Conservation physiology of two closely related, sympatric lemur species, the fat-tailed dwarf lemur (*Cheirogaleus medius*), and the gray mouse lemur (*Microcebus murinus*)

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CONTENTS

CHAPTER 1

CHAPTER 2

CHAPTER 3

SUMMARY

The detrimental effects of human-induced habitat loss and degradation on wildlife are pervasive and represent a primary concern for conservation biologists. Understanding how organisms accurately recognise and respond to these challenges is therefore an important goal of conservation-related research. Species are known to respond differently to anthropogenic disturbance and while some are relatively good at coping with a certain degree of perturbation, some face drastic population decline. This heterogeneity in coping abilities has been generally connected to various biological attributes such as life history characteristics, the degree of diet specialization, and geographical range size. Yet, the underlying mechanisms that drive this species-specific variability in the ability to respond to environmental change and challenges in wild populations remain largely unknown.

The aim of this thesis was to examine how two sympatric lemur species, the grey mouse lemur (*Microcebus murinus*) and the fat-tailed dwarf lemur (*Cheirogaleus medius*) - that differ in various aspects of their life histories and their apparent susceptibility to habitat disturbance - were physiologically affected by anthropogenic disturbances, and in turn, how physiological parameters impacted on general health and fitness output. Specifically, I first investigated how the link between habitat disturbance and various health indicators such as the stress level (measured as hair cortisol concentration, HCC), general body condition (measured as scaled mass index, SMI), and patterns of parasitism (prevalence, morphotype richness, multiple infection) might differ in the two species. I selected four populations of *M. murinus* and *C. medius* that were subjected to a gradient of anthropogenic disturbance in Kirindy Forest, western Madagascar, and compared these health indicators among sites. Secondly, I strived to identify the nature and magnitude of the interrelationships among those health indicators by investigating how HCC influenced SMI and parasite infection using a confirmatory path analysis. Finally, by using a mark-capture-recapture modelling approach, I evaluated the power of the health indicators to predict survival. This last part could only be conducted for *M. murinus* and for the most suitable habitat.

Contrary to the assumption of a deteriorating health (increased HCC and parasitism, and decreased SMI) with increasing level of disturbance, I discovered in the first study that all health indicators were comparable among sites in *C. medius*. In *M. murinus*, neither HCC, nor parasitism patterns (prevalence, parasite species richness and rate of multiple infections) varied across the gradient of anthropogenic disturbance, yet body condition was highest in the most disturbed site. Those results suggest that levels of habitat disturbance may not always be reflected in the physiological health indicator values, probably due to the capacity of animals to develop a range of behavioural adaptations in order to deal with suboptimal conditions. However, the difference in relative density among the study sites and the constantly lower density of *C. medius* in comparison to *M. murinus* imply that, to some extent, both species are negatively affected by environmental changes, with a higher impact on *C. medius.*

Moreover, I found in the second study that there were no associations among HCC, SMI and parasite infection in the fast-living *M. murinus*. In the slow living *C. medius*, I detected a direct positive influence of HCC on parasite morphotype richness, in accordance with the assumption of a reduced immunity in highly stressed individuals. Surprisingly, SMI also showed a positive relationship with HCC especially prior to hibernation. Glucocorticoids (GCs) seem to play a key role in mediating the trade-off between energetic requirements prior to hibernation and an increased susceptibility to parasite infection in *C. medius.*

I finally observed that, following predictions, mouse lemurs with high HCC values experience lowered survival probabilities both at the semi-annual and monthly scale. Individuals with very good body condition (high SMI values) also benefited from higher semi-annual survival in comparison to individuals with mid to low SMI values, but this advantage was not detected at the monthly scale. Survival was not affected by pattern of parasitism. Those last results highlight the negative impact of long-term elevated GC levels on survival and hence population dynamics, and suggest differential strength of selection acting on GCs, body condition, and parasite infection.

In conclusion, the results of this thesis suggest that while they might not always accurately report differences in levels of habitat disturbance, GC hormones can be central in mediating energetic trade-offs, and more importantly, variations of GC levels can predict fitness. The degree of flexibility of the stress response to environmental perturbations might, at least partly, dictate the ability of a given species to cope with harsh condition. Moreover, these findings emphasize the importance of conducting an assessment of the effect of GCs on fitness when using physiological data in conservation-related research.

ZUSAMMENFASSUNG

Die nachteiligen Effekte des durch den Menschen verursachten Verlusts und der Degradierung von Habitaten auf wildlebende Tierarten sind tiefgreifend und ein vorrangiges Tätigkeitsfeld von Naturschutzbiologen. Das Verständnis, wie Organismen präzise diese Herausforderungen erkennen und darauf reagieren, ist daher ein wichtiges Ziel naturschutzrelevanter Forschung. Arten sind dafür bekannt unterschiedlich auf anthropogene Beeinträchtigungen zu reagieren. Während es einige Arten schaffen relativ gut mit Störungen zurecht zu kommen, verzeichnen andere drastische Populationsrückgänge. Diese Heterogenität in der Fähigkeit zur Bewältigung negativer Einflüsse wird allgemein in Verbindung zu verschiedenen biologischen Eigenschaften wie *Life-History*-Charakteristika, Grad der Nahrungsspezialisierung und/oder der Ausdehnung der geographischen Verbreitung gesetzt. Bisher sind die zugrunde liegenden Mechanismen, welche die artenspezifische Variabilität der Reaktionsfähigkeit auf Umweltveränderungen und -anforderungen steuern, jedoch weitgehend unbekannt.

Das Ziel der vorliegenden Arbeit war es, zu untersuchen, wie zwei sympatrische Lemurenarten, der Graue Mausmaki (*Microcebus murinus*) und der Westliche Fettschwanzmaki (*Cheirogaleus medius*) – die sich in verschiedenen Aspekten ihrer *Life-Histories* und ihrer scheinbaren Empfindlichkeit gegenüber Habitatstörungen unterscheiden – physiologisch durch anthropogene Störungen beeinflusst werden, und im Gegenzug, wie sich physiologische Parameter auf den allgemeinen Gesundheitszustand und die Fitness der Tiere auswirken. Im Besonderen untersuchte ich zunächst, wie die Verknüpfung zwischen Habitatstörungen und verschiedenen Gesundheitsindikatoren wie Stressniveau (gemessen als Haar-Cortisolkonzentration, HCC), allgemeiner körperkondition (gemessen als skalierter Masseindex, SMI) und Muster des Parasitismus (Prävalenz, Morphotypenvielfalt, multiple Infektion) zwischen den beiden Arten variieren könnte. Ich wählte vier Populationen von *M. murinus* und *C. medius* im Wald von Kirindy im westlichen Madagaskar aus, die einem Gradienten anthropogener Störungen ausgesetzt waren und verglich die genannten Gesundheitsindikatoren zwischen den Standorten. Zweitens versuchte ich Art und Ausmaβ der Wechselwirkungen zwischen diesen Gesundheitsindikatoren zu identifizieren, indem ich mittels einer konfirmatorischen Pfadanalyse untersuchte, wie die HCC den SMI und die Parasitenbelastung beeinflusste. Durch einen Fang-Wiederfang-Modellierungsansatz beurteilte ich schlieβlich die Aussagekraft der Gesundheitsindikatoren für die Vorhersagbarkeit des Überlebens. Dieser letzte Teil konnte nur für *M. murinus* und das am besten geeignete Habitat durchgeführt werden, da hier der Datensatz am umfangreisten war.

Im Gegensatz zur Annahme eines sich verschlechternden Gesundheitszustandes (erhöhte HCC und erhöhter Parasitismus, und verringerter SMI) mit zunemhenden Grad der Störung fand ich im ersten Teil der Studie heraus, dass alle Gesundheitsindikatoren bei *C. medius* zwischen den Standorten vergleichsweise ähnlich waren. Bei *M. murinus* variierten weder die HCC noch das Parasitenmuster (Prävalenz, Parasitenartenreichtum und Rate multipler Infektionen) entlang des Gradienten der anthropogenen Störungen, wobei die körperliche Verfassung an dem am stärksten beeinträchtigten Standort sogar am besten war. Diese Ergebnisse deuten darauf hin, dass sich der Grad der Habitatstörung nicht immer in den Werten der physiologischen Gesundheitsindikatoren widerspiegelt. Dies hängt vermutlich mit der Fähigkeit von Tieren zur Entwicklung einer Bandbreite an Verhaltensanpassungen zusammen, um mit suboptimalen Bedingungen zurecht zu kommen. Die Unterschiede in der relativen Dichte zwischen den Untersuchungsstandorten deuten jedoch in gewissem Maβe darauf hin, dass beide Arten durch Umweltveränderungen negativ beeinflusst werden, mit stärkeren Auswirkungen auf *C. medius*.

Des weiteren fand ich im zweiten Teil der Studie heraus, dass keine Zusammenhänge zwischen HCC, SMI und Parasiteninfektion bei *M. murinus* mit seiner schnellen *Life-History* bestanden. Bei dem durch eine langsame *Life-History* charakterisierten *C. medius* stellte ich einen direkten positiven Einfluss der HCC auf den Parasitenreichtum fest, in Übereinstimmung mit der Annahme einer verminderten Immunität bei stark gestressten Individuen. Überraschenderweise zeigte der SMI insbesondere vor dem Winterschlaf auch eine positive Beziehung zur HCC. Glucocorticoide (GCs) scheinen eine Schlüsselrolle bei der Vermittlung des Ausgleichs zwischen energetischen Anforderungen vor dem Winterschlaf und einer erhöhten Anfälligkeit für Parasiteninfektionen bei *C. medius* zu spielen.

Schlieβlich beobachtete ich, entsprechend der Vorhersagen, dass Mausmakis mit hohen HCC-Werten verringerte Überlebenswahrscheinlichkeiten sowohl im halbjährlichen als auch im monatlichen Maβstab zeigten. Individuen mit sehr guter körperlicher Verfassung (hohen SMI-Werten) profitierten von einer höheren halbjährlichen Überlebensrate im Vergleich zu Individuen mit mittleren bis niedrigen SMI-Werten. Dieser Teil war jedoch nicht im monatlichen Maβstab feststellbar. Das Überleben war nicht beeinflusst durch das Muster des Parasitismus. Diese Ergebnisse unterstreichen den negativen Einfluss von langfristig erhöhten GC-Spiegeln auf das Überleben und damit die Populationsdynamik, und deuten auf eine unterschiedliche Stärke der Selektion hin, die auf GCs, Körperkondition und Parasiteninfektion wirkt.

Schlussfolgernd lässt sich sagen, dass die Ergebnisse darauf hindeuten, dass GC-Hormone, obwohl sie nicht immer die Unterschiede der Habitatstörung exakt widerspiegeln, von zentraler Bedeutung bei der Vermittlung des Energiehaushalts sind. Noch bedeutsamer ist die Tatsache, dass sich anhand der GC-Spiegel die Fitness vorhersagen lässt. Der Grad der Flexibilität der Stressantwort auf Umweltstörungen könnte zumindest teilweise die Fähigkeit einer bestimmten Art bestimmen, mit widrigen Bedingungen zurecht zu kommen. Darüber hinaus unterstreichen die Ergebnisse die Bedeutung der Bewertung des Effekts von GCs auf die Fitness, wenn physiologische Daten für naturschutzbezogene Forschung herangezogen werden.

GENERAL INTRODUCTION

Habitat loss and degradation represent the most severe threat to wildlife and permanently alter landscapes in ways that can jeopardize the persistence of animal species (Tilman *et al.*, 1994). Being able to accurately recognise and respond to these challenges is pivotal for organisms, and this ability is particularly important under the current increasing and rapid transformation of natural habitats by anthropogenic activities (Steffen *et al.*, 2004). However, species are known to differ considerably in their susceptibility to habitat disturbance and several factors such as strict dietary requirements, restricted distribution ranges or slow life histories were shown to be associated with an elevated extinction risk (Cardillo *et al.*, 2005, Henle *et al.*, 2004, Purvis *et al.*, 2000). Yet, the study of the underlying mechanisms that govern this species-specific variability in the ability to respond to environmental change and challenges in wild populations has lagged remarkably behind. The present study makes a contribution to this field of research by examining the effects of different levels of habitat modification on various health indicators and their consequences in two primate species.

Suites of correlated behavioural and physiological mechanisms can be invoked in order to cope with changing environmental conditions, with potential influences on fitness (Sih *et al.*, 2011, Willmer *et al.*, 2005). The main adaptive mechanism in this context is the physiological stress response mounted by vertebrates when faced with social or environmental stressors. The physiology of stress is relatively well known (Chrousos, 1998, Dallman, 2003, McEwen and Wingfield, 2003, Selye, 1950). However, while the physiological processes associated with a stress response are known to be involved in the mediation of transitions among life-history stages and life history trade-offs (Crespi and Denver, 2005, Denver, 2009, Wada, 2008), it remains poorly understood how this mechanism varies across species that differ in their life history strategies.

The overall goal of this thesis is to determine how a difference in life history characteristics is reflected by the physiological response of two lemur species to habitat disturbance and to examine the potential effects of the stress response on general health and a fitness component. Here, I will first describe the relevant features of the physiological stress response. Then, I will highlight the challenge of interpreting the relationship between an environmental disturbance and the physiological stress response and the possible fitness outcome for an organism by giving an overview of what is known about these two aspects. For a better understanding of the underlying process of the physiological stress response, I will explain the importance of using particular health indicators along with the measure of stress level. Thereafter, I will present evidence for a link between the physiological stress response mechanism and life history characteristics to justify a comparative approach in the study of the disturbance-stress relationship. I will further introduce the two study species (*Cheirogaleus medius* and *Microcebus murinus*), and will outline why these lemurs are suitable for this study. Finally, I will present the specific goals and structure of this thesis, whose overall goal is to determine how a difference in life history characteristics is reflected by the physiological response of two lemur species to habitat disturbance and to explore the potential effect of the stress response on general health and a measure of fitness.

PHYSIOLOGICAL STRESS

The stress response induced by unpredictable and/or noxious stimuli is a common coping mechanism found across all vertebrates and consists of various physiological, hormonal and behavioural components aimed at re-establishing homeostasis (Romero, 2004) Physiologically, the stress response includes the stimulation of the hypothalamic-pituitary-adrenal (HPA) axis to produce neurotransmitters and hormones such as glucocorticoids (GCs) that play an important role in the regulation of other physiological functions, including the immune system and metabolism. Since different types of stressors (e.g physical, psychological or environmental stressors) can trigger such a physiological stress response, it was first thought to be non-specific (Selye, 1950). However, further research suggested that the amplitude of the response primarily depends on the perception of the stressor by the organism (reviewed in Chrousos, 1998, McEwen and Wingfield, 2003, Romero, 2004, Weiss, 1968).

Glucocorticoids (cortisol or corticosterone depending on the taxon) are known to have different functions, depending on the targeted system and their concentration. Indeed, at baseline concentration, GCs have mainly a permissive effect but can have a suppressive, stimulatory or preparative effect in response to stressful events (Sapolsky *et al.*, 2000). Therefore, in the event of a temporary stressor, the elevation of GC concentration has a stimulatory and permissive effect on glucogenesis (Dallman *et al.*, 1993, DeFronzo *et al.*, 1980), but a suppressive effect on long-term survival functions, such as reproduction (Bambino and Hsueh, 1981, Hayashi and Moberg, 1990, Sapolsky, 1985) and immune function (Bateman *et al.*, 1989, McEwen *et al.*, 1997, Reichlin, 1993). The termination of this cascade of reactions is initiated via a negative feedback loop after an effective response to a stressor (Munck *et al.*, 1984). Thus, the stress response fundamentally allows vertebrates to cope with energetically challenging conditions.

STRESS, FITNESS, AND DISTURBANCE

Due to the simultaneous effect of GCs on various functions, much of the literature that focuses on stress physiology asserts that variations in GC levels mediate fitness trade-offs. In an emergency situation (e.g. predation event, unpredicted environmental disturbance), GCs are known to allow the reallocation of energy to enhance immediate survival at the expense of long-term functions such as reproduction (Almasi *et al.*, 2013, Cote *et al.*, 2006, Wingfield and Sapolsky, 2003).

Despite the adaptive value of acute stress responses, the effects of GCs may become maladaptive under long-term activation of the HPA axis, i.e. chronic stress (Romero, 2004, Sapolsky *et al.*, 2000). Due to their suppressive effect on reproduction, prolonged elevation of GC levels may cause anovulation and impotence and, therefore, can impair reproductive output (Breuner *et al.*, 2008, Sapolsky, 1985). As GCs inhibit immune function, chronic stress may increase animals´ susceptibility to disease and infection which in turn can affect energy expenditure and ultimately survival (Dhabhar, 2009, Muehlenbein, 2006, Wayland *et al.*, 2002). Prolonged stress was also shown to negatively impact growth, brain function and cognitive ability (Bernier *et al.*, 2004). As human-induced habitat disturbance often results in long-term adverse changes of environmental conditions, it can negatively affect animal health and fitness.

On account of these observations, three major hypotheses have been proposed to describe how GCs may regulate fitness components: the Cort-trade-off hypothesis (Wingfield and Sapolsky, 2003), the Cort-fitness hypothesis (Bonier *et al.*, 2009b), and the Cort-adaptation hypothesis (Bonier *et al.*, 2009a). Under the Cort-trade-off hypothesis, the acute stress response (short-term elevation of GC levels) should promote survival but impair reproductive success. In contrast, the Cort-fitness hypothesis assumes that elevated baseline GC levels negatively impact both reproduction and survival. Therefore, a chronically stressed individual is considered to be subjected to a less optimal environment and is expected to have a lower fitness in comparison to a non-stressed individual. Under the Cort-adaptation hypothesis, a positive association between baseline GC levels and fitness is assumed due to the required increase of GCs during periods of high investment in reproduction. Thus, GC elevation will enhance reproductive output.

These hypotheses have received mixed support (reviewed in Bonier *et al.*, 2009a, Breuner *et al.*, 2008, Crespi *et al.*, 2013), which might be due to the fact that the link between GCs and fitness is not necessarily a linear one. For instance, field data fail to provide strong support for the "Cort-Fitness Hypothesis" (Bonier *et al.*, 2009b). Various studies have reported the predicted positive association between baseline GC levels and mortality (Blas *et al.*, 2007, Pride, 2005b), but evidence of increased survival probabilities with moderate chronic elevation of GC levels was also found (Cabezas *et al.*, 2007). Several causes were suggested to explain these diverging observations. First, when faced with chronic stressors, an attenuation of the HPA axis activity can allow an individuall to avoid the detrimental effects of long-term elevation of GC levels (Cyr and Romero, 2007, Romero *et al.*, 2009). Moreover, a moderately elevated GC level associated with chronic anticipatory stressors such as predation risk was proposed to be adaptive, depending on the species´ life history.(Boonstra, 2013, Cabezas *et al.*, 2007).

A similar inconsistency has been found for the relationship between GC levels and habitat disturbance. The disturbance-GC association has been documented in several vertebrate taxa [birds (Suorsa *et al.*, 2004, Wasser *et al.*, 1997); mammals (Creel *et al.*, 2002, Martinez-Mota *et al.*, 2007); amphibians (Homan *et al.*, 2003, Janin *et al.*, 2011); reptiles (Romero and Wikelski, 2002, Wikelski *et al.*, 2002)]. The variation in stress load can be connected directly to anthropogenic disturbance (tourism: Fowler, 1999, road density: Wasser *et al.*, 1997) or with multiple direct or indirect effects of habitat disturbance, such as increased predation pressure (Boonstra *et al.*, 1998, Clinchy *et al.*, 2004), change in food availability (Jenni-Eiermann *et al.*, 2008, Kitaysky *et al.*, 1999) or change in habitat quality (Marra and Holberton, 1998, Suorsa *et al.*, 2003). However, while most studies have found the expected elevations in GC levels in disturbed habitats (Behie *et al.*, 2010, Jaimez *et al.*, 2012, Martinez-Mota *et al.*, 2007, Rangel-Negrín *et al.*, 2009), others have reported negligible changes (Muehlenbein *et al.*, 2012) or even decreased GC levels (Tecot, 2013).

Instead of the traditional linear relationship that is assumed between GC levels and fitness, and GC levels and disturbance, Busch and Hayward (2009) proposed that the form of these associations may rather be log-quadratic and may also be species specific. These findings do not only emphasize the need to understand how different species respond to and are affected by environmental disturbance, but also show that considering other health indicators may be required to enhance the interpretability of GC levels.

STRESS LEVEL AND OTHER HEALTH INDICATORS

Anthropogenic disturbance does not only affect stress load but often have simultaneous impacts on various health components such as parasite load or general body condition (Chapman *et al.*, 2006, Chapman *et al.*, 2007, Homyack, 2010). These biomarkers are closely interrelated and can have direct and indirect as well as bidirectional effects that could impact individual fitness (see also Fig. 1). Body condition is known to have a considerable influence on animals´ susceptibility to infection in a way that individuals in poor condition are more vulnerable to infection by pathogens (Beldomenico *et al.*, 2008). Subsequently, infected individuals may in turn show a reduced body condition, creating a vicious circle (Beldomenico *et al.*, 2009). Host condition and infection can act in synergy to further intensify the polarisation between weak and robust individuals (Beldomenico and Begon, 2010). Indeed, the severity of an infection is generally exacerbated in poor condition individuals, with aggravated consequences on health and survival (Garner *et al.*, 2009), whereas parasite infections often have a benign effect on individuals in good condition (Milton, 1996, Munger and Karasov, 1989). Additionally, the cumulative effects of multiple stressors along with potential occurrence of parasite coinfection can have a non-negligible influence on host dynamics (Graham *et al.*, 2007, Marcogliese and Pietrock, 2011).

The immunosuppressive effect of stress hormones is involved, at least partially, in the negative impacts of parasite infection and host condition on fitness (Stratakis and Chrousos, 1995). Population declines could be triggered via the described negative loop in disturbed habitat if the stress caused by the suboptimal environmental conditions leads to a population with a large proportion of individuals in poor condition which in turn could lead to higher prevalence of infection (Beldomenico and Begon, 2010). However, at the species level, these interactions are known to be highly variable and may constitute a proximate mechanism underlying the species-specific variability in the sensitivity of animals with different life history characteristics to environmental disturbance.

HPA AXIS ACTIVITY AND LIFE HISTORY TRAITS

A central tenet of life history theory is that individuals investing heavily in current reproduction are experiencing lower levels of survival or future reproduction (Gadgil and Bossert, 1970, Roff, 2002, Stearns, 1992, Williams, 1966). The physiological basis of this trade-off consists of an alteration of the energy allocation to somatic maintenance and immune function in favour of reproduction, and was suggested to be mediated by changes in secretion of testosterone and GCs (Corlatti *et al.*, 2012, Cox *et al.*, 2010, Ezenwa *et al.*, 2012, Knapp, 2003). While an increase in testosterone levels promotes reproductive effort, it simultaneously, along with GCs, suppresses immune function, which in turn can compromise an individual´s tolerance and/or resistance to parasite infection and ultimately survival (Cox *et al.*, 2010, French *et al.*, 2007, Hirschenhauser and Oliveira, 2006).

In some taxa, comparative analyses of the mechanism of GC modulation revealed that the stress response can be a component of the life-history strategy (reviewed in Crespi *et al.*, 2013). Yet, a simple general pattern fails to emerge when the effect of GC level variation on reproduction and survival is considered for species with different life-history traits. For instance, Hau *et* *al*.(2010) found that in passerine birds, stress-induced GC levels correlated positively with survival rates. However, in an extended review in birds, baseline GC levels were found to negatively correlate with the breeding season length and body mass, and the stress-induced GC levels seem to validate the “brood value hypothesis” (Bokony *et al.*, 2009). Species with low survival probability until the next breeding opportunity showed an attenuated HPA axis activity in response to potential stressors. In mammals, an increase in free GC levels was recorded around the breeding season in some semelparous marsupials (Bradley *et al.*, 1980, Bradley, 1987), but other studies failed to detect a similar pattern in other species with similar life history strategies (Delehanty and Boonstra, 2011).

Moreover, the HPA axis was found to play a key role in the mediation of the transition between life-history stages within a species. In many vertebrates, birth or hatching is preceded by an increase in GC levels, and the same trend was also observed before metamorphosis in amphibians and fish (Crespi and Denver, 2005, Wada, 2008). This elevation of GCs is triggered by environmental cues and allows adequate seasonal timing of these early life-history transitions. Unpredicted changes in the environmental conditions can affect this timing by accelerating or slowing down the developmental stage and the HPA axis is therefore considered to mediate the intra-specific plasticity of vertebrates in early life stages (Denver, 2009, Weiss *et al.*, 2007).

Life history pace is commonly assumed to be linked with a species extinction risk, with slow-living species being more vulnerable to unpredictable threats (Cardillo *et al.*, 2005, Henle *et al.*, 2004, Owens and Bennett, 2000, Purvis *et al.*, 2000, Woodroffe and Ginsberg, 1998). As the HPA axis is also directly involved in the individual response to extrinsic stressors, a comparison of the stress response between species showing different life history traits could provide a powerful tool to elucidate the proximate mechanisms underlying inter-specific variation in vulnerability to environmental disturbance. Yet, this approach often requires controlling for various intrinsic and extrinsic factors, which can be challenging for species that do not co-occur in the same environment.

CHEIROGALEIDS AS STUDY SPECIES: SLOW-LIVING SPECIALIST VERSUS FAST-LIVING GENERALIST

Tropical forests are threatened by anthropogenic activities, despite the fact that they usually host a rich biodiversity coupled with a high level of endemism. Madagascar is no exception to this situation, and has already lost almost 90% of its original forest cover (Green and Sussman, 1990, Harper *et al.*, 2007). The major causes of forest loss in the island range from slash-and-burn agriculture and selective logging for construction and commercial woods to large-scale mining projects (Godfrey and Irwin, 2007, Patel, 2007, Scales, 2012, Watson *et al.*, 2010). Deforestation accelerates the creation of forest fragments, and, together with hunting, constitutes a serious threat to the endemic lemur species of Madagascar. Recurrent natural disasters, such as cyclones and droughts, might be considered as aggravating factors to the existing anthropogenic threats (Gould *et al.*, 1999, Johnson *et al.*, 2011). This situation is particularly worrying since lemurs play a key role in forest regeneration by acting as seed dispersers (Ganzhorn *et al.*, 1999, Razafindratsima and Martinez, 2012).

The cheirogaleid family is currently composed of 34 species distributed among 5 genera (Groves, 2000, Mittermeier *et al.*, 2008, Rasoloarison *et al.*, 2013). They are small bodied (30 – 500 g) nocturnal lemurs and are found in all forest types in Madagascar. Species of the cheirogaleid family can occur sympatrically but are particularly diverse in life history characteristics. At Kirindy forest, for example, the fat-tailed dwarf lemur (*Cheirogaleus medius*) and the gray mouse lemur (*Microcebus murinus*) co-occur in a range of different habitats such as the forest core area, the forest edge or in disturbed forest fragments. However, *C. medius* and *M. murinus* differ in several aspects of their life history, which might affect their respective abilities to cope with potential environmental stressors.

Indeed, several biological attributes of *C. medius* are suggestive of a high sensitivity of this species to challenging environmental conditions. First, it has a slow life history strategy characterized by a small litter size, a late sexual maturity (within 2 years of age), and only one breeding season per year, while reproduction is frequently skipped; (Fietz *et al.*, 2000, Muller and Thalmann, 2002). Second, the fat-tailed dwarf lemur is an obligate hibernator that can be inactive for up to seven months per year (Dausmann *et al.*, 2004). In order to meet the energetic requirements for the long hibernation period and to enable a fast deposition of large quantities of fat, *C. medius* has to rely strongly on specific food items with high sugar content (Fietz and Ganzhorn, 1999, Lahann and Dausmann, 2011). Moreover, *C. medius* hibernates in tree holes in large trees with high insulation capacity that are rare in disturbed habitats (Dausmann, 2013). Despite the relative plasticity of *C. medius* life history that is observed across its range, Lahann & Dausmann (2011) suggested that the extended hibernation period and the frequent skipping of reproduction are crucial for this species in Kirindy due to the highly seasonal variation in resources availability. Therefore, additional environmental constrains arising from anthropogenic disturbance might aggravate this constrain. In fact, *C. medius* was found to occur at low densities in degraded forests in this region (Schäffler *et al.*, 2015) and corroborating the above mentioned theoretical considerations, Rakotoniaina (2011) reported an apparent decline of *C medius* population at Kirindy Forest while showing higher densities at forest parts containing more potential shelter trees.

Unlike the fat-tailed dwarf lemur, *M. murinus* has life-history attributes suggestive of a lower sensitivity to challenging environmental conditions, such as short nursing time, one or two litters per year, and earlier sexual maturity reached at only 10 months of age (Eberle and Kappeler, 2006, Martin, 1972, Perret, 1982, Schmelting *et al.*, 2000). Grey mouse lemurs were also suggested to improve their reproductive success by adopting cooperative breeding among kin (Eberle and Kappeler, 2006). Additionally, *M. murinus* can adopt varying energy-conserving physiological strategies, such as hibernation or short torpor bouts, during the dry season (Schmid and Ganzhorn, 2009). *Vuarin et al*. (2013, 2015) showed a high individual flexibility in the use of daily torpor by *M. murinus* that could be linked to food availability, further proving their strong capacity to survive unexpected environmental fluctuations. In accordance with the theoretical considerations above, *Ganzhorn et al*. (2013) found that the MHC diversity, parasitism pattern, and long-term population dynamics of *M. murinus* were only minimally affected by habitat fragmentation. In summary, despite the wide, mostly sympatric distribution of both species, the fast living, more flexible *M. murinus* can be expected to be less sensitive to human-induced environmental disturbance than the slow living specialist *C. medius.*

OBJECTIVES AND PREDICTIONS OF THE PRESENT STUDY

The overall aim of this study is to link physiological responses of two lemur species with contrasting life history traits to different habitat characteristics and to assess the predictive power of physiological stress on short-term fitness outcomes. Little information is available about physiological responses to habitat destruction in natural habitats and its effects on fitness for lemurs (Irwin *et al.*, 2010a, Pride, 2005b). The inter-specific comparison of health status relative to habitat quality contributes to the understanding of species-specific adaptation, coping mechanisms and response patterns to environmental constraints. Moreover, this investigation will add to existing studies that establish hormonal techniques as an effective tool to assess the general health status of wildlife populations. The validation of such health indicators can equip conservation biologists with a valuable means to evaluate the status of wild populations but also to predict population dynamics. All aspects and interactions that will be addressed in this thesis are shown in Figure 1.

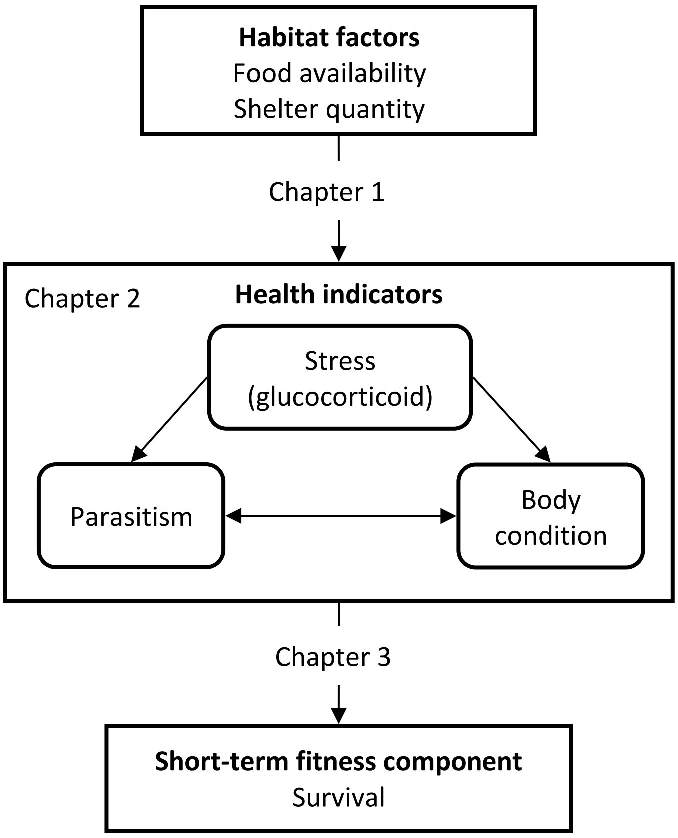

Figure 1. Schematic depiction of the structure of this thesis

In Chapter 1, I first compare the relationship between human disturbance and long-term stress load and two other health indicators (body condition and parasitism) in the specialist *C. medius* and the generalist *M. murinus* in order to assess how habitat changes due to anthropogenic activities affect both species. In detail, I focus on populations of *C. medius* and *M. murinus* inhabiting four different habitats subjected to different levels of anthropogenic disturbance. The first two sites are located in the forest core, but only one of them is regularly visited by tourists. One site is located at the forest edge, adjacent to a savannah, and is frequently subjected to the intrusion of local people from surrounding villages for firewood gathering and hunting. The last site is a forest fragment traversed by several trails that are used daily as paths between villages. It is a source of construction- and firewood and a hunting area for the villagers. I therefore predict that the health indices of the study species positively (stress load and parasitism) or negatively (body condition) correlate with the degree of habitat disturbance, but also that the effect is more pronounced in *C. medius*. This study will add to the rare available data connecting habitat disturbance and physiological stress response in lemur species, and will contribute to a better understanding of the contradictory results obtained so far (Irwin *et al.*, 2010a, Tecot, 2013).

The potential detrimental effect of long-term stress load on general health makes it crucial to study the relationships among GC levels and different health indicators. However, as stress hormones are also known to mediate life history trade-offs, their effects on health should vary between species with contrasting life history traits. In Chapter 2, I investigate the interrelationships among stress load, body condition, and parasite infection in *M. murinus* and *C. medius*. This approach allows a better evaluation of the importance of considering life history characteristics when assessing the impact of stress load on health. Body condition (in terms of nutrient storage) is widely used as a measure of overall health, and it has been shown to be associated with survival as well as reproduction (Stevenson and Woods, 2006). I expect to find higher parasite burdens (prevalence and incidence) coupled with a lower average body condition in individuals showing a chronically high level of GC, but the nature of this association may differ between species with differing life history traits.

Finally, in Chapter 3, I test the hypothesis that increased stress load is associated with decreased survival in *M. murinus*. The accuracy of an indicator to report health status can be assessed by its correlation with fitness. Additionally, I also compare the power of a body condition index and parasitism to predict survival. For the estimation of demographic rates, I combined previously collected long-term data with data collected during this study. This aspect provides valuable information not only for our study system, but is also of general interest regarding the validity of stress as a biomarker of health due to the paucity of studies connecting health indicators to actual fitness outcomes in a wild population (Cooke and O'Connor, 2010). While the link between fitness and stress levels could only be assessed in the most suitable habitat and only for mouse lemurs, a negative relationship between them would reflect a conservative trend that could be applied in more disturbed habitats and in a more sensitive species.

CHAPTER 1

"Does habitat disturbance affect stress, body condition and parasitism in two sympatric lemurs?"

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ABSTRACT

Understanding how animals react to human-induced changes in their environment is a key question in conservation biology. Due to their potential correlation with fitness, several physiological parameters are commonly used to assess the effect of habitat disturbance on animals´ general health status. Here, we studied how two lemur species, the fat-tailed dwarf lemur (*Cheirogaleus medius*) and the grey mouse lemur (*Microcebus murinus*), respond to changing environmental conditions by comparing their stress levels (measured as hair cortisol concentration), parasitism and general body condition across four habitats ordered along a gradient of human disturbance at Kirindy Forest, Western Madagascar. These two species previously revealed contrasting responses toward human disturbance; whereas *M. murinus* is known as a resilient species, *C. medius* is rarely encountered in highly disturbed habitats. However, neither hair cortisol concentrations, nor parasitism patterns (prevalence, parasite species richness and rate of multiple infections) and body condition varied across the gradient of anthropogenic disturbance. Our results indicate that the effect of anthropogenic activities at Kirindy Forest is not reflected in the general health status of both species, which may have developed a range of behavioural adaptation to deal with suboptimal conditions. Yet, a difference in relative density among sites suggests that the carrying capacity of disturbed habitat is lower and both species respond differently to environmental changes, with *C. medius* being more negatively affected. Thus, even for behaviourally flexible species, extended habitat deterioration could hamper long-term viability of populations.

INTRODUCTION

Habitat loss and degradation as a result of anthropogenic activities are major causes of species decline, and identifying their effect on the health and viability of wildlife populations is pivotal to conservation biology (Acevedo-Whitehouse and Duffus, 2009, Wikelski and Cooke, 2006). Anthropogenic disturbances can negatively affect individuals´ general health status, for instance, by altering resources availability, enhancing predation and hunting pressure or facilitating the spread of parasites (Allan *et al.*, 2003, Keyser *et al.*, 1998, Rode *et al.*, 2006). However, despite this general trend, the sensitivity of an organism to changing environmental condition is known to be highly species-specific and biological attributes, such as a slow life history and diet specialization, are good predictors of extinction risk (Cardillo *et al.*, 2005, McKinney, 1997, Purvis *et al.*, 2000, Stark *et al.*, 2004).

Evaluating the physiological responses of animals to environmental changes can help to detect, monitor, and – in the best case – prevent conservation problems (Cooke and O'Connor, 2010, Cooke *et al.*, 2013). Yet, most studies that focused on this aspect essentially investigated a single response variable, such as stress level or pattern of parasitism (Arroyo-Rodriguez and Dias, 2010, Gillespie *et al.*, 2005, Rimbach *et al.*, 2013). Despite the considerable utility of this approach for conservation biologists, it remains extremely arduous to predict how a given species will respond to disturbances or even to identify the intrinsic factors that can potentially initiate a population decline (Wasser *et al.*, 1997 but see Creel *et al.*, 2002). This difficulty is due to the fact that such a complex phenomenon likely involves multiple explanatory factors, as indicated by several long-term studies (Gulland, 1992, Milton, 1996), highlighting the need for using multiple indicators in health assessment studies.

Due to their potential correlation with fitness, stress hormones (glucocorticoids, i.e cortisol for most mammals), general body condition and parasite infection status are commonly used as health indicators and to assess the ability of wild populations to cope with environmental challenges (Chapman *et al.*, 2007, Wikelski *et al.*, 2002). Indeed, the increase in glucocorticoid (GC) level is known to be adaptive when being exposed to an acute stressor (Charmandari *et al.*, 2005, Sapolsky *et al.*, 2000). Under chronically stressful conditions, prolonged hypothalamic-pituitary-adrenal (HPA) axis activity and elevated GC levels may induce various pathological effects and may eventually affect individual fitness negatively by reducing investment in reproduction and immune function (Dhabhar, 2009, Romero, 2004). As habitat degradation constitutes a permanent stressor for wild populations, studies that attempt to identify chronically stressed animals commonly assume GC concentrations to be higher in disturbed habitats than in undisturbed ones, although chronic stress could also lead to HPA hypoactivity. The evidence so far is inconclusive, as increases, decreases or no changes in GC levels have been observed when comparing animals in disturbed and undisturbed habitats (reviewed in Dickens and Romero, 2013).

General body condition is commonly assumed to have an impact on animals´ fitness and health and has been defined as a gauge of an individual´s energy reserves (Krebs and Singleton, 1993, Schulte-Hostedde *et al.*, 2001). Maintenance and reproduction during periods of food scarcity are energetically challenging and therefore make metrics of body condition of primary interest for conservation biologists. Chronic stress consistently leads to a declining body mass, although the magnitude of this effect is species-specific (reviewed in Dickens and Romero, 2013).

Infection with helminths and protozoa can also impair host fitness by affecting survival and/or reproduction. Parasites can severely alter hosts´ health in various ways, ranging from tissue damage, blood loss to death or, more commonly, interfere with basic functions, for example, by decreasing nutrient absorption or increasing energy expenditure (Behnke, 1990, Nunn and Altizer, 2006). Yet, the link between infectious disease risk and habitat disturbance remains unclear and the “dilution effect”, the main hypothesis that was proposed to explain this relationship, lacks consistent empirical support (Young *et al.*, 2013). Indeed, an increase of infection risk with both a decrease (“dilution effect”) and an increase (“amplification effect”) in biodiversity have been found in wild populations (Hechinger and Lafferty, 2005, Jones *et al.*, 2008, Keesing *et al.*, 2006).

Here, we investigated the effect of human disturbance on GC level, general body condition and patterns of parasitism in two small-bodied sympatric lemur species, the fat-tailed dwarf lemur (*Cheirogaleus medius*) and the grey mouse lemur (*Microcebus murinus*), in western Madagascar. These closely related nocturnal species exhibit contrasting life history characteristics and apparently differ in their vulnerability to habitat disturbance. *M. murinus* is a disturbance-tolerant species and can persist in every forest type, even in small forest fragments (Ganzhorn *et al.*, 2003, 2013). During periods of food scarcity, *M. murinus* can adopt multiple energy saving strategies such as flexible daily torpor or hibernation, and is able to switch between both strategies (Schmid and Ganzhorn, 2009, Vuarin *et al.*, 2013). Moreover, *M. murinus* has a relatively fast life history characterized by one or two litters per year (Schmelting *et al.*, 2000), a short lactation length and sexual maturity after 10 months (Eberle and Kappeler, 2006, Martin, 1972, Perret, 1982) that might enhance the species´ resilience to unpredicted or long-term stressors.

Unlike the grey mouse lemur, *C. medius* has life history attributes that render this species more sensitive to challenging environmental conditions such as later sexual maturity (within two years of age), and lower frequency of breeding cycle (once per year) with frequent skipping of reproduction (Fietz *et al.*, 2000, Muller and Thalmann, 2002). *C. medius* are absent in very small forest fragments (Ganzhorn *et al.*, 2003) and occur at low densities in degraded habitat (Schäffler *et al.*, 2015), likely due to their specific dietary and shelter quality (high insulation capacity tree holes) requirements as a strict hibernator (Dausmann, 2013, Fietz and Ganzhorn, 1999). Their slower pace of life and habitat requirements might exacerbate their vulnerability to altered resource availability. Despite being both classified as “least concern” (IUCN, 2015), the assessment of the effects of human activities on the health of these two species can help to detect and understand the proximate mechanisms causing this difference in response to challenging conditions.

In this comparative field study on the specific links between anthropogenic disturbance and several health components, we therefore aimed at determining how these two lemur species respond physiologically to different levels of habitat disturbance. We predicted that a decrease in habitat quality would be linked with an increase in GC concentrations and parasitism levels and a decrease in general body condition. This effect should be more pronounced in *C. medius* compared to *M. murinus* for which low or even no effect of anthropogenic disturbance might be expected.

MATERIALS AND METHODS

Study sites and populations

The study was carried out in the forest concession of Kirindy/CNFEREF (Centre National de Formation, d´Etudes et de Recherche en Environnement et Foresterie) which is part of a dry deciduous forest complex in central western Madagascar (central Menabe region). Four sites, locally known as N5, CS7, Savanna (SV) and Kirindy Village (KV), were selected due to their varying relative levels of current human disturbance. N5 and CS7 belong to the core area of the forest concession and have been used as long-term study sites for research activities since 1993. While N5 is exclusively used for research, CS7 is also regularly frequented by small groups of tourists, both day and night. The SV constitutes the eastern border between the core forest area and a natural savannah, rendering it subject to edge effects. It is occasionally subjected to uncontrolled incursion as the forest constitutes a potential source of food and firewood for the local population. The KV study area is a forest fragment located close to a village and crossed by a path that is used daily by locals as it connects neighbouring villages. This forest fragment is rarely used for research activities and was identified as a suitable site for this study after a survey in February 2012 that confirmed the presence of both study species. Kirindy Village is the site most subjected to human incursion owing to its use as a source of fire and construction wood. The characteristics of the four study sites are summarized in table 1.

Three of the study sites (N5, CS7 and SV) are equipped with a grid system of small foot trails at variable intervals (N5 and CS7: 25 x 25 m; SV: 100 x 25 m). All research activities in KV were conducted along a 1.3 km transect (between 20°4´29.40´´S, 44°37´10.50´´E and 20°5´10.01´´S, 44°37´0.47´´E) passing through this forest fragment.

Habitat structure characterization: density of shelter and food trees

Besides the level of anthropogenic disturbance among study sites, the habitats have not been previously characterized in terms of habitat parameters critical to the study species. To determine whether the level of human disturbance translates into a gradient of habitat quality, we first evaluated the apparent resource availability at each site by estimating densities of trees used for food and shelter by the two species. Trees with a diameter at breast height (DBH) > 10 cm can be considered as a potential refuge (hibernation tree) for *C. medius* and *M. murinus* (Dausmann, 2013, Schmid, 1998). Big trees are also particularly targeted for construction woods by the local population, and we witnessed frequent illegal logging activities in KV. The density of potential shelter trees among study sites was assessed with the point-centred quarter method (Ganzhorn *et al.*, 2011). In N5, CS7 and SV, path intersections were used as centre points and the distance of the nearest tree with DBH > 10 cm from a centre point was measured in each of the four quarters formed by these intersections. In KV, centre points were selected every 25 m on either side of the path, and quarters were obtained in all four compass directions. The number of sampling points differed between sites depending on the sampling design and area size (N5=453, CS7=80; SV=90, KV=106).

The density of food trees for both species was assessed by using plots (5 x 5 m). Each centre point used in the estimation of big tree density was taken to be one corner of the food tree plot. Within each plot, the number of tree species known to be consumed by either species was recorded (see Supplementary Table S1). A list of plant species eaten by *C. medius* is provided in Fietz & Ganzhorn (1999), and the feeding ecology of *M. murinus* is described in Dammhahn & Kappeler (2008).

Table 1 Characteristics of the four study sites in Kirindy Forest.

Site	Habitat characteristic	Human activities	Distance to the closest village (km)	Distance to the closest clear area (km)
N5	Forest core	Research	7.88	5.78
CS7	Forest core	Research, tourism	9.00	5.19
SV	Forest edge	Research, food and firewood gathering	8.97	3.88
KV	Forest fragment	(Research), food, fire and construction wood gathering	3.59	2.3

Sample collection and analysis

Populations of *C. medius* and *M. murinus* have been monitored using long-term live capturing in N5, CS7 and SV (Eberle and Kappeler, 2002, Fietz, 1999, Fietz and Ganzhorn, 1999), and an identical protocol was established in KV for this study. In brief, Sherman live traps baited with banana were set at each of the centre points used for habitat characterization late in the afternoon and checked early in the following morning for three consecutive days. Captured animals were individually tagged with a subcutaneous transponder (Trovan EURO ID, Germany), sexed at first capture and morphometric measurements and body mass were recorded at subsequent monthly captures. All samples used to assess health indicators were collected during four distinct capture sessions: between September - December in 2012 and 2013 (hereafter “dry season”), which is a transition period from dry to rainy season, and January – May in 2013 and 2014 (hereafter “rainy season”), which includes a transition period from rainy to dry season. The September - December period is characterized by low resource availability and covers the mating season of both species. Therefore, it is presumably an energetically demanding period for *M. murinus* and *C. medius* in contrast to the January – May period and that could lead to a contrasting effect on individuals´ health between both seasons. For instance, the chosen periods were shown to be extremes in terms of body mass for our study species (Fietz and Ganzhorn, 1999, Hämäläinen *et al.*, 2014a). Details of the number of individuals captured and samples used for all analysis are given in the supplementary material Table S2 and S3.

Hair sampling for cortisol analysis

We collected one hair sample from the dorso-caudal region per individual and season to avoid potential variation of hair cortisol concentration (HCC) from different body regions (Carlitz *et al.*, 2015, Macbeth *et al.*, 2010). We used a pet grooming clipper (Aesculap Isis GT 420) to cut hair as close as possible to the skin. Samples were then kept at ambient temperature in 2 ml screw cap Sarstedt tubes until shipping to the laboratory. Washing and extraction for hair cortisol analysis was performed at the University of Dresden (Germany), using minor modification from the protocol described by Gao *et al* (2013). In brief, samples were washed twice in 3 ml isopropanol for 3 min and dried under a fume hood. For cortisol extraction 7.5 mg of hair were incubated with 40 µl internal standard and 2.4 ml methanol for 18 h at room temperature in a glass vial. Afterwards, samples were spun in a centrifuge at 10000 rpm for 3 min and 1.6 ml of the clear supernatant was dried at 50°C under a constant stream of nitrogen and re-suspended using 175 µl double-distilled water. Of the final product, 100 µl was used for cortisol concentration determination. This assessment was performed using a Shimadzu HPLC-tandem mass spectrometry system (Shimadzu, Canby, Oregon) coupled to an ABSciex API 5000 Turbo-ion-spray triple quadrupole tandem mass spectrometer (AB Sciex, Foster City, California) with purification by on-line solid-phase extraction (Gao *et al.*, 2013). In total, we collected and analyzed 502 *M. murinus* (N5=236, CS7=125, SV=104, KV=37) and 184 *C. medius* (N5=143, CS7=16, SV=10, KV=15) hair samples obtained during the four field seasons.

Unlike other matrices traditionally used in GC level assessment (plasma, faeces, urine, saliva), hair offers a unique opportunity to measure cortisol accumulated during a wider time window (Stalder and Kirschbaum, 2012). Hair grows slowly; hence GC levels in hair integrate several aspects of HPA axis activity, baseline values, magnitude of the stress response and duration of elevated GC levels over a period of several weeks to months. Only free (i.e. biologically active) GC is thought to be incorporated into the hair shaft (Davenport *et al.*, 2006). There is growing evidence from direct and indirect validation studies that HCC is a useful measure of long-term stress load (Carlitz *et al.*, 2014, Grass *et al.*, 2015, Stalder and Kirschbaum, 2012). Recent studies also found that HCC shows high intra-individual consistency (Stalder *et al.*, 2012). Even though the long-term nature, the minimally invasive sampling and the long stability of HCC (Gonzalez-de-la-Vara *et al.*, 2011, Webb *et al.*, 2010) render this method especially suitable for the field of conservation physiology, only few studies have so far applied the technique in this context (Bechshoft *et al.*, 2012b, Brearley *et al.*, 2012, Carlitz *et al.*, 2016, Macbeth *et al.*, 2010, Martin and Reale, 2008).

Body condition assessment

Individual body condition was estimated by using the scaled mass index (SMI) which reflects internal energy reserves of animals by taking into account the scaling relationship between body mass (BM) and a distinct measurement of body size (Peig and Green, 2009). This index therefore yields an individual value of body mass after standardizing it to the mean body size of all individuals present in the population. As recommended by Peig and Green (2009), head width (HW, the bizygomatic breadth), which is the body size measurement that has the highest correlation with body mass for our study species, was used as a size measurement (see also Vuarin *et al.*, 2013). The scaled mass index for every individual *i* was calculated using the formula:

$$SMI_i = BM_i(HW_0/HW_i)^{bSMA}$$

where HW_0 is the arithmetic mean of HW for our study population (*M. murinus*=21 mm; *C. medius*=26 mm). The scaling exponent bSMA (*M. murinus*=4.976; *C. medius*=4.997), which is the slope of the standardized major axis regression of BM to HW, was obtained using the software RMA (Bohonak and Van der Linde, 2004). The scaled mass index was calculated for 845 *M. murinus* (N5=534, CS7=159, SV=116, KV=36) and 166 *C. medius* (N5=127, CS7=14, SV=11, KV=14) individuals.

Faecal sampling for parasitology

Faecal samples were collected opportunistically from handling bags or traps and directly homogenized and stored in 2 ml screw cap Sarstedt tubes with 10% formaldehyde after being weighed. Subsequent parasite analysis was conducted at the laboratories of the Deutsches Primatenzentrum (Germany) and the Institut Pasteur (Madagascar) using a slightly modified Ritchie´s ether sedimentation method (Ritchie, 1948). Parasite eggs and oocysts were later retrieved from microscopic examination of faecal smears and their shape, size and internal structure were used for parasite identification up to the genus level (Irwin and Raharison, 2009, Raharivololona, 2006, 2009).

To describe the pattern of parasitism, we considered prevalence, parasite morphotype richness and the proportion of a population showing multiple infections (infected by more than one species of parasite), which have been linked to mortality and morbidity (Behnke, 1990, Raso *et al.*, 2004). Parasite prevalence was calculated as the percentage of infected individuals among all examined animals from a population. To identify factors that can influence prevalence and multiple-species infection, we further considered them as response variables in models and coded as 1 if a given parasite morphotype was present (or if more than one species was recorded for multiple infection) and 0 if not. Morphotype richness was defined as the number of egg/oocyst definite types recorded in one individual and could indicate higher morbidity if it increases (Chapman *et al.*, 2005). For a successful application of the method on *M. murinus*, see Hämäläinen *et al.* (2015b). During the field study period, 1167 *M. murinus* (N5=786, CS7=202, SV=148, KV=31) and 186 *C. medius* (N5=144, CS7=16, SV=14, KV=12) faecal samples were collected and analysed.

To control for potential observer bias, we used blind observation by coding samples prior to laboratory analysis of hair cortisol levels and faecal parasites.

Statistical analysis

Habitat structure

Site differences in potential shelter density and food tree density were assessed with Kruskal-Wallis ANOVA, followed by pair-wise post hoc Mann-Whitney U tests. To account for multiple testing, we used false discovery rate correction (Benjamini and Hochberg, 1995) at a threshold level $q = 0.05$. Statistical significance threshold was set at $p < 0.05$.

Health indicators

To examine between-site variation in HCC we used a linear mixed model (LMM; Baayen, 2008) with site identity as a fixed factor for each species. Given the highly seasonal activity pattern of both species (Dausmann *et al.*, 2004, 2005, Schmid and Kappeler, 1998), we included the fixed factor season in all subsequent models. Additionally, the factor sex was also taken into account, and since both sexes are subject to different pressures in different seasons due to their reproductive schedules, we accounted for the potential interaction between season and sex. This effect is expected to be more pronounced in *M. murinus,* where males terminate their torpor period before females (Schmid, 1999, Schmid and Kappeler, 1998) and may therefore experience higher stress during the dry season, whereas females might be energetically more stressed during gestation and weaning periods in the rainy season. Finally, age (juvenile, i.e. < 1 year or adult) and its interaction with sex were added as fixed factors since the sensitivity of juveniles and adults to stressful conditions are known to differ significantly for many non-human primate species (Fourie and Bernstein, 2011, Hämäläinen *et al.*, 2015a). Since we obtained multiple samples from many individuals, identity was used as a random factor. Thus, the general model included the fixed factors site, season, sex, age, the interaction terms season x sex and age x sex. Log-transformation was applied to HCC prior analyses to improve model fit.

The effect of anthropogenic disturbance on body condition was determined by computing a LMM on the log-transformed SMI. Like previous models, the general model included the fixed factor site, sex, age, the interaction terms season x sex and age x sex. For both HCC and SMI models, error variance homogeneity and normality were assessed using visual examination of residual plots of the full models.

The site difference in parasite prevalence and multiple-species infection were assessed using a binomial generalized linear mixed model (GLMM) with a logit link function for each study species. Factors season, sex, age and the interaction terms season x sex and age x sex were also included as fixed terms. Additionally, we controlled for the potential effect of faecal sample mass on the probability to find gastro-intestinal parasites. Sample mass was log-transformed, centred and scaled. We only could estimate predictors of prevalence for the four most common helminths (*Hymenolepis, Subulura*, *Trichuris* and *Ascaris*) due to the very low infection rate with other morphotypes. However, every distinct morphotype was taken into account when assessing morphotype richness. Furthermore, due to the low recapture rate of *C. medius* during rainy seasons, we could only use data collected during dry seasons for the fat-tailed dwarf lemur. Only dry season data were also used (factor season excluded) for the assessment of pattern of infection with *Trichuris* in *M. murinus* due to its low prevalence during the rainy season, raising issues of non-convergence and complete separation in the model. The low infection rate with *Trichuris* and *Ascaris* did not allow us to assess the effect of the interaction term sex x age when modelling the determinants of *Trichuris* prevalence in both species and *Ascaris* prevalence in *C. medius*.

Morphotype richness was analysed using a Poisson GLMM with a log-link function and using the same fixed factors as with prevalence. Only data from the dry season were again used for *C. medius* due to the low recapture rate of *C. medius* during rainy seasons. General models of parasite morphotype richness were neither overdispersed (assessed using Pearson residuals; overdispersion parameter ϕ: *M. murinus* = 1.059; *C. medius* = 0.866) nor zero-inflated (assessed using frequency plots).

Generally, to obtain better estimates of the fixed parameters, non-significant interaction terms and factors ($p > 0.1$) except for site were removed successively from the full models and model comparisons were performed using likelihood ratio tests. Parameter estimates for factors that have a significant effect on health indicators are therefore reported from the reduced model. For all models, a random intercept structure was preferred over a random intercept and slope structure after a model selection based on AIC values. Models were fitted in R (R, version 3.2.2; RCoreTeam, 2016) with the lme4 R-package (Bates *et al.*, 2015) and *p*-values were estimated using the Satterthwaite approximation implemented in the lmerTest package (Kuznetsova *et al.*, 2015). Multiple comparisons between sites were performed with the R add-on package multcomp (Hothorn *et al.*, 2008). Statistical significance threshold was set at $p < 0.05$. The reduced models for all analyses are given below and the full models are reported in the supplementary material (Table S4, S5, S6).

RESULTS

Habitat structure

Habitat structure characterization revealed that the gradient of anthropogenic disturbance between the four study sites was reflected in the density of potential shelter trees and partly observed in the density of food trees of *M. murinus* and *C. medius*. Indeed, the density of trees that could be used as a refuge (DBH > 10 cm) differed significantly among the different sites (Kruskal-Wallis ANOVA: $\chi^2_{(3)} = 235.69$, $p < 0.001$; for all pair-wise Mann-Whitney U tests $p < 0.05$) with a mean density of 1027 trees/ha for N5, 658 trees/ha for CS7, 596 trees/ha for SV and 186 trees/ha for KV (Figure 1a). Moreover, although no difference was detected between N5 and CS7, significantly higher overall densities of food trees used by both species were found in N5 and CS7, compared to SV and KV but also in SV compared to KV (Kruskal-Wallis ANOVA: $\chi^2_{(3)} = 118.42$, $p < 0.001$; N5 vs. CS7, $p = 0.77$ and for all other pair-wise Mann-Whitney U tests $p < 0.05$) with a mean of 11858 trees/ha (N5), 13060 trees/ha (CS7), 8716 trees/ha (SV) and 5864 trees/ha (KV) (Figure 1b). However, large variation in the abundance of each tree species among the sites existed (supplementary material, Table S1). Based on these data, N5 can be considered as the most suitable habitat for *M. murinus* and *C. medius*.

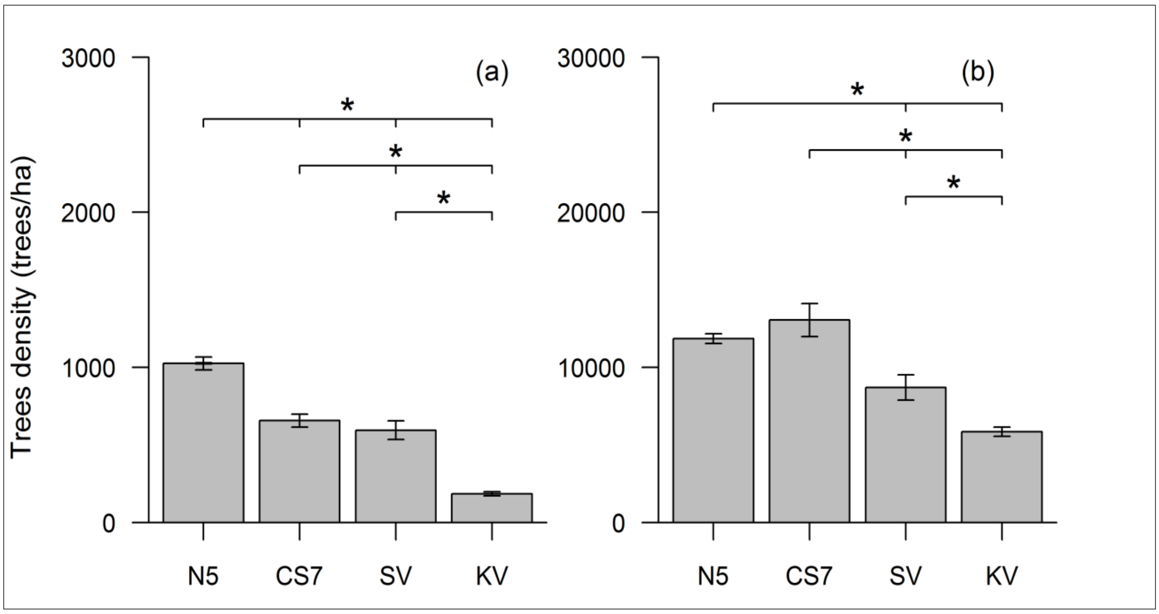

Figure 1 Density of potential shelter trees (a) and food trees (b) of *M. murinus* and *C. medius* at the four study sites in Kirindy Forest. *$p < 0.05$.

Hair cortisol concentration

As expected, the level of human disturbance had no effect on HCC levels in *M. murinus,* (LMM, $\chi^2_{(3)} = 6.95$, $p = 0.07$; Figure 2a). Contrary to our predictions, the difference in habitat condition caused by anthropogenic activities was not reflected by HCC levels in *C. medius* either (LMM, $\chi^2_{(3)} = 6.58$, $p = 0.11$; Figure 2b). Seasonal variation of HCC in both *M. murinus* and *C. medius* corresponded with our predictions based on their natural history. Significantly higher HCC values coupled with higher variation was observed in *C. medius* for the rainy season compared to the dry season (Table 2). For *M. murinus*, we found a sex difference in HCC which seemed to be influenced by season (significant interaction term sex x season): males had a higher average HCC levels than females in the dry season but the trend was reverse in the rainy season. Furthermore, we found higher average HCC concentrations in juvenile mouse lemurs compared to adults (Table 2). Neither sex nor age had a significant effect on mean HCC levels in *C. medius* (see Supplementary Table S4).

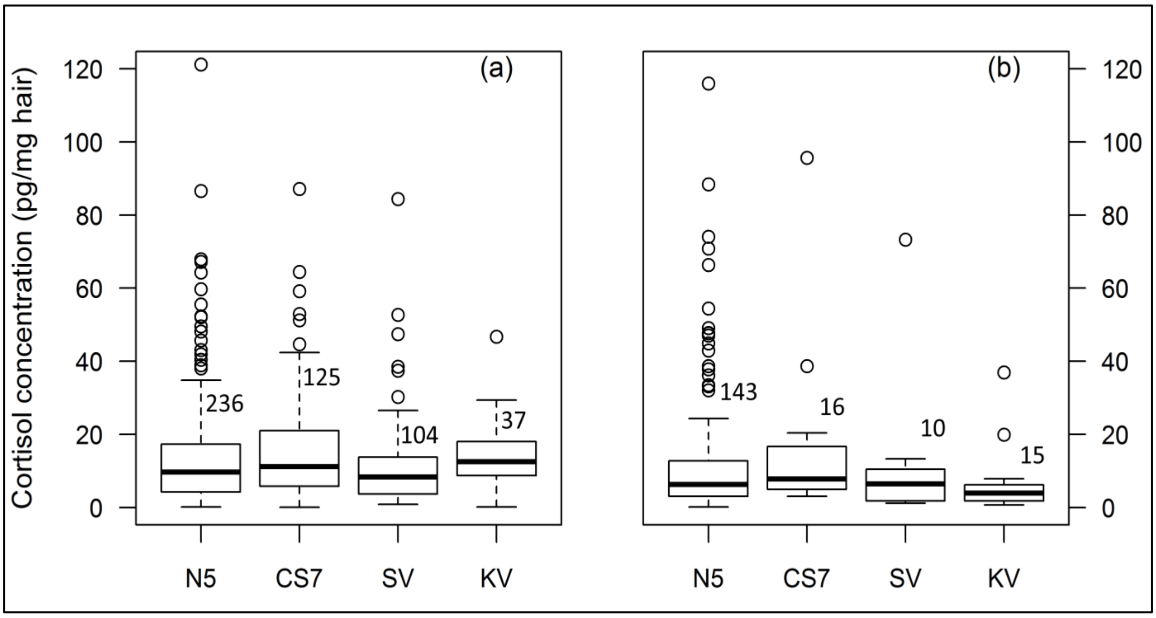

Figure 2 Variation in hair cortisol concentrations in (a) *M. murinus* and (b) *C. medius* among the four study sites. Numbers indicate the total number of hair samples analyzed for each site.

Body condition

We found no significant difference in body condition among study sites in fat-tailed dwarf lemurs (LMM, $\chi^2_{(3)}$ = 1.99, $p = 0.57$; Figure 3b). However, mouse lemurs at KV showed significantly higher average SMI values than at the other sites (N5 vs. KV, $p < 0.001$; CS7 - KV, $p < 0.001$; SV - KV, $p < 0.001$; Figure 3a, Table 2). Not surprisingly, this indicator of internal energy reserves was greater during the rainy season for both species but this difference was more pronounced in *C. medius*. Again, a sex difference in body mass following a seasonal pattern (higher values for males in the dry season but reverse trend in the rainy season) was detected for *M. murinus*. Furthermore, adult mouse lemurs had a significantly higher body condition than juveniles (Table 2).

Table 2 Parameter estimates from reduced linear mixed models assessing variations of log [Hair cortisol concentration (HCC)] and log [general body condition (calculated as scaled mass index)] in *M. murinus* and *C. medius.*

	Microcebus murinus				*Cheirogaleus medius*			
	Estimate	SE	*t*	*p*	Estimate	SE	*t*	*p*
Hair cortisol concentration								
Intercept	2.263	0.115	19.643	**<0.001**	1.850	0.155	11.897	**<0.001**
Site (ref N5) CS7	0.250	0.113	2.202	**0.028**	0.293	0.265	1.105	0.271
SV	-0.344	0.123	-0.279	0.780	0.073	0.325	0.223	0.823
KV	0.224	0.181	1.238	0.216	-0.345	0.272	-1.269	0.206
Season (ref. dry)	0.265	0.138	1.922	0.055	1.260	0.188	6.707	**<0.001**
Sex (ref. female)	0.325	0.123	2.643	**0.008**				
Age (ref. juvenile)	-0.623	0.095	-6.571	**<0.001**	-0.314	0.167	-1.884	0.061
Sex*Season	-0.561	0.185	-3.031	**0.002**				
Body condition (Scaled mass index)								
Intercept	4.041	0.015	268.058	**<0.001**	4.810	0.016	304.126	**<0.001**
Site (ref. N5) CS7	0.006	0.014	0.447	0.728	-0.076	0.045	-1.685	0.094
SV	-0.022	0.016	-1.368	0.230	-0.001	0.050	-0.035	0.972
KV	0.131	0.027	4.807	**<0.001**	-0.017	0.045	-0.383	0.702
Season (ref. dry)	0.091	0.016	5.704	**<0.001**	0.233	0.031	7.591	**<0.001**
Sex (ref. female)	0.056	0.018	3.005	**0.002**				
Age (ref. juvenile)	0.051	0.017	3.032	**0.002**				
Sex*Season	-0.101	0.022	-4.622	**<0.001**				
Sex*Age	-0.043	0.022	-1.929	0.054				

Bold values indicate statistically significant results at the significance threshold $p<0.05$

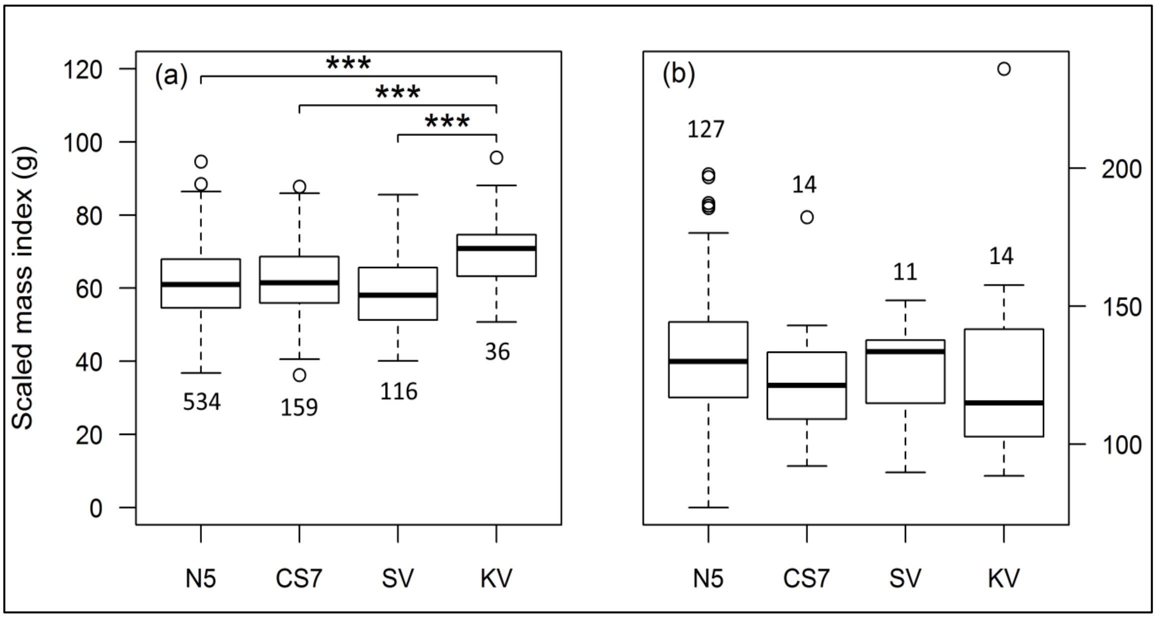

Figure 3 General body condition (measured as scaled mass index) of (a) *M. murinus* and (b) *C. medius* across the four study sites. ****p* < 0.001. Numbers indicate the sample size for each site.

Parasitism

In total, we identified 11 distinct egg morphotypes with two of them (*Oesophagostomum spp.* and *Capillaria spp.*) only found in *M. murinus* (Table 3). Multiple-species infection was rather common in *M. murinus* and *C. medius*, and we detected a maximum of 6 distinct egg morphotypes in a single individual of both species. The occurrence of multiple-species infection (LMM, $\chi^2_{(3)} = 6.70$, $p = 0.08$; Table 4) and morphotype richness (LMM, $\chi^2_{(3)} = 4.49$, $p = 0.21$; Table 4) did not differ among sites for *M. murinus*. None of the components used to assess parasitism showed significant variation between sites for *C. medius* but an age-specific sex difference in parasite morphotype richness was observed in *C. medius* (higher values for adult males; Table 4).

Table 3 Gastrointestinal parasites of *M. murinus* and *C. medius* in four different sites within Kirindy forest.

	Microcebus murinus				*Cheirogaleus medius*			
	N5	CS7	SV	KV	N5	CS7	SV	KV
Total number of individuals	305	95	80	23	98	14	11	11
Multiple infections (%)	47.9	52.6	41.2	39.1	32.6	21.4	18.2	18.2
Prevalence (%) of Cestoda								
Hymenolepis	57.0	49.5	40.0	43.5	35.7	0.0	18.1	27.2
Nematoda								
Subulura	49.5	57.9	62.5	43.5	32.6	21.4	36.4	27.3
Trichuris	21.3	23.1	18.7	13.0	13.3	14.3	18.1	0.0
Ascaris	9.5	17.9	13.7	26.1	8.1	14.3	0.0	18.2
Oxyuridae	6.2	9.5	5.0	4.3	5.1	7.1	9.1	9.1
Lemuricola	1.6	0.0	1.2	0.0	1.0	0.0	0.0	9.1
Oesophagostomum	0.0	1.0	1.2	0.0	-	-	-	-
Capillaria	0.6	0.0	0.0	0.0	-	-	-	-
Strongylida	22.3	15.8	18.7	4.3	7.1	7.1	0.0	0.0
Trematoda								
Opisthorchis	0.0	1.0	0.0	0.0	1.0	0.0	0.0	0.0
Metagonimus	2.9	2.1	3.7	0.0	3.1	0.0	0.0	0.0

The overall parasite prevalence (defined regardless of the helminth morphotype recorded) of the grey mouse lemur did not significantly differ among study sites (Table 5). However, among-site variation was observed for three out of the four most common morphotypes. In fact, the prevalence of *Hymenolepis* spp. eggs was significantly lower in SV compared to N5 ($z = -2.99$, $p = 0.01$) while *Subulura* spp. eggs were more frequently found in CS7 and SV compared to N5 (N5 vs. CS7, $z = 4.03$, $p < 0.001$; N5 vs. SV, $z = 3.31$, $p = 0.004$) and *Ascaris* spp. eggs had a significantly higher prevalence in the CS7 and KV populations compared to the N5 population (N5 vs. CS7, $z = 2.72$, $p = 0.03$; N5 vs. KV, $z = 3.70$, $p = 0.001$; Table 5). Moreover, *Hymenolepis* and *Trichuris* eggs had a higher prevalence in adult mouse lemurs and sex and/or seasonal differences in prevalence were noticed for *Hymenolepis, Subulura*, *Trichuris* and *Ascaris* eggs (Table 5). Furthermore, the season-specific sex differences exhibited by *M. murinus* (higher values for males in the dry season but opposite trend in the rainy season) in other health indicators (HCC and SMI) were also observed for overall prevalence, morphotype richness and rate of multiple infections (Table 4, Table 5).

Table 4 Parameter estimates from reduced generalised linear mixed models assessing variation in multiple-morphotype infection rate and parasite morphotype richness in *M. murinus* and *C. medius.*

	Microcebus murinus				*Cheirogaleus medius*			
	Estimate	SE	*z*	*p*	Estimate	SE	*z*	*p*
Multiple-morphotype infection								
Intercept	-1.478	0.178	-8.295	**<0.001**	-1.000	0.207	-4.838	**<0.001**
Site (ref. N5) CS7	0.463	0.194	2.391	**0.017**	-0.204	0.690	-0.295	0.768
SV	0.019	0.225	0.084	0.933	-0.705	0.796	-0.885	0.376
KV	0.444	0.430	1.031	0.302	-0.504	0.809	-0.623	0.533
Season (ref. dry)	-0.165	0.228	-0.726	0.468	-	-	-	**-**
Sex (ref. female)	0.801	0.172	4.655	**<0.001**				
Age (ref. juvenile)	0.289	0.151	1.914	0.056				
Sex*Season	-0.927	0.334	-2.772	**0.005**	-	-	-	-
Parasite morphotype richness								
Intercept	-0.247	0.077	-3.220	**0.001**	0.039	0.282	0.140	0.888
Site (ref. N5) CS7	0.154	0.086	1.792	0.073	-0.223	0.403	-0.554	0.579
SV	0.035	0.098	0.355	0.723	-0.419	0.403	-1.038	0.299
KV	0.167	0.186	0.896	0.370	-0.295	0.415	-0.709	0.478
Season (ref. dry)	-0.185	0.101	-1.826	0.068	-	-	-	**-**
Sex (ref. female)	0.354	0.075	4.728	**<0.001**	-0.557	0.534	-1.080	0.280
Age (ref. juvenile)	0.182	0.065	2.786	**0.005**	-0.517	0.329	-1.578	0.115
Sex*Season	-0.321	0.145	-2.203	**0.028**	-	-	-	**-**
Sex*age					1.188	0.588	2.020	**0.043**

Bold values indicate statistically significant results at the significance threshold *p*<0.05

Table 5 Parameter estimates from reduced generalised linear mixed models assessing variation of parasite prevalence in *M. murinus* and *C. medius.*

	Microcebus murinus				*Cheirogaleus medius*			
	Estimate	SE	*z*	*p*	Estimate	SE	*z*	*p*
Overall prevalence								
Intercept	0.038	0.155	0.245	0.806	0.009	0.198	0.483	0.629
Site (ref. N5) CS7	0.272	0.195	1.393	0.164	-0.251	0.618	-0.407	0.684
SV	0.102	0.218	0.467	0.640	-0.260	0.624	-0.418	0.676
KV	-0.032	0.420	-0.076	0.939	-0.272	0.667	-0.408	0.683
Season (ref. dry)	-0.288	0.201	-1.435	0.151				
Sex (ref. female)	0.756	0.169	4.482	**<0.001**				
Age (ref. juvenile)	0.339	0.142	2.388	**0.017**				
Sex*Season	-0.635	0.294	-2.163	**0.031**				
Hymenolepis								
Intercept	-0.715	0.141	-5.066	**<0.001**	-0.916	0.239	-3.828	**<0.001**
Site (ref. N5) CS7	-0.042	0.170	-0.249	0.803	-3.618	2.163	-1.673	0.094
SV	-0.622	0.207	-2.999	**0.003**	-0.855	0.834	-1.025	0.305
KV	0.025	0.387	0.064	0.949	-0.701	0.850	-0.824	0.410
Season (ref. dry)	-0.295	0.145	-2.029	**0.042**	-	-	-	-
Sex (ref. female)	0.232	0.129	1.802	0.071				
Age (ref. juvenile)	0.336	0.133	2.525	**0.011**				

Bold values indicate statistically significant results at the significance threshold $p<0.05$

Table 5 Continued

	Microcebus murinus				*Cheirogaleus medius*			
	Estimate	SE	*z*	*p*	Estimate	SE	*z*	*p*
Subulura								
Intercept	-1.205	0.143	-8.450	**<0.001**	-1.000	0.207	-4.838	**<0.001**
Site (ref. N5) CS7	0.767	0.190	4.035	**<0.001**	-0.204	0.690	-0.295	0.768
SV	0.715	0.215	3.319	**<0.001**	0.189	0.635	0.298	0.766
KV	0.458	0.425	1.079	0.280	0.019	0.708	0.227	0.978
Season (ref. dry)	-0.096	0.219	-0.440	0.660	-	-	-	-
Sex (ref. female)	0.926	0.168	5.501	**<0.001**				
Sex*Season	-1.193	0.325	-3.665	**<0.001**	-	-	-	-
Trichuris								
Intercept	-3.139	0.384	-8.173	**<0.001**	-2.954	1.858	-1.589	0.112
Site (ref. N5) CS7	0.071	0.375	0.190	0.850	0.267	1.101	0.243	0.808
SV	-0.562	0.417	-1.347	0.178	0.645	1.184	0.545	0.586
KV	0.182	0.822	0.222	0.825	-2.423	2.539	-0.854	0.340
Sex (ref. female)	1.016	0.295	3.440	**<0.001**				
Age (ref. juvenile)	0.538	0.268	2.006	**0.045**				
Ascaris								
Intercept	-3.031	0.194	-15.585	**<0.001**	-10.026	1.568	-6.393	**<0.001**
Site (ref. N5) CS7	0.929	0.319	2.913	**0.003**	0.333	2.639	0.126	0.900
SV	0.597	0.366	1.635	0.102	-0.893	3.392	-0.263	0.792
KV	1.952	0.504	3.877	**<0.001**	0.480	2.703	0.178	0.859
Season (ref. dry)	-1.174	0.412	-2.852	**0.004**	-	-	-	-
Sample mass	-0.225	0.127	-1.775	0.076				

Bold values indicate statistically significant results at the significance threshold *p*<0.05

DISCUSSION

In this study, we assessed the effect of anthropogenic disturbance on the health of two species that differ in their coping ability to changing conditions and therefore added to the growing number of research focusing on the impact of human disturbance on lemur health (Bublitz *et al.*, 2015, Irwin, 2008a). Concordant with its status as a disturbance-resistant species, *M. murinus* did not show signs of health deterioration in habitats subjected to greater human disturbance. Firstly, a gradual increase of HCC along the gradient of human disturbance was not observed in *M. murinus*. Moreover, general body condition of grey mouse lemurs was best in the most disturbed habitat. Unlike in previous studies (Raharivololona and Ganzhorn, 2009), the overall parasitism pattern observed among the four populations of *M. murinus* were comparable. Surprisingly, the three health indicators were comparable among the fat-tailed dwarf lemur populations exposed to different levels of anthropogenic activities. However, as reported for several other species, season, sex and age seem to play a role in influencing health indicators in both species (ground squirrels: Boswell *et al.*, 1994, mammals: Schalk and Forbes, 1997, non-rodent mammals: Tilbrook *et al.*, 2000).

The higher prevalence observed for male mouse lemurs during the late dry season could be associated with a higher exposure to parasites due to their elevated activity (Eberle and Kappeler, 2004) and/or a higher susceptibility due to the immunosuppressive effect of high steroid hormone levels during this period (Perret, 1985). Although a seasonal comparison of the pattern of parasitism could not be achieved for *C. medius*, it is known for several species that the immune function declines during hibernation and can therefore increase individual susceptibility but also the virulence of pathogens (reviewed in Martinez-Bakker and Helm, 2015). However, at the population level, the effect of a reduced immunity could be compensated by a reduced exposure of hibernators to parasite infections. Moreover, the constantly higher prevalence seen in *M. murinus* in comparison to *C. medius* could be attributed to the fact that while *C. medius* hibernates, mouse lemurs remain partially active during the cold dry season and thus have a higher parasite encounter propability throughout the year.

Invariant levels of the health indicators between sites may primarily result from selective disappearance (Romero, 2004). It is possible that high selective pressure could have caused death or emigration of the more sensitive individuals. In a previous study of the N5 and CS7 populations, Hämäläinen *et al*. (2014a) confirmed that a relatively high body condition was required for *M murinus* to reach an old age in the wild. Anthropogenic disturbance may affect the required threshold level for survival and this may explain the better body condition of *M. murinus* in KV. Several other factors that could explain the observed results such as dietary adaptation, interspecific competition or the social context are discussed below.

Dietary adaptation

Dietary adaptation may explain the lack of difference in health indicators observed among the study populations as well as the better body condition of KV mouse lemurs. When faced with a decrease in food availability, animals can alter their feeding behaviour to meet energetic demands either by broadening their dietary spectrum or by exploiting a subset of their original diet more extensively (Gibson, 2011, Nakagawa *et al.*, 2007, Onderdonk and Chapman, 2000). Animals that are not able to adjust their diet may experience a rapid decline in population size (Chapman *et al.*, 2006). On the physiological level, one potential explanation for this outcome is the synergistic effect of parasitism and dietary stress on hosts.

While the immunosuppressive effect of prolonged food shortage will increase parasitism, high parasite loads could consecutively increase energy demands on host and aggravate the effect of food scarcity. Changes in nutritional habits have been widely noted in wild populations under changing conditions. For instance, diademed sifakas (*Propithecus diadema*) consume a greater amount of mistletoe (*Bakerella*) in fragmented areas compared to continuous forest to compensate for the low availability of suitable fruiting tree species in fragments (Irwin, 2008a). Cheirogaleids were also shown to undergo dietary shifts in fragmented habitats: mouse lemurs increased their arthropod consumption, and dwarf lemurs (*Cheirogaleus sibreei and C crossleyi*) shifted from a frugivorous to a more omnivorous diet in fragmented forest (Crowley *et al.*, 2013). Therefore, the abundance of arthropods near the forest edge was proposed to stabilise population density in edge habitats (Lehman *et al.*, 2006).

The intra-specific variation of food tree density among sites may have facilitated a shift in diet. Fat-tailed dwarf lemurs were observed to feed extensively on *Phyllanthus casticum* pulp at the end of the rainy season in KV and SV, while this tree species is found in low density in N5 and absent in CS7. Additionally, a lower density in disturbed sites may further facilitate the access to resources by the remaining individuals. However, we further acknowledge the fact that the inventory of tree species eaten by *M. murinus* and *C. medius* (Dammhahn and Kappeler, 2008, Fietz and Ganzhorn, 1999) was established in a continuous forest. Therefore, tree species that are potentially suitable for consumption in fragments and disturbed areas might be missing in this list. Thus, a detailed description of the feeding behaviour of both species across habitats with different level of disturbance will be needed in further studies.

Interspecific competition

Interspecific interactions such as competition for resources or predation can be perceived as non-negligible stressors and can trigger a similar effect as human disturbance for wildlife populations (Frid and Dill, 2002). Among the members of the cheirogaleid family, *C. medius* was observed to partially displace *M. murinus* on a local scale and the latter was found to avoid direct competition with the closely related *M. berthae* by spatial separation in an undisturbed habitat (Schwab and Ganzhorn, 2004). Schäffler *et al.* (2015) further emphasized that interspecific interactions within the cheirogaleid family played an important role in shaping their community composition and that the relative abundance of each species depended on the degree of environmental disturbance. They demonstrated that despite an overlap in their diet, *M. murinus* and *M. berthae* could coexist in intact habitat due to predation by *Mirza coquereli* on *M. murinus*. Therefore, *M. berthae* benefits from the spatial avoidance of predation pressure by avoiding competition. In return, *M. murinus* avoid competition and intraguild predation by occupying habitats with high levels of human disturbance. Consequently, the physiological stress caused by human activities in disturbed areas might be balanced with the high pressure set by interspecific competition in more suitable habitats. In order to test this hypothesis, a study of the overall predation pressure between sites and a quantification and differentiation of human-induced stressors seems indicated.

Social factors

Ranging patterns and population density are also known to affect parasitism patterns (Hudson *et al.*, 2002, Nunn *et al.*, 2003). Parasite prevalence, diversity and infection rates correlate positively with host density and home range size (Morand and Poulin, 1998, Packer *et al.*, 1999). The variation in capture success of mouse lemurs and dwarf lemurs among sites might indicate a constantly higher density of *M. murinus* in comparison to *C. medius* and decreasing population density with increased habitat disturbance in both species. This conclusion is concordant with previous findings of Schäffler *et al.* (2015) in the central Menabe region. However, the patterns of parasitism observed in *M. murinus* and *C. medius* do not seem to be associated with density-dependent factors, as our data indicated no association between parasitism and host density. For both species, the importance of density in parasite spread could be trumped by social factors that could influence patterns of parasite transmission by direct contact between individuals. *M. murinus* have a promiscuous mating system and females are known to regularly share sleeping sites (Radespiel, 2000). Despite the fact that they live in permanent pairs, an increase in contact rates between individuals of the fat-tailed dwarf lemur were observed during the mating season, when a high rate of extra-pair copulations was recorded as well (Fietz *et al.*, 2000). The importance of social contacts in parasite transmission was highlighted by several studies (reviewed in Kappeler *et al.*, 2015) for both directly and environmentally transmitted parasites (Drewe, 2010, MacIntosh *et al.*, 2012, Rimbach *et al.*, 2015).

A major limitation of our study is the relatively low and unbalanced sample size collected per site. Nonetheless, the limited capture success resulting in the restricted samples size gathered in the disturbed sites could be an indicator of the indirect long-term negative consequences of human activities and may imply that population size are lower where habitats are suboptimal (Schäffler *et al.*, 2015). The actual significance of human presence may have a limited detrimental impact on the health of the study species since direct encounter rates with humans are probably low for those small nocturnal animals which also do not suffer from direct hunting. Moreover, the forest concession is a reasonably continuous habitat and no small fragment was listed among our study sites. However, the substantial differences in habitat structure observed among sites are at least partially due to human use of the areas. Our results thus suggest that even moderate habitat alteration may influence the population viability of flexible species.

CONCLUSIONS

This study demonstrates that moderate human disturbance may have negligible influence on the general health status of species that are capable of adjusting to suboptimal conditions by behavioural or dietary flexibility. However, the relatively lower density of these species in disturbed environments indicates that human activities may negatively affect long-term population viability of even resilient species. These findings highlight the need to limit human activities in natural areas and prioritize continuous pristine forests in conservation actions. While health parameters are often easier to measure than population density or population decline, this study emphasizes the degree of uncertainty associated with such shortcuts. While an assessment of the general health of wild populations can be informative of their potential sensitivity to environmental change, our results indicate that basing conservation decisions solely on health information may overestimate the resilience of the population, thus raising the risk of misinformed conservation decisions. We thus propose that, for the purposes of political decision-making processes, information on health parameters should be coupled with a study of their fitness consequences as well as other indicators of population viability.

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Supplementary material of chapter 1

Table S1 List and estimated density of trees consumed by *C. medius* and *M. murinus* in Kirindy C.N.F.E.R.E.F. The list was retrieved from Fietz and Ganzhorn (1999) and Dammhahn and Kappeler (2008). An update of classification and scientific nomenclature of the trees species was obtained from a phonological study conducted in Kirindy forest in 2013.

			Density (Trees/ha)			
Species	Family	Local name	N5	CS7	SV	KV
Terminalia boivinii	Combretaceae	Amaninomby	258	745	133	355
Strychnos henningsii	Loganiaceae	Tsivoanino	484	395	840	59
Strychnos decussata	Loganiaceae	Hazomby	1493	4680	3244	131
Rothmannia sp	Rubiaceae	Manamakanamy	159	0	4	0
Phylloctenium decaryanum	Bignoniaceae	Pitikala	250	50	62	10
Phyllarthron subumbellatum	Bignoniaceae	Tohiravy	49	140	27	6
Phyllanthus casticum	Euphorbiaceae	Sanira petite feuille	14	0	178	92
Peponidium sp	Rubiaceae	Kitatamalando	49	20	49	7
Ochna pervilleana	Ochnaceae	Boramena	882	2705	71	120
Noronhia sp	Oleaceae	Tsilaitsy	21	105	58	0
Memecylon sp	Melastomataceae	Tsivoany	284	45	1431	29
Macphersonia gracilis	Sapindaceae	Tsingena	2927	15	778	3
Hyperacanthus sp	Rubiaceae	Piripitsokala	484	225	129	17
Hymenodictyon occidentale	Rubiaceae	Beholitsy	205	390	80	37
Hilsenbergia capuronii	Boraginaceae	Hazomboenga	27	320	4	38
Grewia sp	Malvaceae	Sele nala	170	0	0	3
Grewia sp	Malvaceae	Sele sele	63	110	31	93
Grewia lavanalensis	Malvaceae	Sele be	23	30	289	37
Grewia cyclea	Malvaceae	Latabarika	210	270	31	41
Grewia aff. voloina	Malvaceae	Sele pitiky	286	285	329	116
Euonymus elaeodendroides	Celestraceae	Maronono	552	105	13	7
Diospyros tropophylla	Ebenaceae	Maintifototsy	355	250	111	80
Commiphora aprevalii	Burseraceae	Sarisakoambanditsy	40	245	89	59
Clerodendrum aff. involucratum	Verbenaceae	Ripiky	48	180	58	24
Carphalea kirondron	Rubiaceae	Menavony	0	205	13	39
Capuronia benoistii	Lythraceae	Mamiaho	598	315	31	54
Canthium sp	Rubiaceae	Fatekahitsy	120	485	324	18
Brachylaena sp	Asteraceae	Vahirano	653	375	71	49
?	?	Belohaliky	13	95	13	33
?	?	Hazonkondoky	529	275	222	1
?	?	Manoro	18	0	0	0
?	?	Vahipindy mena	594	0	0	1

Table S2 Number of distinct individuals of different sex-age classes captured at each site (AM: Adult male; AF: Adult female; JM: Juvenile male; JF: Juvenile female) along with the number of samples for HCC (hair samples), SMI (morphometric and body mass measurements) and parasitism (fecal samples) assessment.

	Numbers of individuals (hair samples/morphometric measurements/feacal samples)							
	Microcebus murinus				*Cheirogaleus medius*			
	N5	CS7	SV	KV	N5	CS7	SV	KV
AM	104 (55/139/203)	38 (29/40/68)	35 (32/38/46)	07 (07/07/09)	41 (52/46/52)	03 (02/02/03)	05 (01/02/07)	06 (07/06/06)
AF	127 (67/175/265)	35 (40/49/81)	31 (31/35/47)	11 (11/10/10)	45 (53/46/65)	09 (07/05/09)	06 (06/06/04)	07 (06/07/05)
JM	131 (75/139/193)	37 (38/50/33)	29 (21/24/33)	09 (09/09/05)	12 (10/08/07)	02 (03/02/02)	-	01 (01/00/00)
JF	87 (39/81/125)	21 (18/20/20)	23 (20/19/22)	10 (10/10/07)	29 (28/27/21)	05 (04/05/02)	03 (03/03/02)	01 (01/01/01)

Table S3 Number of distinct individuals of each sex per season captured at each site (DM: Male for the dry season; DF: Female for the dry season; RM: Male for the rainy season; RF: Female for the rainy season) along with the number of samples used for HCC, SMI and patterns of parasitism assessment.

	Numbers of individuals (hair samples/morphometric measurements/feacal samples)							
	Microcebus murinus				*Cheirogaleus medius*			
	N5	CS7	SV	KV	N5	CS7	SV	KV
DM	136 (72/168/300)	49 (32/54/74)	46 (40/49/67)	08 (09/09/10)	44 (57/50/54)	04 (04/03/04)	05 (01/02/07)	07 (08/06/06)
DF	163 (55/138/275)	37 (24/34/64)	39 (39/43/60)	11 (12/12/11)	52 (57/48/66)	09 (08/07/09)	08 (08/08/05)	07 (06/07/05)
RM	104 (58/110/97)	33 (35/36/27)	13 (13/13/11)	07 (07/07/04)	06 (05/04/05)	01 (01/01/01)	-	-
RF	103 (51/118/114)	31 (34/35/37)	12 (12/11/10)	09 (09/08/06)	25 (24/25/20)	03 (03/03/02)	01 (01/01/01)	01 (01/01/01)

Table S4 Parameter estimates from full linear mixed models assessing variations of log [Hair cortisol concentration (HCC)] and log [general body condition (calculated as scaled mass index)] in *M. murinus* and *C. medius.*

	Microcebus murinus				*Cheirogaleus medius*			
	Estimate	SE	*t*	*p*	Estimate	SE	*t*	*p*
Hair cortisol								
Intercept	2.203	0.130	16.998	**<0.001**	1.808	0.187	9.680	**<0.001**
Site (ref. N5) CS7	0.248	0.113	2.187	**0.003**	0.348	0.268	1.298	0.196
SV	-0.026	0.123	-0.212	0.832	0.157	0.330	0.475	0.635
KV	0.231	0.181	1.276	0.203	-0.356	0.273	-1.306	0.193
Season (ref. dry)	0.245	0.139	1.758	0.079	1.366	0.217	6.288	**<0.001**
Sex (ref. female)	0.419	0.154	2.712	**0.006**	0.025	0.325	0.078	0.938
Age (ref. juvenile)	-0.517	0.141	-3.654	**<0.001**	-0.436	0.204	-2.139	**0.038**
Sex*Season	-0.532	0.187	-2.842	**0.005**	-0.248	0.474	-0.524	0.601
Sex*Age	-0.191	0.190	-1.005	0.315	0.297	0.360	0.824	0.401
Body condition								
Intercept	4.041	0.015	268.058	**<0.001**	4.829	0.030	158.170	**<0.001**
Site (ref. N5) CS7	0.006	0.014	0.447	0.728	-0.088	0.046	-1.916	0.057
SV	-0.022	0.016	-1.368	0.230	-0.009	0.051	-0.180	0.857
KV	0.131	0.027	4.807	**<0.001**	-0.014	0.046	-0.312	0.756
Season (ref. dry)	0.091	0.016	5.704	**<0.001**	0.206	0.035	5.818	**<0.001**
Sex (ref. female)	0.056	0.018	3.005	**0.002**	-0.001	0.055	-0.019	0.985
Age (ref. juvenile)	0.051	0.017	3.032	**0.002**	-0.001	0.033	-0.055	0.956
Sex*Season	-0.101	0.022	-4.622	**<0.001**	0.106	0.083	1.283	0.201
Sex*Age	-0.043	0.022	-1.929	0.054	-0.042	0.061	-0.688	0.492

Bold values indicate statistically significant results at the significance threshold $p<0.05$

Table S5 Parameter estimates from full generalised linear mixed models assessing variations of multiple-morphotype infection rate and parasite morphotype richness in *M. murinus* and *C. medius.*

	Microcebus murinus				*Cheirogaleus medius*			
	Estimate	SE	*z*	*p*	Estimate	SE	*z*	*p*
Multiple-morphotype infection								
Intercept	-1.550	0.223	-6.954	**<0.001**	-0.476	0.521.	-0.914	0.361
Site (ref. N5) CS7	0.456	0.195	2.239	**0.019**	0.052	0.713	0.074	0.941
SV	0.021	0.225	0.095	0.924	-0.907	0.811	-1.118	0.264
KV	0.448	0.432	1.037	0.297	-0.575	0.824	-0.698	0.485
Season (ref. dry)	-0.172	0.229	-0.751	0.452	-	-	-	-
Sex (ref. female)	0.900	0.263	3.425	**<0.001**	-1.374	1.198	-1.147	0.251
Age (ref. juvenile)	0.397	0.238	1.666	0.096	-0.893	0.611	-1.461	0.144
Sample mass	0.025	0.070	0.356	0.721	-0.237	0.215	-1.100	0.271
Sex*Season	-0.901	0.335	-2.689	**0.007**	-	-	-	-
Sex*Age	-0.172	0.308	-0.558	0.576	2.087	1.280	1.631	0.103
Parasite morphotype richness								
Intercept	-0.269	0.097	-2.272	**0.005**	0.048	0.280	0.173	0.863
Site (ref. N5) CS7	0.147	0.087	1.683	0.092	-0.216	0.401	-0.538	0.591
SV	0.034	0.099	0.341	0.733	-0.413	0.400	-1.031	0.302
KV	0.164	0.188	0.874	0.382	-0.268	0.415	-0.643	0.520
Season (ref. dry)	-0.190	0.102	-1.860	0.063	-	-	-	-
Sex (ref. female)	0.384	0.115	3.333	**<0.001**	-0.534	0.535	-0.999	0.317
Age (ref. juvenile)	0.217	0.105	2.056	**0.040**	-0.513	0.325	-1.578	0.115
Sample mass	0.015	0.029	0.508	0.611	-0.081	0.111	-0.723	0.469
Sex*Season	-0.312	0.146	-2.137	**0.033**	-	-	-	-
Sex*Age	-0.057	0.134	-0.423	0.672	1.138	0.588	1.933	0.053

Bold values indicate statistically significant results at the significance threshold p<0.05

Table S6 Parameter estimates from full generalised linear mixed models assessing variations of parasite prevalence in *M. murinus* and *C. medius.*

	Microcebus murinus				*Cheirogaleus medius*			
	Estimate	SE	*z*	*p*	Estimate	SE	*z*	*p*
Overall prevalence								
Intercept	0.106	0.184	0.576	0.564	0.458	0.532	0.860	0.390
Site (ref. N5) CS7	0.239	0.197	1.212	0.225	-0.061	0.640	-0.095	0.925
SV	0.085	0.219	0.391	0.696	-0.429	0.654	-0.656	0.512
KV	-0.069	0.422	-0.164	0.870	-0.298	0.687	-0.434	0.665
Season (ref. dry)	-0.287	0.202	-1.417	0.156	-	-	-	-
Sex (ref. female)	0.670	0.237	2.828	**0.004**	0.193	0.938	0.206	0.837
Age (ref. juvenile)	0.275	0.204	1.345	0.179	-0.763	0.600	-1.273	0.203
Sample mass	0.089	0.067	1.333	0.182	-0.205	0.198	-1.035	0.301
Sex*Season	-0.648	0.296	-2.193	**0.028**	-	-	-	-
Sex*Age	0.120	0.285	0.420	0.674	0.582	1.028	0.566	0.571
Hymenolepis								
Intercept	-0.738	0.180	-4.088	**<0.001**	-0.019	0.556	-0.034	0.973
Site (ref. N5) CS7	-0.063	0.171	-0.367	0.713	-3.452	2.207	-1.564	0.118
SV	-0.626	0.208	-3.015	**0.002**	-1.044	0.871	-1.199	0.231
KV	0.003	0.387	-0.010	0.992	-0.671	0.881	-0.761	0.446
Season (ref. dry)	-0.118	0.199	-0.595	0.552	-	-	-	**-**
Sex (ref. female)	0.301	0.226	1.333	0.182	-0.813	1.027	-0.792	0.428
Age (ref. juvenile)	0.317	0.201	1.576	0.115	-1.304	0.664	-1.964	**0.049**
Sample mass	0.069	0.062	1.101	0.271	-0.288	0.243	-1.185	0.236
Sex*Season	-0.383	0.289	-1.323	0.186	-	-	-	-
Sex*Age	0.012	0.268	0.046	0.964	1.364	1.140	1.197	0.231

Bold values indicate statistically significant results at the significance threshold $p<0.05$

Table S6 continued.

	Microcebus murinus				*Cheirogaleus medius*			
	Estimate	SE	*z*	*p*	Estimate	SE	*z*	*p*
Subulura								
Intercept	-1.261	0.207	-6.103	**<0.001**	-0.703	0.564	-1.245	0.213
Site (ref. N5) CS7	0.719	0.193	3.729	**<0.001**	-0.052	0.768	-0.067	0.946
SV	0.713	0.217	3.292	**<0.001**	0.035	0.727	0.048	0.961
KV	0.426	0.427	0.997	0.319	0.020	0.792	0.026	0.980
Season (ref. dry)	-0.113	0.221	-0.514	0.607	-	-	-	-
Sex (ref. female)	0.914	0.250	3.658	**<0.001**	0.260	0.955	0.273	0.785
Age (ref. juvenile)	0.119	0.224	0.533	0.594	-0.857	0.651	-1.318	0.188
Sample mass	0.105	0.068	1.537	0.124	-0.231	0.231	-1.002	0.316
Sex*Season	-1.156	0.327	-3.537	**<0.001**	-	-	-	-
Sex*Age	0.0006	0.296	0.002	0.998	0.529	1.061	0.499	0.618
Trichuris								
Intercept	-3.118	0.386	-8.067	**<0.001**	-8.018	1.752	-4.575	**<0.001**
Site (ref. N5) CS7	0.008	0.379	0.020	0.984	-2.296	3.097	-0.741	0.459
SV	-0.572	0.419	-1.364	0.172	0.169	2.237	0.076	0.940
KV	0.101	0.826	0.123	0.902	-1.317	3.107	-0.424	0.672
Season (ref. dry)	-	-	-	-	-	-	-	-
Sex (ref. female)	1.002	0.297	3.373	**<0.001**	-0.188	1.539	-0.122	0.903
Age (ref. juvenile)	0.526	0.269	1.954	0.051	-0.389	1.498	-0.260	0.795
Sample mass	0.174	0.114	1.523	0.128	0.418	0.577	0.725	0.468
Sex*Season	-	-	-	-	-	-	-	-
Sex*Age	-	-	-	-	-	-	-	-

Bold values indicate statistically significant results at the significance threshold $p<0.05$

Table S6 continued.

	Microcebus murinus				*Cheirogaleus medius*			
	Estimate	SE	*z*	*p*	Estimate	SE	*z*	*p*
Ascaris								
Intercept	-3.862	0.528	-7.320	**<0.001**	-10.263	2.220	-4.624	**<0.001**
Site (ref. N5) CS7	0.875	0.324	2.697	**0.007**	0.315	2.621	0.120	0.904
SV	0.600	0.369	1.627	0.103	-0.881	3.409	-0.258	0.796
KV	1.999	0.511	3.915	**<0.001**	0.498	2.697	0.185	0.854
Season (ref. dry)	-1.702	0.747	-2.279	**0.023**	-	-	-	-
Sex (ref. female)	0.802	0.590	1.361	0.173	-1.151	1.841	-0.082	0.935
Age (ref. juvenile)	0.891	0.560	1.591	0.111	0.443	1.950	0.227	0.820
Sample mass	-0.236	0.127	-1.855	0.063	-0.283	0.896	-0.315	0.753
Sex*Season	0.926	0.896	1.033	0.301	-	-	-	-
Sex*Age	-0.667	0.661	-1.009	0.313	-	-	-	-

Bold values indicate statistically significant results at the significance threshold p<0.05

CHAPTER 2

"Interrelationships among stress, body condition and parasitism relative to life history in two sympatric nocturnal lemurs"

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ABSTRACT

Stress hormones (mainly glucocorticoids, GCs), pattern of parasite infection and general body condition are commonly used as health indicators in wild animal populations as they can reflect the effect of environmental factors at the individual level. However, as they are also assumed to play a key role in mediating life history trade-offs, the interrelationships among those indices of health should differ between species that adopt contrasting life history strategies. In this study, we investigated the nature and magnitude of the associations that might exist among those health indices in natural populations of two sympatric lemur species (*Microcebus murinus* and *Cheirogaleus medius*) that differ in several aspects of their life history traits. Our results show that there are no relationships among these indices in the fast-living *M. murinus*. However, using path models, we found that hair cortisol concentration had a direct positive influence on body condition, but also resulted in high parasite species richness in the slow-living *C. medius*. Thus, in the mouse lemur, the susceptibility to parasite infection might be influenced by short-term variations of GCs that are not captured by our long-term measurements (hair cortisol concentration). In contrast, GCs seem to play a key role in mediating the trade-off between body condition requirements prior to hibernation and an increased susceptibility to parasite infection in *C. medius*. Overall, this study indicates that the relationships among stress, parasitism and body condition could depend on the species-specific life history strategy.

INTRODUCTION

Long-term stress has long been known to have detrimental effects on the health of humans and animals alike (Cyr and Romero, 2007, Juster *et al.*, 2010). One mechanism in this context is the negative impact of hypothalamic-pituitary-adrenal (HPA) axis activation and the associated release of stress hormones (primarily glucocorticoids, GCs) on the immune system (Stratakis and Chrousos, 1995). Indeed, GCs mediate the reallocation of resources away from the maintenance of the immune system and, hence, can induce changes such as a reduction in the number of lymphocytes, in the natural killer cell number and activity, or a degradation of the antibody response (Glaser *et al.*, 1985, Webster *et al.*, 2002, Webster Marketon and Glaser, 2008). Therefore, animals that are subjected to stressful conditions have been commonly assumed to be more susceptible to parasite infections (Lafferty and Holt, 2003).

However, beside stress, various intrinsic factors such as life history stage, sex, or body condition can also affect the individual risk of parasite infection. Animals in poor condition were reported to be more susceptible to infections (Beldomenico *et al.*, 2008), and an elevated parasite infection can in turn further aggravate host condition (Beldomenico *et al.*, 2009). This synergy between host condition and infection risk as well as intensity has recently been suggested to feed a vicious circle driving the health gap between robust and weak individuals (Beldomenico and Begon, 2010). Indeed, as long as individuals are in good condition, parasite infections often seem to have little adverse effects (Milton, 1996, Munger and Karasov, 1989), whereas individuals in poor condition are more likely to succumb to infectious disease (Garner *et al.*, 2009).

As chronic stress was consistently shown to be correlated with reduced body condition (Dickens and Romero, 2013), the link between stress load, parasite infection and individual condition is very likely complex, with direct and indirect effects that can define the overall health state of an individual. As those physiological mechanisms are also assumed to play a key role in mediating life history trade-offs (Corlatti *et al.*, 2012, Cox *et al.*, 2010, Ricklefs and Wikelski, 2002), the interrelationships among these health indices should therefore vary between species with differing life history traits. Yet, this notion remains largely untested.

The purpose of this study was to illuminate the associations among those health indicators in natural populations of the grey mouse lemur (*Microcebus murinus*) and the fat-tailed dwarf lemur (*Cheirogaleus medius*). Specifically, we investigated whether associations existed among long-term stress load (measured as hair cortisol concentration, HCC), body condition and parasite infection and how they might differ depending on the life history strategies adopted by the species. Moreover, we were interested in defining the nature and magnitude of direct and indirect effects of HCC on body condition and species susceptibility to parasite infection using path analysis.

The grey mouse lemur and the fat-tailed dwarf lemur are closely related small strepsirrhine primates that differ in their life history traits. *M. murinus* has a faster life history (earlier sexual maturity, short nursing time; (Martin, 1972, Perret, 1982). Cooperative breeding among closely related females is possibly enhancing reproductive success in this species (Eberle and Kappeler, 2006). In good years, they can produce a second litter (Schmelting *et al.*, 2000). Additionally, *M. murinus* can adopt various energy saving strategies (heterothermy) during the cold dry season (Schmid and Ganzhorn, 2009, Vuarin *et al.*, 2013). In contrast, *C. medius* exhibits a slow life history strategy characterized by a small litter size, a late sexual maturity, only one breeding season per year, and a frequent skipping of reproduction; (Fietz *et al.*, 2000, Muller and Thalmann, 2002). Moreover, the fat-tailed dwarf lemur is an obligate hibernator (Dausmann *et al.*, 2004) with specific diet requirements (Fietz and Ganzhorn, 1999, Lahann and Dausmann, 2011). We generally predict that these measures of health should co-vary with each other, i.e. we expect high levels of stress (HCC) to be associated with low body condition and high parasite loads, but the nature of this association might differ between the fast-living *M. murinus* and the slow-living *C. medius*.

MATERIALS AND METHODS

Sample collection and analysis

We conducted our study on populations of *M. murinus* and *C. medius* in Kirindy Forest, western Madagascar, which is a forest concession operated by the Centre National de Formation, d´Etudes et de Recherche en Environnement et Foresterie Morondava (CNFEREF). Mouse lemurs and fat-tailed dwarf lemurs at Kirindy have been routinely monitored following a protocol of a long-term capture-mark-recapture study (Eberle and Kappeler, 2002, Fietz, 1999). Our capture activities were carried out in four different areas - locally known as N5, CS7, Savanna (SV) and Kirindy Village (KV) (detailed in Rakotoniaina *et al.*, 2016) - on a monthly basis, and with each capture session lasting for three consecutive nights. Traps were set at 25 m intervals and were baited with banana in the late afternoon and checked early next day. Captured individuals were individually marked with a subcutaneous transponder (or indentified at subsequent recaptures), sexed and standard field measures were taken before being released at the site of capture the following afternoon. For the purpose of identifying health indicator values, we sampled hair (for cortisol analysis) and faeces (for parasitology) during handling. Hair samples were obtained from the animal´s dorso-caudal region by using a pet grooming clipper (Aesculap Isis GT 420) and faeces were collected opportunistically in traps or handling bags, weighed, homogenized and stored in sample tubes with 10% formaldehyde. Overall, the data used in the present study were collected between March 2012 and March 2014, during 16 months of field work and a simultaneous record of all health indicators (HCC, body condition and parasitism) was achieved in 536 cases for *M. murinus* and 140 cases for *C. medius*.

The concentration of cortisol contained in hair samples was assessed using a high performance liquid chromatography-mass spectrometry (HPLC-MS) technique with purification by on-line solid-phase extraction (Gao *et al.*, 2013), after an adequate washing and extraction process. In brief, samples were washed twice in 3 ml isopropanol for 3 min and dried under a fume hood. For cortisol extraction 7.5 mg of hair were incubated with 40 µl internal standard and 2.4 ml methanol for 18 h at room temperature in a glass vial. Afterwards, samples were spun in a centrifuge at 10000 rpm for 3 min and 1.6 ml of the clear supernatant was dried at 50°C under a constant stream of nitrogen and re-suspended using 175 µl double-distilled water. Of the final product, 100 µl was used for cortisol concentration determination.

Individual body condition was estimated by using the scaled mass index (SMI) in order to control for the allometric relationship between body mass and body size (Peig and Green, 2009). This index is defined as the body mass of an individual after standardizing it to the mean body size of all individuals present in the population. We used head width (HW) as the morphometric measurement for body size. The SMI for every individual *i* was calculated using the formula:

$$SMI_i = BM_i \left[\frac{HW_0}{HW_i}\right]^{b_{SMA}}$$

where HW_0 is the arithmetic mean of HW for our study population (*M. murinus*=20.90 mm; *C. medius*=26.02 mm), and b_{SMA} (*M. murinus*=4.999 *C. medius*=3.869) is the slope of the standardized major axis regression of BM to HW.

For parasite analysis, faecal samples previously stored in 10% formalin solution were processed using a slightly modified Ritchie´s ether sedimentation method (Ritchie, 1948). Parasite eggs and oocysts were later counted and identified up to the genus level on the basis of their shape, size and internal structure (Irwin and Raharison, 2009, Raharivololona, 2006, 2009). To describe parasite infection, we considered prevalence, parasite morphotype richness and multiple infections. Parasite prevalence was calculated as the proportion of individuals infected by a particular parasite among all examined animals and high parasite prevalence has been correlated to declining populations (Chapman *et al.*, 2006). The proportion of individuals infected by more than one species of parasite can also be an informative index as multiple species infections have been linked to mortality and morbidity (Behnke, 1990, Raso *et al.*, 2004). Parasite richness is defined as the number of distinct parasite morphotypes recorded in each sample and could be suggestive of higher morbidity (Davidar and Morton, 2006).

To control for potential observer bias, hair and faecal samples were analysed blindly with respect to species and study site by coding them prior to laboratory analysis.

Statistical analyses

For each species, to first examine if changes in parasite infection and individual body condition were related to HCC variation, we used linear mixed models (LMMs; Baayen, 2008) or generalized linear mixed model (GLMMs), depending on the type of the dependent variable. Therefore, LMMs were used to explore the association between SMI and HCC. For those models, error variance homogeneity and normality were assessed using visual examination of residual plots of the full models. In all models, HCC and SMI values were standardized. To incorporate them in models as a binomial response, prevalence and multiple-species infection were coded as 1 if a given parasite morphotype was detected (or if more than one morphotype was recorded for multiple infection) and 0 if not. Thus, the association between those indices of parasite infection and HCC were assessed using a binomial GLMM with a logit link function for each study species. Given the very low prevalence of most of the recorded morphotypes, we did not consider individual parasite indices but rather modelled the overall prevalence. Additionally, morphotype richness was used as response variable in Poisson GLMMs with a log-link function, to define its relationship with HCC. General models of parasite morphotype richness were not overdispersed (assessed using Pearson residuals; overdispersion parameter ϕ: *M. murinus* = 1.107; *C. medius* = 0.896). For all models, we added the variables sex x season and age (juvenile, i.e. < 1 year or adult) as fixed factors since they were previously shown to influence the chosen health indicators (Hämäläinen *et al.*, 2015a, 2015b, Rakotoniaina *et al.*, 2016) and controlled for the capture area. However, in order to obtain better estimates for the variable of interest, non-significant terms ($p > 0.1$) were successively removed from the full models and likelihood ratio test were performed to compare models. Reduced models are presented in the results and full models are given in the supplementary material. Individual identity was further used as a random factor because of the multiple sampling of several individuals. The significance level was set at $p < 0.05$.

Next, we used path analysis to identify how the relationships among variables may be structured. We included in the path models only indices of health that showed a significant relationship with HCC. We started by constructing path diagrams that illustrate plausible hypotheses of causal relationships among dependent and independent variables based on a priori knowledge. Therefore, our diagrams were first based upon the hypothesis that HCC can influence parasite infection directly or indirectly by influencing individual body condition (Figure 1a). Secondly, we tested whether HCC is directly impacting on body condition or indirectly through parasite infection (Figure 1b). For the indirect paths, the interaction effect of the two cause variables was also included in the model. Then, we tested all logical combination of paths and variables within these hypotheses in order to identify the causal links among variables. Since our data contain repeated sampling of individuals and not only continuous dependent variables (e.g. prevalence and multiple infection are binomial), we could not apply the standard maximum likelihood χ^2 approach for structural equations models. Instead, we used Shipley´s d-sep test to test the path models (Shipley, 2009, 2016). Within path models, all null hypotheses of conditional independence, which are implied from the k independent elements of Shipley´s d-separation basis set of conditional independencies, were tested using mixed models. Models were fitted with the lme4 package (Bates *et al.*, 2015) implemented in R (version 3.3.0; RCoreTeam, 2016). The null probabilities (p_i) derived from each test were then combined using Fischer´s $C = -2 \Sigma \ln(p_i)$ and we checked if C followed a χ^2 distribution with $2k$ degrees of freedom. Finally, we rejected the model if the null probability of Fisher´s C was below 0.05 or otherwise, concluded that the data are consistent with the causal hypothesis. Path (beta) coefficients (standardized regression coefficients) were used to illustrate the relationships among variables since they allow for a comparison of the relative effects of independent variables measured in different units.

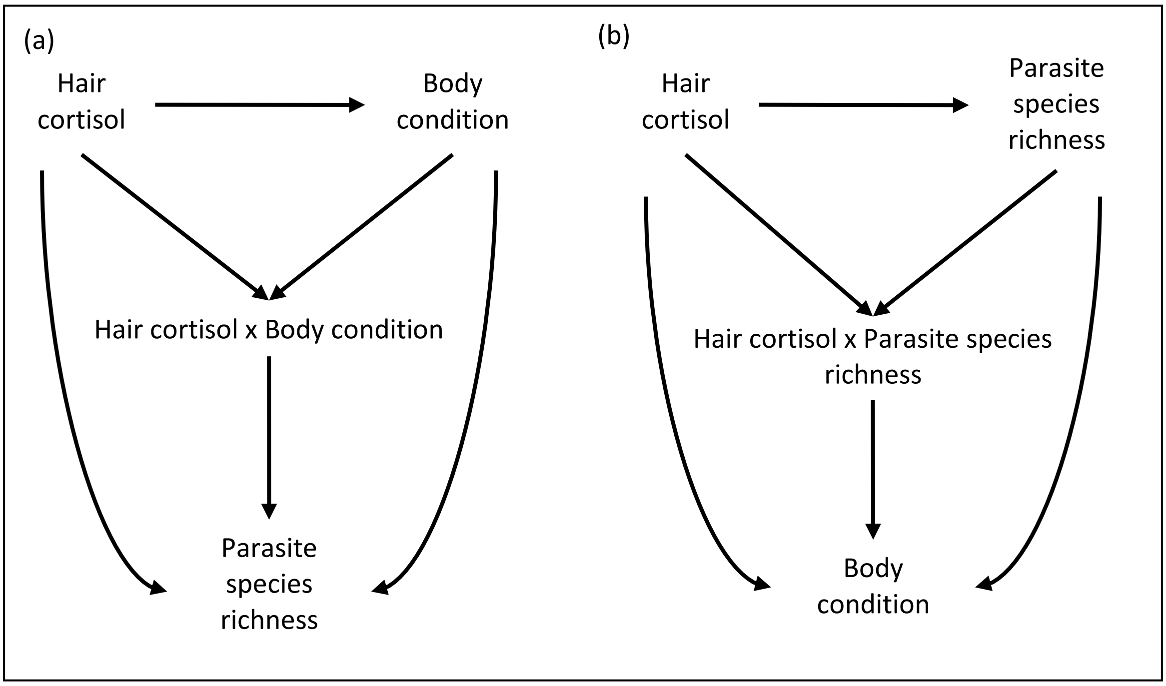

Figure 1 Path models depicting hypothesized links among health indicators for *C. medius* where cortisol is assumed to (a) impact parasite species richness through body condition or (b) impact body condition through parasite species richness.

RESULTS

For *M. murinus*, neither body condition nor the indices of parasite infection were related to HCC (Table 1), despite an overall parasite prevalence of 56.5%. Multiple species infection was also common in mouse lemurs and occurred in almost 26% of the total population. Almost identical parasite infection indices were also recorded for *C. medius* (overall prevalence: 50%; multiple parasite infection rate: 35%). However, parasite species richness was positively linked to HCC in the fat-tailed dwarf lemur (Table 1). Moreover, body condition also showed a strong association with HCC but contrary to our prediction, high HCC levels were associated with high SMI values.

Table 1 Parameter estimates and statistics of reduced (generalised) linear mixed models assessing variation of body condition and parasite infection in *M. murinus* and *C. medius.*

	Microcebus murinus				*Cheirogaleus medius*			
	Estimate	SE	*t / z*	*p*	Estimate	SE	*t / z*	*p*
Body condition (Scaled mass index)								
Intercept	57.470	0.986	58.262	**<0.001**	113.444	3.513	32.290	**<0.001**
Site (ref. N5) CS7	1.746	1.036	1.685	0.093				
SV	-2.775	1.159	-2.395	**0.017**				
KV	8.844	2.018	4.382	**<0.001**				
Season (ref. dry)	4.152	1.099	3.777	**<0.001**	26.666	4.781	5.578	**<0.001**
Sex (ref. female)	3.061	1.107	2.765	**0.006**	-5.854	3.556	-1.646	0.102
Age (ref. juvenile)	3.446	0.831	4.145	**<0.001**	15.340	3.678	4.170	**<0.001**
Sex*Season	-7.669	1.551	-4.945	**<0.001**	23.365	9.650	2.421	**0.017**
HCC	-0.396	0.432	-0.917	0.359	4.130	1.406	2.938	**0.004**
Overall prevalence								
Intercept	0.371	0.158	2.356	**0.018**	0.389	0.225	1.723	0.085
Season (ref. dry)	-0.603	0.181	-3.321	**<0.001**	-2.004	0.655	-3.059	**0.002**
Age (ref. juvenile)	0.343	0.185	1.854	0.064				
HCC	-0.044	0.096	-0.455	0.649	0.180	0.186	0.970	0.332
Multiple-morphotype infection								
Intercept	-1.213	0.250	-4.852	**<0.001**	-0.775	0.213	-3.640	**<0.001**
Season (ref. dry)	-0.481	0.295	-1.632	0.103	-2.743	1.096	-2.504	**0.012**
Sex (ref. female)	0.567	0.272	2.084	**0.037**				
Age (ref. juvenile)	0.396	0.219	1.805	0.071				
Sex*Season	-0.842	0.431	-1.950	0.051				
HCC	0.055	0.108	0.511	0.609	0.151	0.199	0.759	0.448

Table 1 Continued

	Microcebus murinus				*Cheirogaleus medius*			
	Estimate	SE	*t* / *z*	*p*	Estimate	SE	*t* / *z*	*p*
Parasite morphotype richness								
Intercept	-0.180	0.113	-1.593	0.111	-0.036	0.134	-0.266	0.790
Season (ref. dry)	-0.301	0.134	-2.240	**0.025**	-1.723	0.434	-3.970	**<0.001**
Sex (ref. female)	0.285	0.122	2.326	**0.020**				
Age (ref. juvenile)	0.207	0.100	2.075	**0.038**				
Sex*Season	-0.314	0.191	-1.649	0.099				
HCC	0.002	0.050	0.038	0.970	0.178	0.085	2.085	**0.037**

Path analyses applied to determine the structural relationships among HCC, body condition and parasite species richness in *C. medius*, revealed that a potential causal link which assumes an influence of HCC on body condition through parasite richness is not supported by the data ($\chi^2 = 6.247$, $df = 2$, $p = 0.044$; Table 2). Instead, our data are consistent with the hypothesis that HCC has a direct impact on parasite richness and body condition and an indirect impact on parasite richness through its effect on body condition ($\chi^2 = 0.267$, $df = 2$, $p = 0.875$; Table 2). Yet, the indirect effect of varying HCC on parasite richness is relatively weak (0.207 x 0.266 = 0.055, Figure 2) in comparison to the direct effect (0.183). Moreover, a causal link involving an effect of the interaction of HCC and body condition on parasite richness was not supported by the data ($\chi^2 = 10.617$, $df = 4$, $p = 0.031$).

Table 2 Basis sets retrieved from the path models in figure 1a, b and corresponding tests of conditional independence. Variables used are hair cortisol concentration (1), body condition (2), hair cortisol concentration x body condition (3), parasite species richness (4), hair cortisol concentration x parasite species richness (5). Notation follows Shipley (2016) where $X \perp\!\!\!\perp Y|\{W\}$ means that *X* and *Y* are independent, given the conditioning set *W.*

Basis set	*t* value	Partial *r*	Null probability
Figure 1a: model assuming an effect of HCC on parasite richness through body condition			
$4 \perp\!\!\!\perp 3|\{1,2\}$	-0.157	-0.019	0.875
Figure 1b: model assuming an effect of HCC on body condition through parasite richness			
$2 \perp\!\!\!\perp 5|\{1,4\}$	-2.036	-0.045	0.044

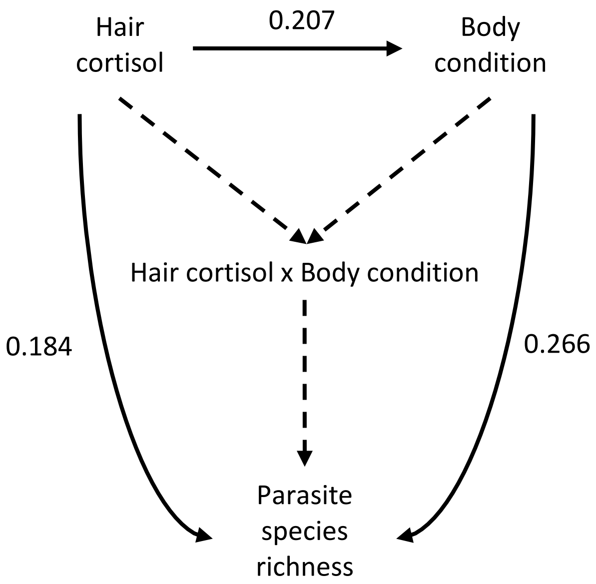

Figure 2 Path model linking hair cortisol, body condition and parasite species richness of *C. medius* with the standardized path coefficients of significant paths. The dashed line indicates a non-significant path.

DISCUSSION

In this study, we investigated whether and how cortisol level, parasite infection and general body condition co-vary in two small nocturnal lemur species that differ in various aspects of their life history traits. We did not find associations among HCC, pattern of parasitism and body condition in the fast living *M. murinus*. In contrast, HCC was associated with parasite species richness and body condition in *C. medius*. Surprisingly, high HCC values were linked with good body condition but also with elevated numbers of distinct parasite morphotypes recorded in the fat-tailed dwarf lemur. Additionally, these variations in body condition and parasite species richness seem to be directly caused by variation in hair cortisol levels.

Our failure to find associations among these health indicators in *M. murinus* does not necessarily indicate that they are fully independent. Variation in stress load, parasite infection and body condition can be caused by various extrinsic factors such as inclement weather, predation events or resource availability. For instance, parasitism could be influenced by parasite encounter rate, population density or ranging patterns and thus, increased exposure to infection (Hudson *et al.*, 2002). However, our findings are in line with previous studies that showed higher overall prevalence and parasite morphotype richness during the dry season for *M. murinus* (Hämäläinen *et al.*, 2015b, Rakotoniaina *et al.*, 2016, Springer and Kappeler, 2016). As environmental stages of parasites are assumed to survive better under warm and moist condition (Ramos *et al.*, 2013, Turner and Getz, 2010), Springer and Kappeler (2016) suggested that the seasonal pattern of parasitism being observed for mouse lemurs in Kirindy may rather be caused by increased host susceptibility to parasite infection than increased exposure. Moreover, the high levels of faecal glucocorticoid metabolites (FGCM) of *M. murinus* found during this period (Hämäläinen *et al.*, 2015a) may further indicate that this increased susceptibility is mediated by hormonal changes. The dry season is particularly challenging for mouse lemurs since it is a period of reduced resource availability (fruits, leaves and insects) and comprises the short mating season which is characterized by increased individual activities (Eberle and Kappeler, 2002). Moreover, a particularly high male mortality rates was recorded during this period (Kraus *et al.*, 2008). Under such conditions, the hormone-mediated immunosuppression could be aimed at reallocating the limited resources to costly behaviours associated with reproduction (Sheldon and Verhulst, 1996, Wedekind and Folstad, 1994) and/or to avoid immunopathology relative to a hyperactivation of the immune system during stressful events (Besedovsky and Del Rey, 1996, Råberg *et al.*, 1998). Therefore, the failure of HCC to explain the high levels of parasitism may imply that the stress-induced immunosuppression in *M. murinus* occurs through trends of short-term fluctuation and peaks of GC release that are not captured by the overall concentration of cortisol accumulated in the hair shaft during long time periods, and that might explain the high values of FGCM results obtained during the dry season (Hämäläinen *et al.*, 2015a). Yet, those short-term increases in response to a challenging condition such as during the mating season still seem to have a higher survival cost for long-term stressed individuals (Rakotoniaina *et al*, unpublished data).

The lack of association between body condition and cortisol levels might be due to the direct link of body mass to food intake. *M. murinus* is known to have a high plasticity in its diet (Dammhahn and Kappeler, 2008) and food should not be a primary source of stress for a species that does not have strict dietary requirements. While GC levels were shown to be related to diet quality in several species (Carnegie *et al.*, 2011, Foerster *et al.*, 2012, Foerster and Monfort, 2010), it does not necessarily have a direct impact on body condition. For instance, the use of fallback foods which are of relatively poor nutritional value, but are found in high abundance, can help animals with a flexible diet to maintain good condition during periods of low abundance of preferred food items (Harrison and Marshall, 2011, Wrangham *et al.*, 1998). Particularly, Rakotoniaina *et al*. (2016) found that habitat disturbance did not affect HCC in mouse lemurs. Instead, individuals in better condition were found in disturbed habitat, probably due to the capacity of *M. murinus* to adapt its diet depending on environmental condition and to take advantage of the high abundance of alternative food items, such as arthropods, that are present in those habitat types (Lehman *et al.*, 2006). Thus, in *M. murinus*, the costs associated with a high energetic investment during the mating season might be mitigated by highly flexible feeding behaviour.

In contrast, the positive association of HCC with parasite richness in *C. medius* seems to reflect, as suggested by the path analysis, the direct negative influence of long-term stress on the immune system. This link between increased susceptibility to parasite infection and increased stress hormone level was previously reported for various taxa (tree frogs: Belden and Kiesecker, 2005, mice: Malisch *et al.*, 2009, chimpanzees: Muehlenbein, 2006).

Moreover, short-term elevation of GC hormones are known to act directly or indirectly on appetite and stimulate the increase of energy intake in amphibians, fish and mammals (Bernier *et al.*, 2004, Crespi *et al.*, 2004, Dallman *et al.*, 1999, Tataranni *et al.*, 1996). The direct positive effect of HCC on body condition observed in *C. medius* implies that even a sustained increase in cortisol levels can promote food intake and highlight the importance of meeting energetic demands before the hibernation period in this species. Accordingly, Rakotoniaina *et al*. (2016) reported high HCC levels prior to hibernation (rainy season) in comparison to the dry season for the fat-tailed dwarf lemur. Those results are concordant with observations of seasonal GC fluctuations in various hibernator species which are known to show a constant increase of GC concentration until the onset of hibernation (yellow-bellied marmots: Armitage, 1991, Golden-mantled ground squirrel : Boswell *et al.*, 1994, little brown myotis: Reeder *et al.*, 2004, European ground squirrels: Shivatcheva *et al.*, 1988). Thus, GCs might play an important role in mediating the trade-off between accumulating enough energy reserves to face hibernation and an increased susceptibility to parasite infection in the fat-tailed dwarf lemur.

In summary, these results suggest that the nature and strength of the interrelationships among stress load, parasite infection and body condition are tightly linked with the species-specific difference in life history strategies in a natural setting. In a fast-living species, under a highly seasonal environment, the association between GCs and parasitism might be controlled by short-term rapid variations of hormone concentrations. In contrast, slow-living species seem to undergo long-term gradual changes in the level of hormones that in turn affect individual susceptibility to parasite infection and energy intake. This reinforces the view of stress-induced immunosuppression as a potential proximate mechanism underlying the life-history trade-off between an investment on reproduction and survival (Corlatti *et al.*, 2012, Cox *et al.*, 2010). Moreover, as GCs play a key role in physiological responses to stressful conditions, studies that attempt to understand the link between extrinsic stressors (e.g. environmental challenges, human disturbance) and GS levels should take into account the life history characteristic of animals when interpreting results.

Supplementary material of chapter 2

Table S1 Parameter estimates and statistics from full (generalised) linear mixed models assessing variation of body condition and parasite infection in *M. murinus* and *C. medius.*

	Microcebus murinus				*Cheirogaleus medius*			
	Estimate	SE	*t / z*	*p*	Estimate	SE	*t / z*	*p*
Body condition (Scaled mass index)								
Intercept	57.470	0.986	58.262	**<0.001**	113.535	3.678	30.872	**<0.001**
Site (ref. N5) CS7	1.746	1.036	1.685	0.093	-5.079	5.409	-0.939	0.349
SV	-2.775	1.159	-2.395	**0.017**	4.571	6.439	0.710	0.479
KV	8.844	2.018	4.382	**<0.001**	8.914	6.075	1.467	0.145
Season (ref. dry)	4.152	1.099	3.777	**<0.001**	26.969	4.797	5.622	**<0.001**
Sex (ref. female)	3.061	1.107	2.765	**0.006**	-5.909	3.610	-1.637	0.104
Age (ref. juvenile)	3.446	0.831	4.145	**<0.001**	14.483	3.696	3.918	**<0.001**
Sex*Season	-7.669	1.551	-4.945	**<0.001**	24.769	9.682	2.558	**0.012**
HCC	-0.396	0.432	-0.917	0.359	4.096	1.406	2.913	**0.004**
Overall prevalence								
Intercept	-0.017	0.222	-0.075	0.940	0.479	0.462	1.038	0.299
Site (ref. N5) CS7	0.321	0.230	1.397	0.162	-0.189	0.673	-0.280	0.779
SV	0.336	0.265	1.266	0.205	-0.975	0.842	-1.158	0.247
KV	0.264	0.459	0.575	0.565	-0.250	0.742	-0.337	0.736
Season (ref. dry)	-0.323	0.253	-1.275	0.202	-1.909	0.730	-2.615	**0.009**
Sex (ref. female)	0.467	0.257	1.813	0.070	0.497	0.451	1.101	0.271
Age (ref. juvenile)	0.349	0.188	1.848	0.065	-0.238	0.467	-0.509	0.611
Sex*Season	-0.481	0.361	-1.331	0.183	-0.494	1.358	-0.364	0.716
HCC	-0.052	0.098	-0.531	0.596	0.182	0.190	0.961	0.336

Table S1 Continued

	Microcebus murinus				*Cheirogaleus medius*			
	Estimate	SE	*t / z*	*p*	Estimate	SE	*t / z*	*p*
Multiple-morphotype infection								
Intercept	-1.365	0.276	-4.941	**<0.001**	-0.653	0.460	-1.421	0.155
Site (ref. N5) CS7	0.421	0.261	1.614	0.106	0.357	0.680	0.525	0.599
SV	0.103	0.292	0.353	0.724	-0.284	0.869	-0.326	0.744
KV	0.685	0.490	1.397	0.162	-1.335	1.089	-1.226	0.220
Season (ref. dry)	-0.476	0.299	-1.589	0.112	-2.759	1.109	-2.487	**0.013**
Sex (ref. female)	0.571	0.273	2.091	**0.036**	0.430	0.433	0.992	0.321
Age (ref. juvenile)	0.413	0.221	1.864	0.062	-0.328	0.492	-0.667	0.505
Sex*Season	-0.855	0.433	-1.973	**0.048**				
HCC	0.050	0.110	0.451	0.652	0.151	0.199	0.759	0.448
Parasite morphotype richness								
Intercept	-0.252	0.122	-2.058	**0.039**	0.011	0.248	0.045	0.964
Site (ref. N5) CS7	1.180	0.121	1.494	0.135	-0.056	0.371	-0.150	0.880
SV	0.122	0.131	0.929	0.353	-0.508	0.489	-1.037	0.299
KV	0.223	0.227	0.982	0.360	-0.331	0.447	-0.740	0.459
Season (ref. dry)	-0.286	0.135	-2.211	**0.035**	-1.844	0.516	-3.574	**<0.001**
Sex (ref. female)	0.281	0.122	2.301	**0.021**	0.213	0.230	0.927	0.354
Age (ref. juvenile)	0.206	0.100	2.059	**0.039**	-0.091	0.252	-0.363	0.717
Sex*Season	-0.317	0.190	-1.667	0.096	0.514	0.905	0.568	0.570
HCC	0.002	0.051	0.041	0.967	0.183	0.086	2.111	**0.035**

CHAPTER 3

“Hair cortisol concentrations correlate negatively with survival in a wild primate population”

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ABSTRACT

Background

Glucocorticoid hormones are known to play a key role in mediating a cascade of physiological responses to social and ecological stressors and can therefore influence animals´ behaviour and ultimately fitness. Yet, how glucocorticoid levels are associated with reproductive success or survival in a natural setting has received little empirical attention so far. Here, we examined links between survival and levels of glucocorticoid in a small, short-lived primate, the grey mouse lemur (*Microcebus murinus*), using for the first time an indicator of long-term stress load (hair cortisol concentration). Using a capture-mark-recapture modelling approach, we assessed the effect of stress on survival in a broad context (semi-annual rates), but also under a specific period of high energetic demands during the reproductive season. We further assessed the power of other commonly used health indicators (body condition and parasitism) in predicting survival outcomes relative to the effect of long-term stress.

Results

We found that high levels of hair cortisol were associated with reduced survival probabilities both at the semi-annual scale and over the reproductive season. Additionally, very good body condition (measured as scaled mass index) was related to increased survival at the semi-annual scale, but not during the breeding season. In contrast, variation in parasitism failed to predict survival.

Conclusion

Altogether, our results indicate that long-term increased glucocorticoid levels can be related to survival and hence population dynamics, and suggest differential strength of selection acting on glucocorticoids, body condition, and parasite infection.

BACKGROUND

Identifying the links between physiological traits and fitness is vital for understanding the proximate mechanisms of selection that regulate natural populations. Glucocorticoid (GC) hormones are commonly employed as a biomarker of health or relative condition, both at the individual and the population level, since they mediate an array of physiological processes that can directly or indirectly impact fitness (Walker *et al.*, 2005, Wikelski and Cooke, 2006). As part of the hypothalamic-pituitary-adrenal (HPA) axis activity, GCs play a key role in the reallocation of resources in response to actual or perceived ecological challenges, such as inclement climatic conditions or predation pressure, that are associated with increased energetic needs (Boonstra *et al.*, 2014, Romero, 2002, Sapolsky *et al.*, 2000). Whereas the increase in GCs during acute stress is generally adaptive, chronic elevation of GC levels can compromise reproduction, immune function, and ultimately survival, thus reducing fitness (Breuner *et al.*, 2008, Pride, 2005b, Romero, 2004, Wayland *et al.*, 2002, Wingfield and Sapolsky, 2003). For instance, individuals of various taxa have been found to potentially suffer fitness consequences of high GC concentrations through their increased susceptibility to parasite infection because of the immunosuppressive effects of GC (Charbonnel *et al.*, 2008, French *et al.*, 2006, French *et al.*, 2010, Muehlenbein, 2006, Rojas *et al.*, 2002). The negative relationship between GC levels and fitness is at the core of the "Cort-fitness hypothesis", which posits that high levels of baseline GCs indicate poor individual or population condition (Bonier *et al.*, 2009a, 2009b).

However, to date, tests of the Cort-fitness hypothesis in wild populations are rare (Bonier *et al.*, 2009a, Breuner *et al.*, 2008), and in some of the few studies that attempted to do so, this hypothesis failed to receive empirical support (reviewed in Bonier *et al.*, 2009a). Plausible explanations of this failure include the lack of repeatability of both the cort-fitness relationship and GC measures. Indeed, the cort-fitness relationship has been frequently described to be context-dependent, and factors such as sex and reproductive strategy may influence the nature of this correlation (Lancaster *et al.*, 2008, Wingfield *et al.*, 1999). Furthermore, GCs are known to show strong fluctuations over time (Ouyang *et al.*, 2011a, Romero, 2002) and within-individual variability can potentially mask the effect of GC variation on fitness among individuals (Ouyang *et al.*, 2011a). The majority of studies that have examined this correlation were based on a single sampling of GCs, using established biomarkers of acute stress (i.e., serum, saliva or urine), potentially biasing estimates of overall individual condition. Hence, when multiple sampling is not possible, the use of a biomarker of chronic stress might better illuminate this relationship.

Recently, the measurement of hair cortisol concentration (HCC) has emerged as a promising new tool for monitoring long-term HPA axis activity (Meyer and Novak, 2012, Russell *et* *al.*, 2012, Sharpley *et al.*, 2012). Free circulating GCs are thought to be incorporated into the hair shaft throughout its growth (Davenport *et al.*, 2006, Sauve *et al.*, 2007). Therefore, HCC is unaffected by potential short-term fluctuation in cortisol secretion and allows an assessment of accumulated cortisol levels over a wider time window compared to traditionally used matrices. Moreover, cortisol levels in hair were proven to be highly stable and consistent within individuals (Bechshoft *et al.*, 2012a, Stalder *et al.*, 2012, Webb *et al.*, 2010). While HCC has been applied to monitor individuals´ response to adverse conditions in several species (e.g, humans: Dettenborn *et al.*, 2010; chimpanzees: Carlitz *et al.*, 2016; vervet monkeys: Fairbanks *et al.*, 2011, Fourie *et al.*, 2015; marmosets: Clara *et al.*, 2008; squirrels: Brearley *et al.*, 2012; cows: Comin *et al.*, 2011; wolves: Bryan *et al.*, 2015), to our knowledge, no attempt was previously made to connect this indicator to measures of fitness in a wild population.

Studies of small, short-lived species can be advantageous when assessing the relationship between GCs and fitness in a wild setting, because in such study systems, physiological state can be measured repeatedly throughout the individual lifespan. The grey mouse lemur (*Microcebus murinus*), a small-bodied nocturnal primate (Lemuriformes: Cheirogaleidae), presents several key features allowing investigations of the potential relationship between individual condition and fitness. First, its average lifespan in the wild is 2-3 years, with a high annual turnover of around 50% (Hämäläinen *et al.*, 2014a, Kraus *et al.*, 2008). Second, in its highly seasonal habitat, strong fluctuation of water and food availability affects the feeding behaviour of *M. murinus* (Dammhahn and Kappeler, 2008), but also several health indicators such as body mass, parasitism and levels of faecal GC metabolites (Hämäläinen *et al.*, 2014a, 2015a, 2015b). Condition-dependent mortality is suggested to occur in mouse lemurs (Hämäläinen *et al.*, 2014a), and risky behaviour significantly influences male mortality during the breeding season (Kraus *et al.*, 2008). While predation has been invoked as the probable leading cause of mortality (Goodman *et al.*, 1993), the proximate physiological mechanisms accompanying this non-random mortality remain unknown.

In this study, we test the hypothesis that high HCC (as a measure of long-term stress level) is related to individual survival in a wild population of *M. murinus*. Additionally, we assess the power of two other health indicators (size-adjusted body mass and patterns of parasitism) to predict survival. Specifically, we evaluate the potential link between these three health indicators and survival at two different levels. First, in order to define a general pattern, we test whether they can predict survival by assessing their relationship on a semi-annual basis, following the seasonal fluctuation of environmental conditions (Sorg and Rohner, 1996). Second, we estimate the significance of individual condition under a specific context of high energetic demands by focusing on survival rates at the end of the dry season. The short mating season occurs during this period (Eberle and Kappeler, 2002, 2004), and it is associated with the lowest body mass and highest faecal GC metabolite levels (Hämäläinen *et al.*, 2014a, 2015a, Schmid and Kappeler, 1998). We therefore predicted that individuals with high HCC suffer from lower survival. Furthermore, as individuals in poor health should be more vulnerable to ecologically adverse conditions, we expect survival to be positively correlated with general body condition, while individuals that exhibit a high degree of parasitism should face higher mortality.

MATERIALS AND METHODS

Study site and population

The study was conducted in Kirindy Forest, which is a concession operated by the CNFEREF (Centre National de Formation, d´Etudes et de Recherche en Environnement et Foresterie), located approximately 60 km north of Morondava and a part of a dry deciduous forest in central western Madagascar (Sorg and Rohner, 1996). We focused on a population of *M. murinus* from a 25 ha area (500 x 500 m) locally known as N5. This population has been continuously monitored since 2002.

Capture-mark-recapture

As a part of the long-term live capturing protocol, we conducted monthly capture sessions during the mouse lemurs´ active period (from September to April) between 2012 and 2014. Trapping sessions consisted of three consecutive nights of capturing, using Sherman live traps baited with banana. Traps were set at 25 m intervals, at the intersections of a grid system of foot trails, in the late afternoon at 40 – 200 cm height, and checked in the early morning the next day. After being anesthetized with 0.02 ml ketamine (Ketavet®, Pfizer, Germany), captured animals were individually marked (or only identified without anesthetization if recaptured) with a subcutaneous transponder (Trovan EURO ID, Germany) and sexed, and standard field measurements, such as head width and body mass, were recorded. Hair samples for cortisol analysis and faecal samples for parasitology were also collected during capture sessions. While hair samples and morphometric measurements were collected semi-annually (in September-October and March-April), faecal samples were obtained opportunistically at a monthly rate.

To assess the relationship between HCC and semi-annual survival probabilities, we used the results of capture sessions held in October 2012, 2013, 2014, April 2013, and March 2014, during which a total of 171 individuals (74 females, 97 males) were captured. The same dataset, except the October 2014 session, was used to assess the effect of body condition on semi-annual survival probabilities, for a total of 149 individuals (63 females, 86 males). The link between survival probabilities and the health indicators (HCC, body condition, and parasitism) over the reproductive period was assessed by using data collected during monthly trapping sessions between September 2012 and April 2013. This dataset included 48 individuals (16 females and 32 males).

Sample collection and analysis

Assessment of hair cortisol concentration

In order to avoid potential variation of hair cortisol concentration (HCC) from different body regions (Carlitz *et al.*, 2015, Macbeth *et al.*, 2010), we collected hair samples consistently from the animals' dorso-caudal region, where pelage coloration was reported to vary little across individuals and sexes (Rasoloarison *et al.*, 2000), using a pet grooming clipper (Aesculap Isis GT 420). The detailed protocol for washing and extracting hair cortisol is described by Gao *et al* (2013), and all laboratory analyses were conducted at the University of Dresden (Germany). As a minor modification to the original protocol, because the hair structure of *M. murinus* prohibited the measurement of individual hair, we extracted cortisol using 7.5 mg of sample after washing (twice in 3 ml isopropanol for 3 min) and drying. The sample was further incubated with 40 µl internal standard and 2.4 ml methanol for 18 h at room temperature in a glass vial. After centrifugation at 10000 rpm for 3 min, 1.6 ml of the clear supernatant was dried at 50 °C under a constant stream of nitrogen. The dry residue was re-suspended using 175 µl double-distilled water, 100 µl of which was used for cortisol concentration determination with liquid chromatography tandem mass spectrometry (LC-MS/MS). This assessment was performed using a Shimadzu HPLC-tandem mass spectrometry system (Shimadzu, Canby, Oregon) coupled to an ABSciex API 5000 Turbo-ion-spray triple quadrupole tandem mass spectrometer (AB Sciex, Foster City, California) with purification by on-line solid-phase extraction (Gao *et al.*, 2013).

Although the mode (synchronous or asynchronous) and rate of hair growth are unknown for mouse lemurs, we are confident that the amount of hair we used for the HCC analysis is sufficient to accurately reflect a mean baseline cortisol concentration for this species. Indeed, as *M. murinus* has very dense fur, consisting of very thin hair. The number of hairs in a 7.5 mg sample (containing whole strands) is largely above 50-100 hairs, the number recommended by Fourie *et al.* (Fourie *et al.*, 2016) when studying medium-sized primates. Also, we observed that hair did not fully regrow after a month, motivating us to sample hair only at a semi-annual rate.

Assessment of general body condition

Instead of using body mass (BM) per se, we computed the scaled mass index (SMI) to assess body condition, thus controlling for the allometric relationship between body mass and body size (Peig and Green, 2009). This index yields an individual value of body mass standardized to the mean body size of all individuals present in the population. We used head width (HW) as a body size measurement due to its strong positive correlation with body mass in this species (see also Vuarin *et al.*, 2013). The scaled mass index for every individual *i* was calculated as follows:

$$SMI_i = BM_i \left[\frac{HW_0}{HW_i} \right]^{b_{SMA}}$$

where HW_0 (= 21.92 mm) is the arithmetic mean of HW for the population and b_{SMA} (= 3.888) the slope of the standardized major axis (SMA) regression of ln (*BM*) on ln (*HW*). We used the software RMA (Bohonak and Van der Linde, 2004) to calculate the value of b_{SMA}.

Assessment of parasitism pattern

Fresh faeces collected opportunistically from handling bags or traps were weighted, directly homogenized with 10% formaldehyde and stored in 2 ml screw cap Sarstedt tubes. Parasite eggs and oocysts were identified under microscopic examination following a slightly modified Ritchie´s ether sedimentation method (Ritchie, 1948). Parasites were further classified up to the genus level based on egg or oocyst shape, size and internal structure (Irwin and Raharison, 2009, Raharivololona, 2006, 2009), and prevalence, morphotype richness and occurrence of multiple morphotype infections were used to characterize the pattern of parasitism, as detailed in Rakotoniaina *et al*. (2016).

To control for potential observer bias, we used blind observation by coding samples prior to laboratory analysis of hair cortisol levels and faecal parasites.

Modelling outline and candidate set of models

Semi-annual survival

In order to statistically estimate the link between HCC and SMI, as well as semi-annual survival (Φ), we used multistate capture-mark-recapture models (Brownie *et al.*, 1993, Hestbeck *et al.*, 1991, Neil Arnason, 1972, 1973, Schwarz *et al.*, 1993) implemented in the program MARK version 8.0 (White and Burnham, 1999), which account for recapture (p) and state-transition (ψ) probabilities. For each capture session, each individual was first assigned to a high or low HCC and SMI state, using the population median HCC or SMI value of the considered session as a cut-off point. Afterwards, in order to check if the correlation with survival is stronger at high ends of the health indicator values, we explored models where the categorization cut-off was based on the third quartile of HCC and SMI values. We could not use actual HCC and SMI values because modelling individually time-varying covariates (a different covariate value per individual at each recapture event) in MARK requires including a value of the covariates at each capture event even for missing (not recaptured) animals. Therefore, the multistate approach (using HCC/SMI categories) allows us to incorporate a variable value of the covariates between capture events (by accounting for ψ) while controlling for missing individuals (by accounting for p). Unlike HCC and SMI, the effect of parasitism on survival could not be modelled using this approach since we could not obtain faecal samples for every single individual at every single capture event. Therefore, indices of parasite infection were only considered for the assessment of reproductive season survival (see below).

Following Burnham and Anderson (2002), we constructed *a priori* a candidate set of biologically plausible models (see Additional file 1). We assessed the goodness-of-fit of the global models and obtained an estimation of the variance inflation factor $\hat{c}$ with the median-$\hat{c}$ approach implemented in MARK. This method suggested that our data were slightly overdispersed (models with categorization set using the median: $\hat{c}(HCC) = 1.204$, $\hat{c}(SMI) = 1.432$; models with categorization set using the third quartile: $\hat{c}(HCC) = 1.213$, $\hat{c}(SMI) = 1.432$), thus model selection statistics were adjusted accordingly. Owing to the rather small sample size, we based our model selection on AICc (or QAICc in the presence of overdispersion) (Anderson *et al.*, 1994), which is an adjusted variant of the Akaike´s information criterion (AIC). The difference (Δ_i) between the AICc of the most parsimonious model and a given model *i* and the normalized Akaike weights (w_i) were used to interpret the results of model selection. Hence, models with $\Delta_i \leq 2$ were considered to have a strong support while models with $4 < \Delta_i < 7$ have intermediate support and models with $\Delta_i > 10$ have negligible support (Burnham and Anderson, 2002). The relatively low values of the

Akaike weights of our top models (< 0.9) for the HCC and the SMI datasets indicated model selection uncertainty and therefore, we adopted multi-model inference techniques over a confidence subset of models (all models with relative likelihood > 0.05; see Additional file 2 and 3). Thus, the importance of a variable (given as w_+ (variable)) was determined by summing the Akaike weights of models containing the variable of interest. Parameter estimates and their unconditional standard errors were calculated by averaging over all models in our confidence subset of models (Buckland *et al.*, 1997, Burnham and Anderson, 2002).

We established all our candidate model sets by including factors known to influence mouse lemurs' survival (Kraus *et al.*, 2008) and consequently considered the factors sex (s) and time (t) in addition to our measure of the animal condition (c; high/low HCC or SMI). As a global model, we used $\Phi(c*s+t)$ $p(s+t)$ $\psi(c*t)$ (*: interactive effect, +: additive effect). Subsequently, all possible additive combinations of c, s and t and their single effects were used to model survival probability (Φ). Recapture probability (p) was additionally considered to be time dependant or constant over time. The condition index (c) was not included to model recapture probability since previous studies have reported a lack of a link between stress responses and previous capture experience (Hämäläinen *et al.*, 2014b), but also an increasing recapture probability ("trap happiness") of most individuals in this mouse lemur population (Kraus *et al.*, 2008), suggesting no long-term physiological cost of capture activities. Finally, we further modelled state-transition probability (ψ) to depend only on c. We fitted a total of 54 models for each condition index by considering all combinations of parameterization used for Φ, p and ψ.

Breeding season survival

We further estimated the potential association between our health indicators (HCC, SMI and parasitism) and survival (Φ) and recapture probabilities (p) over the breeding season by using the "Cormack-Jolly-Seber" model for open populations (Cormack, 1964, Jolly, 1965, Seber, 1965) implemented in MARK. In order to get an accurate estimation of (Φ) and (p) over the reproductive season, we used data from monthly trapping sessions conducted between the end of the dry season (September 2012) and the end of the rainy season (April 2013). However, as data for January and February 2013 were missing, we controlled for the bias that this gap may have induced to the estimation of (Φ) and (p) by manually adjusting the time interval of trapping sessions between December and March (three months instead of one) in our models. Thereafter, we proceeded in two steps. First, we established a starting set of models by using $\Phi(s*t)$ $p(s+t)$ as the global model and further comparing all possible permutation of models with an effect of $(s+t)$, s and t on survival probability and s and t on recapture probability along with a constant Φ and p

(see Additional file 4). Then, our health parameter values (HCC, SMI, parasite morphotype richness, overall prevalence and multiple species infection) were successively included as an individual covariate (for the first month only) to the most parsimonious model (basic model) among the starting set to assess if their inclusion improved the fit of the model. Additionally, we checked for potentially normalizing selection that might favour optimal HCC and body mass values and therefore tested for a quadratic effect of HCC and SMI on survival probabilities. We also fitted models including natural log-transformed HCC and SMI values to our monthly capture data. As above, the goodness-of-fit of the global model was assessed using the median-$\hat{c}$ approach (all $\hat{c}$ were < 1) and model selection was based on the information theoretical approach (Burnham and Anderson, 2002).

Additionally, we tested for potential intercorrelations among HCC and the other health indicators. If existing, those correlations might mask or interfere with the assessment of their independent links to survival. Yet, no association was detected; neither between HCC and SMI ($r^2 = 0.006$, $df = 227$, $p = 0.247$) nor between HCC and parasitism pattern (species richness: $r^2 = 0.053$, $df = 46$, $p = 0.112$; overall prevalence: $r^2 = 0.068$, $df = 46$, $p = 0.073$; multiple infection: $r^2 = 0.062$, $df = 46$, $p = 0.087$).

RESULTS

Semi-annual survival relative to HCC values

Multistate models applied on HCC revealed that survival is lower for mouse lemurs with elevated levels of hair cortisol. The gap in survival probability between low and high HCC individuals is larger at high ends of HCC values (Fig. 1a and 1b). Individuals with low HCC had on average (based on geometric mean across years regardless of sex) a 9.8% higher chance to survive than those with high HCC when the categorization was set using the median HCC value. This gap increased to up to 13.9% when the categories were defined using the third quartile value (Fig. 1a and 1b). In both cases, in addition to the HCC effect, the best-supported models ($\Delta_{i} < 2$) also suggested a sex difference in survival (Table 1). Females survived relatively better than males (Fig. 1a and 1b; geometric means over time where the median is used as categorization cut-off: $\Phi_{\text{low HCC F}} = 0.758$, $\Phi_{\text{low HCC M}} = 0.724$, $\Phi_{\text{high HCC F}} = 0.664$, $\Phi_{\text{high HCC M}} = 0.622$; geometric means over time where the third quartile is used as categorization cut-off: $\Phi_{\text{low HCC F}} = 0.729$, $\Phi_{\text{low HCC M}} = 0.694$, $\Phi_{\text{high HCC F}} = 0.594$, $\Phi_{\text{high HCC M}} = 0.550$). Yet, multi-model inference emphasized that the relative importance of the effect of HCC on survival was higher than the effect of sex independently of the method used to set the categories (median cut-off: $w_+(HCC) = 0.701$; $w_+(sex) = 0.504$; third quartile cut-off: $w_+(HCC) = 0.756$; $w_+(sex) = 0.487$), further highlighting the strong support for a lowered survival of individuals with high HCC values.

In both approaches, our candidate set of models showed limited support for between-season variation in survival (median cut-off: $w_+(t) = 0.170$; third quartile cut-off: $w_+(t) = 0.108$) but instead a strong variability of recapture probabilities through time (median cut-off: $w_+(t) = 0.805$; third quartile cut-off: $w_+(t) = 0.756$). All models in the confidence sets supported that a transition to a given state (high or low HCC) depended mainly on the current state of the individual (Table 1).

Table 1 Model selection statistics (multistate approach) for semi-annual survival (Φ), recapture (p) and state-transition (ψ) probabilities of *M. murinus* depending on hair cortisol concentration and general body condition (measured as scaled mass index).

Rank	Model	K	QDEV	QAICc	Δ_i	w_i
Hair cortisol concentration (*categorization cut-off: median*)						
1	$\Phi(c)\ p(t)\ \psi(c)$	8	200.78	515.10	0	0.147
2	$\Phi(s)\ p(t)\ \psi(c)$	8	201.90	516.23	1.13	0.142
3	$\Phi(c + s)\ p(t)\ \psi(c)$	9	199.96	516.44	1.34	0.126
4	$\Phi(c)\ p(.)\ \psi(c)$	5	208.91	516.88	1.77	0.120
53	**$\Phi(c * s + t)\ p(s + t)\ \psi(c * t)$**	**20**	**192.77**	**534.29**	**19.19**	**0.0001**
Hair cortisol concentration (*categorization cut-off: third quartile*)						
1	$\Phi(c)\ p(t)\ \psi(c)$	8	146.94	465.86	0	0.230
2	$\Phi(c + s)\ p(t)\ \psi(c)$	9	146.08	467.16	1.30	0.120
3	$\Phi(c)\ p(.)\ \psi(c)$	5	154.75	467.31	1.45	0.111
4	$\Phi(s)\ p(t)\ \psi(c)$	8	148.66	467.59	1.73	0.097
54	**$\Phi(c * s + t)\ p(s + t)\ \psi(c * t)$**	**20**	**138.21**	**484.33**	**18.47**	**0.00002**
Scaled mass index (*categorization cut-off: median*)						
1	$\Phi(s)\ p(t)\ \psi(c)$	7	114.35	330.82	0	0.272
2	$\Phi(c)\ p(t)\ \psi(c)$	7	115.43	331.90	1.08	0.158
54	**$\Phi(c * s + t)\ p(s + t)\ \psi(c * t)$**	**16**	**109.73**	**346.67**	**15.85**	**0.0004**
Scaled mass index (*categorization cut-off: third quartile*)						
1	$\Phi(c)\ p(t)\ \psi(c)$	7	102.53	323.87	0	0.229
2	$\Phi(c + s)\ p(t)\ \psi(c)$	8	101.34	324.85	0.99	0.140
3	$\Phi(s)\ p(t)\ \psi(c)$	7	103.72	325.06	1.19	0.127
4	$\Phi(c)\ p(s + t)\ \psi(c)$	8	102.03	325.55	1.68	0.099
54	**$\Phi(c * s + t)\ p(s + t)\ \psi(c * t)$**	**16**	**96.91**	**338.72**	**14.85**	**0.0001**

Only models with $\Delta_i \leq 2$ and the global model (in bold) are shown here with the number of parameters (K), the quasi-likelihood adjusted deviance (QDEV), the quasi-likelihood adjusted AIC for small sample size (QAICc), the difference between the QAICc of the top model and a given model *i* (Δ_i) and the Akaike weights (w_i). Variables considered are the condition index (*c,* which can indicate HCC or SMI values), sex (*s*) and time (*t*). Constant parameters are noted (.). Interactions are indicated by (*) and additive effects by (+).

Semi-annual survival relative to SMI values

We found only weak support for an effect of SMI on survival in comparison to the effect of sex when categories were established according to the median SMI value (w_+ (*sex*) = 0.625; w_+ (*SMI*) = 0.441). On average (based on the geometric mean across seasons), females had around 6% higher chance to survive to the next season than males but the difference of survival probability between conditions was negligible (Fig. 1c). However, our results also suggested that *M. murinus* in very good body condition (categories based on the third quartile regardless of sexes) survive on average 13.7% better than low condition animals (Fig. 1d), and strong support for a positive effect of SMI on survival was obtained with those models (w_+ (*SMI*) = 0.744; w_+ (*sex*) = 0.513).

Additionally, a time varying recapture probability structure was strongly supported by our confidence set of models in both scenarios (median cut-off: w_+ (*t*) = 0.895; third quartile cut-off: w_+ (*t*) = 0.884).

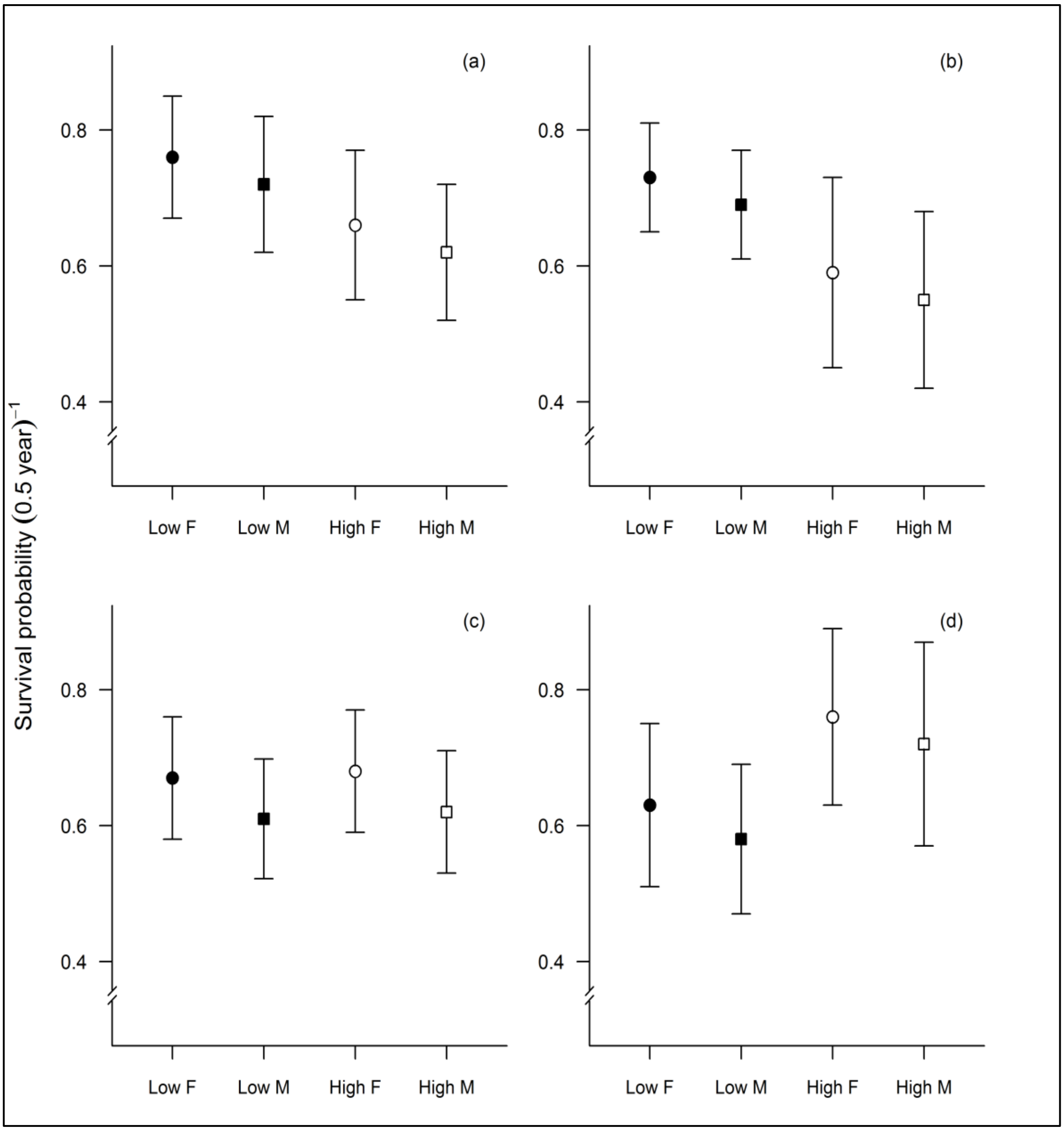

Figure 1 Semi-annual survival probabilities of *M. murinus.* Estimates are relative to: hair cortisol concentration (a and b), where the categorization cut-off is the median (a), or the third quartile (b); and the scaled mass index (c and d), where the categorization cut-off is the median (c) or the third quartile (d). Presented are model-averaged maximum likelihood estimates and unconditional standard errors (Filled symbols/Low: low value of the condition index; Open symbols/High: high value of the condition index; Circles/F: females; Squares/M: males). Estimates are averaged (geometric mean) over capture sessions.

Health indicators and survival over the breeding season

The most parsimonious model (basic model) among the starting set contained time varying survival and recapture probabilities (*Φ(t) p(t)*). All models including HCC as a predictor of survival had a better fit than the basic model (Table 2). The top model (with natural log-transformed HCC) was more than four times better supported than the basic model (w(ln(HCC)) = 0.366, w(t) = 0.0.83; 0.366/0.083 = 4.404). In the two best-supported models ($\Delta_i < 2$) we found a negative relationship between HCC and survival (ln (HCC), Fig. 2a; HCC, Fig. 2b). In contrast, we found no evidence of a link between survival and SMI or parasitemia, as the basic model performed better than all the models containing the other health indicators as covariates (SMI, multiple parasite species richness, parasite morphotype richness and overall prevalence; Table 2).

Table 2 Model selection statistics for monthly survival (Φ) and recapture (p) probabilities of *M. murinus*.

Rank	Model	K	DEV	AICc	Δ_i	w_i
1	$\Phi(t + ln(HCC))\ p(t)$	10	193.11	215.04	0	0.366
2	$\Phi(t + HCC)\ p(t)$	10	193.83	215.76	0.72	0.256
3	$\Phi(t + HCC + HCC^2)\ p(t)$	11	193.65	217.98	2.94	0.084
4	$\boldsymbol{\Phi(t)\ p(t)}$	**9**	**198.44**	**218.01**	**2.97**	**0.083**
5	$\Phi(t + mult)\ p(t)$	10	196.51	218.44	3.40	0.067
6	$\Phi(t + rich)\ p(t)$	10	197.70	219.63	4.59	0.037
7	$\Phi(t + ln(SMI))\ p(t)$	10	197.84	219.77	4.73	0.034
8	$\Phi(t + SMI)\ p(t)$	10	197.95	219.88	4.84	0.033
9	$\Phi(t + prev)\ p(t)$	10	198.41	220.34	5.30	0.026
10	$\Phi(t + SMI + SMI^2)\ p(t)$	11	197.15	221.49	6.45	0.015

In this Cormack-Jolly-Seber approach, monthly survival (Φ) and recapture (p) probabilities are assessed depending on hair cortisol concentration (*HCC*), general body condition (measured as scaled mass index, *SMI*) and patterns of parasitism (multiple species infection: *mult;* parasite morphotype richness: *rich;* overall prevalence: *prev*). The basic model (in bold) and models containing covariates are shown with the number of parameters (K), the deviance (DEV), the adjusted AIC for small sample size (AICc), the difference between the AICc of the top model and a given model *i* (Δ_i) and the Akaike weights (w_i).

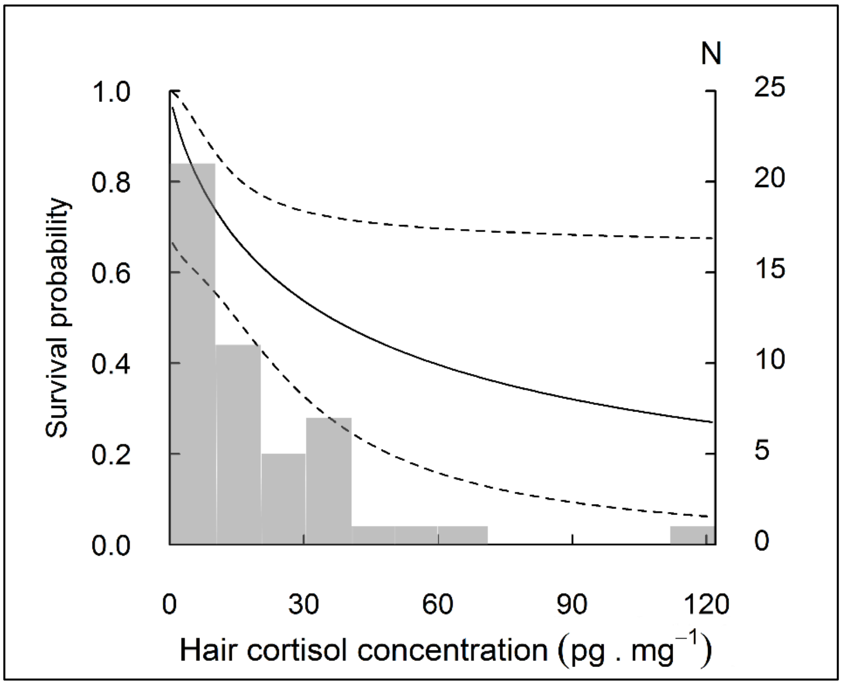

Figure 2 Monthly survival probabilities of *M. murinus* relative to the hair cortisol concentration. Considered is linear effect on the natural log-transformed HCC (values presented here are back transformed to the original scale). Presented are main effect (solid line) and 95% CI (dashed lines). Histograms represent the sample size for each category.

DISCUSSION

In this study, we tested the hypothesis that high HCC (as a measure of long-term activation of the HPA axis) translates into reduced survival. Using wild mouse lemurs previously known to face condition-dependent mortality (Hämäläinen *et al.*, 2014a), we further tested the correlation of two other health indicators (body condition and patterns of parasitism) with survival in order to compare their predictive power for fitness outcomes. As predicted by the Cort-fitness hypothesis (Bonier *et al.*, 2009a), both semi-annual survival and survival over the reproductive period were negatively associated with the level of accumulated hair cortisol. The first approach revealed that the relationship between HCC and survival is particularly strong at the high end of HCC values. Furthermore, our result suggested that individuals in extremely good condition enjoy higher survival probabilities than the ones with mid to low SMI values. In contrast, there was little support for the effects of SMI and measures of parasitism (multiple parasite species richness, parasite morphotype richness and overall prevalence) on survival over the reproductive period.

Our results provide general support for the Cort-fitness hypothesis which posits that elevated GCs are associated with a decline in one component of fitness (Bonier *et al.*, 2009a). Under this hypothesis, individuals in poor quality are assumed to perceive their environment as challenging and therefore secrete higher levels of GC than good quality individuals. However, similar to what has been reported by several other studies (Brown *et al.*, 2005, Patterson *et al.*, 2014), our data suggest that the range of GC concentration can be wide and thus, emphasize the need for caution when interpreting differences in GC concentrations without a proper assessment of their biological significance (Busch and Hayward, 2009). Individual significant differences in GC concentrations do not necessarily translate into significant diverging biological effects and conversely, slight changes in hormonal levels could be important. For instance, Pride (2005b) found that GC can be a sensitive indicator of survival probabilities but especially at very high values. Although natural selection can strongly operate on GC regulation, the high inter-individual variability for this trait seen in the wild could be maintained if the divergent stress responses offer alternative strategies with differing payoffs depending on environmental conditions (Koolhaas *et al.*, 1999, Korte *et al.*, 2005).

Furthermore, the assessment of how HCC correlates with survival of *M. murinus* over the reproductive season supported the notion that the relationship between GC and fitness can be context-dependent (Angelier *et al.*, 2009, Breuner *et al.*, 2008). Indeed, the benefit of having a relatively low stress load seems to be maximized prior to entering the breeding season as previously observed for various bird species (Bonier *et al.*, 2009b, Ouyang *et al.*, 2011b). Individuals that are already strongly affected by challenging conditions during the dry season might not be able to cope with the additional costs related to the mating season. This brief period is particularly challenging for male mouse lemurs, which show a drastic increase in mortality coupled with significant body condition deterioration over the mating season (Kraus *et al.*, 2008, Schmid and Kappeler, 1998) that might be proximately mediated by the adverse physiological consequences of the stress accumulated over the dry season. Unlike females, which hibernate for several months, male mouse lemurs stay active and only undergo daily torpor during the dry winter (Schmid and Kappeler, 1998, Schmid, 1999). Staying active and the need to be energetically prepared to face the mating season (Schülke and Ostner, 2007) seems to physiologically affect males, which showed a higher HCC than females at the end of the dry season (Rakotoniaina *et al.*, 2016). However, we could not detect this sex difference in the present data set, probably due to the limited sample size. This limitation emphasizes the importance of chronologically isolating specific processes in the life cycle in order to comprehend the proximate mechanisms that impact the survival of a given population.

High mortality rate as a cost of high reproductive success was described for several study systems (Reznick *et al.*, 1986, Williams, 1966), and GC hormones were suggested to be central in mediating this trade-off (Boonstra and Boag, 1992). Lee and Cockburn (1985) proposed that during the mating period, animals (especially males) may exhibit an adaptive stress response, which can compromise their survival but promotes reproductive fitness by permitting a redirection of energy to reproduction. For instance, such terminal investment was detected in male arctic ground squirrels (Boonstra *et al.*, 2001), and it was evoked that it may occur in mammalian species with similar life history traits characterized by a single annual breeding opportunity per year coupled with high between-year mortality (Boonstra and Boag, 1992). This physiological adaptation might occur in male grey mouse lemurs facing strong intrasexual competition over access to receptive females (Eberle and Kappeler, 2004) and thus, it could severely affect individuals that are showing already signs of high stress load at the beginning of the mating season. An estimation of the reproductive success of animals showing high GC levels will help to test this hypothesis.

Several reasons could lead to the high mortality of chronically stressed individuals, including impaired immune and inflammatory responses leading to impaired resistance to diseases (Dhabhar, 2009), or a maladaptive adrenocortical response to additional unpredictable stressors that might impair the animals´ coping ability (Norris *et al.*, 1999). However, our results seem to argue against the hypothesis of increased mortality due to increased parasitism. All models that included an indicator of parasitism failed to support a monthly survival trend and suggested that multiple parasite species infection, parasite morphotype richness, and overall prevalence were poor predictors of individual survival. Acquired immunity against helminth infections (Hämäläinen *et al.*, 2015b) might further explain the relatively low selective pressure on parasitism. As parasite virulence and host tolerance might also be highly variable, these findings highlight the degree of uncertainty associated with the use of basic measures of parasitism as biomarker of health without information on parasite pathogenicity.

Additionally, chronically stressed individuals could fail to mount an adaptive HPA activity response to an acute stressor such as predation which might increase their vulnerability during the mating season. For instance, the grey mouse lemur is known to be preyed upon by several predators such as snakes, owls or another lemur (*Mirza coquereli*), and although they face a continuously high predation risk (Eberle and Kappeler, 2008, Goodman *et al.*, 1993, Kappeler and Rasoloarison, 2003), this threat might be maximal at the peak of the activity period of both predators and prey. In this case, an inappropriate physiological response to the presence of a predator could be translated into reduced reaction time or escaping ability of the high-stress individuals. Several studies have reported that GC responses to acute stressors were down-regulated in animals exposed to chronic stress (Rich and Romero, 2005, Sapolsky *et al.*, 2000). For instance, in lemurs, Tecot (2013) found that *Eulemur rubriventer* showed an attenuated GC response to known seasonal environmental challenges in altered habitats. While this response could be aimed at reducing the detrimental effects of chronic elevation of GC levels, it may negatively affect the capacity of an animal to face acute life-threatening stressors.

Age is a factor that could influence the GC-survival relationship, but it could unfortunately not be addressed in this study. Previous studies suggested that both survival and GC profile are age-dependent in grey mouse lemurs. For instance, Kraus *et al* (2008) reported lower survival of juveniles over the dry winter but no significant difference between juvenile and adult survival probabilities over the summer. Also, while older individuals were found to have higher faecal GC metabolites during the breeding season (Hämäläinen *et al.*, 2015a), Rakotoniaina *et al* (2016) showed that HCC was higher in juvenile *M. murinus*. The contradicting results found in those studies might have arisen from the different matrices used to assess physiological stress but also from the definition of age: while the first study used estimates of individual age, the latter applied age categorization. This age-GC link was also detected in various study systems (Elliott *et al.*, 2014, Goncharova and Lapin, 2004, Goutte *et al.*, 2010, Gust *et al.*, 2000, Heuser *et al.*, 1994) and is mainly assumed to be associated with the impaired ability of aged individuals to cope with challenges (Sapolsky *et al.*, 1987). Considering age is therefore recommended in future studies examining the link between GC and fitness.

Body condition, stress and survival

As suggested previously (Hämäläinen *et al.*, 2014a), we found that mortality is condition-dependent in *M. murinus*. In fact, very good condition (measured as scaled mass index) was associated with a high semi-annual survival probability. Poor quality individuals can be more vulnerable to diseases (Beldomenico and Begon, 2010) and predation (Wirsing *et al.*, 2002) but also have a lower capacity to face competition (Kokko, 1999). While body mass has been reported to consistently decline in chronically stressed individuals (Dickens and Romero, 2013), it is still necessary to disentangle and define the causal effect of body condition and stress hormones on the survival output at high values of these health indicators. As body condition and GC levels were not correlated in our study population, selection on either of these traits should not influence the detection of survival selection on the other one (Patterson *et al.*, 2014).

Also, body condition was not correlated with monthly survival, which may indicate that different selective pressures acting on this trait might have opposite effects during the mating season. For instance, if individuals in good condition are more active than weak individuals in this period, this may increase their probability to encounter predators (Biro *et al.*, 2004, Lima and Dill, 1990, Magnhagen, 1991). The benefits of being in better condition (e.g. low susceptibility to disease, high success in resource competition) could therefore be curtailed by increased predation risk. Yet, if body condition does not correlate with survival from low to mid values, as suggested by the multistate analysis, this failure of body condition to explain monthly survival could also arise from the limited sample size being used, which does not allow us to detect this trend. Overall, our results indicate the possibility that physiological traits are under stronger selection in terms of survival consequences than body condition. Additional studies of the heritability and effects of these health indicators on reproductive success would be needed, however, to confirm the overall selective potential for these traits.

Hair cortisol concentration as a reliable health indicator

The gold standard of validating a biological indicator of health is to show that it correlates with fitness. Here, we demonstrate for the first time that HCC exhibited such a correlation in a wild population. Although the exact mechanism of incorporation of cortisol in the hair shaft is not yet well understood (Boumba *et al.*, 2006, Pragst and Balikova, 2006, Raul *et al.*, 2004), it is mostly assumed that cortisol contained in hair is representative of free systemic concentration (For a review see Meyer and Novak, 2012, Russell *et al.*, 2012, but see Ito *et al.*, 2005, Sharpley *et al.*, 2009. Similar to our study, Patterson *et al*. (2014) found that free GC hormone levels may be more relevant than morphological traits as a predictor of survival in white-crowned sparrows.

The fundamental advantage of using hair as a matrix to assess cortisol levels is the broadness of the time window reflected by HCC. While HCC is assumed to account for up to several months of stress load, traditionally used biomarkers of stress (blood, faeces, urine, saliva) are point estimators and could fail to describe the true individual condition. Under natural conditions where animals cannot be continuously observed and sampled, it is difficult to obtain a reliable measure of the baseline GC level with such biomarkers. However, most studies that investigated this aspect in the wild rely solely on limited sampling of indicators of short-term stress responses that are likely affected, for instance, by daily level fluctuations or individual stressful events experienced prior to sampling (e.g. Brown *et al.*, 2005, Madliger and Love, 2016). Overall, this might explain the large inconsistency in the results so far reported from studies that attempted to link stress and fitness (Bonier *et al.*, 2009a). At present, the lack of precise information on hair growth rate in *M. murinus* limits our estimation about the period of accumulation of cortisol recorded with the hair samples. However, when an animal was captured and sampled early in September, we observed that the hair had fully regrown after a subsequent recapture of the same individual in December. Thus, we are confident that HCC in our study reflected the mean cortisol load over, at least, a substantial part of each period between sampling sessions.

Moreover, HCC has been shown to be heritable and reported to represent an individual trait that is affected by genes and environment (Fairbanks *et al.*, 2011). Since cortisol is known to be closely linked to a series of other phenotypic traits (Koolhaas *et al.*, 1999, Øverli *et al.*, 2007), levels of hair cortisol may indicate individual quality, where low quality individuals that might perceive their environment as more challenging secrete higher cortisol levels (Husak and Moore, 2008). For instance in our study population, considering that HCC reflects an accumulation of cortisol over an extended period of time, and that individuals are assumed to face similar extrinsic pressures, it is very likely that individual differences in HCC reflect true differences in condition rather than a potential difference in exposure to various stressors. However, consistent individual behavioural responses to external stimuli, also refered to as "personality" (sensu Dall *et al.*, 2004) could interfere with this individual quality-GC profile relationship. Indeed, several studies have demonstrated that specific personality traits such as boldness can strongly correlate with HPA axis responses (Baugh *et al.*, 2013, Clary *et al.*, 2014, Cockrem, 2013, Oswald *et al.*, 2006). An investigation of such a relationship by conducting personality tests combined with HCC measurements might enrich the interpretation of information obtained from hair cortisol levels.

CONCLUSIONS

This study provides support for the Cort-fitness hypothesis by demonstrating that survival is negatively associated with levels of hair cortisol concentrations in a wild grey mouse lemur population. This study therefore provides a first confirmation of the predictive power of HCC variation on individual fitness in a wild setting. Moreover, we demonstrate that, while GC, body condition and parasite resistance could all influence individual survival, their effects might differ in strength. Thus, we emphasize that care must be taken when interpreting such indices without prior knowledge of their effect on fitness. Although our approach is correlational and does not permit the identification of the exact causes of mortality, it suggests that variation in GC hormone concentrations alone may underlie demographic fluctuations of natural populations. Thus, these results highlight the need to consider environmental pressures that can affect GC levels as potential threats to survival. Since population decline is often hard to measure, the assessment of an individual health indicator such as long-term stress levels could, therefore, provide an easier alternative for detecting issues emerging at the population level and ultimately predicting wild populations’ responses to environmental challenges.

LIST OF ABBREVIATIONS

GC: Glucocorticoid
HCC: Hair cortisol concentration
HPA axis: Hypothalamic-pituitary-adrenal axis
BM: Body mass
SMI: Scaled mass index

DECLARATIONS

Ethics approval

All research activities conducted in Madagascar got an official approval from the Ministère de l´Environnement, de l´Ecologie, de la Mer et des Forêts and comply with animal care national legislations of Madagascar.

Availability of data and materials

The dataset supporting the results of this article is available in the Figshare repository, https://dx.doi.org/10.6084/m9.figshare.5259415.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

CKraus and PK originally formulated the idea. CKirschbaum developed and implemented the hormone analyses. JR, EK, and AH collected field data. JR analyzed the data and wrote the manuscript with input from PK, AH, EK, and CKraus. All authors read and approved the final manuscript.

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Supplementary material of chapter 3

Table S1 Full set of starting candidate models. We considered the following factors: condition index (*c*, which can indicate HCC or SMI values), sex (*s*), and time (*t*). Constant parameters are noted (.). Interactions are indicated by (*) and additive effects by (+). All possible combinations of parameters given below were modelled and tested. Global models are in bold.

Survival probability *Φ*	Recapture probability *p*	State-transition probability *ψ*
Semi-annual survival (*Multistate models*)		
c * s + t	***s + t***	***c * t***
c + s + t	*t*	*c*
*c * s*	(.)	
c + s		
c + t		
s + t		
c		
s		
t		
Monthly survival (*Cormack-Jolly-Seber models where explanatory variables were later added as an individual covariates*)		
s * t	***s + t***	
s + t	*s*	
s	*t*	
t	(.)	
(.)		

Table S2 Confidence set of models (all models with relative likelihood > 0.05) for semi-annual survival of *M. murinus* depending on hair cortisol concentration. Presented are the number of parameters (K), the deviance (QDEV), the quasi-likelihood adjusted second order AIC (QAICc), the difference between the QAICc of the top model and a given model i (Δ_i) and the Akaike weights (w_i).

Rank	Model	K	QDEV	QAICc	Δ_i	w_i
Categorization cut-off: median						
1	$\Phi(c)\ p(t)\ \psi(c)$	8	200.78	515.10	0	0.208
2	$\Phi(s)\ p(t)\ \psi(c)$	8	201.90	516.23	1.13	0.119
3	$\Phi(c + s)\ p(t)\ \psi(c)$	9	199.96	516.44	1.34	0.107
4	$\Phi(c)\ p(.)\ \psi(c)$	5	208.91	516.88	1.77	0.086
5	$\Phi(c)\ p(s + t)\ \psi(c)$	9	200.73	517.21	2.11	0.072
6	$\Phi(s)\ p(.)\ \psi(c)$	5	210.01	517.98	2.87	0.050
7	$\Phi(t)\ p(t)\ \psi(c)$	10	199.40	518.06	2.96	0.048
8	$\Phi(c + t)\ p(t)\ \psi(c)$	11	197.29	518.15	3.05	0.045
9	$\Phi(c + s)\ p(.)\ \psi(c)$	6	208.16	518.23	3.12	0.044
10	$\Phi(s)\ p(s + t)\ \psi(c)$	9	201.84	518.32	3.22	0.042
11	$\Phi(c + s)\ p(s + t)\ \psi(c)$	10	199.86	518.52	3.42	0.038
12	$\Phi(c * s)\ p(t)\ \psi(c)$	10	199.88	518.54	3.44	0.037
13	$\Phi(s + t)\ p(t)\ \psi(c)$	11	198.81	519.67	4.57	0.021
14	$\Phi(t)\ p(s + t)\ \psi(c)$	11	198.96	519.81	4.71	0.020
15	$\Phi(c + s + t)\ p(t)\ \psi(c)$	12	196.76	519.84	4.74	0.020
16	$\Phi(c + t)\ p(s + t)\ \psi(c)$	12	197.09	520.16	5.06	0.017
17	$\Phi(c * s)\ p(.)\ \psi(c)$	7	208.10	520.29	5.19	0.016
18	$\Phi(c * s)\ p(s + t)\ \psi(c)$	11	199.80	520.66	5.56	0.013
Categorization cut-off: third quartile						
1	$\Phi(c)\ p(t)\ \psi(c)$	8	146.94	465.86	0	0.230
2	$\Phi(c + s)\ p(t)\ \psi(c)$	9	146.08	467.16	1.30	0.120
3	$\Phi(c)\ p(.)\ \psi(c)$	5	154.75	467.31	1.45	0.111
4	$\Phi(s)\ p(t)\ \psi(c)$	8	148.66	467.59	1.73	0.097
5	$\Phi(c)\ p(s + t)\ \psi(c)$	9	146.88	467.97	2.11	0.080
6	$\Phi(c + s)\ p(.)\ \psi(c)$	6	153.97	468.63	2.77	0.058
7	$\Phi(c + s)\ p(s + t)\ \psi(c)$	10	145.98	469.24	3.38	0.042
8	$\Phi(c * s)\ p(t)\ \psi(c)$	10	146.00	469.26	3.40	0.042
9	$\Phi(s)\ p(.)\ \psi(c)$	5	156.72	469.28	3.42	0.042
10	$\Phi(t)\ p(t)\ \psi(c)$	10	146.17	469.44	3.57	0.038
11	$\Phi(s)\ p(s + t)\ \psi(c)$	9	148.60	469.69	3.82	0.034
12	$\Phi(c + t)\ p(t)\ \psi(c)$	11	144.88	470.34	4.47	0.025
13	$\Phi(c * s)\ p(.)\ \psi(c)$	7	153.89	470.68	4.81	0.021
14	$\Phi(s + t)\ p(t)\ \psi(c)$	11	145.60	471.06	5.19	0.017
15	$\Phi(t)\ p(s + t)\ \psi(c)$	11	145.74	471.20	5.33	0.016
16	$\Phi(c * s)\ p(s + t)\ \psi(c)$	11	145.91	471.37	5.51	0.015
17	$\Phi(s + t)\ p(.)\ \psi(c)$	8	152.78	471.71	5.84	0.012

Table S3 Confidence set of models (all models with relative likelihood > 0.05) for semi-annual survival of *M. murinus* depending on scaled mass index. Presented are the number of parameters (K), the deviance (QDEV), the quasi-likelihood adjusted second order AIC (QAICc), the difference between the QAICc of the top model and a given model i (Δ_i) and the Akaike weights (w_i).

Rank	Model	K	QDEV	QAICc	Δ_i	w_i
Categorization cut-off: median						
1	$\Phi(s)\ p(t)\ \psi(c)$	7	114.35	330.82	0	0.272
2	$\Phi(c)\ p(t)\ \psi(c)$	7	115.44	331.90	1.09	0.158
3	$\Phi(s)\ p(s + t)\ \psi(c)$	8	114.31	332.96	2.14	0.093
4	$\Phi(c + s)\ p(t)\ \psi(c)$	8	114.35	332.99	2.18	0.092
5	$\Phi(c)\ p(s + t)\ \psi(c)$	8	115.01	333.65	2.84	0.066
6	$\Phi(t)\ p(t)\ \psi(c)$	8	115.36	334.01	3.19	0.055
7	$\Phi(c * s)\ p(t)\ \psi(c)$	9	113.72	334.56	3.75	0.042
8	$\Phi(t)\ p(.)\ \psi(c)$	6	120.63	334.94	4.12	0.035
9	$\Phi(s)\ p(.)\ \psi(c)$	5	122.92	335.10	4.29	0.032
10	$\Phi(s + t)\ p(t)\ \psi(c)$	9	114.28	335.13	4.31	0.031
11	$\Phi(c + s)\ p(s + t)\ \psi(c)$	9	114.31	335.15	4.34	0.031
12	$\Phi(t)\ p(s + t)\ \psi(c)$	9	114.98	335.83	5.01	0.022
13	$\Phi(c)\ p(.)\ \psi(c)$	5	123.82	336.00	5.18	0.020
14	$\Phi(c + t)\ p(t)\ \psi(c)$	9	115.29	336.14	5.32	0.019
15	$\Phi(s + t)\ p(.)\ \psi(c)$	7	119.72	336.19	5.37	0.019
16	$\Phi(c * s)\ p(s + t)\ \psi(c)$	10	113.67	336.74	5.92	0.014
Categorization cut-off: third quartile						
1	$\Phi(c)\ p(t)\ \psi(c)$	7	102.53	323.87	0	0.229
2	$\Phi(c + s)\ p(t)\ \psi(c)$	8	101.34	324.85	0.99	0.140
3	$\Phi(s)\ p(t)\ \psi(c)$	7	103.72	325.06	1.19	0.127
4	$\Phi(c)\ p(s + t)\ \psi(c)$	8	102.03	325.55	1.68	0.099
5	$\Phi(c + s)\ p(s + t)\ \psi(c)$	9	101.28	327.00	3.13	0.048
6	$\Phi(c * s)\ p(t)\ \psi(c)$	9	101.34	327.05	3.19	0.047
7	$\Phi(s)\ p(s + t)\ \psi(c)$	8	103.68	327.20	3.33	0.043
8	$\Phi(c)\ p(.)\ \psi(c)$	5	110.64	327.69	3.83	0.034
9	$\Phi(c + t)\ p(t)\ \psi(c)$	9	102.51	328.22	4.36	0.026
10	$\Phi(t)\ p(t)\ \psi(c)$	8	104.73	328.24	4.38	0.026
11	$\Phi(c)\ p(t)\ \psi(c * t)$	11	98.10	328.28	4.42	0.025
12	$\Phi(c + t)\ p(.)\ \psi(c)$	7	107.43	328.77	4.90	0.020
13	$\Phi(c + s)\ p(.)\ \psi(c)$	6	109.63	328.81	4.95	0.019
14	$\Phi(t)\ p(.)\ \psi(c)$	6	110.00	329.18	5.31	0.016
15	$\Phi(c * s)\ p(s + t)\ \psi(c)$	10	101.28	329.22	5.36	0.016

Table S3 continued

Rank	Model	K	QDEV	QAICc	Δ_i	w_i
Categorization cut-off: third quartile						
16	$\Phi(c + s + t)\ p(t)\ \psi(c)$	10	101.33	329.27	5.40	0.015
17	$\Phi(s)\ p(.)\ \psi(c)$	5	112.29	329.34	5.48	0.015
18	$\Phi(s)\ p(t)\ \psi(c * t)$	11	99.16	329.35	5.48	0.015
19	$\Phi(s + t)\ p(t)\ \psi(c)$	9	103.65	329.37	5.50	0.015
20	$\Phi(c + t)\ p(t)\ \psi(c * t)$	12	97.01	329.47	5.60	0.014
21	$\Phi(c + s + t)\ p(.)\ \psi(c)$	8	106.24	329.76	5.89	0.012

Table S4 Starting set of models for monthly survival of *M. murinus*. Presented are the number of parameters (K), the deviance (DEV), the second order AIC (AICc), the difference between the AICc of the top model and a given model *i* (Δ_i) and the Akaike weights (w_i) for the total set including the global model (in bold).

Rank	Model	K	DEV	AICc	Δ_i	w_i
1	$\Phi(t)\ p(t)$	9	16.06	218.01	0	0.562
2	$\Phi(t)\ p(s + t)$	10	15.89	220.20	2.19	0.188
3	$\Phi(s + t)\ p(t)$	10	15.97	220.28	2.28	0.180
4	$\Phi(s + t)\ p(s + t)$	11	15.89	222.60	4.60	0.056
5	$\Phi(.)\ p(t)$	6	32.52	227.61	9.60	0.005
6	$\Phi(s * t)\ p(t)$	14	13.51	227.71	9.70	0.004
7	$\Phi(s)\ p(t)$	7	32.45	229.78	11.78	0.002
8	$\Phi(.)\ p(s + t)$	7	32.51	229.84	11.84	0.002
9	$\boldsymbol{\Phi(s * t)\ p(s + t)}$	**15**	**13.49**	**230.27**	**12.27**	**0.001**
10	$\Phi(s)\ p(s + t)$	8	32.44	232.06	14.05	0.001
11	$\Phi(t)\ p(.)$	4	51.38	242.09	24.09	0.000
12	$\Phi(t)\ p(s)$	7	51.22	248.55	30.55	0.000
13	$\Phi(s + t)\ p(.)$	7	51.38	248.72	30.71	0.000
14	$\Phi(s + t)\ p(s)$	8	51.21	250.83	32.83	0.000
15	$\Phi(s * t)\ p(.)$	11	51.26	257.98	39.97	0.000
16	$\Phi(s * t)\ p(s)$	12	51.12	260.29	42.28	0.000
17	$\Phi(.)\ p(.)$	2	83.32	269.79	51.79	0.000
18	$\Phi(.)\ p(s)$	3	83.19	271.77	53.77	0.000
19	$\Phi(s)\ p(.)$	3	83.32	271.89	53.89	0.000
20	$\Phi(s)\ p(s)$	4	83.19	273.90	55.90	0.000

GENERAL DISCUSSION

Understanding how animals perceive and react to anthropogenic disturbance is pivotal in conservation biology. In the present thesis, I aimed at understanding how species that differ in various aspect of their life history might physiologically respond to human-induced changes of the environment. Moreover, I strived to define how stress load might affect various health indices and ultimately survival, in order to comprehend the proximate mechanisms that might govern the species-specific difference in vulnerability to environmental challenges.

In the following, I will first summarize the main findings of the three studies that have been seen in earlier chapters before comparing the results with what is known about the physiological stress response to disturbance in other taxa. Then, I will briefly describe a coping mechanism that could be vital for animals while facing environmental perturbations. Afterwards, I will examine the potential basis of the inter-specific difference in coping ability to environmental constraints by relating the life history traits of the study species to their GC profile. I will further highlight the utility and care to be taken when using stress hormone measures in conservation-related studies and finally, I will present various prospective directions of future studies that could enhance our understanding of how animals might deal with human-induced environmental changes.

HABITAT DISTURBANCE, HEALTH, AND FITNESS

How organisms react to changing environmental circumstances is becoming of primary concern in the light of the increasing human global impact on wildlife (Sih, 2013, Visser, 2008). Stress levels, parasitism, and general body condition (measured generally as a scaled mass index; SMI) are commonly used to evaluate the impact of habitat disturbance on animal´s general health status due to their potential correlation with fitness. In **Chapter 1**, I investigated how different levels of human disturbance (found across four study sites: N5, CS7, Savanna, Kirindy Village) affected those health indicators in *M. murinus* and *C. medius*. I discovered that while neither stress levels (measured as hair cortisol concentrations; HCC), nor parasitism patterns (prevalence, parasite species richness and rate of multiple infections) varied across the gradient of anthropogenic disturbance for both species, body condition was higher for *M. murinus* in the most disturbed site. Covariates such as season, sex, and age were also important in predicting the health indicators. For instance, HCC were elevated prior to hibernation in *C. medius,* whereas HCC values were higher in male *M. murinus* during the mating season. The counterintuitive results on the health indicator profiles among sites suggest that these species may have developed a range of behavioural adaptations to deal with suboptimal conditions. Yet, a difference in relative density among sites suggests that, to some extent, both species are negatively affected by environmental changes, with a higher impact on *C. medius.* Thus, despite the fact that the health indicators did not reflect the extent of anthropogenic activities, extended habitat deterioration could still hamper long-term viability of populations, even for behaviourally flexible species.

One of the main functions of GCs is their suppressive effect on the immune system (Stratakis and Chrousos, 1995). While this link can negatively impact individual´s general health in the event of chronic stress, it is also known to permit a reallocation of resources and, thus, help an animal to meet energetic demands during challenging periods (Sapolsky, 2000). This relationship between GCs and immunity is assumed to be central in the mediation of life history trade-offs but also in the physiological stress responses that are mounted by vertebrates under harsh conditions (Corlatti *et al.*, 2012, Cox *et al.*, 2010). The influence of GCs on other health indicators might also vary depending on the species´ life history characteristic. Thus, in **Chapter 2**, I strived to determine the nature and magnitude of the interrelationships among HCC, body condition and parasite infection in *M. murinus* and *C. medius*. I found that there were no relationships among these indices in the fast-living *M. murinus*. Using path models, I detected a direct positive influence of HCC on body condition and on the parasite species richness in the slow-living *C. medius*. Thus, GCs seem to play a key role in mediating the trade-off between body condition requirements prior to hibernation and an increased susceptibility to parasite infection in *C. medius*. Corroborating this conclusion, I uncovered in **Chapter 1** that higher levels of cortisol were recorded prior to hibernation in comparison to the mating season in *C. medius*. The nature of these relationships in *M. murinus* is not yet clear but might involve rapid short-term fluctuations of GCs that are not detected by the long-term measurements (hair cortisol concentration). Overall, this study indicated that the relationships among stress, parasitism and body condition could depend on the species-specific life history strategy.

The negative effect of chronic stress on fitness is the main assumption of the "Cort-fitness hypothesis". In **Chapter 3**, I used a capture-mark-recapture modelling approach to assess the impact of high stress load, along with parasite infection and SMI, on survival in *M. murinus*. I tested the Cort-fitness hypothesis on two different time scales: first on a semi-annual basis, following the seasonal pattern of resource availability, and on a monthly basis, during a period of high energetic demands during the reproductive season. Following predictions, I found that high levels of hair cortisol were associated with reduced survival probabilities both at the semi-annual and monthly scale. Moreover, very good body condition (high SMI values) was predictive of increased survival at the semi-annual but not at the monthly scale. In contrast, variation in parasitism was not related to survival. Altogether, these results indicate that long-term increased GC levels can impact survival and hence population dynamics, and suggest differential strength of selection acting on GCs, body condition, and parasite infection.

STRESS PHYSIOLOGY AND DISTURBANCE

Responding appropriately to environmental perturbations such as extreme climatic conditions, high predation pressure, and habitat degradation is a vital process that involves various physiological, morphological, and behavioural mechanisms (Sapolsky *et al.*, 2000). When an organism has to deal with a permanent perturbation factor such as human-induced habitat degradation, some adjustments must take place in order to avoid the detrimental effect of long-term activation of the physiological stress response (Romero, 2004). Failure to do so might result in death or chronic impairment of the ability to sustain daily routines and ultimately in reduced fitness (McEwen and Wingfield, 2003, Wingfield *et al.*, 2011). Assessing the relationships among disturbance, the physiological stress response to a given disturbance, and the potential resulting fitness output is, however, not straightforward due to the fact that disturbance can impact the ecological environment in various ways that do not necessarily all have a negative impact on the animal. Here I will focus on the effect of exposure to humans and food availability on the stress levels of *C. medius* and *M. murinus*.

Exposure to humans

One of the main goals of this thesis was to relate the increased gradient of anthropogenic disturbance to the stress response of *C. medius* and *M. murinus*, and each study site was characterized by a different probability of exposure to humans (**Chapter 1**). The effect of tourism on animals´ GC levels is particularly well documented across taxa (Gàlapagos marine iguana: Romero and Wikelski, 2002; yellow-eyed penguins: Ellenberg *et al.*, 2007; Barbary macaques: Maréchal *et al.*, 2011; capercaille Thiel *et al.*, 2011; Mountain hares: Rehnus *et al.*, 2014), with positive or negative relationships depending on the species. Acclimation (down-regulation of the HPA axis activity) to repeated exposure to tourists was also observed and certain species adapt rapidly to human presence (Millspaugh *et al.*, 2001, Muehlenbein *et al.*, 2012, Romero and Wikelski, 2002, Walker *et al.*, 2006). Acclimated animals then showed signs of compromised ability to cope with future stressors (Busch and Hayward, 2009). The lack of difference in GC levels among our study sites (**Chapter 1**) might suggest that *C. medius* and *M. murinus* became acclimated to exposure to humans. Touristic activities might also have a limited effect on both species since the touristic season is during the dry season when they have reduced activities. For the two nocturnal lemurs, human encounters are also very likely rare in the forest fragments nearby Kirindy village and the Savanna.

Resource availability

In *C. medius*, the importance of food resources was highlighted by the positive association between HCC and SMI (**Chapter 2**), and the elevated HCC prior to hibernation (**Chapter 1**) which is associated with an increased energy intake in several organisms (Crespi *et al.*, 2004, Dallman *et al.*, 1999). The hormonal profiles seem to indicate that food was not a major source of stress for either species, despite the different food tree densities among the study sites (**Chapter 1**). However, as indicated by the good condition of *M. murinus* at the most disturbed site, they probably underwent a dietary adaptation in order to meet their energetic demands. Feeding behavior adaptations are common in nature when animals are faced with suboptimal habitat conditions (Gibson, 2011, Onderdonk and Chapman, 2000).

Previous studies have reported that the direction of the relationship between food abundance and GCs can depend on the conditions the animals are facing. While GCs and food abundance are generally negatively correlated when the animal is not experiencing starvation, GC levels increase only after the individual reaches a bottom limit of body condition under chronic malnutrition (Clinchy *et al.*, 2004, Dallman *et al.*, 1993, 1999, Romero and Wikelski, 2001, Schoech *et al.*, 2007). However, the SMI results in *C. medius* and *M. murinus* do not coincide with this latter observation. Thus, the disturbed sites seem to provide sufficient alternative resources for both species.

PHYSIOLOGICAL FLEXIBILITY: COPING MECHANISM

Recently, the degree of flexibility of the physiological stress response has been proposed as a potential major mechanism that might influence the individual capacity to cope with stochastic environmental changes (Angelier and Wingfield, 2013). Having a highly flexible GC stress response, which accommodates reversible modifications (Piersma and Van Gils, 2011), is considered to be beneficial as it permits an organism to constantly adjust the stress response in order to maximize fitness when facing unpredictable stressors (Piersma and Drent, 2003, Wingfield, 2013). Specifically, physiological flexibility should allow individuals to moderate the stress response (habituation) if it was over-activated when initially encountering a given stressor, or to enhance it (sensitization) in case of under-activation (Berger *et al.*, 2007, Rödl *et al.*, 2007, Romero and Wikelski, 2002). In fact, it has been shown that inflexible physiological processes could lead to a suboptimal life history strategy and impaired fitness (Dawson, 2008, Pörtner and Farrell, 2008). For instance, it was observed that the physiological processes triggering the migration of the pied flycatcher rely on day length, and can cause a late arrival of the migratory bird at their breeding site when a time shift in food availability is influenced by rises of temperature (Both and Visser, 2001).

The stress response flexibility has two major components: the magnitude or scope and the speed of the response (Taff and Vitousek, 2016). While examining physiological states along environmental gradients, as done in **Chapter 1**, can be useful for evaluating the scope of the stress response, the assessment of the speed will require small scale time-series of samples. The range of the GC response flexibility that an organism can display is likely limited by the HPA axis activity and can be dependent on its regulatory mechanism (Wingfield, 2013). Therefore, between-species differences in GC response flexibility could emerge at three levels: during hormone secretion (Wingfield and Romero, 2001), transport (Breuner and Orchinik, 2002), and the response of the targeted cells (Breuner *et al.*, 2003).

Although the study of the stress response flexibility is still in its infancy (Taff and Vitousek, 2016), evidence for the modulation of the stress response in order to maximise fitness is pervasive in wild populations. For instance, males of the western sandpipers show reduced GC responses to stress during the breeding season due to their high involvement in territorial defence and incubation (O'Reilly and Wingfield, 2003). Moreover, adult male Arctic ground squirrels maintain prolonged high levels of baseline GC during the breeding season in order to maintain aggression level while competing for mates (Boonstra *et al.*, 2001). Finally, in olive baboons, the dominant males have a lower basal GC levels than subordinates and develop a resistance to acute stress to avoid the stress-induced suppression of testicular function (Sapolsky, 1985).

HPA AXIS ACTIVITY, LIFE HISTORY, AND SUSCEPTIBILITY TO DISTURBANCE IN TWO SYMPATRIC LEMURS

Glucocorticoid hormones are known to be involved in the acute response to perturbations of the environment but also in the regulation of various daily life routine functions (Landys *et al.*, 2006, Romero, 2002). The species-specific circadian and seasonal change in GC concentrations was proposed to be pivotal in the expression of the stress response and its consequence on fitness outputs (Romero, 2002). The concept of allostasis and the reactive scope model (McEwen and

Wingfield, 2003, Romero *et al.*, 2009) offer the possibility to assess the relative coping ability of an animal depending on its basal physiological state. Indeed, Romero *et al*. (2009) defined four ranges within which the level of GC should have divergent effects. First, the predictive homeostasis represents the normal circadian and seasonal range that is needed to cope with predictable environmental changes. The reactive homeostasis is defined as the range of GC levels required to maintain or return to homeostasis after an unpredictable stressful event. Therefore, the normal reactive scope of an organism is encompassed within these two ranges. Next, the homeostatic failure is delineated by the range below the predictive homeostasis when normal physiological functions cannot be maintained, and finally the homeostatic overload is the range above the reactive homeostasis when the GC values become pathological. The key concept of the reactive scope model is to assume that maintaining the GC levels in the reactive homeostasis range is costly and results in a decrease of the ability of an animal to cope.

The grey mouse lemur and the fat-tailed dwarf lemur showed clear patterns of seasonal variation in their basal HCC (**Chapter 1**). HCC values of *C. medius* were significantly higher prior to hibernation (late rainy season) than during the breeding season (late dry season) independently of sex whereas HCC levels were elevated only in males during the late dry season in *M. murinus* (Figure 1). Those differences in patterns of fluctuation of GC levels coincide with the differing life history strategies adopted by the two species. The high HCC levels in male *M. murinus* during the late dry season likely reflect their increased activity during the mating season and shorter torpor bouts in comparison to the females (Schmid and Ganzhorn, 2009, Schmid and Kappeler, 1998). In contrast, the involvement of both sexes in parental care and the energy requirements for hibernation (Dausmann *et al.*, 2004, Fietz and Dausmann, 2003) could explain the high GC values observed for *C. medius* in late rainy season. Those patterns are in accordance with what is observed generally in mammals where baseline GCs can be elevated during and post-breeding, or prior to hibernation (Amirat *et al.*, 1980, Gustafson and Belt, 1981, Kenagy and Place, 2000, Kenagy *et al.*, 1999, Monamy, 1995).

Following the reactive scope model (Romero *et al.*, 2009), the difference in GC profiles in the two species could translate into a difference of susceptibility to disturbance. For instance, in *M. murinus*, the likelihood of entering the homeostasis overload might be higher for males than females during the breeding season while facing a same stressor (Figure 1b). Therefore, at the population level, the negative effect of a potential source of stress might be buffered by the difference in susceptibility of males and females. However, in *C. medius*, both sexes will equally bear the negative effects of a potential stressor which could be particularly critical prior to hibernation when the difference between homeostasis overload and reactive homeostasis is at its lowest (Figure 1a).

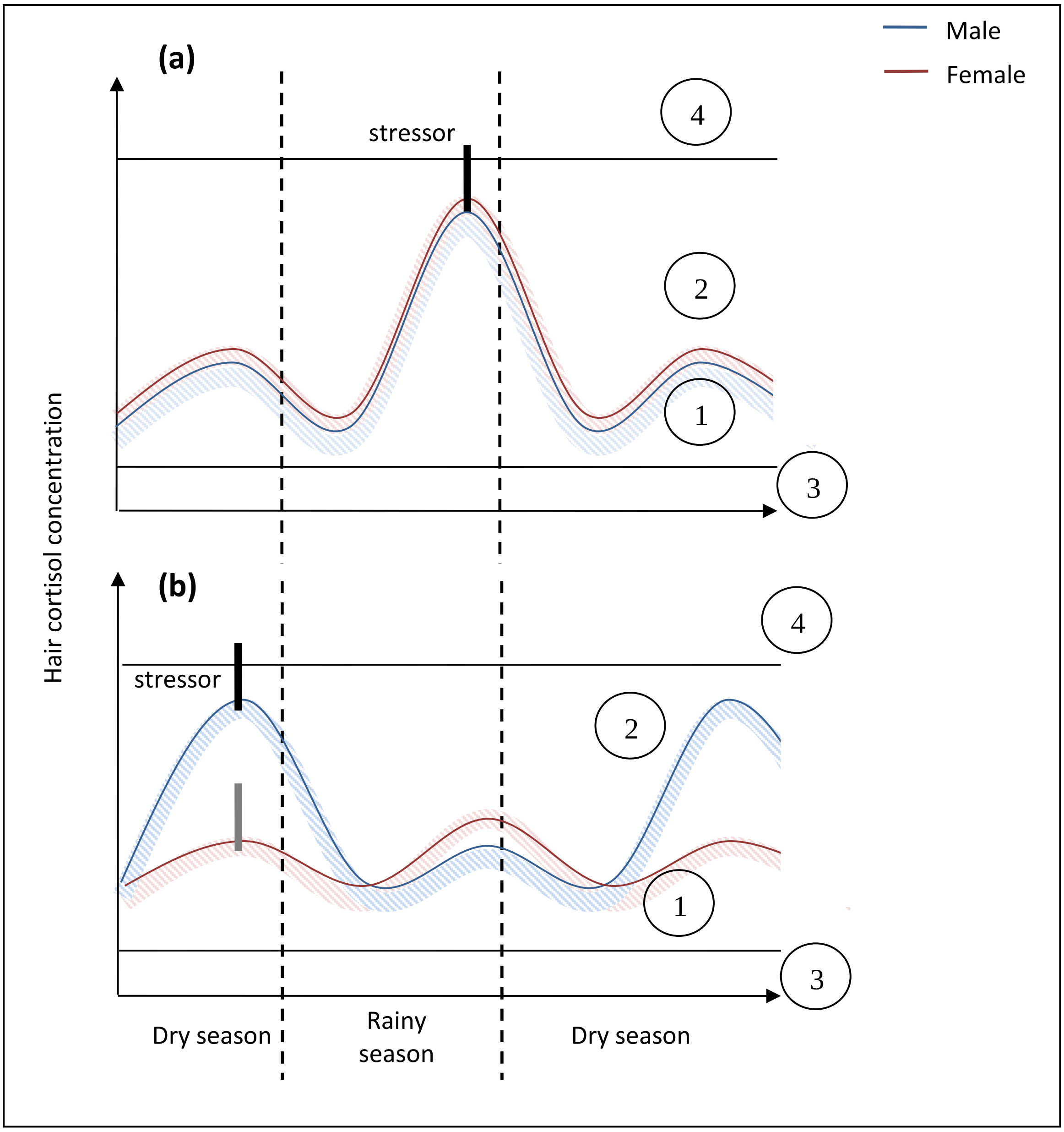

Figure 1. The graphical reactive scope model applied to the seasonal variation of basal hair cortisol concentration of *C. medius* (a) and *M. murinus* (b). The four ranges within which the HCC values exist: 1. the predictive homeostasis (crosshatched area), 2. the reactive homeostasis, 3. the homeostatic failure, 4. the homeostatic overload. The vertical bars represent acute stressors that could lead to homeostatic overload (black) or not (grey).

For the mouse lemur, the prediction that could be derived from such a model is concordant with the observation of an increased mortality rate and a deteriorated body condition in males during the mating season. This high male mortality has been associated with risky male behaviour aimed at increasing reproductive success (Kraus *et al.*, 2008, Schmid and Kappeler, 1998). Although the exact cause of increased death of males in this period remains unknown, such terminal investment (Clutton-Brock, 1984) might, at least partially, be hormone-mediated (**Chapter 3**). Almost all indices of parasitism were higher in the late dry season compared to the late rainy season (**Chapter 1**), but surprisingly, no association between parasitism and HCC was found (**Chapter 2**). I did not detect a sex-specific mortality rate during the breeding season (**Chapter 3**), probably due to the limited amount of data in comparison with the broader analysis conducted by Kraus *et al*. (2008). Concordant with the assumption of a hormone-mediated investment in reproduction, previous comparative studies on birds have shown that acute GC stress responses differ in species with contrasting life-history strategies (Angelier *et al.*, 2011, Bokony *et al.*, 2009, Hau *et al.*, 2010) where long-lived animals show a stronger GC stress response than short-lived ones, probably due to the high value of future reproduction for animals that still have several breeding opportunities. Similarly, the long-lived red squirrels were observed to have a stronger acute stress response than the arctic ground squirrels (Boonstra and McColl, 2000). For male mouse lemurs, a down-regulation of the acute response during the mating season might help to reduce the detrimental effect of a high baseline GC level.

For *C. medius*, GCs seem to play an important role in mediating the trade-off between immunity and resource acquisition in order to fulfil energetic demands (**Chapter 2**). Indeed, elevation of GC levels is known to promote energy intake in various taxa (human: Tataranni *et al.*, 1996, goldfish: Bernier *et al.*, 2004, African clowed frog: Crespi *et al.*, 2004), which is crucial for obligate hibernators such as *C. medius* (Boonstra *et al.*, 2014, Dausmann *et al.*, 2005, Schülke and Ostner, 2007). A dysfunction of the HPA activity prior to hibernation might negatively affect individual fitness in various ways. If energetic needs are not fulfilled, too short hibernation bouts will result in a suboptimal arousal time when resources might still be scarce (Lahann and Dausmann, 2011). An impaired immune system prior to hibernation might also expose animals to increased parasite infection risk (Glaser *et al.*, 1985). As immunity is generally reduced during hibernation, regular arousals are assumed to activate the dormant immune system (Bouma *et al.*, 2010, Luis and Hudson, 2006). However, arousals are energetically costly and can consume up to 80-90% of the energy allocated for hibernation (Kayser, 1965, Thomas *et al.*, 1990). As observed with bats, infection with the white fungus (*Geomyces destructans*), responsible for the White-

Nose Syndrom, prior to hibernation was associated with abnormally high rates of arousal that leads to death in the infected individual (Reeder *et al.*, 2012).

STRESS PHYSIOLOGY AND CONSERVATION

Monitoring the effect of human-induced environmental changes is an important goal of conservation (Van Dyke, 2008). Lately, GC hormones have become widely used as a biomarker of stress and considered as indicators of habitat quality, disturbance levels, and potential fitness in wild populations (e.g. Hopkins and DuRant, 2011, Leshyk *et al.*, 2012, Rehnus *et al.*, 2014, **Chapter 1 and 3**). The use of stress physiology in conservation-related studies (one aspect of “conservation physiology”; Wikelski and Cooke, 2006), can constitute a powerful mechanistic approach that allows the assessment of cause-and-effect relationships, and provides conservation biologists with an evidence-based approach to tackle conservation problems (Carey, 2005). It can have broad applications starting from a general assessment of the conservation status of a given population to a prediction of future population dynamic trends. It can also aid in evaluating the effectiveness of management techniques (Wikelski and Cooke, 2006) and has already helped in generating various conservation-oriented changes in management and policy (reviewed in Cooke *et al.*, 2013, Madliger *et al.*, 2016). Yet, as highlighted by the results of this thesis, conservation practitioners should be aware of various aspects that might render GC data difficult to obtain or to interpret, such as the type of measurements adopted or the context-dependent nature of the GC release.

First, despite the value of GC response measurements in informing about an individual´s capacity to cope with acute challenges, they might not be optimal for conservation-oriented studies. The implementation of acute GC measures typically requires multiple sampling of blood combined with a stress-inducing protocol, which can be too invasive and unpractical when studying natural populations (Sheriff *et al.*, 2011). The assessment of baseline or integrated GC levels (saliva, faeces, hair) constitutes a better alternative to acute measures (Madliger and Love, 2014). Baseline GCs reflect the effect of both intrinsic and extrinsic stressors that have been experienced by an animal, and represent the overall costs of maintaining energetic balance (homeostasis) (Love *et al.*, 2013, McEwen and Wingfield, 2003). Hence, they can be used as a powerful index of an organism general state since conservation-relevant disturbances often translate into an energetic imbalance by impacting on individuals’ energy expenditure and/or intake. The use of the hair matrix (**Chapter 1, 2, 3**) is a promising approach as it gives the exclusive advantage of accumulating GC hormones over a long time period and therefore can be adopted as a means of assessing chronic stress (Fourie *et al.*, 2016, Koren *et al.*, 2002, Russell *et al.*, 2012). Hair also gives the possibility to obtain an estimation of the baseline GC value with a single sample (Davenport *et al.*, 2006) and can be collected non-invasively, for instance in sleeping nests (Carlitz *et al.*, 2016) or from hair traps (Valderrama *et al.*, 1999). As hair is a stable environment for steroids, it can be stored at ambient temperature and thus, can facilitate the study of wild species in remote areas (Webb *et al.*, 2010, Webb *et al.*, 2015). To my knowledge, this study was the first that linked HCC to a fitness component (**Chapter 3**) and further strengthened the validity of using it as a biomarker of stress when monitoring wild populations.

Furthermore, the general assumption underlying the approach of linking stress load with disturbance levels and fitness relates an increase of GC concentrations to a stressful environment and reduced fitness (Bonier *et al.*, 2009b, Busch and Hayward, 2009). One of the difficulties encountered while interpreting results based on this simplistic assumption is that variations of GC levels can occur independently of environmental changes and can be context-dependent (Bonier *et al.*, 2009a, Dantzer *et al.*, 2014, Madliger and Love, 2014). Thus, despite the appealing aspect of using GCs in conservation-related studies, various parameters such as sex, age, season and life history stage (Romero, 2002) have to be taken into consideration while examining the link between GC levels, habitat disturbance, and fitness. In *M. murinus*, stress load varied with season, sex, and age, and in *C. medius,* a seasonal variation of GCs was also seen (**Chapter 1**). While these patterns seemed to be consistent across habitats with different levels of disturbance in both species, several studies reported a change in GC profiles in response to stressful events depending on these factors (Reeder and Kramer, 2005, Romero, 2002). For instance, in Gambel's white-crowned sparrow (*Zonotrichia leucophrys gambelii*), stress-induced GCs were higher during breeding stages in comparison to other stages (Romero *et al.*, 1997).

Additionally, in order to be informative for conservation biologists, GC measures should reliably reflect environmental conditions, and more importantly, correlate with measures of fitness. Yet, only few studies have examined GC levels, variation in habitat quality and fitness simultaneously (D’Alba *et al.*, 2011, Lanctot *et al.*, 2003, Madliger and Love, 2016, Riechert *et al.*, 2014). Without demographic data, the interpretation of GC data can be challenging (reviewed in Busch and Hayward, 2009). For instance, in **Chapter 1**, the lack of difference in GC levels detected among the increased gradient of human disturbance could be interpreted as a lack of effect of disturbance on *M. murinus* and *C. medius*. However, the difference in relative density among the sites along with the previous observation of reduced density of both species in disturbed habitats (Schäffler *et al.*, 2015) suggest that potential differences in fitness components among habitats might have not been detected by our GC measures. It could be possible under strong selective pressure acting on GC levels that would have caused immigration or death of weak individuals. This selective disappearance might explain the high body condition of grey mouse lemurs captured at the most disturbed site in comparison with the ones at the undisturbed sites (**Chapter 1**) since being able to survive under such conditions might require better health. Accordingly, selection on GCs was found for *M. murinus,* and very good condition individuals (high SMI values) benefited from a higher survival rate (**Chapter 3**). I could only test the link between survival and physiological parameters at one site, but since it was considered the most suitable site for our study species, the uncovered relationship could be even stronger in habitats that are subjected to human disturbance.

The few studies on wild populations that controlled for the relevant variables and simultaneously accounted for the disturbance-GCs and the GC-fitness relationships, could improve the interpretation and the usefulness of GC data for conservation. For instance, female elephants that were subjected to high poaching risk showed higher faecal GC levels and lower reproductive output than those facing lower poaching risk (Gobush *et al.*, 2008). Moreover, in roe deer, both the Cort-fitness hypothesis and the Cort-adaptation hypothesis were confirmed depending on season (Escribano-Avila *et al.*, 2013). Strasser and Heath (2013) further warned against the negative effect of noise from high levels of human activity even for human-tolerant species while relating this disturbance to high baseline corticosterone levels and low reproductive output in female American kestrels.

OUTLOOK

This thesis contributed to the better understanding of the potential proximate physiological mechanisms underlying the species-specific variation in coping ability when facing environmental challenges. However, various aspects still remain to be investigated:

- Long-term data in all the gradients of habitat disturbance for both species will allow a broad assessment of the species-specific variation of the strength of selection acting on the health indicators depending on the environmental condition.
- In order to fully understand the proximate mechanisms that dictate the responses of organisms to habitat changes, the evolving study of physiological flexibility is particularly promising (Taff and Vitousek, 2016). This approach could be applied at different levels and for instance, when applied between species, it would help to define to what extent physiological flexibility impact on the species-specific difference in coping ability.

- As suggested by the results of this thesis, an assessment of the feeding behaviour of *C. medius* and *M. murinus* across the gradient of habitat disturbance will help to clarify the failure of the health indices to reflect the level of environmental perturbations.

- The life history traits of *M. murinus* and *C. medius* were shown to be flexible (Lahann and Dausmann, 2011, Lahann *et al.*, 2006), depending on environmental conditions. Testing this association at a regional scale will help to define whether the observed demographic difference among sites is caused by an increased mortality rate and/or a reduced reproductive output.

- Extending the use of physiological parameters along with demographic data on various lemur taxa to assess population status will give conservation biologists a tool to conduct science-based conservation actions. So far, only limited lemur-based studies have used GCs to monitor the degree of habitat perturbations, and only one of them has related GCs with measures of fitness (Pride, 2005a, Tecot, 2013, Tecot *et al.*, 2013). Most studies on the effect of habitat disturbance on lemur populations focus on their behavioral response (reviewed in: Irwin *et al.*, 2010b).

- Despite the advance of methods to assess stress for wild populations, relatively little is known about selection on this physiological trait, especially in mammals. A thorough understanding of the HPA activity will only emerge after accumulating data for various mammalian species that are subjected to different environmental conditions and at various life-history stages.

CONCLUSION

In this thesis I have demonstrated that the influence of moderate anthropogenic disturbance on the general health status of behaviourally flexible species may be negligible. While facing suboptimal conditions, species that can show behavioural or dietary flexibility might be capable of mitigating the potential negative effects of harsh conditions on health indices, up to a certain limit. However, beyond this limit, human activities may be negatively affecting long-term population viability even for resilient species. Although the use of various health indicators when assessing differences in habitat quality is appealing due to the relatively straightforward way to obtain them, this study emphasizes the degree of uncertainty associated with such shortcuts.

Using a comparative approach, I found that variations of stress hormone levels can have a species-specific effect on other health indicators. Especially, the direct positive effect of GC levels on body condition and parasite infection uncovered in *C. medius* highlighted the important role of GCs in mediating energetic trade-offs. As these trade-offs are also part of the mechanisms that allow an organism to respond to both predictable and unpredictable environmental perturbations, these findings suggest that GC hormones might, at least partly, be involved in the species-specific variability in coping ability to environmental challenges.

With this thesis, I further gave the first confirmation of the predictive power of HCC variation on individual fitness in a wild population. The results confirmed the fundamental prediction of the Cort-fitness hypothesis of a reduced survival associated with high GC levels. Consequently, all environmental pressures that can affect GC levels should be considered as potential threats to survival and might therefore cause population decline.

Altogether, I propose that in order to produce sound political decisions, the disturbance-stress-fitness relationship should, whenever possible, be considered simultaneously in future conservation-related studies. Moreover, since population decline is often hard to assess, the evaluation of an individual health indicator such as long-term stress load could provide an easier alternative for detecting issues emerging at the population level and ultimately predicting wild populations’ responses to environmental challenges.

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