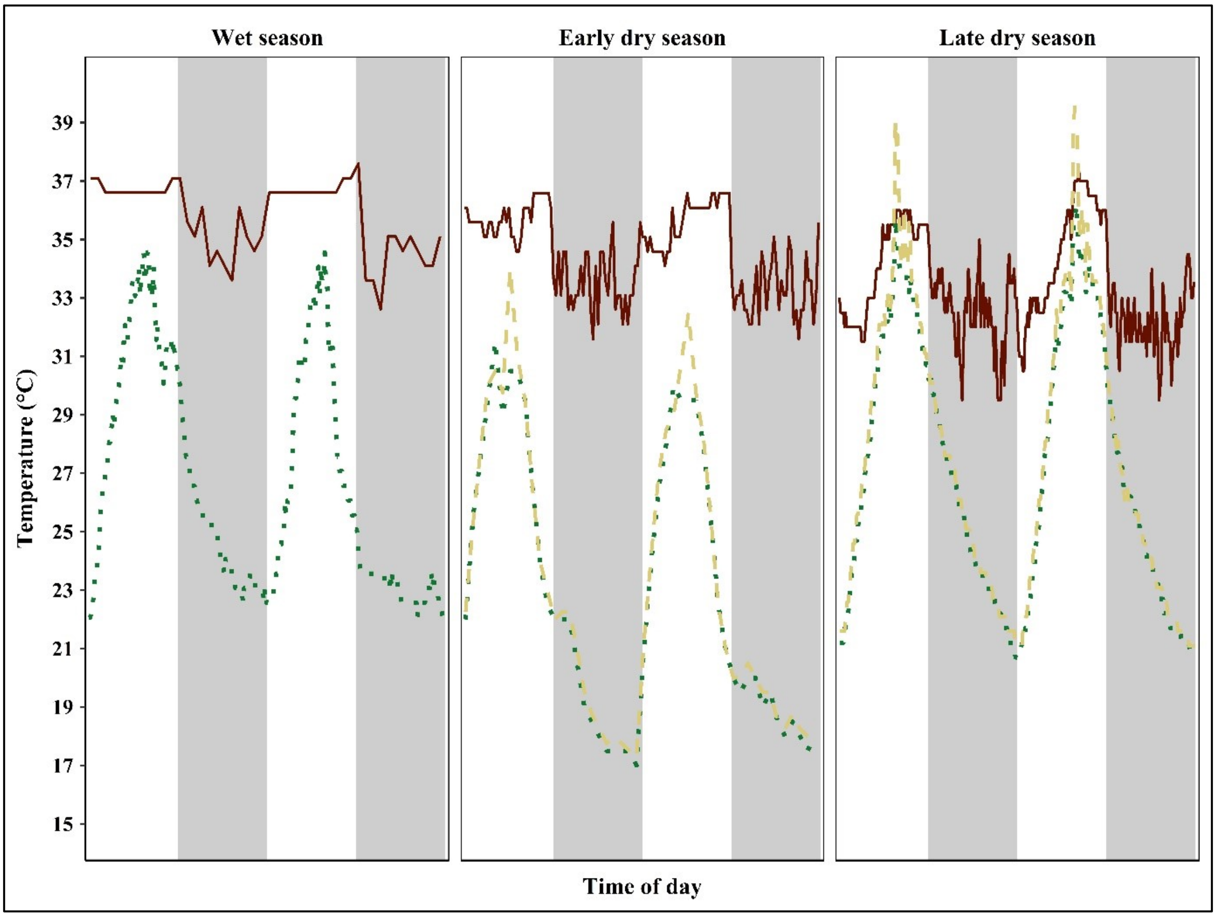

Fig. 3: Fluctuations of resting site temperatures and of skin temperature of *Lepilemur edwardsi* during the three sampling periods. Skin temperature (red, solid line) and ambient temperature outside (green, dotted line) and inside (yellow, dashed line) of the resting site of the same individual of *Lepilemur edwardsi* on two consecutive days during the wet season, early dry season and late dry season. Grey blocks show the dark phase and hence phase of activity of *L. edwardsi.*

Seasonal differences in RMR and T_{skin}

There was no evidence of torpor episodes in any of the individuals during the wet and the early dry season as T_{skin} never dropped below 25°C (Table 1), neither during the RMR measurements, nor in the free-ranging individuals. However, in the late dry season one individual (Lepilemur 15) showed a sudden drop of T_{skin} from 31°C to 26°C at around

6:00 hours and a $T_{chamber}$ of 19°C, followed by a distinct drop in RMR during the measurements, while being curled up in a resting position (Fig. 4). T_{rectal} of this individual was below 33°C at the end of the RMR measurement (unfortunately, the handheld thermometer could not measure temperatures below 33°C).

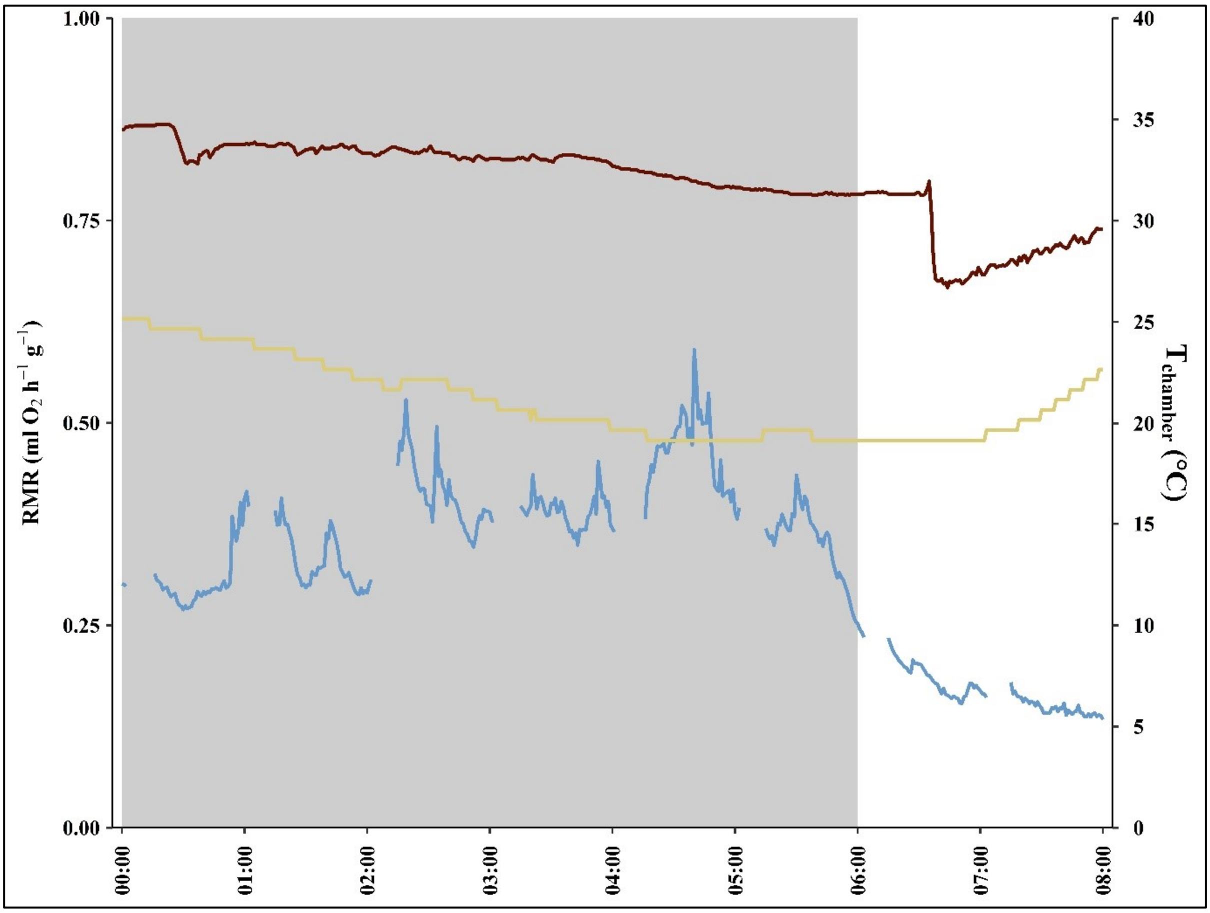

Fig. 4: "Torpor-like" episode of one individual towards the end of respirometry. Resting metabolic rate (RMR; blue line), skin temperature (T_{skin}; red line) and ambient box temperature ($T_{chamber}$; yellow line) of one individual of *Lepilemur edwardsi* during the late dry season showing a brief "torpor-like" episode. The grey block shows the dark phase.

Generally, T_{skin} during the RMR measurements averaged around 36.1 ± 0.8°C (N = 11) in the wet season, 36.8 ± 0.8°C (N = 12) in the early dry season and 35.4 ± 2.1°C (N = 17) in the late dry season (Table 1). Whereas T_{chambe}r fluctuated between 24.6 – 31.1°C in the wet season, 21.6 – 32.0°C in the early dry season and 19.1 – 40.1°C in the late dry season. T_{recta}l measured right before the beginning of the RMR-measurements (with an approximate time lag of <2 minutes before the first T_{skin} measurement), did not differ from the first data point of the T_{skin} measurements of all individuals during respirometry

(Pearson's Chi-squared test; p = 0.31). At the same $T_{chamber}$, individuals had the lowest mean MR in the wet season (0.06 ± 0.04ml O2 h^{-1} g^{-1}), intermediate mean RMR in the early dry season (0.17 ± 0.10ml O2 h^{-1} g^{-1}) and the highest mean RMR in the late dry season (0.25 ± 0.07ml O2 h^{-1} g^{-1}; Table 2; Fig. 5, 6).

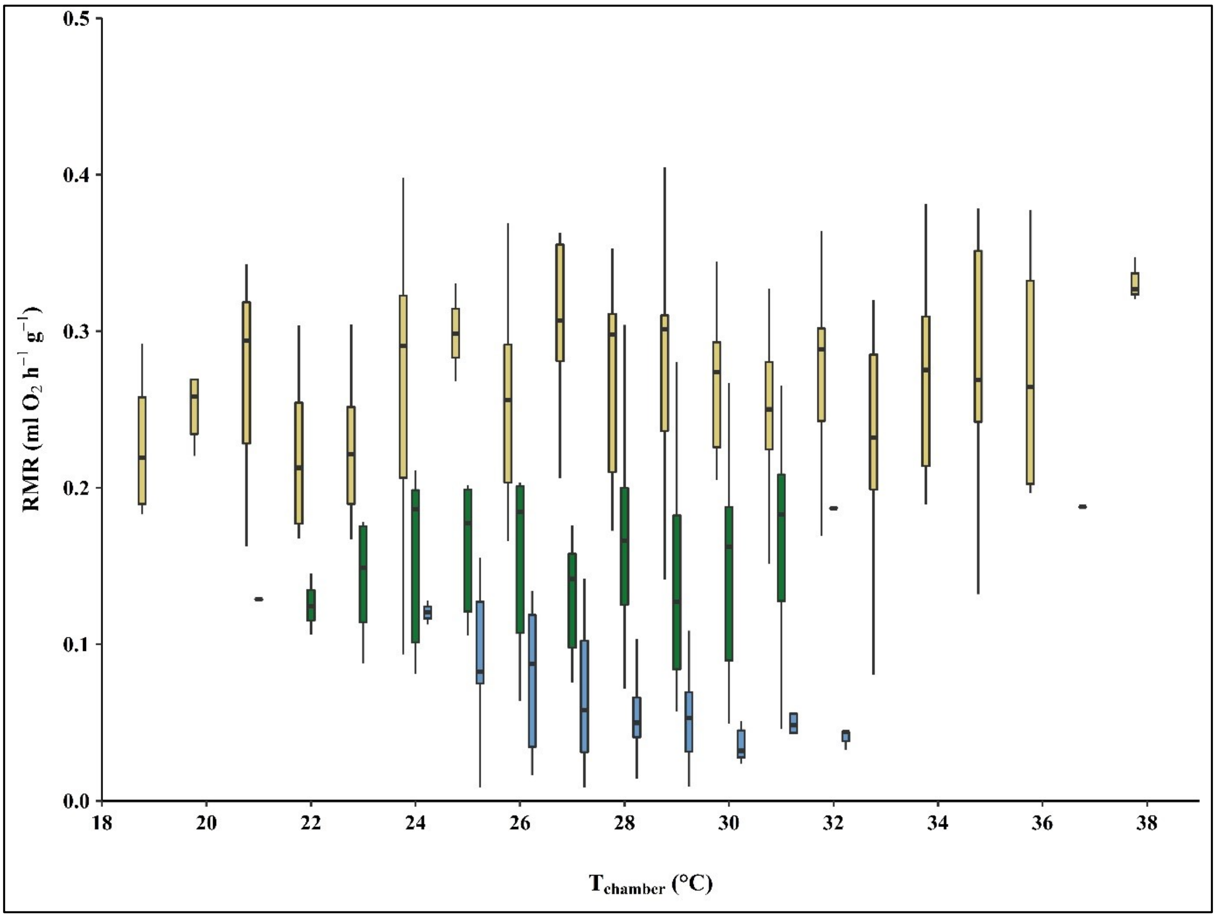

Fig. 5: Seasonal differences in resting metabolic rate of *Lepilemur edwardsi* at different ambient temperatures (respiratory chamber). Resting metabolic rates (RMR) of *Lepilemur edwardsi* at different ambient chamber temperatures ($T_{chamber}$) during the wet season (blue; N = 11), early dry season (green; N = 12) and late dry season (yellow; N = 17). The boxplots show medians and quartiles, whiskers show maxima and minima.

To examine the seasonal influence and the influence of ambient $T_{chamber}$ on the RMR of *L. edwardsi* we modelled the data with a GAMM, which showed an approximate significant influence of all smooth terms, i.e. explanatory variables ($T_{chamber}$, month) on the response variable (RMR), indicating a high influence of month, i.e. season and $T_{chamber}$ on the RMR of *L. edwardsi* (Table 3).

Table 2: Mean daily resting energy expenditure (DREE), mean resting energy expenditure per hour (REE) and mean daily oxygen consumption, i.e. metabolic rate (MR) and mean mass-specific metabolic rate MR of *Lepilemur edwardsi* during the different seasons.

Season	Mean DREE (kJ 24h^{-1})	Mean REE (J^{1} h^{1} g^{-1})	Mean MR (ml O$_2$ 24h^{-1})	Mean MR (ml O$_2$ h^{-1} g^{-1})
Wet season 2018	25.1 ± 16.22	1.25 ± 0.78	1232.24 ± 796.3	0.06 ± 0.04
Early dry season 2019	75.56 ± 39.50	3.49 ± 2.05	3709.36 ± 1939.28	0.17 ± 0.10
Late dry season 2018	106.61 ± 30.65	5.13 ± 1.36	5233.5 ± 1504.87	0.25 ± 0.07

Table 3: Results of the final generalized additive mixed model (GAMM) with metabolic rate (MR) as response variable: Types of smooth terms, maximum possible degrees of freedom for the smooth terms (k'), effective degrees of freedom (EDF), index for pattern in the residuals (k-index) and p-value (calculated, based on distribution of k-index after randomizing order of residuals) for each explanatory variable. The asterisks represent the respective significance levels with $p < 0.001$***, $p = 0.001$**, $p = 0.01$* for each variable.

Explanatory variables	Smooth terms	*k'*	EDF	*k*-index	*p*-value
Ambient chamber temperature ($T_{chamber}$)	Cubic regression spline	19	4.480	0.59	0.000817***
Interaction term $T_{chamber}$: Month	Cyclic cubic regression spline	19	3.397	0.59	< 0.001***
Month	Cyclic cubic regression spline	4	2.202	0.22	< 0.001***
Individuals	Random effect	1	0.773	0.24	0.0238
Time	Random effect	1	0.898	0.85	0.0013**
Sex	Factor	-	-	-	0.453

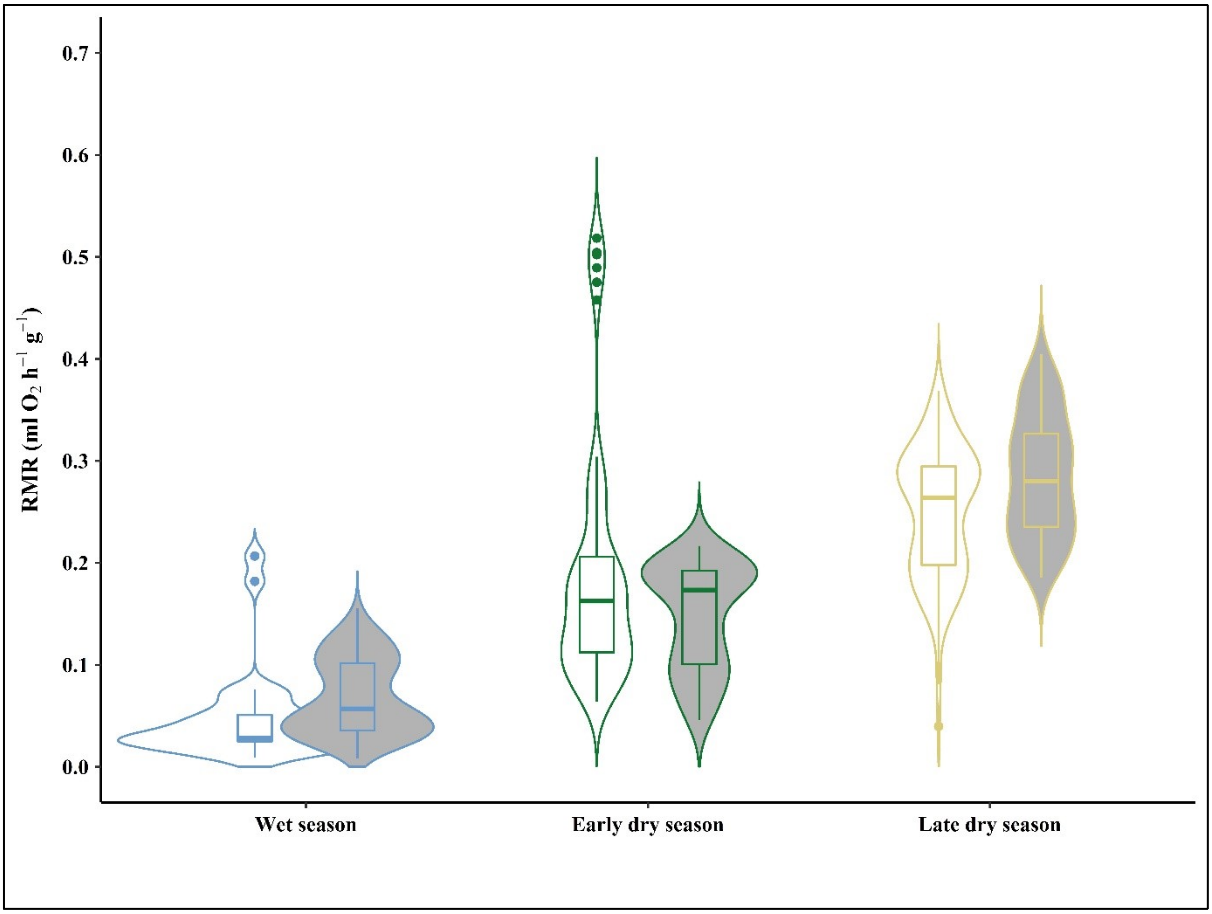

Fig. 6: Resting metabolic rate of males and females of *Lepilemur edwardsi* during the three sampling periods. Resting Metabolic rates (RMR) of males (white; wet season: N = 9, early dry season: N = 6, late dry season: N = 7) and females (grey; wet season: N = 2, early dry season: N = 6, late dry season: N = 10) of *Lepilemur edwardsi* during the wet season (blue, N = 11), early dry season (green, N = 12) and late dry season (yellow, N = 17). The boxplots show medians and quartiles, whiskers show maxima and minima. Violin shapes illustrate the distribution and density of the data.

Daily resting energy expenditure

After calculating the "typical" temperature profile for a day in each sampling period with T_{forest} (average temperature for each hour), we calculated the average DREE of *Lepilemur edwardsi* for each sampling period with the RMR determined in the respiratory measurements for this temperature and sampling period. Hourly resting energy expenditure (REE) during the early dry season was more varied than in the wet and late dry season, particularly during the hottest time of the day (Fig. 2). *L. edwardsi* showed a comparatively low DREE in the wet season of 25.1 ± 16.22kJ $24h^{-1}$ which increased more than three-fold in the early dry season to 75.56 ± 39.50kJ $24h^{-1}$. DREE in the late dry season was even higher, almost five-fold that of the wet season and 1.5fold that of the early dry season with a DREE of 106.61 ± 30.65kJ $24h^{-1}$ (Table 2).

The mean energy expenditure of *L. edwardsi* amounted to 1.05 ± 0.42kJ h^{-1} in the wet season, to 3.44 ± 1.78kJ h^{-1} in the early dry season and 4.57 ± 1.07kJ h^{-1} in the late dry season. Thus, accounting for approximately 15%, 41% and 58% of the mass-specific BMR value expected by the scaling equation in the wet season, early dry season and late dry season, respectively.

2.5 Discussion

Our measurements of the RMR of the Malagasy primate species *L. edwardsi* showed that this species has to increase its generally low mass-specific RMR to cope with seasonally changing conditions, from the food abundant wet season to the scarce dry season.

Low metabolism allows for a folivorous, potentially toxic, diet

By adopting a mainly sedentary lifestyle and a generally low metabolism, folivores living in dry habitats with unfavorable conditions (e.g. high seasonality, unpredictability of precipitation etc.) are able to save energy and particularly water (Lovegrove 2000, 2003; McNab 2008; Swanson et al. 2017). However, daily and seasonally fluctuating T_a can be a key physiological stressor, which can affect many aspects of physiology, most evidently those concerning thermoregulation, but also less obvious ones, such as hormonal responses (e.g. Weingrill *et al.*, 2004; Cristóbal-Azkarate *et al.*, 2016). Our study showed that the RMR of the small folivorous *L. edwardsi* was significantly influenced by T_a as well as generally by season and presumably by the associated changes in food and water availability. Basal energy expenditure of *L. edwardsi* during the wet season was extremely low (1.05 ± 0.42 kJ h^{-1}; Table 2), matching the generally low resting metabolism of other roughly same-sized sportive lemur species: *L. petteri* (Bethge et al. 2017) and *L. ruficaudatus* (Schmid and Ganzhorn 1996). This value is much lower (about 15% of the expected value) than the value predicted based on the BMR scaling equation for primates (McNab 2008), which would be 7.63kJ h^{-1} for primates of comparable body mass. However, these allometric scaling equations are widely debated (see for example: Kozlowski and Konarzewski, 2004; Glazier, 2005; White *et al.*, 2007) as they often do not account for ecological factors of the species, such as home range sizes (Nunn and Barton 2000). During the wet season, temperatures are quite benign in Ampijoroa, and daily fluctuations in T_a are comparatively small. Furthermore, precipitation is highest during this time of the year and food is abundant. Low metabolism might be essential for a folivorous diet, due to its relatively low quality and, in the case of sportive lemurs, toxicity (Ravelomandrato 2006). To minimize the need to invest in detoxification, sportive lemurs might decrease their general food intake, which is only possible when MR is low (McNab, 1978b; Moore *et al.*, 2015) and energetic investments (e. g., behavior, reproduction) are well economized. *L. edwardsi* is possibly able to subsist on this extremely low resting metabolism by decreasing general activity and maintaining only comparatively small home-ranges (Warren and Crompton 1997).

It was previously hypothesized that sportive lemurs use heterothermic responses, such as daily torpor (Ganzhorn 1988; Schmid and Ganzhorn 1996) to counter increased energy and water demands during the challenging dry seasons of Madagascar. However, during our study we could not detect regular employment of heterothermy. We only observed one individual showing such a physiological response (Lepilemur 15): a sudden drop in RMR and also T_{skin} (as low as 26°C; Suppl. Fig. 2). The lack of a comparable drop in other individuals suggests that maybe this “torpor-like” episode was not a regular seasonal response, but rather possibly an emergency response to low body condition, T_a and food availability, as it has been observed in the African bushbaby *Galago moholi*, and the least gerbil *Gerbillus pusillus* (Buffenstein 1985; Nowack et al. 2013). In *G. moholi* only subadult individuals with poor body condition entered daily torpor on rare occasions and individuals that showed a $T_{skin} \leq 19$°C had problems to return to normothermy without external, passive heating by T_a (Nowack et al. 2013). In the case of *G. pusillus*, food and water deprived individuals increased their torpor frequency and a critical T_a threshold during torpor was reached below 15°C (Buffenstein, 1985). The observed individual of *L. edwardsi* also had a comparatively low body mass (655g; Table 1) and was probably a sub-adult offspring from the previous breeding season. Entry into torpor commenced towards the end of the measurement (between 06:00 and 07:00 hours) when the individual had already been deprived of food for about 24h. As starvation has an effect on the use of heterothermy (Morhardt and Hudson 1966; Buffenstein 1985; Bozinovic et al. 2007), this could have been the trigger for this “torpor-like” episode.

Increased metabolism during the challenging dry season presumably allows for detoxification of low-quality food, while securing adequate water uptake

Compared to the wet season, individuals increased their resting metabolism during the dry season conditions independently of the changes in T_a (Fig. 3), especially at the end of the dry season, which probably is the harshest and most challenging time of the year for survival, as also evidenced by the significant weight loss of the individuals during this time. Over the course of the year, the RMR of *L. edwardsi* tripled from the wet to the early dry season and increased approximately five-fold from the wet to the late dry season, even at the same T_a (Table 2; Fig. 1, 3). This is analogous to the pattern found in *L. petteri*; this species was also found to increase its RMR in the dry season presumably due to increased detoxification efforts of its potentially toxic diet and the hot daytime temperatures during the dry season of southern Madagascar. During the wet season, when food is abundant, *L. petteri* has a much lower RMR and is presumably able to compensate the increased heat production by its sedentary behavior. However, due to cooler nights during the dry season in the South, *L. petteri* needs more energy to thermoregulate, requiring an increased foraging effort. This in turn results in a higher need for detoxification, which is negatively influenced by the high daytime T_a during this season (Bethge et al. 2017). Similarly, *L. edwardsi* also seems to be more affected by the effect of the hot daytime temperatures towards the end of the lean dry season on their food quality, than low nighttime temperatures. As *L. edwardsi* shows an elevated resting metabolism even at temperatures that also occur during the wet season, thermoregulation cannot be the only factor inducing this increase in resting metabolism during the (late) dry season (Fig. 1, 3). However, as the weight loss of *L. edwardsi* is presumably mostly due to the loss of, metabolic inactive, white adipose tissue, this could also contribute to an apparent increase in mass-specific RMR.

Many plants adjust their nutritional content (e.g. protein) and their plant secondary metabolites (PSMs), depending on leaf age (Coley 1983), herbivore defense (Mithöfer and Boland 2012) and T_a (Ganzhorn and Wright 1994; Ganzhorn 1995; Dearing 2013). Moreover, a folivorous diet is generally characterized by a low caloric intake (McNab, 1978a). In seasonal deciduous forests the quality and availability of leaves fluctuates throughout the year, with young, protein-rich (high-quality) leaves with low fiber in the wet season and mature, protein-poor (low-quality) leaves with high fiber in the dry season. Leaf-quality increases also within a day and is highest in the afternoon (highest protein and sugar content, but also tannin concentrations; Coley, 1983; Ganzhorn and Wright, 1994), advantageous for a species that starts feeding at the beginning of the night. *L. edwardsi* discriminates against condensed tannins, but chooses leaves with a higher alkaloid concentration. Moreover, *L. edwardsi* eats leaves with a lower protein content in comparison to the sympatric-living lemur species *Avahi occidentalis*, potentially as a result of niche-partitioning (Ganzhorn 1988; Thalmann 2001). Since PSM concentrations increase with rising T_a, and simultaneously protein content decreases (Dearing 2013; Beale et al. 2018), it seems possible that *L. edwardsi* needs to increase its food intake which in turn implicates an increase in RMR to provide for energetically expensive detoxification, at the expense of investing energy in other activities, such as territoriality or growth. As detoxification and protein turnover generate heat (Berry et al. 1985; Wang et al. 2010), the inability to dissipate this additional heat sufficiently at high T_a could be an additional factor necessitating *L. edwardsi* to resort to low-quality leaves. Furthermore, renal excretion of PSMs requires water (Foley et al. 1995) and *L. edwardsi* has to budget with this resource, as it covers its water intake solely through its food; rising T_a, particularly unanticipated ones due to climatic changes, could result in a critically insufficient water intake of *L. edwardsi*. To compensate the lower food quality and still meet sufficient nutritional and water intake, especially during the late dry season, *L. edwardsi* presumably has to eat more, which demands a higher foraging effort to satisfy the higher DREE (Coley 1983; Ganzhorn 1995; Beale et al. 2018).In order to meet their nutritional requirements when feeding on an unbalanced diet, which is probably the case for *L. edwardsi* in the dry season, individuals are presumably forced to overeat PSMs to balance undereating of some potentially necessary nutrients or vitamins (Simpson and Raubenheimer 1997, 2001; Felton et al. 2009), resulting in a higher need for detoxification and therefore higher RMR. This seems counterintuitive, but *L. edwardsi* might compensate the need of higher RMR for detoxification by a reduction in, e.g. activities such as travelling, thereby reducing the total amount of energy that is expended. Since *L. edwardsi* loses weight during the dry season, however, it seems that this offset is not completely met. Shifting its activity and digestion to the second half of the night when T_a is lower (Warren and Crompton 1997), firstly lowers the PSM intake for *L. edwardsi* as these concentrations decrease over the course of the night, and secondly could be important for detoxification, as high T_a has a negative influence on liver clearance function (Dearing 2013; Beale et al. 2018), potentially due to a decrease in hepatic enzyme activity and gene expression, resulting in a lower capacity for detoxification (Kurnath and Dearing 2013). Hence, the high fluctuations of T_{skin} in *L. edwardsi* even during its activity phase could mirror different needs for detoxification and therefore enhanced liver function of this species during the night. Particularly, in the late dry season individuals seem to follow T_{forest} resp. $T_{treehole}$ passively as T_{skin} fluctuates strongly between 28°C and 40°C (Fig. 2). Maximal T_{skin}, on the other hand, was higher during the late dry season and daily fluctuation became more pronounced during the dry season. This indicates a higher heat production during this season, presumably due to the increasing need for detoxification. Another possible explanation for the higher maximal T_{skin} during the dry season could be the higher daily T_a during this season and therefore higher amplitudes of T_{skin}. In the early mornings of the dry season, we sometimes found individuals sitting in tree crowns and outside of their tree holes basking in the sun (personal observation). They obviously use solar radiation to passively (and energetically efficiently) support endogenous thermoregulation (Fig. 2). This might enable a lowered level of metabolism, which in turn will help reduce water loss when T_a is high (Mitchell et al. 2018; Fuller et al. 2021). However, small endotherms, that meet their water requirements solely through their food, are unlikely to maintain continuous evaporative cooling, as they often do not have access to free water (Mitchell et al. 2018), as it is the case in *L. edwardsi* during the dry season.

Many herbivores and especially arboreal folivores cover their water requirements exclusively through their food. Water limitations can even influence the distribution of arboreal folivores, as forest canopies provide only few sufficient sources of free water (Krockenberger et al. 2012). When folivores reduce their food intake to avoid PSM intake and therefore costly and heat producing detoxification, they occasionally cannot cover their water requirements. This is known to be the case in the koala (*Phasocolarctos cinereus*): this species uses evaporative cooling for heat dissipation by panting, but also by licking its forearms (Degabriele et al. 1978; Degabriele and Dawson 1979). By reducing food intake to avoid detoxification, koalas may not ingest enough water to use these heat loss strategies and suffer hyperthermia (Beale et al. 2018) forcing this species to climb down from their trees and search for free water, e.g., in urban areas (Gordon et al. 1988; Lunney et al. 2012).

These interrelations of a herbivorous diet, T_a and water and energy budgets might become problematic with the ongoing increase of global T_a, as well as simultaneously extended periods of droughts and higher frequency of heat waves (IPCC 2019). Folivorous species have to spend more energy on thermoregulation and water conservation which will ultimately threaten their reproductive success (Beale et al. 2018).

Conclusion

Despite its generally extremely low RMR and therefore low energetic demands on its habitat, *L. edwardsi* presumable has only very little scope to counter impacts due to global warming, especially towards the end of the dry season, when conditions are already challenging. Similar to its sister species *L. petteri*, *L. edwardsi* seem to struggle with the effects of environmental conditions during the dry season on food and water availability and food quality, particularly towards the end of the dry season: both show increased DREE, presumably due to higher detoxification efforts (Bethge et al. 2017). Consequently, this seems to be a general physiological trait of sportive lemurs, as *L. edwardsi* and *L. petteri* show the same physiological response to seasonality and their diet. Moreover, an anthropogenically induced, further increase of T_a might also cause an increase of PSM concentrations and a decrease of protein content in the, already low quality, folivorous diet of *L. edwardsi*, while simultaneously decreasing the functionality of the liver and increasing the water loss due to heat stress. This may result in additional thermoregulatory challenges and increased energy requirements, ultimately resulting in a very small scope to deal with changing environmental conditions.

2.6 Acknowledgments

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2.8 Appendix

Suppl. Table 1: Modelled generalized additive mixed models (GAMMs) with the different explanatory variables (ambient box temperature ($T_{chamber}$ [°C]), interaction term of $T_{chamber}$ and month ($T_{chamber}$: month, month and sex), random effects (individuals and time) and the two implemented autoregressive structures (autoregressive model (AR1) and autoregressive-moving-average model (ARMA)). Also shown are the degrees of freedom, the Akaike's information criterion (AIC) and the adjusted coefficient of determination (adjusted R^2) for each model. The asterisks represent the respective significance levels with $p < 0.001$***, $p = 0.001$**, $p = 0.01$* for each variable.

Model	Explanatory variables				Random effects		Autoregressive structure	Degrees of freedom	AIC	Adjusted R^2
M1	$T_{chamber}^{*}$	$T_{chamber}$: Month***	Month***	Sex				8	730.75	0.638
M2	$T_{chamber}^{*}$	$T_{chamber}$: Month***	Month***	Sex	Individuals*			9	725.77	0.640
M3	$T_{chamber}^{***}$	$T_{chamber}$: Month***	Month***	Sex		Time***		9	724.72	0.644
M4	$T_{chamber}^{**}$	$T_{chamber}$: Month***	Month***	Sex	Individuals*	Time***		10	719.63	0.646
M5	$T_{chamber}^{**}$	$T_{chamber}$: Month***	Month***		Individuals*	Time***		9	722.80	0.645
M6	$T_{chamber}^{*}$	$T_{chamber}$: Month***	Month***	Sex			AR1	9	732.75	0.638
M7	$T_{chamber}^{*}$	$T_{chamber}$: Month***	Month***	Sex	Individuals*		AR1	10	727.78	0.640
M8	$T_{chamber}^{***}$	$T_{chamber}$: Month***	Month***	Sex		Time***	AR1	10	726.72	0.644
M9	$T_{chamber}^{**}$	$T_{chamber}$: Month***	Month***	Sex	Individuals*	Time***	AR1	11	721.63	0.646
M10	$T_{chamber}^{**}$	$T_{chamber}$: Month***	Month***		Individuals*	Time***	AR1	10	724.80	0.645
M11	$T_{chamber}^{**}$	$T_{chamber}$: Month***	Month***	Sex	Individuals*	Time***	ARMA ($p = 1, q = 1$)	12	723.63	0.646
M12	$T_{chamber}^{**}$	$T_{chamber}$: Month***	Month***	Sex	Individuals*	Time***	ARMA ($p = 2, q = 1$)	13	725.63	0.646

CHAPTER 3:

SEASON AND REPRODUCTIVE ACTIVITY INFLUENCE STRESS LEVEL OF THE MALAGASY PRIMATE *LEPILEMUR EDWARDSI*

3.1 Abstract

Throughout the year, a range of stressors, such as environmental stressors and reproductive activities, affect the stress level of animals with varying durations, intensities and impacts. Measurements of hair cortisol are increasingly used to assess the impact of numerous stressors on the stress levels of wild animal populations. Here, we examined the seasonal influence and the influence of reproduction, body condition, and sleeping site quality on the stress level of individuals of *Lepilemur edwardsi*, a small Malagasy primate species, living in the seasonal dry forest of western Madagascar via hair cortisol concentrations. We found a significant increase of the stress levels from the early wet season to the early dry season, presumably due to a decreasing quality and quantity of the diet of *L. edwardsi*. Additionally, we found a sharp increase of the stress levels of reproducing male individuals during the mating season (early dry season). Female individuals showed no influence of the mating or the birthing season (early wet season) on their stress levels. However, they showed a slight increase in stress levels during the lactating season (late wet season). When additionally considering the elevated resting metabolism of *L.* edwardsi during the dry season, this can have a negative influence on reproductive success and therefore an individual's fitness. Ultimately, our results indicate that *L. edwardsi* might have only a small leeway to cope with declining conditions due to climatic changes and anthropogenic habitat alterations.

3.2 Introduction

Stress is the hormonal response of animals when experiencing a threat to their homeostasis. Prolonged stress or a series of acute stressors can divert resources away from other biological functions (e.g. reproduction, growth) and ultimately lead to death. However, stress is not necessarily adverse for animals, e.g. enhancing flight response to a predation risk, and not every stressor has a long-term negative effect on animals' health and the costs are often not paramount (Johnson et al. 1992; Moberg and Mench 2000). Stress levels in animals change throughout the year due to a multitude of interacting stressors of varying impacts, durations and intensities (Romero 2004; Spiga et al. 2014). Environmental stressors include seasonal changes (Balestri et al. 2014) and natural disasters, such as droughts and cyclones (Sapolsky 1986; Fardi et al. 2018). Additionally, reproductive activities (Brunton et al. 2008), social status (Koren et al. 2008) and nutritional stress (Chapman et al. 2015), as well as parasite infections (Triki et al. 2016) and diseases (Mormède et al. 2007) can affect the hormonal balance of individuals and therefore their fitness (Bonier et al. 2009).

In mammals, the glucocorticoid hormone cortisol (GC) is considered as a good biomarker to measure the response to stressors and normally follows diurnal and ultradian rhythms (Mormède et al. 2007; Spiga et al. 2014; Ralph and Tilbrook 2016), as its production and release is directly triggered by the neuroendocrine stress response cascade (Johnson et al. 1992). In animal conservation efforts, measurements of cortisol have proven useful to monitor the impact environmental or anthropogenic stressors have on the health of wild animals by determining their stress levels (Romero 2004; Ralph and Tilbrook 2016).

Measurements of glucocorticoid concentrations differ in their degree of invasiveness, feasibility of collection and the required amount of tissue. Short-term stress and the reaction to immediate stressors are generally measured in blood, plasma, saliva, urine and feces of species. The release of GC in the subsequent tissues can range from minutes (blood, saliva) to hours (feces, urine) and is species-specific (Mormède et al. 2007). Recently, measurements of glucocorticoid concentrations in hair and feathers and also in scales of reptiles have been used to depict long-term stress in individuals (e.g. long-term stress in brown bears, Cattet et al. 2014; survival and parasite infection in lemurs, Rakotoniaina et al. 2016, 2017; survival in passerine birds, Koren et al. 2012; stress of handling in snakes, Berkvens et al. 2013). All these tissues are recommendable for measurements of stress in wild populations and of threatened species where repeated capture is difficult and invasive sampling is not appropriate (Heimbürge et al. 2019; for examples see: Macbeth et al. 2012; Carlitz et al. 2016).

The endangered, pair-living primate species *Lepilemur edwardsi* (800 – 1000g; head-body length ~27cm) lives in the dry deciduous forests of western Madagascar (Mittermeier et al. 2010; Louis et al. 2020). This region is characterized by a distinct seasonality in precipitation, and thus productivity. Ambient temperature (T_a) during the wet abundant season from November to April rarely drops below 20°C and usually increases to about 30°C and precipitation is high. During the lean dry season, daily maximum T_a increases with the progressing season from May to October and animals have to cope with T_a around 33°C during the day and 23°C during the night, and almost no precipitation for about 6 months (Bethge et al. 2021). These climatic conditions lead to a continuing decrease in quantity and quality of food resources over the dry season, as plant growth is generally reduced and many plants decrease their nutritional content (e.g. protein content) and increase their secondary metabolites in the leaves with increasing T_a (Ganzhorn and Wright 1994; Ganzhorn 1995; Dearing 2013). As *L. edwardsi* is mainly folivorous (Mittermeier et al. 2010), this constitutes an extremely and increasingly challenging period of the seasonal cycle for this species. Moreover, reproductive activities potentially add to the stress level of *L. edwardsi* during this time, as the mating season occurs at the beginning of the dry season (May / June), and the females are pregnant throughout the lean dry season until they give birth in the early wet season (October / November; Randrianambinina et al. 2007; Mittermeier et al. 2010). Additional stressors for this species are the current and continuing habitat loss due to anthropogenic impacts, as well as climatic changes due to global warming (Craul et al. 2009; Steffens and Lehman 2018; Bethge et al. 2021). Even relatively low-impact logging might be detrimental to this species, as it is reliant on tree hollows in mature trees for the diurnal resting phase (Rasoloharijaona et al. 2003; Mittermeier et al. 2010), providing protection from predators and thermal insulation (Rakotomalala et al. 2017; Wulff 2020).

In this study, we investigated if the challenging climatic, nutritional and reproductive conditions of the dry season are reflected in the stress levels of *L. edwardsi* by measuring hair cortisol concentrations (HCC) of a wild population of *L. edwardsi* in western Madagascar. We hypothesize that the stress level increases at the beginning of the dry season, when food quantity and quality start to decline, especially in individuals with poorer body condition. Secondly, we assume that pregnant and lactating females have a higher stress level than non-pregnant females and males, because of the energetic and hormonal challenges of pregnancy. And thirdly, we assume that individuals that inhabit tree hollows in larger, mature trees have lower stress levels, as bigger tree hollows presumably provide a better thermal insulation and protection from predation.

3.3 Materials & Methods

Study site, animal captures and sampling

We conducted the study in the Ankarafantsika National Park (S 16° 19’, E 46° 48’) in Jardin Botanique A, a 30.6 ha research area within the dry deciduous forest of western Madagascar. We captured 34 individuals of *Lepilemur edwardsi* during three capture periods in 2018 and 2019. We located the individuals in their sleeping sites in the mornings (9:00 – 12:00h), captured the individuals by hand and briefly anesthetized them with 0.1mL/kg ketamine hydrochloride (Ketamidor® 100mg/mL, WDT, Garbsen, Germany). We weighed the individuals to the nearest 1g (Clatronic® International GmbH, Kempen, Germany), sexed them and took body measurements, before we individually marked them with subcutaneously injected passive integrated transponders (Trovan, EURO I.D. Usling GmbH, Weilerswist, Germany). We took 47 hair samples in the dorsocaudal region of 14 individuals (9 males, 5 females) in February 2018, 15 individuals (8 males, 7 females) in May – June 2019 and 18 individuals (7 males, 11 females) in

August – September 2018. The hair was cut by hand as close as possible to the skin, with an industrial razorblade (Gillette Platinum) and stored dry at surrounding temperature in little sealable plastic bags. Through recaptures we were able to assess the hair growth rate of *L. edwardsi*, which was approximately 0.5 – 1cm in 6 months. The sampled full-length hair had a length of about 1 – 2cm, therefore depicting the previous 6 – 12 months. Hence, we expected that the measured HCC represents approximately 1 – 6 months before sampling, reflecting the early wet season (sampling in February 2018), the late wet season (sampling in May / June 2019) and the early dry season (sampling in August / September 2018; Table 1). Using HCC to assess stress levels has the advantage of depicting long-term stress responses of several months, as is necessary to evaluate continuing (environmental) stressors. Other matrices used to measure cortisol (e.g. blood, plasma, urine, saliva, feces) are solely reflecting a single stressful event and are therefore not suitable to obtain baseline GC levels (Heimbürge et al. 2019). As HCC varies between sample areas on the body (Acker et al. 2018; Lavergne et al. 2020), we always sampled the hair in the dorsocaudal region.

Finally, we measured the diameter of their sleeping site trees at breast height (dbh) after each capture and recapture. As *L. edwardsi* has a high sleeping site fidelity limited to 2- 3 tree hollows in its territory (Rasoloharijaona et al. 2003), we calculated the mean dbh of the different known sleeping site trees for each individual.

Lab analysis

The analysis of the hair cortisol was performed in the lab of Prof. Dr. C. Kirschbaum at the Technical University of Dresden (Germany) following the protocol by Gao et al. 2013. Hair samples were washed in isopropanol and dried for at least 12h. Afterwards a subsample of 7.5mg was taken and 40µL internal standard and 2.4mL methanol were added, followed by an incubation for 18h to extract the steroids. After centrifugation, the clear supernatant was separated and the samples dried under a constant stream of nitrogen and at high temperatures of 65°C until the alcohol was completely evaporated. Finally, the residues were re-suspended with 175µL distilled water and an aliquot of 100µL was used for cortisol concentration determination with liquid chromatography tandem mass spectrometry (LC-MS/MS; LC-20AD HPLC unit with SIL-20AC autosampler and CTA-20AC column temperature oven, Shimadzu, Canby, OR, USA) coupled to a Turbo-ion-spray® triple quadrupole tandem mass spectrometer (API 5000, ABSciex, Foster City, CA, USA) with purification by on-line solid-phase extraction.

Data analysis and statistics

We analyzed the data using Cran R (Version 4.0.3, R Core Team 2019) and the packages “plyr” (Wickham 2020), “dplyr” (Wickham et al. 2019) and “ggplot2” (Wickham et al. 2020). After thorough data exploration, we excluded the extremely low HCC-value of one individual in the early dry season from the model, as it is below biologically acceptable values and cannot be explained (Suppl. Table 1). We examined and modeled the influence of different explanatory variables (season, sex, mean dbh of sleeping site trees, and body condition) on the HCC of *L. edwardsi*. Body condition was defined as the quotient of body mass (g) and tarsus length (cm). Due to a high variance in the data, we log10 transformed the cortisol data and applied a generalized linear mixed model (GLMM) with all explanatory variables using the package “glmmTMB” (Magnusson et al. 2020). We modelled 64 different GLMMs with individual ID as random effect, as the HCC of some individuals were measured in multiple seasons. After model selection using the dredge()-function in package “MuMIn” (Barton 2020), we selected the “best models” based on delta Akaike’s information criterion ($\Delta AIC < 2$) and performed a model averaging approach (Table 2). We followed the recommendation of Burnham and Anderson (Burnham and Anderson 2002) and calculated the sum of the Akaike weight of the models (models with a $\Delta AIC < 2$) which included a certain explanatory variable to determine the relative importance. The sum of the Akaike weights for the response variables season, sex (and therefore also the interaction term) and body condition were highest and hence explained the HCC of *L. edwardsi* best (Table 2).

Body condition and the sleeping site data were non-independent (some individuals were measured more than once in different seasons) and showed non-normality (Shapiro-Wilk Test: $p = 0.007$ and $p < 0.001$). We therefore applied an unpaired Wilcoxon rank sum test with a Bonferroni correction to test for differences in the body condition.

Table 1: Capture period and time of incorporation of hair cortisol concentrations (HCC) of *Lepilemur edwardsi*. Median HCC with interquartile range during the early wet season (N = 14; 9 males, 5 females), late wet season (N = 14; 7 males, 7 females) and early dry season (N = 18; 7 males, 11 females).

Incorporation of HCC	**Capture period**	**Median HCC** (pg*mg^{-1})
Early wet season	January – February 2018	3.81(1.56)
Late wet season	May – June 2019	4.37(1.74)
Early dry season	August – September 2018	5.3(11.08)

Table 2: Model selection output of the four models with the lowest corrected Akaike information criterion (AICc) and a ΔAIC ≤ 2. The values for the intercept and the explanatory variables body condition and diameter at breast height (dbh) of the sleeping site trees, are the estimates (i.e. the beta coefficients) for the terms, when they are present in the model. The factor explanatory variables season and sex (and the interaction term for season and sex) are marked with a "+", when they are included in the model and a "–", when not included in the model.

Model rank	**Intercept**	**Body condition**	**Season**	**Sex**	**Dbh of sleeping site trees**	**Interaction term season : sex**	**df**	**logLik**	**AICc**	**ΔAIC**	**Akaike weight**
1	0.38	1.60x10^{-3}	+	+	-	+	9	8.87	6.7	0.00	0.23
2	0.32	2.38x10^{-3}	+	+	-	-	7	5.20	7.3	0.64	0.17
3	0.57	-	+	+	-	+	8	5.99	7.9	1.22	0.12
4	0.36	1.53x10^{-3}	+	-	-	-	6	3.23	8.3	1.56	0.11

3.4 Results

Body condition slightly decreased from 129.13 (11.94)g*cm^{-1} in the early wet season to 127.42 (12.29)g*cm^{-1} in the late wet season (Wilcoxon rank sum test; early wet season vs. late wet season, $p = 1.0$) and significantly decreased to 116 (21.33)g*cm^{-1} in the early dry season (Wilcoxon rank sum test; early wet season vs. early dry season, $p = 0.36$; late wet season vs. early dry season, $p = 0.56$; Fig. 1). Furthermore, females had a slightly better body condition than males with 128.53 (14.6)g*cm^{-1} and 124.1 (19.59)g*cm^{-1} respectively.

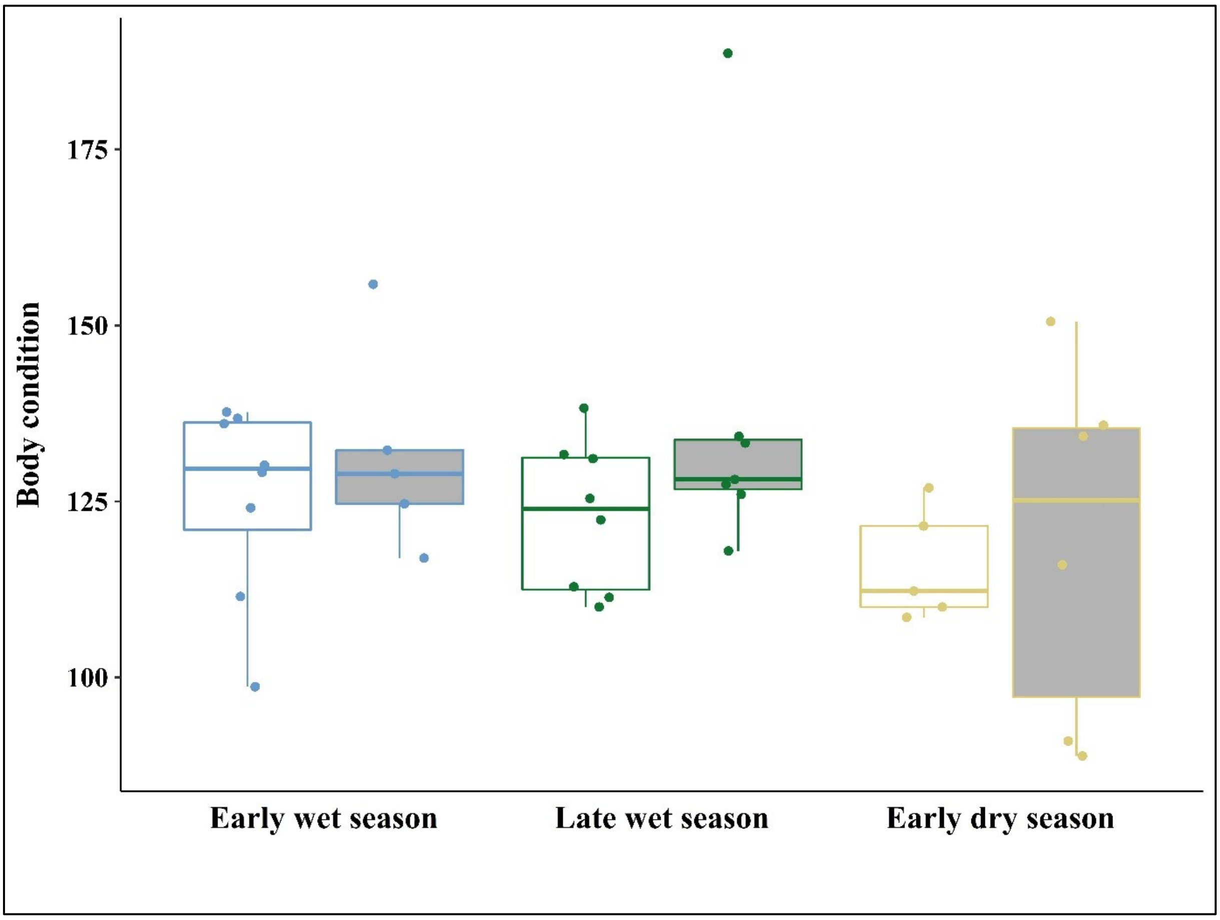

Fig. 1: Body condition (quotient of body mass and tarsus length) of male (white) and female (grey) individuals of *Lepilemur edwardsi* during the early wet season (blue; N = 14; 9 males, 5 females), late wet season (green; N = 14; 7 males, 7 females) and early dry season (yellow; N = 18; 7 males, 11 females; bold line, median; box limits, first and third quartiles; whiskers, 1.5x interquartile range; all data points are included).

Median HCC of *L. edwardsi* in the early wet season (N = 14; 9 males, 5 females) amounted to 3.81 (1.56)pg*mg^{-1}; in the late wet season (N = 14; 7 males, 7 females) to

4.37 (1.74)pg*mg^{-1} and in the early dry season (N = 18; 7 males, 11 females) to 5.3 (11.08)pg*mg^{-1} (Table 1; Fig. 2). The females of *L. edwardsi* showed a slightly higher median of HCC with 4.7 (2.32)pg*mg^{-1}, whereas the males showed a high variance in HCC with a median HCC of 4.47 (4.91)pg*mg^{-1}. Five males showed particularly high HCC during the early dry season with HCC three to eleven times higher than the median during this period.

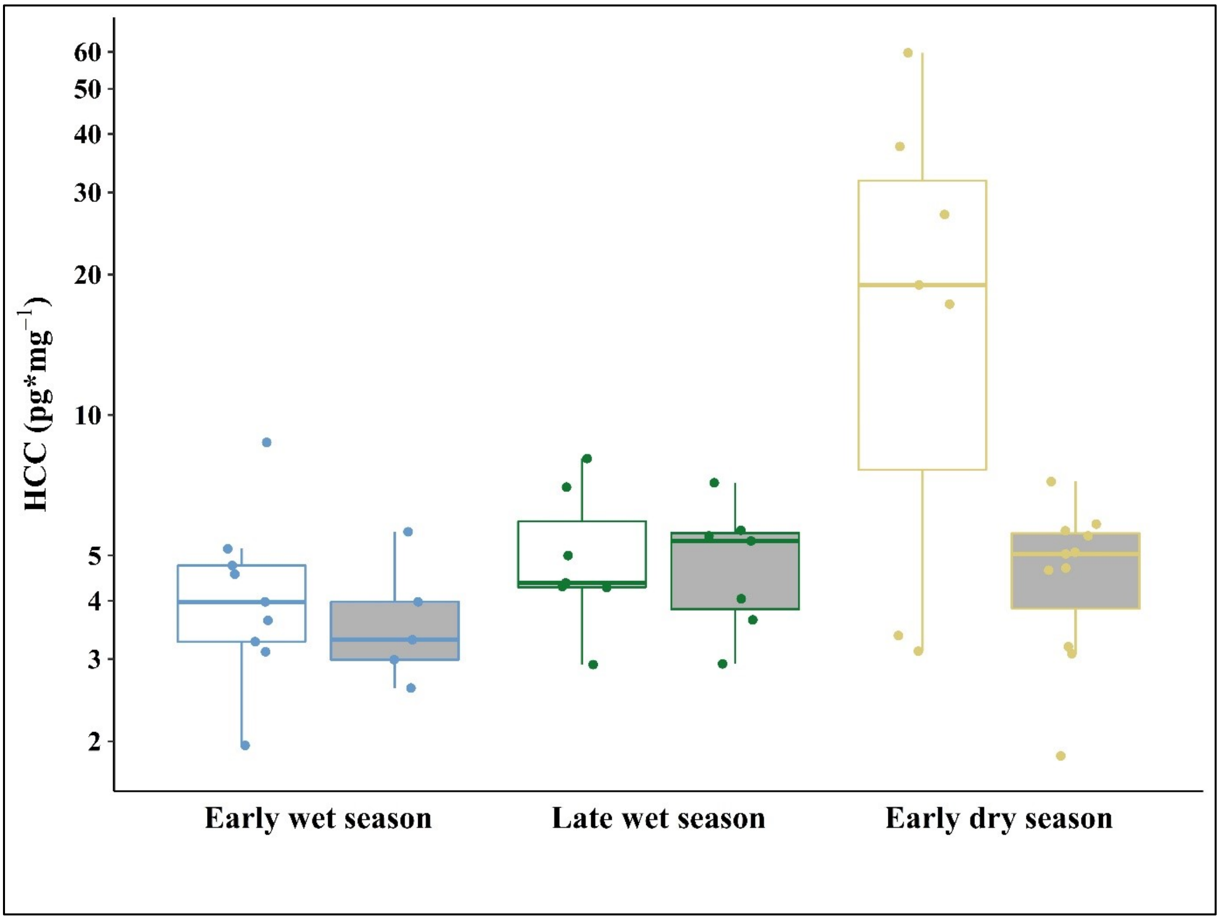

Fig. 2: Hair cortisol concentration (HCC) of male (white) and female (grey) individuals of *Lepilemur edwardsi* during the early wet season (blue; N = 14; 9 males, 5 females), late wet season (green; N = 14; 7 males, 7 females) and early dry season (yellow; N = 18; 7 males, 11 females; bold line, median; box limits, first and third quartiles; whiskers, 1.5x interquartile range; all data points are included) plotted on a log-scale.

Furthermore, three females were pregnant or already lactating during the early wet season and the median of the HCC of these females was lower (3.19 (2.34)pg*mg^{-1}; N = 3) than the HCC of the non-reproducing females during this season (5.04 (1.53) pg*mg^{-1}; N = 2; Fig. 2). The model averaging process showed a strong influence of season on the response variable HCC and all models calculated an increase in HCC on a log10 scale, from the early wet season to the wet season and a stronger increase from the wet to the early dry season. On a log10 scale, the HCC was 0.16 times higher in the early wet season, than in the late wet season and 0.40 times higher in the early dry season. Furthermore, the HCC of the females in the early dry season was lower than the HCC of the males on a log10 scale. The GLMM showed no significant influence of body condition on the HCC of *L. edwardsi* but body condition explained some of the variance of the data, as the AIC of the model with body condition as explanatory variable was lower and sum of the Akaike weights of this variable was high (Table 3). Conversely, the sum of the Akaike weights for the models that included the mean diameter at breast height of the sleeping site trees was low, hence the relative importance of this variable on HCC was low (Table 3).

Table 3: Results of the full model averaging with a subset of the generalized linear mixed effects models (GLMMs, ΔAIC < 2)) and the log-transformed hair cortisol concentration (HCC) as response variable: Estimate, standard error (SE), adjusted standard error, *z*-value, *p*-value, the 95% confidence intervals (CI) and the sum of the Akaike weight for each explanatory variable included in the four models with the lowest corrected Akaike information criterion. The asterisk represents the respective significance levels with *p* < 0.001***, *p* = 0.001**, *p* = 0.01* for each variable.

Explanatory variable	**Estimate**	**SE**	**Adjusted SE**	***z* value**	***p* value**	***CI 2.5%***	***CI 97.5%***	***Sum of Akaike weight***
Intercept	0.40	0.26	0.28	1.44	0.15	-0.14	0.94	1.0
Late wet season	0.15	0.09	0.09	1.60	0.11	-0.04	0.34	0.75
Early dry season	0.38	0.16	0.16	2.32	0.02*	0.06	0.70	0.75
Body condition	0.00	0.00	0.00	0.69	0.49	-0.00	0.01	0.75
Females	-0.06	0.11	0.12	0.50	0.62	-0.28	0.17	0.72
Late wet season : females	-0.01	0.11	0.11	0.10	0.92	-0.23	0.21	0.47
Early dry season : females	-0.24	0.24	0.25	0.99	0.32	-0.72	0.24	0.47

3.5 Discussion

Sportive lemurs inhabit almost all habitats in Madagascar and can survive even in the driest and harshest environments on this island, such as the extremely dry spiny forest in southern Madagascar and the seasonal deciduous dry forest in the West. In this study, we present the first data on the stress levels of free-ranging sportive lemurs. We show a seasonal increase of HCC of *Lepilemur edwardsi*, from the early wet to the progressing dry season, as well as a strong influence of sex (Fig. 3, Table 3). Body condition, on the other hand, had no discernible influence on HCC (Table 2, 3). Nevertheless, the HCC of *L. edwardsi* was generally extremely low, compared to other lemur species such as the diurnal ring-tailed lemurs (*Lemur catta*) where the HCC ranged from 407 to 2258pg*mg^{-1}, depending on weather events, age and body mass (Fardi et al. 2018). Also other diurnal primate species such as two baboon species (*Papio anubis* and *P. hamadryas*) showed distinctly higher stress levels (median HCC: 26.9 pg*mg^{-1} and 29.69 pg*mg^{-1} respectively; Fourie et al. 2015). This is in concordance with previous findings that plasma GC decreases with decreasing mass-specific resting metabolic rates of mammals (Haase et al. 2016), as sportive lemurs have one of the lowest mass-specific resting metabolic rates measured in folivorous mammals so far (Schmid and Ganzhorn 1996; Bethge et al. 2017, 2021). Other nocturnal lemur species, such as the fat-tailed dwarf lemur (*Cheirogaleus medius*) and the gray mouse lemur (*Microcebus murinus*), which also have comparatively low metabolic rates, also showed low stress levels ranging from 0.15 to 24.33 pg*mg^{-1} and 0.15 to 42.35 pg*mg^{-1} HCC respectively (Rakotoniaina et al. 2016).

HCC increases in the dry season

L. edwardsi lives in a highly seasonal habitat where environmental conditions change every few months. Both, male and female individuals of *L. edwardsi* showed higher stress levels in the late wet season and early dry season than in the early wet season, as evidenced by the increase of HCC (Fig. 2; Table 1, 3), presumably due to reproductive activity and decreasing food quantity and quality. This conforms to one of our earlier studies, where we showed that *L. edwardsi* also increases its metabolic rate strongly and independently of Ta from the wet to the dry season (Bethge et al. 2021). We assume this is due to the increased need of detoxification of plant secondary metabolites in their diet, making the diet of *L. edwardsi* even more unfavorable during the progressing dry season (Ganzhorn 1992; Ganzhorn and Wright 1994; Dearing 2013; Bethge et al. 2021). Furthermore, the body condition of *L. edwardsi* slightly, but significantly, decreased from the early wet to the early dry season indicating that they might struggle with the beginning of the aggravating environmental conditions and food availability (Fig. 1). Taken together, this does indeed demonstrate that the dry season is a very challenging season for *L. edwardsi*. Presumably this is also true for other sportive lemur species, as the same pattern of seasonal changes in metabolic rate was also found in *L. petteri*, sister species of *L. edwardsi*, living in the spiny forest of southern Madagascar. This study on *L. petteri* demonstrated only a small scope of physiological response at the upper end of the Ta range, limiting its possibilities to adapt to increasingly unfavorable conditions (Bethge et al. 2017). HCC measurements of *C. jacchus*, another primate species living in a dry (semi-desert) seasonal habitat, also revealed a much higher HCC during the dry than the wet season, presumably due to food constraints and the associated water stress (Garber et al. 2020). Conversely, the lemur species *C. medius*, which lives sympatrically with *L. edwardsi*, showed a significantly higher HCC in the wet and resource abundant season compared to the dry and low-resource season (Rakotoniaina et al. 2016). However, *C. medius* hibernates during the dry season for seven to nine months and thus circumvents the most challenging times with this physiological response (Dausmann et al. 2004). Interestingly, *M. murinus*, living in the same habitat as *C. medius* with the ability to show daily torpor and hibernation, showed no influence of season on HCC (Rakotoniaina et al. 2016). This apparent lack of stress response to the dry season might be caused be the diets of *C. medius* and *M. murinus*, which is more opportunistic compared to the folivorous diet of *L. edwardsi*, as these species are mainly frugivorous and insectivorous. Folivory is generally associated with the intake of food with a low energy content, which is additionally decreasing with leave age, while increasing in secondary metabolites, making the diet of *L. edwardsi* even less nutritious towards the end of the dry season (Coley 1983; Ganzhorn 1992; Dearing 2013). HCC seems to depend strongly on the habitat of species and other environmental factors, such as water and food availability, and ambient temperature (Chapman et al. 2015; Heimbürge et al. 2019). Furthermore, life-history parameters appear to play a crucial role in the release of stress hormones, as it was shown in *M. murinus*, where males had a higher HCC than females in the dry season. However, this effect was reversed in the wet season (Rakotoniaina et al. 2016).

Males are more stressed than females – sometimes

The HCC of female individuals of *L. edwardsi* was slightly higher than that of the male individuals and males showed a particularly strong increase of HCC from the late wet to the early dry season. Five male individuals showed extremely elevated HCC levels during the early dry season, presumably due to the mating period (Fig. 2). Elevated HCC levels in male individuals during the mating period are also common in other primates, such as the muriquis (*Brachyteles arachnoides*; Strier et al. 1999) or mangabeys (*Lophocebus albigena*; Arlet et al. 2009). In black bears (*Ursus americanus*) male individuals have a generally higher HCC than females and experience greater inter-individual variations (Lafferty et al. 2015). However, this is not the case in all mammalian species, as for example in the golden lion tamarins (*Leontopithecus rosalia*) where their group hierarchy seems to affect cortisol levels of males. Mating does not affect fecal cortisol levels of reproducing dominant golden lion tamarin males and unrelated subordinate males, as the latter presumably suppress their androgen secretion and therefore reproduction to avoid aggressions from the dominant males (Bales et al. 2006).

Female individuals of *L. edwardsi* showed an increase of HCC from the birthing season (early wet season) to the lactating season (wet season) and a slight decrease from the lactating season to the mating season (early dry season; Fig. 2). However, the HCC of reproducing females (either in estrus, pregnant or lactating) was lower than the HCC of non-reproducing females. This is not unique, as, similarly, the HCC of wild Egyptian mongooses (*Herpestes ichneumon*) also showed no effect of female reproduction (Azevedo et al. 2019). During pregnancy and lactation, the neuroendocrine stress response cascade is altered, which might avoid detrimental effects on mother and offspring. Furthermore, the circadian release of glucocorticoids is down-regulated, which secures a stable energy supply to the fetus or young, as it limits the catabolic effects that are generally a result of surges in secretion (Brunton et al. 2008). Conversely, the HCC of female *C. jacchus* was significantly higher than that of males, however, independent of reproduction, potentially due to a combination of environmental and social stressors, e.g. competition with other females (Garber et al. 2020). Ultimately, there are a lot of inter- and intraspecific sex-differences in HCC and the influence of sex on HCC seems to be inconsistent between species and additionally depends on other factors, such as age (Heimbürge et al. 2019). Even in *L. edwardsi* two of the sampled males did not show elevated HCC (Suppl. Table 1). One of the males shared a sleeping site with another pair during the dry season. The other male was sharing multiple sleeping sites with another female; however, this female was also found in a sleeping site which was frequented by another male (personal observation). Combined with the low HCC levels, this indicates that these two males were not breeding during the early dry season.

Conclusion

Measurements of the stress hormone cortisol in hair is a robust non-invasive method and proxy for the long-term activity of the stress response and chronic stress of animals in the wild (Heimbürge et al. 2019). The results of this first study of HCC in sportive lemurs show that *L. edwardsi* is indeed stressed by the challenging dry season and, in the case of the males, by mating. When additionally considering the extremely elevated metabolic rate of this species during the late dry season (Bethge et al. 2021), it seems that *L. edwardsi* has only a small scope to adequately respond to declining environmental conditions, such as by persistent droughts or habitat losses, due to e.g., global climate change or anthropogenic habitat transformations.

3.6 Acknowledgments

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Hamburg, and all procedures comply with the current laws of Madagascar. The research was approved by the Direction du Système des Aires Protégées, Ministère de l'Environnement, the Université d'Antananarivo (Autorisation de recherche no. 305/17/MEEF/SG/DGF/DSAP/SCB.Re, no. 106/18/MEEF/SG/DGF/DSAP/SCB.Re and no. 086/19/MEDD/SG/DGF/DSAP/SCB.Re) and the Bundesamt für Naturschutz in compliance with CITES (no. DE-E-07783/17 and no. 1028C-EA12/MG18, no. DE-E-05323/18, no. DE-E-01666/19 and no. 497C-EA07/MG19). We acknowledge the authorization and support of this study by the Ministère de l'Environnement des Forêts, MNP and the University of Antananarivo. We thank MNP, J. B. Kennedy, T. Andrianasolo, J. Rakotondravony, S. Reher, A. Wulff and J. U. Ganzhorn for their collaboration and support. We thank the Dresden Lab Service GmbH and Prof. Dr. C. Kirschbaum und I. Kaden for performing the laboratory work. This work was funded by the Evangelisches Studienwerk Villigst e.V. and the Universität Hamburg.

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CHAPTER 4:

SEASONAL CHANGES IN THE PARASITE PREVALENCE OF A SMALL MALAGASY LEMUR SPECIES (*LEPILEMUR EDWARDSI*)

4.1 Abstract

Parasitic infections can impact the fitness of individuals and are ultimately a major driver of animals' population dynamics. An individuals' parasite prevalence often changes depending on external or seasonal changes, e.g., rainfall and ambient temperatures, but also on internal changes, e.g., changes in body condition. In this study we aimed to identify the environmental factors that may influence the intestinal parasite and ectoparasite prevalence of the folivorous Malagasy primate species, *Lepilemur edwardsi,* living in a seasonal dry deciduous forest. Species living in this habitat have to adapt to seasonal changes of ambient temperature, with almost no precipitation during the dry season and hence strong fluctuations of resource availability throughout the year. We sampled the feces and ectoparasites of *L. edwardsi* throughout the year. Intestinal parasite prevalence increased from the wet to the dry season and was highest in the late dry season, which might be due to the accompanying decrease in diet-quality. Conversely, ectoparasite prevalence decreased in the dry season, presumably due to the prevailing unfavorable environmental conditions for the development of ectoparasites (i.e., mites and ticks). Paired with the higher resting metabolism and stress level of *L. edwardsi* during the late dry season, it seems that this species may struggle when dry seasons intensify in its habitat.

4.2 Introduction

Parasitic infections affect the health (Woodroffe 1999), reproductive success (Hillegass et al. 2010) and therefore the fitness of individuals and can ultimately regulate the dynamics of wild animal populations, affect the survival and influence evolution of their host species (Sheldon and Verhulst 1996; Daszak et al. 2000; Hudson et al. 2002). In reindeer (*Ostertagia gruehneri*), for example, an antihelminthic treatment increased the calving probability of females in the following year (Albon et al. 2002). In Soay sheep, the infection with nematodes caused malnutrition which in turn caused periodic mass mortality events (Gulland 1992). Parasite prevalence can fluctuate strongly depending on internal factors, such as an individual's body condition (Sánchez et al. 2018) or external factors, such as rainfall (Turner et al. 2012) and ambient temperature (Studer et al. 2010). Moreover, the severity of parasitic infections depends greatly on the hosts' diet quality with a high parasite load when the diet-quality is low and vice versa (Coop and Field 1983; Coop and Holmes 1996; Coop and Kyriazakis 1999; Lange et al. 2014). Primates with a folivorous, and thus lower-quality, diet showed a higher parasite load, as they had to ingest higher amounts of leaves to cover their nutritional requirements (e.g. insectivores; Vitone et al. 2004). Intestinal parasites, such as nematodes reduce the absorption of nutrients in the intestines which leads to malnutrition and a decrease in body condition of the hosts (Coop and Holmes 1996; Coop and Kyriazakis 1999). As a balanced nutrition is required for the immune cells to function optimally, the lower food availability and quality might further negatively influence the immune system and lead to a higher susceptibility to parasite infections or diseases and thus a poorer body condition (Coop and Holmes 1996; Coop and Kyriazakis 1999; Childs et al. 2019). Moreover, as leaf-quality decreases with increasing leaf age and ambient temperatures (Coley 1983; Ganzhorn and Wright 1994; Ganzhorn 1995; Dearing 2013), dry and warm periods with reduced resource availability may promote parasite infection and thus might become even more challenging, particularly with the ongoing climatic changes (i.e., global warming).

Despite having mainly negative consequences on their hosts' health, parasites are crucial for the proper functioning of ecosystems, as they, e.g., influence the energy flow or alter interspecific competition in ecosystems (Hudson et al. 2006; Spencer and Zuk 2016). Furthermore, specialist parasites increase the general biodiversity and can even be important for the conservation of endangered species, stabilizing population dynamics (Hudson et al. 2006; Spencer and Zuk 2016). Mild parasitic infections may even enhance the efficiency of the immune system, and thus might protect species from future infections (Stringer and Linklater 2014; Spencer and Zuk 2016). However, parasitic infections can also weaken the immune defense of the hosts and therefore increase the susceptibility to further infections with parasites or diseases (Bertó-Moran et al. 2013). Furthermore, resting sites of species can have an influence on parasite prevalence and probability of parasite infestation, as they often provide dark and humid microclimates with stable ambient temperatures. For example, in rodents which inhabited deep and long-lasting burrows with a constant microclimate ectoparasite prevalence was higher than in rodent species which used short-lasting burrows or above-ground nests (Krasnov et al. 2010).

Lepilemur edwardsi (800 – 1000 g; ˜27cm) is a nocturnal and folivorous lemur species that lives in a seasonal habitat, with a hot and humid wet season with high resource availability (November – April) and a dry, less resourced season (May – October). During the wet season precipitation is high and ambient temperature fluctuates between 23°C and 36°C, whereas during the dry season there is no precipitation for approximately 6 months and ambient temperature fluctuates between 17°C and 37°C (Randrianambinina et al. 2007). This species lives in the dry deciduous forest of western Madagascar and rests in tree hollows during the day. These tree hollows are an essential resource to shelter from predators and climatic extremes (Rasoloharijaona et al. 2003; Wulff 2020). However, they also seem to be a suitable habitat for several parasite species, as they are dark and humid. Indeed, in comparison to the sympatric living wooly lemur species, *Avahi occidentalis*, which occupies a very similar ecological niche, but rests in open foliage, *L. edwardsi* showed a higher intestinal parasite prevalence (Hokan et al. 2018). Furthermore *L. edwardsi* is pair-living and each pair uses 2-3 different resting sites in a defended territory, either together or alternating (Rasoloharijaona et al. 2003), which facilitates parasite transmission.

In our study, we aimed to elucidate the factors influencing parasite prevalence in *L. edwardsi*. We examined the influence of season, ambient temperature of the resting sites ($T_{\text{tree hollow}}$) and body condition of individuals on the prevalence of intestinal parasite and ectoparasites of this species. We hypothesize firstly that *L. edwardsi* has generally a high parasite prevalence, compared to other folivorous lemur species (e.g., *Eulemur fulvus;* Clough, Heistermann and Kappeler, 2010) as it rests in tree hollows during the day, which promote parasite development and survival, as well as transmission. Secondly, we assume that the individuals show differences in their parasite prevalence between the seasons and particularly that this is influenced by ambient temperatures, as high ambient temperatures seem to have a big impact, especially on ectoparasites (abundance). As there is almost no rainfall in the habitat of *L. edwardsi* during the dry season, we expect a decrease in parasite prevalence during this time. Lastly, we expect individuals with a poorer body condition to have a higher parasite prevalence, as compromised immune systems are often unable to control parasitic infections (Møller et al. 1998).

4.3 Materials & Methods

Study site

We conducted the fieldwork in western Madagascar within the Ankarafantsika National Park (S 16° 19’, E 46° 48’) around the Ampijoroa Forest Station in Jardin Botanique A, a 30.6 ha research area with dry deciduous forest. The region has a pronounced seasonality with a distinct food-abundant wet season from November to April and a lean dry season from May to October. During the wet season, precipitation is high and ambient temperature seldom drops below 20°C and increases to a maximal median of 30°C daily. Ambient temperature during the dry season ranges between a daily maximum median of 33°C and a minimal median of 23°C during the night and there is no rainfall for about six months (Bethge et al. 2021).

Animal captures and parasite sampling

We captured 34 individuals of *Lepilemur edwardsi* during three sampling periods in the wet and late dry season in 2018, with some recaptures (N = 14, January – March; N = 15, August – October) and the early dry season in 2019 (N = 19, May – July). Two individuals were sampled in all three sampling periods (Individuals 1 and 11; Suppl. Table 1). We located the individuals in their resting sites in the morning (9:00 – 12:00h), captured them by hand and anesthetized them with 0.1mL/kg ketamine hydrochloride (Ketamidor 100mg/mL, WDT, Garbsen, Germany). Afterwards, we weighed the individuals to the nearest 1g (Clatronic International GmbH, Kempen, Germany), sexed them and measured the tarsus length, before we individually marked them with a subcutaneously injected passive integrated transponder (Trovan, EURO I.D. Usling GmbH, Weilerswist, Germany).

We collected feces from individuals that were kept in captivity for 24h for another experiment and stored the feces in 90 % ethanol, to determine the prevalence of intestinal parasites eggs. We collected 11 feces samples (from 8 males, 3 females) in the wet season, 13 samples (from 8 males, 5 females) in the early dry season and 18 samples (from 6 males, 12 females) in the late dry season.

To collect smaller ectoparasites, we combed the individuals twenty times along the dorsal area with a fine comb and stored the samples dry in little sealable plastic bags. We obtained 12 samples during the wet season (9 males; 3 females), 15 samples during the early dry season (8 males; 7 females) and 18 samples during the late dry season (7 males; 11 females). Additionally, we visually searched every individual for ticks and collected and stored the ticks in 90% ethanol.

To analyze the influence of $T_{\text{tree hollow}}$ of the sampled individuals of *L. edwardsi*, we placed temperature loggers (Thermochron iButton, Maxim Integrated Products, San Jose, USA; resolution: ±0.1°C) inside their respective resting sites during the three different sampling periods (wet season: N = 13, early dry season: N = 5, late dry season: N = 11), which recorded ambient temperatures in the resting sites every 30 minutes ($T_{\text{tree hollow}}$). We calculated maximum $T_{\text{tree hollow}}$ for each sampled resting site to include in the statistical analysis. To evaluate the influence of other parameters of these tree hollows on the parasite prevalence, we measured the diameter of their resting site trees at breast height (dbh) as a reference for resting site quality, after each capture and recapture. *L. edwardsi* has a high fidelity to its 2-3 resting sites in its territory (Rasoloharijaona et al. 2003), hence we calculated the mean dbh of these resting sites for each individual.

Lab analysis

All parasite samples were transported to the Universität Hamburg, Germany. We determined the intestinal parasite infestation via the McMaster flotation egg counting technique followed by a visual inspection under the microscope. Briefly, feces samples were transferred in tap water to remove the ethanol for 20 – 30 minutes. Afterwards, we subsampled 300mg of feces and used a tea strainer to filter plant material using saturated potassium iodide solution (1.5g mL) as flotation solution out of the samples. A subsample of 500µl was transferred onto a McMasters counting microscope slide (Assistent, Glaswarenfabrik Karl Hecht GmbH & Co KG, Sondheim vor der Rhön, Germany) and inspected visually for parasite eggs. We used the 10x objective of a microscope (Zeiss Axioscope 2, Carl Zeiss AG, Oberkochen, Germany) to check each sample, count and identify parasite eggs, using descriptions from Irwin and Raharison (2009). We identified the parasite eggs to a genus level when feasible, otherwise we identified the suborder. We photographed the parasite eggs with a camera (AxioCam, Carl Zeiss AG, Oberkochen, Germany) and the software Axiovision (Version 4.8, Carl Zeiss AG, Oberkochen, Germany) and calculated the amount of eggs per gramm (epg) for each fecal sample.

The ectoparasite samples were analyzed and ectoparasites were counted with a binocular loupe (Olympus SZ2-ST, Olympus Corporation, Tokyo, Japan). Ticks were identified to the species level, mites sorted into morphospecies.

Data analysis and statistics

We analyzed the data using Cran R (R Core Team 2019) and the packages “plyr” (Wickham 2020), “dplyr” (Wickham et al. 2019) and “ggplot2” (Wickham 2016). We modeled and examined the influence of different explanatory variables (body condition, season, sex, dbh of resting site trees) on the intestinal parasite abundance (eggs per gramm feces) of *L. edwardsi*. Body condition was defined as the quotient of body mass (g) and tarsus length (cm). We applied a generalized linear mixed model (GLMM) with all explanatory variables using the package “glmmTMB” (Magnusson et al. 2020) and individual ID as random effect (as we took some feces samples from the same individuals in multiple seasons). As $T_{\text{tree hollow}}$ correlated with the season we were not able to include $T_{\text{tree hollow}}$ as a response variable in the GLMM. After data exploration we decided to use a negative-binomial distribution, as the data showed a skewed distribution with zero-inflation. We selected the “best model” based on delta Akaike’s information criterion ($\Delta AIC < 2$), and a visual approach using the model_check()-function of the “performance”-package (Lüdecke et al. 2021) and the DHARMa-package (Hartig 2021), after a model selection process using the dredge()-function in package “MuMIn” (Barton 2020). To examine the influence of maximum $T_{\text{tree hollow}}$ on the intestinal parasite prevalence of *L. edwardsi* we calculated a Spearman rank correlation, as the data showed non-normality (two-sample Kolmogorov-Smirnov test: $D = 0.88$, $p = <0.001$).

Data of the body condition of *L. edwardsi* were non-independent, as some individuals were measured in multiple seasons and showed non-normality (Shapiro-Wilk test, $W = 0.92$, $p = 0.007$). Therefore, we tested for differences in the body condition using a paired Wilcoxon rank sum test with a Bonferroni correction.

The difference in ectoparasite prevalence between the seasons was tested with a Kruskal-Wallis-test on a subset of the data that excluded individuals which were measured more than once. We tested a potential impact of maximum $T_{\text{tree hollow}}$ on ectoparasite prevalence of *L. edwardsi* with a Spearman rank correlation, as the combined mites and ticks count data showed non-normality (Shapiro-Wilk test, $W = 0.78$, $p < 0.001$). As the ectoparasite sample sizes were comparatively low, particularly during the early and late dry season, we were not able to calculate further statistics with this data.

4.4 Results

Body condition of all individuals sampled in the three different sampling periods (wet season, early dry season and late dry season) did not differ (pairwise Wilcoxon rank sum test with Bonferroni correction: wet season vs. early dry season, $p = 1$; wet season vs. late dry season, $p = 0.36$; early dry season vs. late dry season, $p = 0.56$; Fig. 1). Furthermore, there was no difference in body condition between male and female individuals of *L. edwardsi* (Wilcoxon signed rank test with continuity correction: V = 55, $p = 0.804$; Fig. 1)

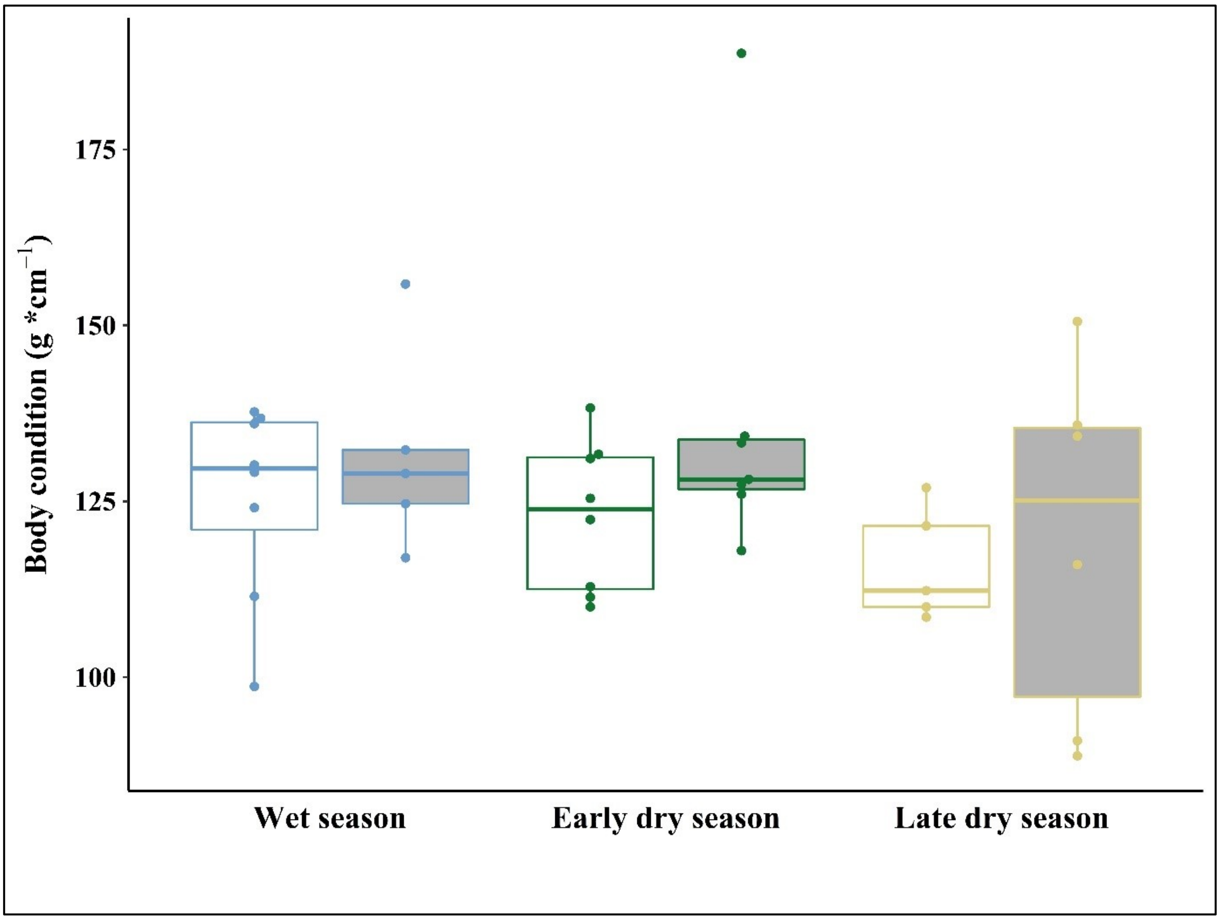

Fig. 1: Body condition of sampled individuals during the three sampling periods. Body condition (quotient of body mass and tarsus length) of male (white) and female (grey) individuals of *Lepilemur edwardsi* during the wet season (N = 11; 8 males, 3 females), early dry season (N = 13; 8 males 5 females) and late dry season (N = 18; 6 males, 12 females; bold line, median; box limits, first and third quartiles; whiskers, 1.5x interquartile range; all data points are included).

Intestinal parasites

Fecal samples of 11 individuals of *Lepilemur edwardsi* (8 males; 3 females) in the wet season (January – March 2018), 13 individuals (8 males; 5 females) in the early dry season

(May – July 2019) and 18 individuals (6 males; 12 females) in the late dry season (August – September 2018) were analyzed. Intestinal parasite prevalence varied substantially between individuals, with egg counts of intestinal parasites varying between samples from 0 to 18700 eggs per gram (epg). Individuals had a mean (± SD) intestinal parasite egg count of 409 ± 688epg in the wet season, 2085 ± 5053epg in the early dry season and 2578 ± 3799epg in the late dry season (Fig. 2).

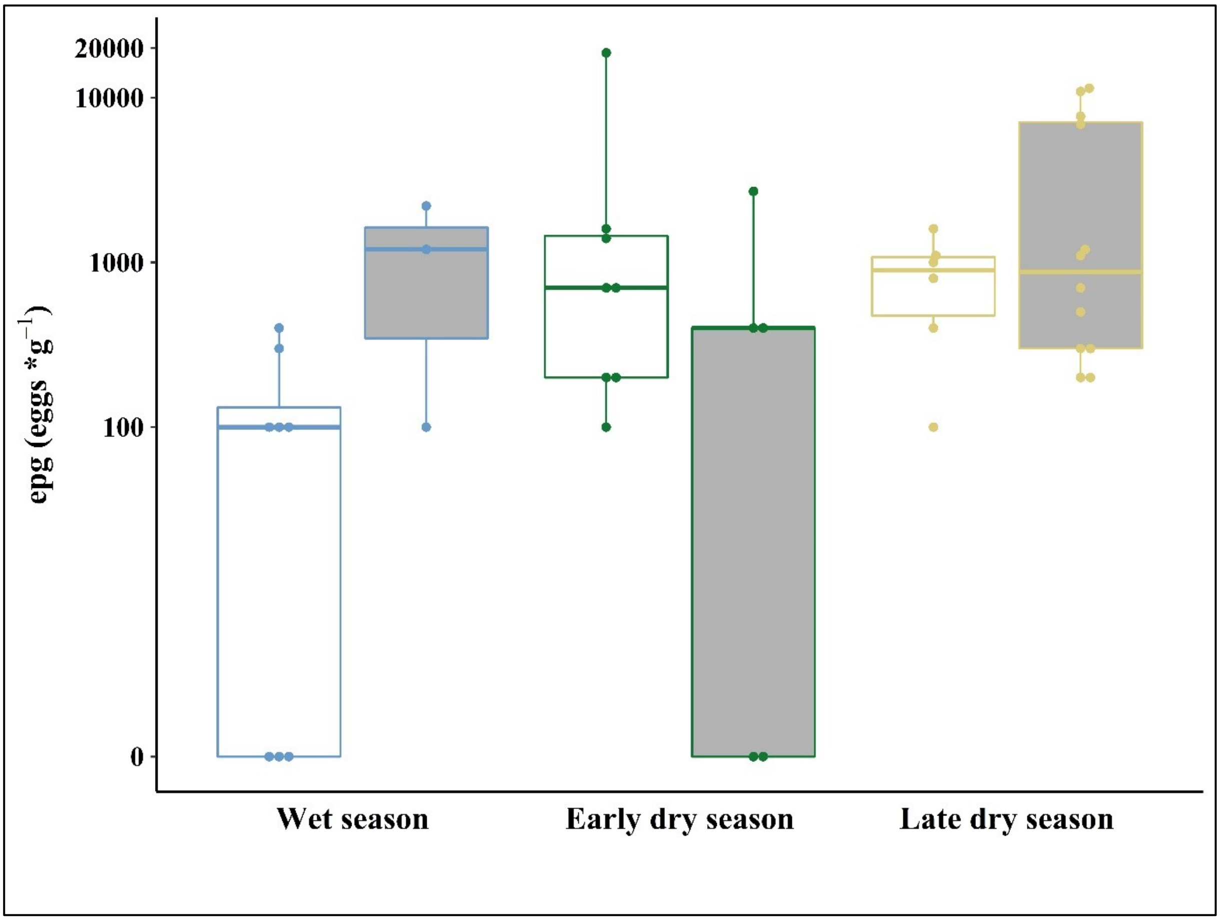

Fig. 2: Intestinal parasite count (eggs per gram; epg) during the three sampling seasons. Intestinal parasites (eggs per gram; epg) of male (white) and female (grey) individuals of *Lepilemur edwardsi* during wet season (N = 11; 8 males, 3 females), early dry season (N = 13; 8 males 5 females) and late dry season (N = 18; 6 males, 12 females; bold line, median; box limits, first and third quartiles; whiskers, 1.5x interquartile range; all data points are included).

In 12 % (in 5 out of 42 samples) of the samples, and only in samples from the wet and early dry season, we could not find any parasite eggs. 92.5% of the analyzed intestinal parasite eggs were from *Lemuricola* sp. (Oxyurida, Oxyuridae; Fig. 3, Table 1). *Lemuricola* spp. prevalence was 45.5% (5 out of 11 samples) during the wet season, 61.5% (8 out of 13 samples) during the early dry season and 83.3% (15 out of 18 samples) during the late dry season. Prevalence of strongyles (nematode; Strongylida, Chabertiidae) and *Ascaris* sp. (Ascaridida, Ascarididae) were 54.5% (6 out of 11 samples) and 9.1% (1 out of 11 samples) in the wet season, 23.1% (3 out of 13 samples) in the early dry season and 50% (9 out of 18 samples) and 27.8% (5 out of 18 samples) in the late dry season, respectively (Fig. 3, Table 1). The total intestinal parasite prevalence of male and female individuals showed no difference (Wilcoxon signed rank test with continuity correction: V = 65, *p* = 0.602; Fig. 2).

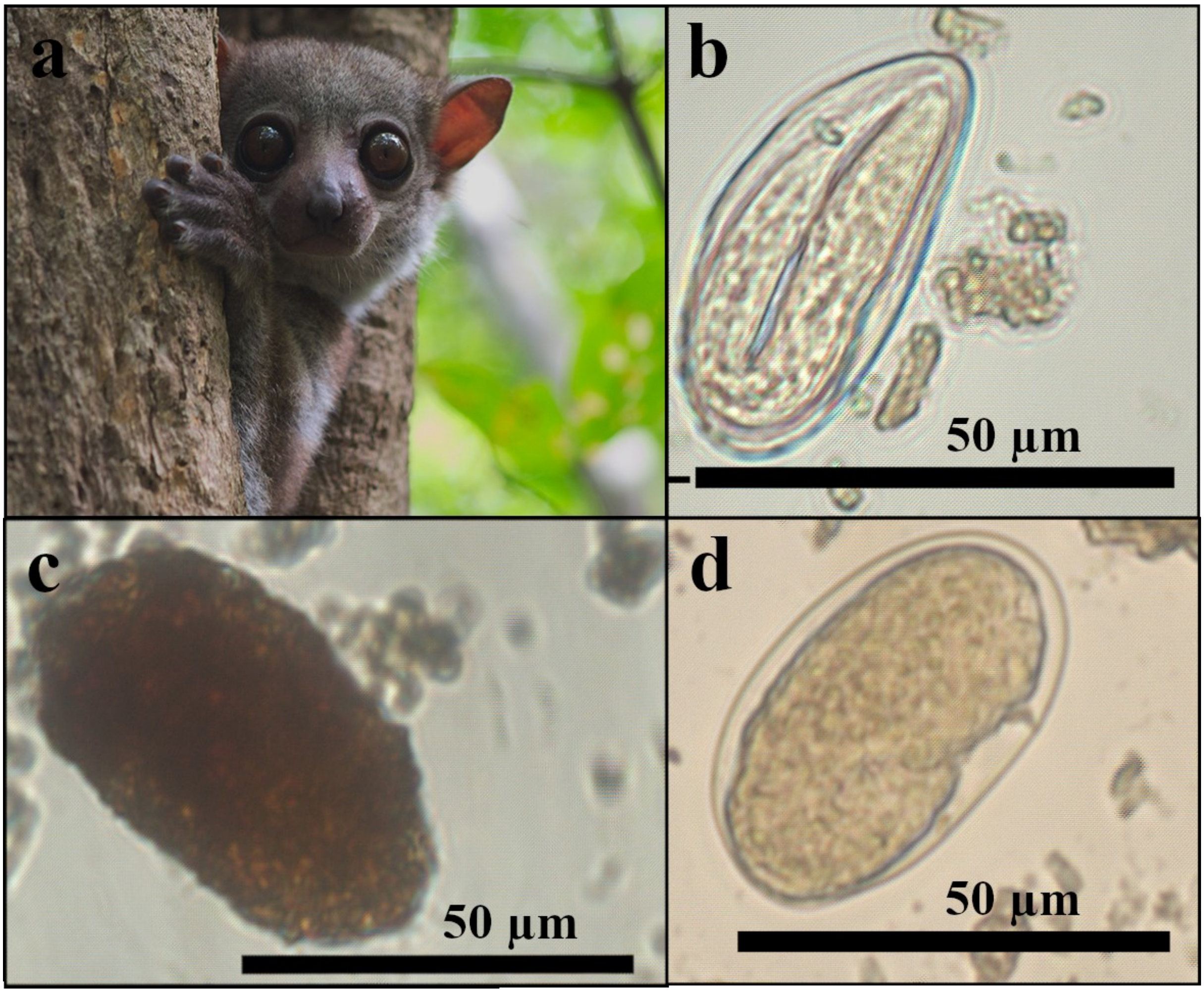

Fig. 3: (a) *Lepilemur edwardsi* and egg states of some of its parasites (b) *Lemuricola* spp. (Oxyurida, Oxyuridae), (c) *Ascaris* sp. (Ascaridida, Ascarididae) and (d) Strongyles (Strongylida, Chabertiidae).

We modelled 28 GLMMs with a negative-binomial distribution to examine the influence of different explanatory variables on the intestinal parasite prevalence of *L. edwardsi*. The model with season and body condition as fixed effects and individual ID as random effect had the lowest AIC and explained 94.2% of the deviance (Table 2). We could detect a significantly higher intestinal parasite prevalence during the late dry season ($p < 0.001$; Table 2). Although body condition showed no significant influence on intestinal parasite prevalence it explained some of the variance, as the model with body condition as a fixed effect performed better than the model without it.

There was no correlation between maximum $T_{\text{tree hollow}}$ and the intestinal parasite prevalence of *L. edwardsi* (Spearman's rank correlation, S = 4132.8, rho = 0.24, $p = 0.18$).

Table 1: Egg count and parasite prevalence of intestinal parasites of *Lepilemur edwardsi* during the wet season (N = 11; 8 males, 3 females), early dry season (N = 13; 8 males, 5 females) and late dry season (N = 18; 6 males, 12 females).

	***Lemuricola* sp.**		**Strongyles**		***Ascaris* sp.**		
Season	**Egg count**	**Prevalence**	**Egg count**	**Prevalence**	**Egg count**	**Prevalence**	**Total egg count**
Wet season 2018	28	45.5%	15	54.5%	2	9.1%	45
Early dry season 2019	268	61.5%	9	46.2%	3	23.1%	280
Late dry season 2018	434	83.3%	23	50%	7	27.8%	464

Ectoparasites

L. edwardsi carried adult ticks of the species *Haemaphysalis lemuris* (Ixodidae) predominantly in the wet season (N = 11) with a prevalence of 42.9%. In the early dry season, we found *H. lemuris* with a prevalence of 6.6% (N = 1). No ticks were found in the late dry season (Table 3, Fig. 4).

We found mites in all sampling periods, however with a higher prevalence of 83.3% (N = 33, found in 10 out of 12 samples) and 80% (N = 25, found in 12 out of 15 samples) in the wet and early dry season, respectively than in the late dry season with a prevalence of 11% (N = 3, found in 2 out of 18 samples). Out of the 45 samples 24 did at least contain one individual of mite. The difference in mite counts between the different seasons was found to be significant for the subset of samples that stemmed from animals that were only sampled once during all of the aforementioned three seasons (N = 21, Kruskal-Wallis chi-squared = 7.3, df = 2, $p = 0.026$; Table 3, Fig. 4). To account for the paired nature of the samples that were taken from the same individual in multiple seasons this data was analyzed separately. A trend in the prevalence of mites over the course of the year is visible with fewer or equal amounts of mites in the dry season samples than in the wet season. No significant difference in mite counts was found between male and female individuals in any of the three seasons (Mann-Whitney-Wilcox-U-test; ws18: N=12, W = 20.5, p = 0.217; ds18: N=18, W = 37, p = 0.868; ds19: N=12, W = 24, p = 0.667; Table 3, Fig. 4). We did not detect any lice in *L. edwardsi*.

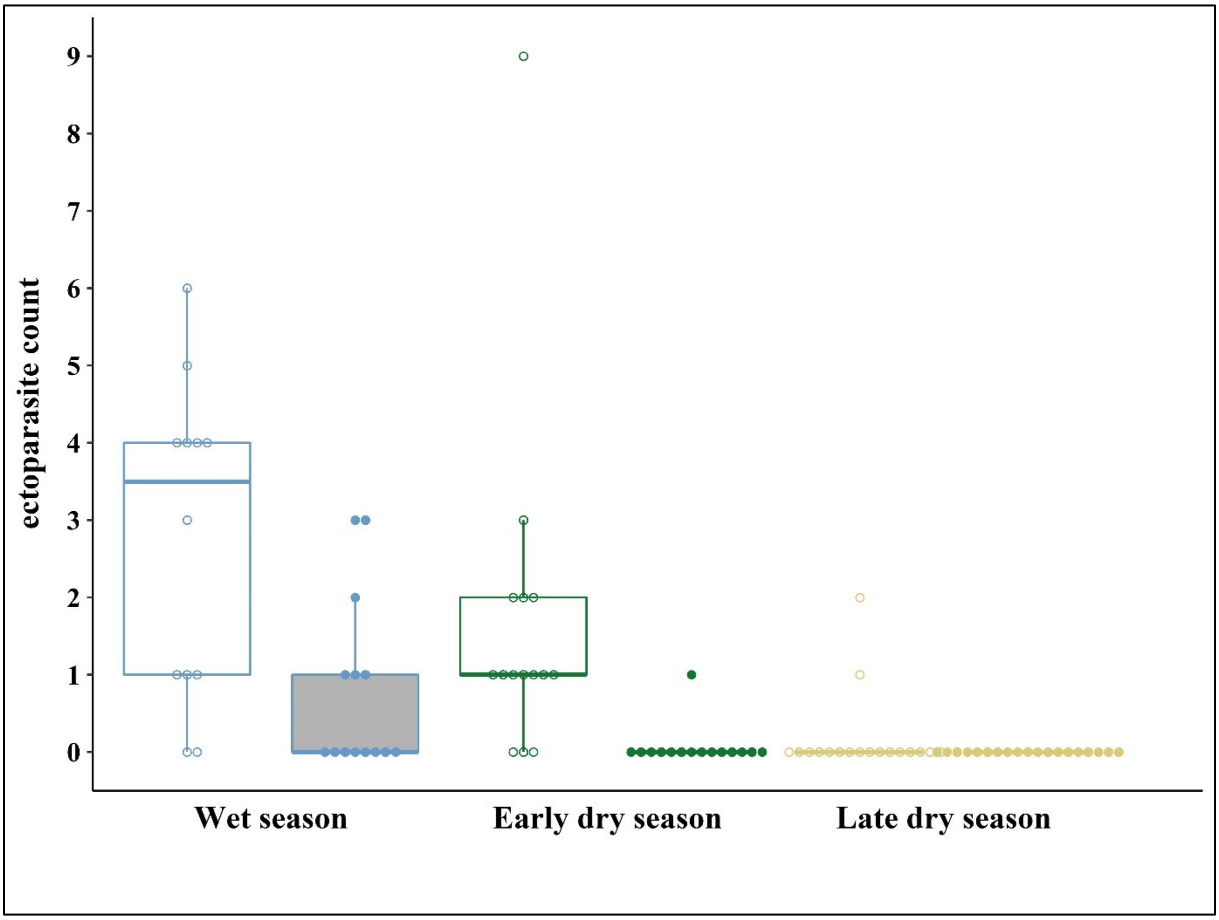

Fig. 3: Ectoparasite count during the three sampling seasons. Number of ticks (grey boxplot; filled circles) and mites (white boxplot; open circles) of *Lepilemur edwardsi* individuals during the wet season (N = 12), early dry season (N = 15) and late dry season (N = 18; bold line, median; box limits, first and third quartiles; whiskers, 1.5x interquartile range; all data points are included).

The Spearman correlation showed a significant decrease of the ectoparasite prevalence of *L. edwardsi* at higher maximum $T_{\text{tree hollow}}$ (Spearman's rank correlation, S = 9413, rho = -0.57, p = <0.001; Fig. 5).

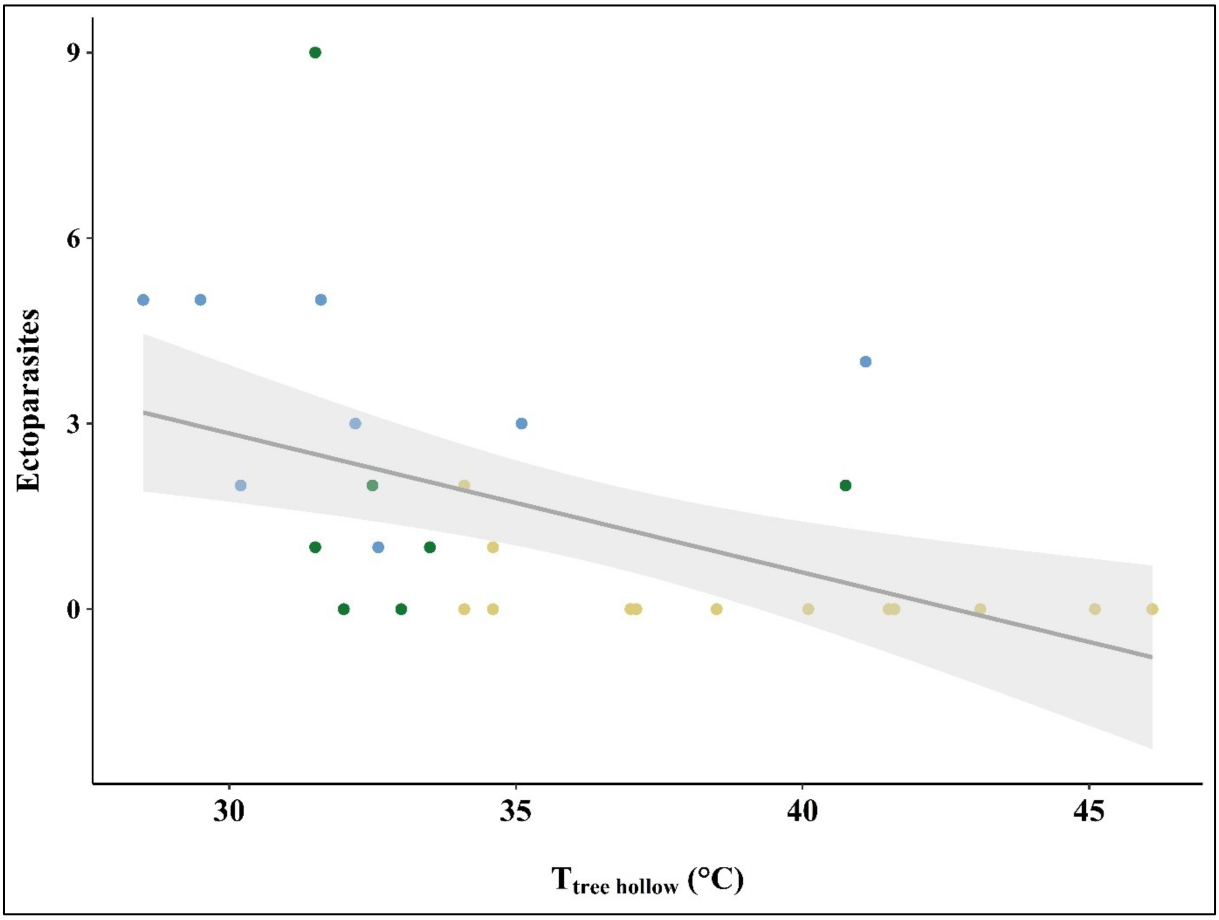

Fig. 4: Seasonal change of ectoparasite count with increasing $T_{\text{tree hollow}}$. Regression line (adjusted $r^2 = 0.24$, $p < 0.001$) and 95% confidence interval of relationship between maximum tree hollow temperature ($T_{\text{tree hollow}}$) and ectoparasite prevalence of all individuals of *Lepilemur edwardsi* during all seasons (wet season: N = 14, early dry season: N = 15, and late dry season: N = 18).

Table 3: Ectoparasite prevalence and count of mites and ticks (*Haemaphysalis lemuris*) of *Lepilemur edwardsi* during the wet season (mites: N = 12, 9 males, 3 females; ticks: N = 14, 9 males, 5 females), early dry season (N = 15; 8 males, 7 females) and late dry season (N = 18; 7 males, 11 females).

	Mites		**Ticks**		
Season	**Count**	**Prevalence**	**Count**	**Prevalence**	**Total count**
Wet season 2018	33	83.3%	11	42.9%	44
Early dry season 2019	25	80%	1	6.6%	26
Late dry season 2018	3	11%	0	0%	3

Table 2: Result of the conditional “best” generalized linear mixed effects model (GLMM) with eggs per gramm (epg) of the intestinal parasites as response variable: Estimate, standard error (SE), *z*-value and the *p*-value for each explanatory variable. The asterisk represents the respective significance levels with $p < 0.001$***, $p < 0.01$**, $p < 0.05$* for each variable.

Explanatory variable	**Estimate**	**SE**	***z* value**	***p* value**
Intercept	5.38	2.67	2.02	0.04*
Late wet season	0.69	0.43	1.61	0.11
Early dry season	1.90	0.57	3.33	<0.001***
Body condition	-0.00	0.02	-0.10	0.92

4.5 Discussion

In this study, we examined the influence of seasonal changes, sex and body condition, resting site characteristics (size and $T_{\text{tree hollow}}$) on the parasite prevalence of the pair-living and arboreal *Lepilemur edwardsi* in western Madagascar. Generally, *L. edwardsi* showed a low diversity of intestinal parasites with only a few different parasite species, compared to other lemur species, e.g., wild red-fronted lemurs (*Eulemur fulvus rufus*) which showed a three times higher diversity of intestinal parasites. *E. fulvus rufus,* however, lives in groups and often changes its vertical habitat which promotes higher parasite species diversity, as social activities, e. g. , grooming, and the consummation of contaminated food or water and greater mobility, facilitate the transfer of parasites (Clough et al. 2010).

During the course of the year, parasite prevalence of *L. edwardsi* fluctuated, albeit in opposing patterns in intestinal and ectoparasites: levels were higher in intestinal parasites, but lower in ectoparasites during the wet season (Fig. 2, 4). Body condition however, did not change seasonally as we also included younger and therefore smaller individuals and hence the data showed a high variance (Suppl. Tab. 1).

Intestinal parasites

In 92.5% of the feces samples of *L. edwardsi* we found mainly pinworm eggs of *Lemuricola* sp.. *Lemuricola* sp. was also found in many other lemur species with different diets such as in the frugivorous *Eulemur* spp., the insectivorous *Daubentonia madagascariensis* and also in other folivorous sportive lemurs (Lepilemuridae)(Junge and Sauther 2006; Hokan et al. 2018). The prevalence of *Lemuricola* sp. might even be underestimated, as the female pinworms excrete their eggs in the perinatal region of the host rather than the intestinal lumen. Transmission of *Lemuricola* sp. could be possible through grooming of the perianal region or through the olfactory inspection of scent marks, a common behavior in lemurs (Irwin and Raharison 2009). Furthermore, the tree hollows that *L. edwardsi* uses for resting during the day seem to be advantageous for *Lemuricola* sp. transmission, as the sympatric and also folivorous lemur species

Avahi occidentalis, which occupies a very similar niche, but rests on open branches rather than in tree hollows, showed no infection with *Lemuricola* sp. (Hokan et al. 2018). *Microcebus murinus*, another lemur species that rests in tree hollows, also showed a higher *Lemuricola* sp. prevalence than the closely related *M. ravelobensis*, which rests in leaf nests during the day (Kiene et al. 2021). The sportive lemurs' tree hollows are an ideal habitat for intestinal parasites and also ectoparasites, as they are often dark and humid and frequently visited by their inhabitants (Rasoloharijaona et al. 2003; Hokan et al. 2018).

In this study, we found a strong variation in *Lemuricola* sp. prevalence between the seasons, from 45.5% in the wet season to 61.5% in the early dry season and nearly a doubling in *Lemuricola* sp. prevalence from the wet to the late dry season (83.3%; Table 1, Fig. 2). This is presumably due to the unfavorable environmental conditions during the dry season, as ambient temperatures in Ankarafantsika are highest during the late dry season after no precipitation for several months (Bethge et al. 2021), resulting in a decrease in food-quality. As *L. edwardsi* elevates its resting metabolism while losing body mass, it seems that this imposes substantial constraints for this species (Bethge et al. 2021). These factors may also influence the immune efficiency and might have an effect on the susceptibility of *L. edwardsi* to parasite infections (Pruett 2003). As shown in several wild African ruminant species which had a higher intestinal parasite loads during droughts than in periods with more rainfall, potentially due to their poor-quality diet during this time of the year (Ezenwa 2004; Shearer and Ezenwa 2020).

$T_{\text{tree hollow}}$ showed no correlation with the intestinal parasite prevalence of *L. edwardsi*. A possible explanation for the increase of intestinal parasites towards the end of the dry season could be the decrease in diet-quality. Individuals need a balanced diet for the immune cells to function optimally (Coop and Holmes 1996; Coop and Kyriazakis 1999; Childs et al. 2019). As the availability and quality of the folivorous diet of *L. edwardsi* strongly decreases during the dry season, due to a decrease in protein and an increase in plant secondary metabolites (Coley 1983; Dearing 2013), the immune system might be impaired. Hence, *L. edwardsi* does not seem to maintain an adequate diet, and as a result is presumably not able to fight off the intestinal parasites. However, as some individuals showed a lower intestinal parasite prevalence in the early dry season in 2019 than in the late dry season in 2018 (Suppl. Table 1), it seems that individuals are able to shed intestinal parasites over the course of the wet season. In another folivorous lemur species, the sifaka (*Propithecus verreauxi verreauxi*), pregnant female individuals selected for tannins in their diet, presumably due to their anti-abortive but probably also due to their anti-helminthic propensities (Athanasiadou et al. 2001; Carrai et al. 2003). In *L. edwardsi*, individuals might also self-medicate by selecting leaves which have a higher concentration of tannins and are therefore able to fight off their parasitic infections.

Conversely to our study, Hokan et al. (2018) could not detect a seasonal effect in the prevalence of intestinal parasites in the same species, but also could not detect any influence of weather. This could be due to the different timing of sampling, as the sampling period of Hokan et al. (2018) differed from the sampling periods in this study.

Lemuricola sp. and other pinworm species have an indirect life cycle with insects as intermediate hosts (Irwin and Raharison 2009). Development of parasites in these intermediate hosts is enhanced at higher ambient temperatures (Altizer et al. 2006), which is why it is suggested that the prevalence of many parasite species will increase in habitats which are particularly affected by climatic changes (Short et al. 2017). Additionally, the resting sites of *L. edwardsi* promote the hatching of parasite eggs, as the conditions of the tree hollows allow an increase the abundance of larvae (Rogers and Sommerville 1963). Further research should include sampling of parasites inside the tree hollows of *L. edwardsi* to verify the importance of these resting sites for parasite transmission. All these factors give rise to the concern of a higher parasite infection rate in the upcoming years as climate change intensifies (Short et al. 2017; IPCC 2019).

Strongyle (nematode) egg excretion in *L. edwardsi* was lowest in the early dry season but highest in the late dry season. However, prevalence was also high in the wet season, with 54.5% for Strongyle eggs, compared to 46.2% and 50% in the early and late dry season, respectively (Table 1). This might be explained by the development of Strongyles, as nematode larvae sometimes arrest their development (hypobiosis) in the intestine of their hosts, when the environmental conditions are unfavorable for external larvae development (i.e., dry conditions)(Schad 1977). However, in *L. edwardsi* infestation with Strongyles seems possible year-around due to the conditions of their resting sites in tree hollows. This is supported by the finding that the branch-resting *A. occidentalis*, did not show Strongyle prevalence in the dry season (Hokan et al. 2018). Prevalence of *Ascaris* sp. increased from 9.1% in the wet season to 23.1% in the early and 27.8% late dry season (Table 1). Hokan et al. (2018) only found Strongyle eggs when the individuals were also infected with *Lemuricola* sp. which we could not confirm in this study, as we found individuals which were only infected with Strongyles. Generally, infection with Strongyles and particularly *Ascaris* sp. was relatively low (Table 1).

Ectoparasites

Resting in tree hollows has numerous advantages, such as protection against predation (Anderson 1998) and insulation from climatic extremes (Schmid 1998). However, tree hollows are not only a suitable environment for intestinal parasites (see above) but also particularly for ectoparasites, especially, when tree hollows are shared between individuals (Hokan et al. 2017; Klein et al. 2018). Nidicolous ticks (i.e. ticks that live near or in their hosts resting sites), such as Ixodidae (hard ticks), prefer the humid, dark and regularly frequented resting sites of *L. edwardsi* (Hokan et al. 2017). We only found the ixodid tick species *Haemaphysalis lemuris* on individuals (Table 3; Fig. 4). This tick species is known to parasitize several lemur species (e.g. *Microcebus rufus* (Durden et al. 2010)) and was previously described to infest *L. edwardsi* (Hokan et al. 2017). *H. lemuris* has a seasonal life cycle with multiple hosts to complete its development (Rodriguez et al. 2015). The larvae of *H. lemuris* develop in the early dry season (May – July), followed by the nymph stadium (June – November) and the adult ticks (December – April). As we were only able to find adult individuals of *H. lemuris* on *L. edwardsi* during the wet season, it is possible that the larvae and nymph stadium feeds on a different lemur species, such as *M. murinus* or *M. ravelobensis* (Rodriguez et al. 2015; Klein et al. 2018).

Mite infection of *L. edwardsi* was lower during the dry season, with almost no mites found in the late dry season (Table 3, Fig. 4). The mites potentially use the protected resting sites of *L. edwardsi* for infestation of individuals (Hokan et al. 2017). This is in concordance with previous findings that *M. murinus*, which rests in resting groups in tree hollows during the day (Radespiel et al. 2003) showed a higher prevalence of mites than the sympatric *M. ravelobensis*, which rest in leaf nests (Thorén et al. 2010; Kiene et al. 2020). Additionally, mite prevalence was lower at the forest edges than in the core area, presumably due to higher ambient temperatures and lower humidity in these areas (Kiene et al. 2020). During the late dry season, the prevailing high ambient temperatures and low humidity might negatively influence the reproduction and development of mites in the resting sites of *L. edwardsi* (Arlian et al. 1999; Kiene et al. 2020) and we did, indeed, find that ectoparasite prevalence decreased with increasing maximum $T_{\text{tree hollow}}$ from the wet to the late dry season in *L. edwardsi* (Fig. 5). As these conditions arrest development and increase mortality of many ectoparasites, such as ticks (Needham and Teel 1991) and mites (Merino and Potti 1996; Castaño-Vázquez et al. 2018), ectoparasite abundance often decreases during drier periods (Moyer et al. 2002).

4.6 Conclusion

Animals living in seasonal habitats have to adapt to annually changing environmental conditions and the accompanying changes in food availability and quality (Pörtner and Farrell 2008), which in turn negatively influences parasite prevalence. Particularly, during the dry season *L. edwardsi* has to cope with the decreasing quality of its folivorous diet. The increased intestinal parasite prevalence and low body mass during the dry season suggests that *L. edwardsi* is struggling with the dry season conditions. This suggests that this species might already be at its adaptive limits and presumably only has few possibilities to react to further changes in its habitat.

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4.9 Appendix

Suppl. Tab. 1: Sampled individuals of *Lepilemur edwardsi* in the early wet season, wet season and early dry season: Sex of the individuals (m = male, f = female), eggs per gramm (epg) of intestinal parasites, counts of mites and ticks, maximum tree hollow temperature ($T_{\text{tree hollow}}$) of the resting sites, body mass (BM), tarsus length and diameter at breast height (dbh) of the resting site trees where the individuals were captured.

			Wet season 2018						Early dry season 2019						Late dry season 2018					
Ind.	**Sex**	**Dbh of sleeping site** (cm)	**epg**	**Mites**	**Ticks**	**Max. $T_{\text{tree hollow}}$** (°C)	**BM** (g)	**Tarsus length** (cm)	**epg**	**Mites**	**Ticks**	**Max. $T_{\text{tree hollow}}$** (°C)	**BM** (g)	**Tarsus length** (cm)	**epg**	**Mites**	**Ticks**	**Max. $T_{\text{tree hollow}}$** (°C)	**BM** (g)	**Tarsus length** (cm)
1	m	80	100	0	3	35.1	671	6.8	100	1	0	31.5	814	7.4	1000	0	0	43.1	786	7.0
2	m	49	NA	0	3	32.2	895	6.5												
3	m	82	300	1	0	32.6	947	NA	1400	1	0	NA	954	6.9						
4	m	57	0	4	1	29.5	819	6.6												
5	f	68	NA	NA	0	35.1	904	5.8							7700	0	0	34.6	1013	NA
6	m	59	100	1	1	30.2	747	6.7												
7	m	76	0	4	1	31.6	857	6.3							400	1	0	34.6	880	8
8	m	52	0	4	0	41.1	924	7.1							1100	0	0	41.6	952	7.5
9	m	101	100	1	0	NA	891	6.9							NA	0	0	38.5	861	NA
10	f	97	100	NA	2	28.5	926	7.0	NA	0	0	33.0	948	7.4	6900	NA	NA	37.0	1068	NA
11	m	101	400	5	0	28.5	944	6.9	700	1	0	33.5	948	7.2	1600	0	0	37.0	914	NA
12	f	80	2200	4	0	NA	772	6.6	2700	0	0	32	814	6.9						
13	f	61	1200	3	0	NA	910	7.3												
14	f	54	NA	6	0	NA	864	6.7							10900	0	0	37.1	910	6.7
15	f	59													500	0	0	41.5	655	7.2
16	f	52													700	0	0	41.6	684	7.7
17	m	102							200	1	1	32.5	970	7.4	100	0	0	38.5	887	7.3
18	f	95													200	0	0	46.1	812	7.0
19	f	74							400	9	0	31.5	945	7.5	300	0	0	37.1	1067	NA
20	f	47													1100	0	0	34.1	600	NA
21	f	44													1200	2	0	34.1	1084	7.2
22	f	46													800	0	0	40.1	814	7.5
23	f	58							NA	1	0	NA	981	5.2	200	0	0	37.1	1074	8
24	f	135													11400	0	0	45.1	NA	NA
25	f	135													300	0	0	45.1	NA	8.4
26	f	52							0	2	0	41.0	1141	8.5						
28	f	78							0	1	0	NA	841	6.6						
30	m	37							700	3	0	NA	813	7.3						
31	m	79							18700	2	0	NA	903	7.2						
32	m	51							200	0	0	NA	869	7.1						
33	m	60							1600	2	0	NA	824	7.3						
34	f	82							400	1	0	NA	973	7.3						

CHAPTER 5:

GENERAL DISCUSSION

5.1 General Discussion

Animals living in Madagascar's western tropical dry deciduous forests have to adapt to seasonally changing fluctuations of ambient temperature and precipitation and hence resource availability (Randrianambinina et al. 2007; Kappeler and Fichtel 2012; Varpe 2017). The extremely elevated resting metabolic rate (Chapter 2), the higher stress levels (Chapter 3) and the increased intestinal parasite prevalence (Chapter 4) of *Lepilemur edwardsi* during the late dry season indicate, that this species struggles with these changes of environmental conditions. In fact, *L. edwardsi* might already be at its adaptive limits, and as climate changes scenarios predict a 2.5 - 4°C rise in air temperatures and an expansion of dry areas and dry periods in coming years (Fig. 1)(IPCC 2021) it seems, that this species only has few possibilities to react to further environmental changes. As, *L. edwardsi* strongly elevates its resting metabolism to cope with the decreasing resource availability and quality during the late dry season, it may not have a big margin of physiological plasticity in adapting to rising ambient temperatures and the associated changes (i.e., changes in resource availability and quality, water scarcity etc.). Indeed, the pattern we found in *L. edwardsi* seems to fit in the "*plastic floor and concrete ceilings*" hypothesis. This hypothesis was first posed in a study of the basal energy requirements of a fish species (i.e., the European perch *Perca fluviatilis*) and describes the physiological plasticity of species, whereby basal energy expenditure is predicted to be physiologically flexible, but maximum metabolism shows only limited physiological plasticity. Particularly, individuals that were already acclimated to high environmental temperatures had reduced possibilities to a further increase in temperatures, such as heat waves ("*concrete ceiling*")(Sandblom et al. 2016). This was also shown in terrestrial ectotherms, particularly in insects of mid-latitude populations, some of which are already quite close to their thermal maxima. These species may only have a limited capacity to cope with changes in their upper thermal limits, making them vulnerable to further climatic changes in the future (Hoffmann et al. 2013). *L. edwardsi* might also have a smaller scope to react to high ambient temperatures ("*concrete ceiling*") as opposed to low ambient temperatures ("*plastic* *floor*"), presumably due to the restricting environmental conditions during the late dry season.

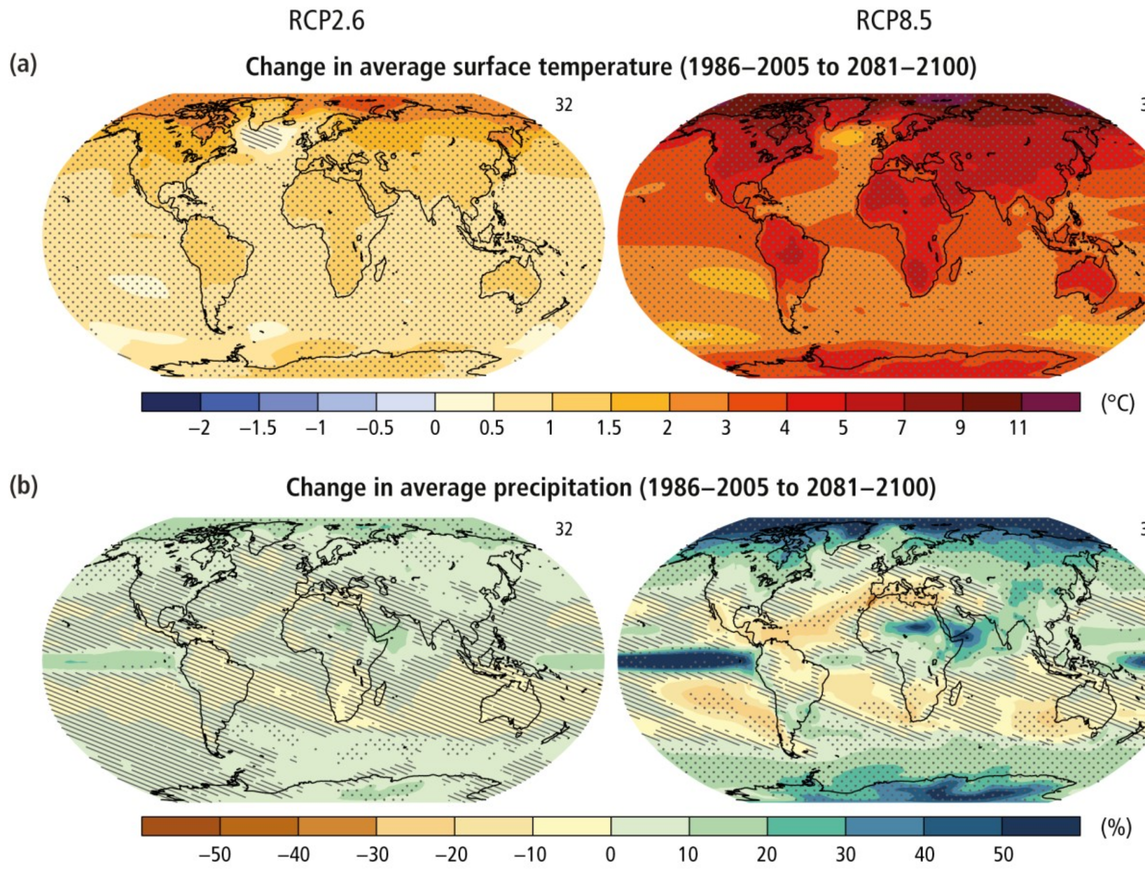

Fig. 1: Modelled projections of two representative concentration pathway (RCP) scenarios. RCP2.6 projects the changes when the global warming stays below 2°C above pre-industrial temperatures and RCP8.5 is a scenario which projects very high greenhouse gas emissions. (a) Change in annual mean surface temperature and (b) change in annual mean precipitation, in percentages. The dots indicate regions where the projected change is larger compared to natural internal variability and where 90% of the models agree on the sign of change. Diagonal lines show regions where the projected change is less than one standard deviation of natural internal variability in 20-year means (Figure adapted from IPCC 2021).

Generally, however, *L. edwardsi* showed a relatively low resting metabolism, with a 15%, 41% and 58% lower mean resting energy expenditure in the wet season, early dry season and late dry season respectively, than expected of the mass-specific basal metabolic rate for a primate with a similar body mass (Chapter 2)(McNab 2008). Therefore, matching the generally low resting metabolism of two other sportive lemur species *L. ruficaudatus* (Schmid and Ganzhorn 1996) and *L. petteri* (as *L. leucopus* in Bethge et al. 2017). Arboreal folivores, such as sportive lemurs, generally adopt a sedentary lifestyle to counteract their poor diet and are therefore able to save energy and particularly water (McNab 1978; Pauli et al. 2016; Swanson et al. 2017). Sportive lemurs, and many other arboreal folivores (e.g., the koala, *Phascolarctos cinerus*) cover their water requirements solely through their food (Degabriele et al. 1978). However, when arboreal folivores cannot meet their water requirements, e.g., during droughts or dry periods, they are often unable to maintain evaporative cooling at high ambient temperatures, and are sometimes even forced to climb down from their trees, to search for free water (Degabriele et al. 1978; Chaves et al. 2021). Brown howler monkeys (*Alouatta guariba clamitans*), for example, sometimes drink free water at streams during the driest time of the year, despite the higher predation risk (Chaves et al. 2021). The arboreal and diurnal common brown lemur, *Eulemur fulvus*, on the other hand, presumably rehydrates during the dry season by consuming the leaves of a succulent plant (*Lissochilus rutenbergianus*), that have no nutritional value but a high water content. This species has a frugivorous diet during the wet season, but needs to cover its water requirements solely through a folivorous diet during the dry season, as fruits are not available (Sato et al. 2014). Furthermore, *E. fulvus* shifts its activity period into the nighttime, either to compensate its low-quality diet by prolonging its foraging time or to avoid the high daytime ambient temperatures and therefore costly evaporative cooling, during the dry season (Sato et al. 2014; Levy et al. 2019). As far it is known, *L. edwardsi* shows no such adaptations to the low water availability in the dry season and therefore has to cover its water requirements solely through its diet. However, evaporative cooling mechanisms of animals are often aggravated at high ambient temperatures with high relative humidity, as it is the case in Ampijoroa during the wet season, as the air is almost saturated with water and cannot absorb any more water (Adolph 1947; Mitchell et al. 2018). Birds that use panting as a mechanism for evaporative cooling, such as the social weavers (*Philetairus socius*; Passerine), for example, dehydrated quickly at high ambient temperatures and humidity when they had no access to free water and showed a higher metabolic rate and body temperature (Gerson et al. 2014). By adapting a generally low resting metabolism during the hot and humid wet season, *L. edwardsi* might reduce its need for evaporative cooling and therefore lower its water requirements (Chapter 2).

Thus, *L. edwardsi* tries to reduce its energy budgeting by defending smaller home ranges and having shorter travel distances during foraging. Furthermore, individuals rest often and return to their tree hollows during foraging and therefore further reduce their energy requirements (Warren and Crompton 1997). In fact, Warren and Crompton (1997) noticed that *L. edwardsi* almost seemed torpid, as individuals often rested for about 2 – 4 hours around midnight. In the sugar gliders, *Petaurus breviceps*, individuals also reduce their energy expenditure when the availability of food in their habitat is low, by reducing their activity time and simultaneously reducing their normothermic body temperature. However, when the unfavorable conditions did not improve, individuals entered daily torpor to save even more energy (Christian & Geiser 2007). *L. edwardsi* also showed strong fluctuations in its normothermic free-ranging skin temperature and at a single occurrence a sharp decline in resting metabolism and skin temperature of one individual towards the end of respirometry (in the early morning at the end of their active phase) during the late dry season. But, we could not detect regular employment of heterothermy in *L. edwardsi*. Therefore, *L. edwardsi* might employ daily torpor as an emergency solution when an individuals' body condition is poor and environmental conditions and particularly food availability are unfavorable. This rare use of torpor is also employed by a closely related primate species from the African mainland the African bushbaby, *Galago moholi*. In this species only non-reproducing individuals with an additional poor body condition enter daily torpor as an "emergency-response" (Nowack et al. 2010, 2013), which seems also to be the case in *L. edwardsi*, as the "torpid" individual was presumably a younger and non-reproducing individual (this year's offspring) with a comparatively low body mass of 655g (Chapter 2).

Apart from this single occurrence and contrary to the assumption that species lower their metabolism during challenging, poor-resourced seasons, *L. edwardsi* elevates its resting metabolic rate from a generally low resting metabolism in the wet season to a five-fold higher resting metabolism in the late dry season, independently of ambient temperature. This could be due to the increased toxicity of the diet of *L. edwardsi* during the dry season, as detoxification of plant secondary metabolites (PSMs) generates heat and can therefore impede thermoregulation, and lead to hyperthermia, especially at high ambient temperatures (Dearing et al. 2008; Beale et al. 2018). Generally, when foraging, primates usually select for high protein leaves, when the average protein content of the leaves in the forest is low, which might be the case during the dry season in the dry forests of Western Madagascar (Ganzhorn et al. 2016). *L. edwardsi*, however, is quite limited in its food-choice, and neither selects for protein nor discriminates against fiber, presumably due to food-competition with the sympatrically living nocturnal woolly lemur species *Avahi occidentalis* (Ganzhorn 1993; Warren 1997; Warren and Crompton 1997; Thalmann 2001). However, the protein content of leaves decreases with leaf age and simultaneously (with the protein content), PSM-content of leaves in tropical forests changes, with a higher PSM-concentration in younger than in mature leaves (Coley 1983; Coley and Barone 1996; Coley and Kursor 1996). Thus, plants defend their younger leaves by reallocating the PSMs from older to younger leaves, making the protein-rich young leaves less attractive for folivorous species to avoid herbivory (Coley 1983; Iwasa et al. 1996). Nonetheless, in mature leaves toughness, i.e., fiber content, is probably more important than PSM-content, as tropical young leaves strongly increase their fiber content with leaf age (Coley and Barone 1996; Coley and Kursor 1996). During the dry season in the tropical dry forests of western Madagascar, however, younger leaves are not available and plants might relocate all their PSMs into the mature leaves. Particularly, alkaloids and tannins are more abundant, divers and more toxic in the tropics than in their temperate counterparts (Levin 1976; Levin and York 1978; Turner 1995; Coley and Barone 1996), whereas phenolic compounds showed no difference in these characteristics (Coley and Barone 1996). Additionally, due to temperature-dependent-toxicity, increasing ambient temperatures negatively influence PSM-content of leaves, resulting in fluctuations of leaf-quality throughout the year and a higher toxicity during warmer periods (Dearing 2013). Ultimately, this leads to a sharp decrease of food-quality for *L. edwardsi* during the dry season, as the protein and water content in the leaves decreases with increasing leaf age and the PSM-content increases with the higher ambient temperatures during the dry season. A possible mechanism to cope with high PSMs in the diet, might be the shift of the thermoneutral zone. Dearing (2013) proposed that animals process PSMs better at ambient temperatures just below the thermoneutral zone (Fig. 2). *L. petteri*, sister species of *L. edwardsi*, shifts its thermoneutral zone from colder ambient temperatures in the warmer wet season (25 – 30°C) to warmer ambient temperatures in the cooler dry season (29 – 32°C), which seems counterintuitively. However, *L. petteri* mainly eats leaves from trees of the *Alluaudia* genus, which are low in protein and high in indigestible fiber and presumably PSMs. By shifting its thermoneutral zone in the dry season to higher ambient temperatures *L. petteri* might be able to process PSMs better (Bethge et al. 2017). Unfortunately, however we could neither detect the thermoneutral zone of *L. edwardsi* in general, nor detect a shift of it, as it was previously described for *L. petteri* (Bethge et al. 2017). This might be due to our respirometry setup, as we used the naturally fluctuating ambient temperatures to measure the resting metabolism of *L. edwardsi* (Chapter 2) rather than experimentally manipulating the ambient temperatures as we did with *L. petteri* (Bethge et al. 2017). In *L. petteri*, we used a climate chamber to expose the individuals to ambient temperatures ranging from 11 – 38°C in the wet season and 15 – 35°C in the dry season (Jolly et al. 2006; Génin 2008), which is in the approximate range of the ambient temperatures of the respective contrary season. Measuring the resting metabolism of individuals using the naturally occurring daily fluctuations in ambient temperatures during the respective seasons instead, as we did with *L. edwardsi*, might however show a more relevant response of individuals (Chapter 2).

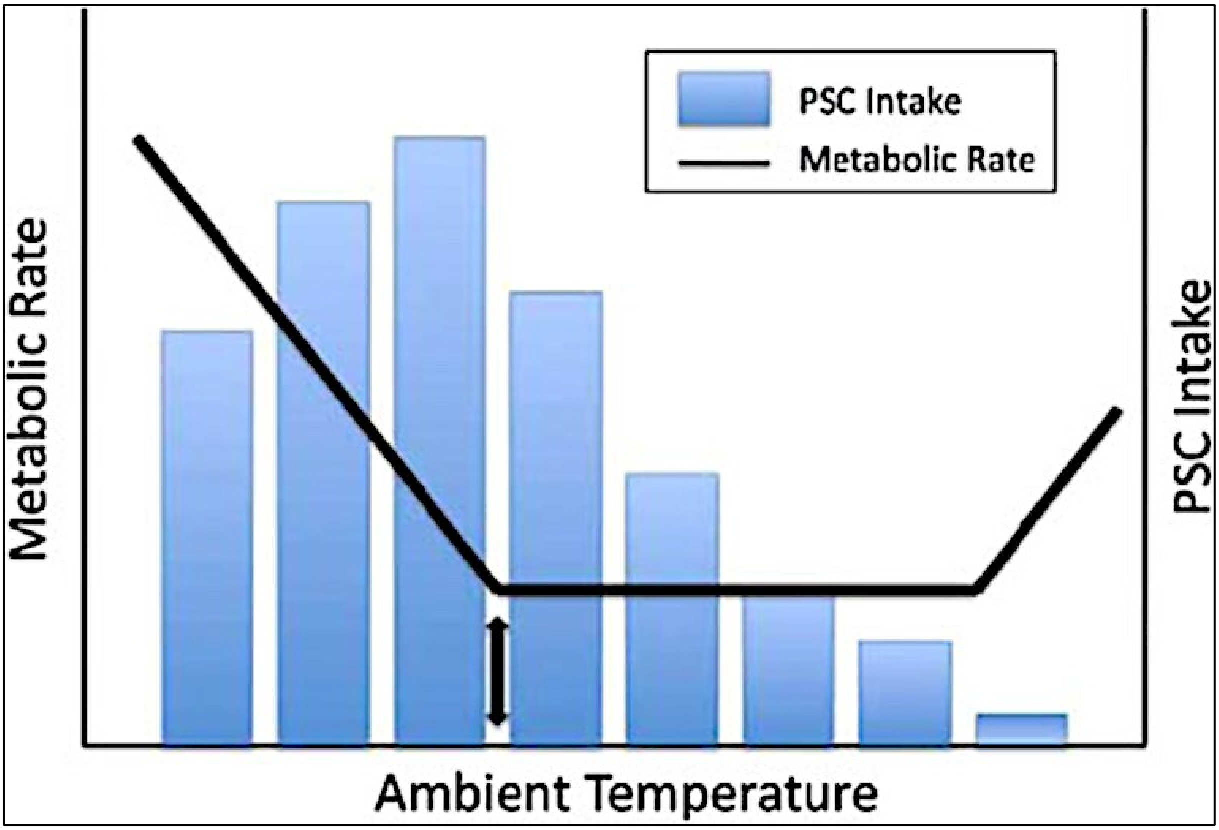

Fig. 2: Relationship between ambient temperature and ingestion of plant secondary metabolites (PSM, here: PSC) hypothesized by Dearing (2013). The arrow indicates the ambient temperature just below the lower critical temperature of the thermoneutral zone where Herbivores are supposed to tolerate the greatest PSM-intake.

Nevertheless, *L. edwardsi* has to cope with the dry season conditions (i.e., higher ambient temperatures, low water and food availability, low food-quality) and the need for detoxification and therefore the need for evaporative cooling and thus metabolic rate increases. As high ambient temperatures additionally negatively influence hepatic enzyme activity and gene expression, the liver clearance function is inhibited, which further exacerbates detoxification of PSMs (Dearing et al. 2008; Dearing 2013; Kurnath and Dearing 2013). Thus, it might be advantageous for *L. edwardsi* to shift its foraging activity and therefore digestion to the second half of the night (Warren and Crompton 1997) when ambient temperatures and hence PSM-concentrations are lower. Furthermore, renal excretion of PSMs requires water and as arboreal folivores, such as *L. edwardsi*, often do not have access to free water and meet their water requirements solely through their food (see above), they might not be able to maintain continuous evaporative cooling to counteract the additional water requirements, due to detoxification (Beale et al. 2018; Mitchell et al. 2018). This creates a trade-off between food intake and water conservation when leaves have a high PSM-content (i.e., leaves at high ambient temperature) but animals also need to gain enough electrolytes, which additionally require water for renal excretion. When an animal needs the majority of its water for evaporative cooling, there is less water available for renal excretion and therefore food intake (resp. water intake) is further constraint (Beale et al. 2018). As many animals strongly reduce their food intake at high ambient temperatures (Youngentob et al. 2021), *L. edwardsi* might therefore not be able to meet its increased energy demands due to the detoxification of its diet. An indication of this imbalance could be the decrease of the body mass of *L. edwardsi* from the wet season to the late dry season, which suggests that the increase of the resting metabolism is not sustainable in the long run (Chapter 2). This is also visible in the elevated stress levels in *L. edwardsi* from the early wet season to the early dry season, which are perhaps linked to the increase of the resting metabolism and the associated decrease in body mass during the dry season (Chapter 3). Presumably, the starting decline in food availability and quality, the accompanying decrease in water availability and the simultaneously increased need for detoxification during the early dry season, initiated a stress response in *L. edwardsi*.

The stress response of vertebrates is regulated by the hypothalamic-pituitary-adrenal (HPA) axis, which is the major neuroendocrine system in mammals (Spiga et al. 2014). Glucocorticoid hormones (corticosterone in rodents, birds, reptiles, amphibians and cortisol in many mammals and fish), regulate many physiological functions, such as the stress response, fat metabolism, immune response and other cognitive functions in vertebrates (Sapolsky et al. 2000; De Guia et al. 2014; Spiga et al. 2014). Usually, the hormone secretion through the HPA-axis is characterized by a circadian and ultradian rhythm. When an individual is exposed to a stressor however, such as environmental stressors, social stressors, and predation pressure, a stress response in the HPA-axis is initiated (Moberg and Mench 2000; Spiga et al. 2014; Spencer and Deak 2017). When experiencing an acute stressor, the response of the HPA-axis varies depending on the stressor, species, age, and sex of individuals (Spencer and Deak 2017). Furthermore, this response is differently in animals that were previously exposed to this specific stressor, as the HPA-axis response to repeated stress from the same stressor weakens. If the HPA-axis then experiences a novel stressor however, it reacts with the “normal” response or it shows a facilitated response (Spencer and Deak 2017). Chronically elevated cortisol levels can eventually lead to a reduced body condition (Boonstra et al. 1998) and a disruption of reproductive success (Moberg 1991; Moberg and Mench 2000).

During the early dry season five of the sampled seven male individuals of *L. edwardsi* showed extremely elevated stress levels. This might be due to mating behavior which takes place in the beginning of the dry season, as females are in estrus in May / June, and males have enlarged testes (Randrianambinina et al. 2007). Sportive lemurs are living in dispersed pairs and are supposed to be monogamous (see Chapter 1) with fixed sleeping groups that share their sleeping sites during the day, either together or alternating in time (Rasoloharijaona et al. 2003). However, when male individuals of the sister species *L. ruficaudatus* had the possibility for extra-copulation with another female, they mated multiple times (Hilgartner 2006; Hilgartner et al. 2012). In almost all Lemuriformes, females are the dominant sex and particularly in pairs of *L. edwardsi* where the males were smaller and lighter than the females, the pairs predominately slept separated from each other. This suggests, that males might not have permanent access to females in sportive lemurs (Rasoloharijaona et al. 2003; Randrianambinina et al. 2007). During fieldwork we captured many, predominantly male, individuals with strong injuries, which might be the result of aggressive behavior of conspecifics towards these individuals (e.g., home range defending behavior; Fig. 3). However, it is also possible that these injuries stemmed from unsuccessful predation attempts (but see my arguments regarding the predation pressure below). In the sister species *L. ruficaudatus,* males defend their home ranges against other males, but never against females. Additionally, during the mating season males travelled more, showed signs of mate guarding and defended their partner with extreme expression of aggressive behavior (Hilgartner et al. 2012). This behavior is energy-costly and stressful, which presumably strongly elevates the cortisol levels of individuals. Ganzhorn (2002) even suggests that, males are more stressed by the mating season than by the nutritional constraints during the dry season. Indeed, the high stress levels of male individuals during the mating season in *L. edwardsi* indicate that this season is probably rather stressful for the males. Which is considerably unusual for a monogamous species, as high cortisol levels are often associated with a higher rank and monopolization of females and high testosterone (Creel 2001, 2005; Reeder and Kramer 2005). In the polygynous wild red deer (*Cervus elaphus*), for example the highest fecal cortisol levels were found in the highest-ranking stags that guarded the largest cumulative harem sizes (Pavitt et al. 2015). During the reproductive season of red deer, testosterone is known to regulate the expression of reproductive and aggressive behavior and the high-ranking individuals showed a more aggressive behavior (Lincoln et al. 1972; Fletcher 1978; Pavitt et al. 2015). Moreover, this positive correlation between high cortisol levels and male dominance is also shown in wild chimpanzees, *Pan troglodytes schweinfurthii*, where dominant male individuals showed higher urinary cortisol levels and also an increased aggressive behavior (Müller & Wrangham 2004). However, this pattern is not uniform in all primate species. In the group-living, wild moustached tamarins, *Saguinus mystax*, testosterone and fecal cortisol levels of male individuals did not change significantly throughout the year (Huck et al. 2005). Interestingly, male individuals of *L. ruficaudatus* dominated females during the mating season and showed mate defense behavior (Hilgartner et al. 2012). If this is also the case in *L. edwardsi*, the high cortisol levels of male individuals in this species might be due to higher testosterone and the regulation of reproductive behavior during mating. Ultimately, reproduction seems rather energy-costly for *L. edwardsi* as two presumably non-breeding males did not show elevated cortisol levels and probably also did not invest in breeding behavior this year (Chapter 3).

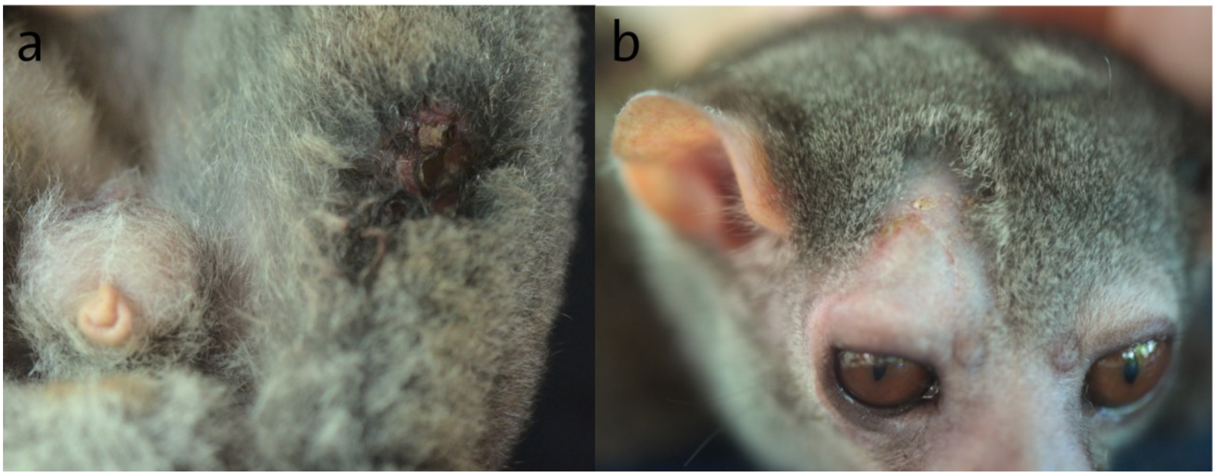

Fig. 3: Injuries of captured male individuals of *Lepilemur edwardsi*, presumably caused by conspecifics that defended their resting sites or home ranges. (Pictures by Arne Wulff)

Female individuals of *L. edwardsi* showed an increase of stress levels during the weaning period, but cortisol concentration during the lactation period was comparatively low. This is in concordance with the assumption of Ganzhorn (2002) who assumed that females are constrained during the lactation and weaning period and prioritize reproduction over food intake. However, as we also measured an increase in resting metabolism with the progressing seasons, we assume, that nutritional stress might be equally stressful. Particularly, as female individuals are also pregnant during the dry season and might have an additional need of energy and nutrients (Chapter 3)(Speakman 2008). The lower food availability and quality might furthermore negatively influence the immune system of *L. edwardsi* and lead to a higher susceptibility to parasite infections or diseases, as a balanced nutrition is required for the immune cells to function optimally (Coop and Holmes 1996; Coop and Kyriazakis 1999; Childs et al. 2019). Furthermore, when the immune system reacts to an intruder (e.g., bacteria, viruses or a parasitic infection), it increases the energy expenditure of individuals and therefore energy demand, which in turn increases the demand of food (Childs et al. 2019). Cortisol is known to have immunosuppressive and anti-inflammatory characteristics (Pruett 2003), therefore weakening the immune response of stressed animals against diseases and parasites. This was also shown in wild male *P. troglodytes schweinfurthii*, which showed a positive association of cortisol with intestinal parasite infections (Muehlenbein and Watts 2010). And also, in red colobus monkeys, *Pilocolobus tephrosceles*, which showed increased fecal cortisol levels in individuals with a higher prevalence of intestinal parasites (Chapman et al. 2006). Chapman et al. (2006) assumed that this is due to the habitat loss the individuals experienced and the associated decline in food availability which in turn negatively impacted the immune response of individuals. And indeed, *L. edwardsi* also showed a higher intestinal parasite prevalence during the more stressful and poor-resourced dry season (Chapter 4). This might be due to the poor nutritional value of their diet (i.e., low protein, high fiber, high PSM-content) and the associated higher stress levels that might impair the immune response of *L. edwardsi*. Individuals from wild impala populations (*Aepyeros melampus*), for example showed a higher intestinal parasite prevalence while simultaneously having lower dietary crude protein levels in their feces in comparison to individuals with higher dietary crude protein levels, which had a lower intestinal parasite prevalence (Ezenwa 2004). We predominately found eggs of the pinworm *Lemuricola* sp. which is known to infect a lot of other lemur species, such as the aye-aye (*Daubentonia madagascariensis*)(Junge and Sauther 2006). *Lemuricola* sp. normally secrets its eggs at the perianal region of the individuals, which is why they can be possibly be transmitted by grooming or via the resting sites of *L. edwardsi* (Chapter 4)(Irwin and Raharison 2009).

Contrastingly, the ectoparasite prevalence was higher in the wet and lower in the dry season, presumably due to the specific life-cycles of the parasite species and their requirements concerning their habitat (i.e. high humidity). *Haemaphysalis lemuris*, the ixodid tick species, we found on *L. edwardsi* prefers dark and humid habitats and adult ticks can only be found from December to April, as the nymph and larvae stadium presumably feeds on other mammalian species during the rest of the year (Rodriguez et al. 2015; Klein et al. 2018). Additionally, mite species, also prefer cooler humid habitats for their development (Arlian et al. 1999; Hokan et al. 2017), which is presumably why the prevalence was much lower during the hot dry season (Chapter 4).

Albeit being a breeding ground for intestinal parasites and ectoparasites, the resting sites of *L. edwardsi* might be a valuable resource for individuals, as they act as protection against predators and the elements or as thermal insulation (Wulff 2020).

These resting site trees are presumably limited in the habitat of *L. edwardsi*, as the trees probably need to be of a certain size and not all tree species may be suitable (Rasoloharijaona et al. 2008; Seiler et al. 2013). Therefore, *L. edwardsi* seems to use any tree hollow that it can physically fit into as a resting site, presumably also a result of the ongoing deforestation of the dry forests (personal observation; Wulff 2020).

The effectiveness of these tree hollows against predation might also be visible in the comparatively low stress levels of *L. edwardsi* during all sampling seasons. The only natural predators that prey on *L. edwardsi* are the fossa (*Cryptoprocta ferox*) whose numbers are declining in the western dry forests, the Malagasy ground boa (*Acrantophis madagascariensis*) and some diurnal and nocturnal birds of prey (i.e., the Madagascan harrier hawk, *Polyboroides radiatus*)(Schülke and Ostner 2001; Mittermeier et al. 2010). Additionally, humans and also introduced species, such as the African wild cats (*Felis silvestris*) prey on sportive lemurs (Borgerson et al., 2021; Dröscher and Kappeler, 2014; García and Goodman, 2003; personal observation of a cat preying on *L. petteri* in Berenty). Sportive lemurs are an easy prey for humans, as they rarely react to them with signs of alert and are comparatively easy to capture in their tree hollows during the day (García and Goodman, 2003; personal observations). In *Lepus americanus*, the snowshoe hares, fecal cortisol levels were greatest in winters with a high risk of predation and low food availability and influenced greatly the reproductive fitness (Sheriff et al. 2011). As we were often able to find the same individuals during all sampling periods over two years and on some occasions even captured individuals that were marked from a previous study in 2013 and 2014 (Hokan et al. 2017, 2018) we assume that the predation pressure is quite low, which could be one of the reasons for the comparatively low stress levels of *L. edwardsi*. Nevertheless, compared to the presumably less stressful wet season, *L. edwardsi* showed elevated stress levels in the early dry season, presumably depicting the decreasing resource availability (Chapter 3).

There are still many unanswered questions regarding the adaptations of the energy budgeting of *L. edwardsi*. Further research of the energy budgeting of *L. edwardsi* should include measurements of the metabolic rate and stress levels of individuals living in forest fragments. The forest at Ampijoroa where we captured my study individuals is a comparatively “healthy” and well-protected part of the national park, as it is close by the administration offices and touristic sites. Individuals living in the forest fragments in more remote areas of the national park might be even more affected by poaching, deforestation and therefore the negative effects of forest edges, such as the high solar radiation and lack of resting sites. Additionally, it would be interesting to evaluate the anthropogenic disturbances on the metabolic rate and especially the stress levels of *L. edwardsi*, by comparing a population in a rather undisturbed forest (e.g., JBA in Ampijoroa) with a population that inhabits a forest with a lot of anthropogenic disturbances (e.g., poaching, logging, agriculture and tourism). For further research it would be advantageous to sample hair of *L. edwardsi* continuously throughout the year, but especially during the early wet season to measure stress levels of the challenging late dry season. Furthermore, a study on the nutritional and PSM-content of the diet, combined with measurements of the field metabolic rate of *L. edwardsi* to calculate DEE would potentially reveal the underlying mechanisms of the elevated resting metabolism during the dry season. Moreover, further research should include sampling of intestinal parasites and ectoparasites inside the resting sites, to verify my assumptions about the impact of the resting sites on the parasite prevalence of this species. To determine the mites on a species level a genetic analysis would be advantageous. Finally, a comparative study of the parasite prevalence in different populations of *L. edwardsi* living in forest fragments would show the influence of anthropogenic disturbances on the parasite prevalence of individuals.

5.2 Conclusion

The current climatic changes and the associated increase of the global ambient surface temperature are increasing the frequency, intensity and duration of climate extremes, such as heat waves, particularly in already dry regions (IPCC 2021). The arid regions of Madagascar, especially the South, are already extremely affected by severe droughts with no rainfall for several years (Tadross et al. 2008; Hannah et al. 2008; Waeber et al. 2015); and global climate change scenarios predict an expansion of dry regions and periods and a decrease in average annual precipitation in coming years (Fig. 1)(IPCC 2021). Animals living in these habitats have to react flexibly to these climatic changes and the accompanying changes in food and water availability (Pörtner and Farrell 2008; Khaliq et al. 2014; Radchuk et al. 2019). *Lepilemur edwardsi* seems to struggle with the environmental conditions during the dry season, particularly toward the end, as its resting metabolic rate and stress levels increase and simultaneously its body mass decreases. Moreover, the changes of the intestinal parasite prevalence of this species show that some individuals show a strong infestation with intestinal parasites during the late dry season. This is cause for concern, as it seems that *L. edwardsi* is already near its "concrete ceiling" and presumably has only a small scope to changing environmental conditions. Should the dry season in western Madagascar get longer and even hotter due to climatic changes, *L. edwardsi* might not be able to sufficiently cover its nutrient and water intake solely through its diet and ultimately the population sizes might decrease.

Additionally, *L. edwardsi* is affected by habitat loss through deforestation, as illegal logging and burning of forests increased dramatically in the last years (Vieilledent et al. 2018; Steffens et al. 2020). Consequently, the habitat of *L. edwardsi* is getting fragmented and the presumably important resting sites of this species are destroyed. In other parts of the Ankarafantsika Nationalpark *L. edwardsi* can only be found in the larger forest fragments, loses genetic diversity and did not form metapopulations in the smaller forest fragments (Craul et al. 2009; Steffens and Lehman 2018). Thus, *L. edwardsi* appears to be more impacted by habitat loss and fragmentation, in comparison to other nocturnal lemur species in this forest, e.g., *Microcebus* spp. (Steffens and Lehman 2018), as *L. edwardsi* relies heavily on the presence of tree hollows, which are only provided by bigger trees. Habitat fragmentation also generates more forest edges, which can lead to environmental changes in the fragments that can reach from the edge in varying degrees of depth into the forest. Especially, abiotic changes at the forest edges, such as changes in ambient temperature, radiation and humidity can cause biotic changes in the vegetation and therefore impact the animals living in these fragments (Gehlhausen et al. 2000; Lehman et al. 2006; Andriatsitohaina et al. 2020). Within these fragments, the restricted availability of suitable resting sites (i.e., tree hollows) or food might lead to an increase in intraspecific-competition leading to higher stress levels (Chapman et al. 2006; Martínez-Mota et al. 2007). Furthermore, forest edges facilitate the access for domestic and invasive animals, which can be hosts for diseases and parasites and can eventually further affect *L. edwardsi* (Ehlers et al. 2019; Kiene et al. 2021). Also, potential predators might get greater access to formerly remote areas, particularly when *L. edwardsi* is lacking sufficient tree hollows for resting and protection against predators, which in turn increases predation pressure (Small and Hunter 1988). One of these predators might be humans, who are known to poach *L. edwardsi*. *L. edwardsi* is highly vulnerable to poaching, as this species shows no flight response when encountering humans, gets consumed regularly and additionally has a slow reproduction rate (one young per year)(García and Goodman 2003; Mittermeier et al. 2010; Borgerson et al. 2021).

Ultimately, my dissertation shows the importance of looking at multiple factors that might influence the energy budgeting and stress response of animals, directly in the field and particularly during different seasons. For example, the slightly elevated stress levels of *L. edwardsi* in the dry season alone might perhaps be not that critical. However, when additionally considering the extremely elevated resting metabolism and higher parasitic burden of individuals during the dry season, compared to the wet season, it seems that *L. edwardsi* might be near its adaptive limits. The consideration of seasonal climatic changes and the associated changes in resource availability and quality, as well as the subsequent constraints that animals face in their habitat, is essential, especially in the light of future climatic changes.

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Eidesstattliche Versicherung

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertationsschrift selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe.

Janina Bethge