A short story: senescence in an annual reptile, Labord’s chameleon (*Furcifer labordi*)

Dissertation
for the award of the degree
"Doctor rerum naturalium" (Dr.rer.nat.)
of the Georg-August-Universität Göttingen

Within the doctoral program GAUSS Basic Program in Biology
of the Georg-August University School of Science (GAUSS)

Submitted by
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Born in
Duisburg, Germany

Göttingen, 2019

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Date of the oral examination: 11.12.2019

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Summary

Among animals, the fast-slow continuum is the dominant axis that shapes life-history variation. As resources are limited a trade-off occurs between a short lifespan, young age at maturity, high reproductive rates and high aging rates or the other way round. At an ultimate level, rates of adult mortality with age are assumed to determine whether a species falls in the one or in the other continuum, at which high rates of extrinsic mortality select for fast life-histories. Within terrestrial vertebrates (tetrapods), the current record holder for short lifespan is the Labord's chameleon, *Furcifer labordi*. These chameleons, inhabiting the seasonal western and southwestern deciduous dry forests of Madagascar, were found to have a post-hatching lifespan of only several months. At the onset of the active respectively rainy season, in November, a cohort of hatchlings emerges. Their early life is determined by rapid growth, resulting in fast sexual maturity and subsequent reproduction in January-February. After the mating season, senescent declines become apparent, and at the beginning of the dry season, a fast die-off has been observed in both sexes. Hereafter, the population only exists as eggs, probably as an adaption to survive the harsh dry season. High adult mortality combined with relatively high juvenile (here egg) survival might have ultimately selected for this annual life-history. It is unique in tetrapods and makes this chameleon an intriguing model species for investigating the proximate and ultimate factors shaping rapid senescence, especially because perennial, sympatric congeners are available for comparative studies.

Semelparity, i.e. the strategy to allocate all energy in one reproduction event, is rarely found in tetrapods and has apart from a very few reptile species only been detected in a few small-sized marsupial species. However, in these marsupial species the die-off following the mating season is restricted to males, while several females survive until the next breeding season. Apart from these species, for which the male die-off is obligate, less extreme cases of semelparity have been documented. Here, facultative male die-off is restricted to some populations and/or only observed in some years and has been linked to variable resource availability. Strong prey seasonality that leads to a short breeding season has been suggested to explain male semelparity in marsupials, at which high levels of sperm competition combined with female reproductive synchrony select for obligate semelparity. Interestingly, males that were captured before engaging in reproductive season, can survive for more than two years. However, while captivity may shield males from early death due to extrinsic factors, they still experience loss in body mass and irreversible regression of sexual organs as observed in their wild conspecifics after the mating season, proposing an annual, intrinsic pacesetter. In contrast

to these marsupials, the proximate causes leading to the short life in this chameleon species remain entirely unknown. Is it the consequence of extrinsic factors, such as intense intra-sexual competition, high predation rates, and low food availability, or are the physiological changes leading to rapid senescence internally “programmed” and thus impending? In the marsupials mentioned above, both factors seem to play a role. Furthermore, so far, only one population in the southernmost and thus driest distribution range of this species has been studied in detail. Thus, the ultimate aim of my thesis is to reflect on the interplay between the external and internal factors that favour the short life-history of this chameleon species.

In the first study, I investigated the variability of lifespan in a population of *F. labordi* in the Kirindy Forest, which has a much longer rainy season compared to the more southern habitat. I documented that the population wide die-off is prolonged in Kirindy several months forward. Moreover, I showed a bias in sex-specific mortality, in which females have a longevity advantage. To exclude most extrinsic factors of mortality, I kept several individuals in captivity and demonstrated that both sexes could survive until the next season of activity. Therefore, this study revealed considerable plasticity of this life-history that is associated to differences in ecological factors. Within the second study, I compared the life-history of *F. labordi* with those of the congeneric and sympatric species *F.* cf. *nicosiai* and *F. oustaleti* in the Kirindy forest and tested for potential niche segregation that might have driven evolutionary selection for this short lifespan. In all three species, I found high interspecific differences in time of hatching, growth rates, age at reproduction, adult body size and lifespan. While the cohort of *F. labordi* was already adult, hatchlings of the *F. oustaleti* and subsequently *F.* cf. *nicosiai* emerged. As hatchlings in these species are similar-sized, these differences indicate dietary niche segregation. However, I found that compared to other similar sized lizards, the lifespans of *F.* cf. *nicosiai* and *F. oustaleti* are considerably short and probably attributed to the ecology of chameleons. In the third study, I examined to which extend parasite infection as a proxy of overall health in *F. labordi* might be connected to the short lifespan. Therefore, I inspected gastrointestinal, - blood - and ectoparasite burden and compared my findings to those of the sympatric and longer living *F.* cf. *nicosiai* and the caged individuals. Within *F. labordi,* I found an intense increase of prevalence in gastrointestinal parasites over the reproductive period. Males showed a higher prevalence that could be caused by frequent male-male combats resulting in higher baseline stress levels and their earlier disappearance. Contrary to our expectations, gastrointestinal, - blood - and ectoparasite burden was higher in adult *F.* cf. *nicosiai*. In captivity, both sexes showed less parasite burden and were longer living than their wild conspecifics. The final study aimed at determining the effect of glucocorticoids (GCs) and telomere shortening as well as their interplay on the acceleration of the aging process in *F. labordi*. To indirectly measure baseline stress levels, I used the ratio between heterophils to lymphocytes (H/L ratio) in blood smears as an indicator of physiological stress since a rise in GC level is closely correlate with a decrease in lymphocytes and increase of heterophils. I quantified telomere length using real-time quantitative PCR (qPCR) in relative telomere length (TL) in blood cells. I found that H/L ratios were higher in wild *F. labordi* compared to *F.* cf. *nicosiai*. TL was longer in *F.* cf. *nicosiai*, but shortened rapidly towards the dry season. The captive specimens showed lower H/L ratios compared to their wild conspecifics.

In summary, I evidenced that the short lifespan of *F. labordi* is not entirely "programmed" but highly variable and linked to ecological factors. Moreover, I found that males were the shorter living sex in the wild, but no significant intersexual differences in lifespan under protected caged conditions were observed. This implies that despite their earlier mortality, males do not obligatory experience faster senescence in components of health. Concerning gastrointestinal parasite infections, I found an increase towards the dry season in both sexes, whereas males showed continuously higher prevalences. Parasite infection was considerably low in the caged individuals, which might have affected their comparatively longer survival. In concordance to these findings, H/L ratios were higher in wild *F. labordi* compared to their captive conspecifics, probably resulting from the isolation of external stressors. The interspecific comparison with the longer living species *F.* cf. *nicosiai* suggests that the relatively slow growing juveniles invest comparatively more energy in self-maintenance. Contrary to our predictions, adult stages seem to suffer from visible senescent declines towards the dry season. Therefore, the disappearance in the wild towards the dry season seems to be a "post-reproductive" phenomenon in both species. In conclusion, the results of this thesis support the fundamental predictions of life-history theory, at which species that experience high extrinsic mortality risk in the wild invest their cellular energy into growth and reproduction at the expense of cellular repair mechanisms. As observed in the semelparous marsupials, both intrinsic and extrinsic mechanisms seem to shape the life-history of *F. labordi*.

Zusammenfassung

Bei Tieren ist das “schnell-langsame Kontinuum“ die dominierende Achse, die die Variation der Life History prägt. Da Ressourcen begrenzt sind, kommt es offenbar zu einem Kompromiss zwischen einer kurzen Lebensdauer, einem frühen Erreichen der Geschlechtsreife, einer hohen Reproduktionsrate und einer schnellen Alterungsrate oder eben genau umgekehrt. Letztendlich wird angenommen, dass die Sterblichkeitsrate von adulten Tieren mit dem Alter bestimmt, ob eine Art in das eine oder andere Kontinuum fällt, wobei hohe extrinsische Sterblichkeitsraten für eine schnelle Life History ausschlaggebend sind. Innerhalb der terrestrischen Wirbeltieren (Tetrapoden) ist das Labord´s Chamäleon, *Furcifer labordi*, der aktuelle Rekordhalter bezüglich Kurzlebigkeit. Es wurde festgestellt, dass diese Chamäleons, die aus den saisonalen Trockenwäldern im Westen und im Südwesten Madagaskars stammen, nur einige Monate nach dem Schlüpfen leben. Zu Beginn der Regenzeit im November schlüpft eine Kohorte von Jungtieren. Ihr frühes Leben ist durch schnelles Wachstum gekennzeichnet, das zu einer schnellen Geschlechtsreife und einer anschließenden Paarungszeit im Januar und Februar führt. Nach der Fortpflanzungsperiode zeigen sich Alterungsprozesse und zu Beginn der Trockenzeit ist bei beiden Geschlechtern ein schnelles Ableben zu beobachten. Hierauf existiert die Population nur noch in Form von Eiern, wahrscheinlich als Anpassung, um die raue Trockenzeit zu überstehen. Eine hohe Sterblichkeit bei aduten Tiere in Kombination mit einer relativ hohen Überlebensrate junger Stadien (hier Eiern) könnte letztendlich für diese annuelle Lebensgeschichte ausschlaggebend sein. Bei Tetrapoden ist diese Life History einzigartig und macht dieses Chamäleon zu einer faszinierenden Modellspezies für die Untersuchung der proximalen und ultimativen Faktoren für eine schnelle Seneszenz, insbesondere, weil längerlebige, sympatrische *Furcifer*-Arten für Vergleichsstudien zur Verfügung stehen.

Semelparität, d. h. die Strategie, die gesamte Energie in eine Reproduktionsperiode zu investieren, ist bei Tetrapoden selten anzutreffen und wurde, abgesehen von wenigen Reptilienarten, fast ausschließlich bei wenigen kleinen Beuteltierarten nachgewiesen. Bei diesen Arten ist das Sterben nach der Paarungszeit jedoch auf die Männchen beschränkt, während die Weibchen häufig bis zur nächsten Fortpflanzungssaison überleben. Abgesehen von diesen Arten, für die das Versterben der Männchen obligat ist, wurden auch weniger extreme Fälle von Semelparität dokumentiert. Hier ist das fakultative Sterben der Männchen auf einige Populationen beschränkt und/oder nur in einigen Jahren zu beobachten und wurde mit einer variablen Ressourcenverfügbarkeit erklärt. Eine starke Saisonalität der Nahrungsverfügbarkeit, die zu einer kurzen Paarungszeit führt, ist wahrscheinlich mitunter der Grund für die

Semelparität bei den männlichen Beuteltieren. Ein hohes Maß an Spermienkonkurrenz kombiniert mit weiblicher Fortpflanzungssynchronität hat wahrscheinlich zu einer obligaten Semelparie geführt. Interessanterweise können Männchen, die vor der Paarungszeit gefangen wurden, ein Alter von über zwei Jahre erreichen. Zwar kann die Gefangenschaft vor äußeren Faktoren schützen, doch es kommt hier immer noch zu einem Verlust an Körpermasse und zu einer irreversiblen Rückbildung der Geschlechtsorgane, wie es bei ihren Artgenossen nach der Paarungszeit beobachtet wurden, was auf einen annuellen, intrinsischen Schrittmacher schließen lässt. Im Gegensatz zu diesen Beuteltieren sind die unmittelbaren Ursachen für das kurze Leben des Labord's Chamäleon völlig unbekannt. Ist es die Folge von extrinsischen Faktoren wie intensiver intra-sexueller Konkurrenz, hohem Prädationsdruck und schwindender Nahrungsverfügbarkeit, oder sind es physiologischen Veränderungen, die zu einer raschen Seneszenz führen, und der Tod somit vorprogrammiert? Bei den erwähnten Beuteltieren scheinen beide Faktoren eine Rolle zu spielen. Darüber hinaus wurde bisher nur eine Population im südlichsten und damit trockensten Verbreitungsgebiet von *F. labordi* detailliert untersucht. Das Hauptziel meiner Doktorarbeit ist es daher, das Zusammenspiel der äußeren und inneren Faktoren zu entschlüsseln, die die kurze Life History dieser Chamäleonart begünstigen. In der ersten Studie untersuchte ich die Variabilität der Lebensdauer einer Population von *F. labordi* im Kirindy-Wald, die im Vergleich zum südlicheren Verbreitungsgebiet eine längere Regenzeit aufweist. Ich konnte zeigen, dass sich das populationsweite Sterben in Kirindy um mehrere Monate verzögerte. Darüber hinaus zeigte sich, dass Weibchen langlebiger sind als Männchen. Um einen Großteil der extrinsischen Mortalitätsfaktoren auszuschließen, habe ich mehrere Individuen in Gefangenschaft gehalten und gezeigt, dass beide Geschlechter bis zur nächsten Aktivitätsperiode überleben können. Diese Studie ergab insgesamt eine erhebliche Plastizität dieser Life History, die mit Unterschieden von ökologischen Faktoren verbunden ist. In der zweiten Studie habe ich die Lebensgeschichte von *F. labordi* mit der von den sympatrischen und längerlebigen, zur selben Gattung gehörenden Arten *F.* cf. *nicosiai* und *F. oustaleti* im Wald von Kirindy verglichen. Hierbei habe ich die Arten auf potentielle Nischentrennung untersucht, die möglicherweise die Evolution einer so kurzen Lebensdauer begünstigt. Bei allen drei Arten fand ich hohe interspezifische Unterschiede vom Schlupfzeitpunkt, Wachstumsraten, Alter bei der Reproduktion, Körpergröße der adulten Tiere und der Lebensdauer. Während die Kohorte von *F. labordi* bereits adult ist, schlüpften die Jungtiere von *F. oustaleti* und anschließend *F.* cf. *nicosiai*. Da Jungtiere bei diesen Arten ähnlich groß sind, deuten diese Unterschiede auf eine zeitliche Nischentrennung in Bezug auf Nahrung hin. Ich stellte jedoch auch fest, dass im Vergleich zu anderen Echsen ähnlicher Größe die Lebenserwartung von *F.*

cf *nicosiai* und *F. oustaleti* vergleichsweise kurz ist, was sich wahrscheinlich auf die Ökologie der Chamäleons zurückführen lässt. In der dritten Studie habe ich untersucht, inwieweit Parasiteninfektionen als Indikator für den allgemeinen Gesundheitszustand von *F. labordi* und deren kurzer Lebensdauer verbunden sein könnte. Aus diesem Grund habe ich die Gastrointestinal, - Blut - und Ektoparasitenbelastung untersucht und die Befunde mit denen des sympatrischen *F.* cf. *nicosiai* sowie den Tieren in Gefangenschaft verglichen. Bei *F. labordi* fand ich einen starken Anstieg der Prävalenz von gastrointestinalen Parasiten im Verlauf der Fortpflanzungsperiode. Männchen wiesen eine höhere Prävalenz auf, die wahrscheinlich durch häufige intrasexuelle Kämpfe und daraus resultierende hohe Stressniveaus begünstigt wurde und letzendlich zu ihrem früheren Verschwinden führte. Entgegen unseren Erwartungen war die Belastung durch gastrointestinale, - Blut - und Ektoparasiten bei adulten Individuen höher als bei *F. labordi*. In Gefangenschaft zeigten beide Geschlechter eine geringe Parasitenbelastung und lebten länger als ihre wildlebenden Artgenossen. Das Ziel der letzten Studie war es, die Wirkung von Glukokortikoiden (GC) und Telomerverkürzung sowie deren Zusammenspiel auf die Beschleunigung des Alterungsprozesses bei *F. labordi* zu untersuchen. Zur Messung des Ausgangsstressniveaus verwendete ich das Verhältnis von Heterophilen zu Lymphozyten (H/L-Verhältnis) in Blutausstrichen als indirektes Maß für den Stress, da dieser eng mit den GC-Niveaus aufgrund des GC-induzierten Leukozytenproduktion korreliert. Ich habe die Telomerlänge mithilfe der quantitativen real time-PCR (qPCR) in Bezug auf die relative Telomerlänge (TL) in Blutzellen quantifiziert. Ich fand heraus, dass das H/L Level in wilden *F. labordi* höher waren als bei F. cf. *nicosiai*. Die TL waren bei *F.* cf. *nicosiai* ursprünglich länger, aber zur trockenen Jahreszeit verkürzten sich die Telomere schnell. Die in Gefangenschaft gehaltenen Exemplare zeigten geringere H/L Level im Vergleich zu ihren Artgenossen in der Natur. Der interspezifische Vergleich mit der länger lebenden Art *F.* cf. *nicosiai* zufolge investieren die relativ langsam wachsenden juvenilen Tiere vergleichsweise mehr Energie in Selbsterhaltungsmechanismen. Entgegen unseren Vorhersagen scheinen die adulten Tiere zur Trockenzeit hin aber auch unter sichtbaren Altersprozessen zu leiden. Daher scheint das Verschwinden in der Wildnis zur Trockenzeit hin bei beiden Arten ein gängiges Phänomen nach der Fortpflanzungperiode zu sein. Zusammenfassend stützen die Ergebnisse dieser Arbeit die fundamentalen Vorhersagen der Life History Theory, bei denen Arten, die in freier Wildbahn einem hohen extrinsischen Mortalitätsrisiko ausgesetzt sind, ihre zelluläre Energie auf Kosten von inneren Reparaturmechanismen in Wachstum und Fortpflanzung investieren. Wie in auch bei den semelparen Beuteltieren beobachtet wurde, scheinen sowohl intrinsische als auch extrinsische Mechanismen die Life History von *F. labordi* zu prägen.

Chapter 1: General Introduction

Lifespan covers the period from the fertilization of an egg cell towards the death of a multicellular organism. However, frequently, the beginning of life is understood as hatching from the egg or birth. This period differs due to the mortality risk of each individual of a species in a population. The statistical mean of a lifetime of individuals of a species is referred to as average lifespan, as opposed to the maximum lifespan is defined by the oldest known specimen. The course of the individual organism consists of several phases: development, maturation, reproduction, and senescence. The mean duration of each phase and the ratio of duration to each other are evolutionarily selected for each species (Rensing and Rippe 2014). In addition, there are phases such as hibernation and diapause, which reduce the mortality risk. Life-history studies the alterations that organisms experience from conception to death, but focuses mainly on the schedule of reproduction and survival (Stearns 1992, Charnov 1993). Life-history analysis is also a tool for examining hypotheses of evolutionary causes of adaptations in general (McNamara and Houston 1996, Nylin and Gotthard 1998). In fact, progressing age involves declines in physiological functioning. In humans, the average lifespan in industrialized countries has increased significantly in the last century, mainly due to medical advances in treating diseases, improved food quality and the avoidance of wars. In contrast, lifespan has declined in many African countries due to diseases such as AIDS, lack of food and armed conflict (Christensen et al. 2009).

Only quite recently, interest in aging as a phenomenon, beyond its effects on humans, has arisen. In fact, senescent decline was long considered a luxury of the sheltered anthropogenic environment reserved for humans and animals in their captivity. It was assumed that in nature, any deterioration would quickly lead to decease by natural selection (Hayflick 2000). An increase of research has followed the realization that actuarial (i.e. demographic senescence, the increasing risk of mortality with proceeding age) and functional senescence (within-individual deterioration of physical or physiological functioning with proceeding age) may be more widespread also in natural populations than assumed before (Nussey et al. 2013). Despite these recent advances, the study of senescence in wild populations, including the ultimate causes, variability among individuals and species, and the shaping of senescence by intrinsic and extrinsic factors is scarce. In my thesis, I will contribute to its progress by exploring the life-history of an annual chameleon by focusing on the extrinsic and intrinsic mechanisms leading to the short lifespan of this species. To the beginning, I will summarize some theories explaining "Why do we age". Further, I will point out "how variable is senescence" and will focus on differences between species and within species. Moreover, I will examine variances of aging under natural vs protected conditions as well as sex specific patterns of senescence and mortality under natural and protected conditions. Additionally, I will explain the phenomena "semelparity" and discuss its occurrence among different animal taxa. I will also introduce the study species used in this thesis, the Labord's chameleon (*Furcifer labordi*). Finally, I will outline the specific aims of my thesis and introduce the four distinct studies undertaken to achieve those aims.

Why do we age?

Many theories have emerged to explain which processes or mechanisms drive the process of senescence, but the distinction between causes and effects is often difficult. That is why, at present, no consensus exists over the specific causes of senescence. However, some theories have attained more empirical support than others have and will be discussed below.

Medawar (1952) and Williams (1957) assumed that senescent declines ultimately arise because natural selection is inefficient at eliminating maladaptive mutations that appear after an individual has already had the opportunity to pass on its genes. While evidence has been found to support their thesis (e.g. Hunt et al. 2006), the particular genetic mechanisms of the physiological changes associated with senescence still remain uncertain (Hughes et al. 2002, Kirkwood 2005, Moorad and Promislow 2009). However, a more general explanation offered for senescent deterioration, the "disposable soma theory" found concordant support (Kirkwood 1977, Kirkwood 2002, Kirkwood 2005). This theory predicts that a limited amount of resources is available for somatic self-maintenance, prevention and repair of cellular damage, as well as other functions, such as reproduction and growth. Here, inevitable trade-offs arise between self-maintenance and the other functions concomitantly shaping the rate of aging (Kirkwood and Austad 2000, Lemaitre et al. 2014). Consequently, the effectiveness of self-maintenance is at the core of senescent declines, and several molecular mechanisms such as the accumulation of reactive oxygen species (e.g. Marnett 2002), and the shortening of telomeres (protective repetitive nucleotide sequences at the ends of chromosomes) (Monaghan 2014) have been identified as potential proximate causes of age-related functional decline. In the absence of appropriate prevention and repair mechanisms (e.g. DNA repair, anti-oxidant action, and telomerase activity), these molecular alterations lead to cellular damage (Kirkwood 2005) that in turn can bring on changes in physiological processes, tissue composition and functional performance at old age. The resulting effect is reflected in age-related diseases, disability and frailty and, in consequence, actuarial senescence in the vast majority of animal species (Gaillard 1994, Ricklefs 1998, Jones et al. 2014). Physiological stress is an essential mediator in the trade-off between survival and reproduction (Boonstra et al. 2001, Ricklefs and Wikelski 2002). Glucocorticoids (GCs) affect the expression of up to 10% of the genome (Le et al. 2005) and some of their effects parallel those observed during aging, suggesting that chronic stress has a high potential to accelerate the aging process (Sapolsky et al. 1987, Veldhuis et al. 2013).

The immunosuppressive effects of chronic GC elevation and their consequences for morbidity and mortality have been studied intensively (e.g. Sapolsky et al. 2000, Dahabhar 2009). Alterations in key immunological parameters during chronic stress parallel those during normal immunosenescence to a large degree (Bauer 2008). One potential pathway shared by stress and aging is an oxidative stress-mediated shortening of telomeres (Haussmann and Marchetto 2010, Monaghan 2014). Elevated GC levels, particularly during long-term physiological or psychological stress, have been linked to increased oxidative stress, shortened telomeres, and reduced telomerase activity (Eple et al. 2004, Constantini et al. 2011).

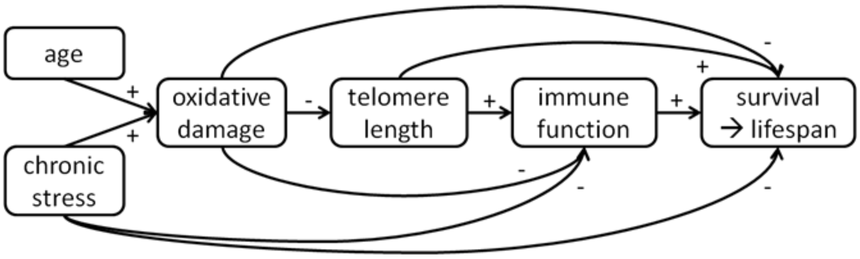

Fig 1. Hypothesized pathways by which age and chronic stress affect lifespan. Simplified and modified after (Vleck et al. 2007) and (Haussmann and Marchetto 2010).

Classical evolutionary models of aging predict that all species ultimately age (Hamilton 1966). However, studies from captivity and in the wild have shown that several species of fishes, amphibians, and reptiles, to name only vertebrates, fail to show signs of aging (Finch and Austad 2001). The authors specifically questioned whether exceptionally long-lived animals showed signs of negligible senescence on physiological and biochemical levels. The term negligible senescence was devised by the biogerontologist Caleb Finch (1990) to describe the very slow aging reported in bivalves, cold-water fish, and chelonians. Many of these organisms that were aged by growth zone analyses of the otolith, ear bone or shell, are assumed to live for more than 100 years. Finch proposed three specific criteria to test the incidence of negligible senescence, explicitly no observable: (1) age-related increase in mortality rate, (2) decrease in reproduction rate after sexual maturity, as well as (3) no age related decline in physiological functioning. Nevertheless, these animals have only been studied for a limited amount of time. Still, it is surprising that long-term studies revealed that females of Blanding's turtle and Painted turtle increased both survivorship and reproductive output with age (Congdon et al. 2001, Congdon et al. 2003). Moreover, Ruby et al. (2018) identified that the naked mole rats achieve negligible senescence that has never been documented in mammals before. Moreover, Buffenstein (2008) suggested that this rodent is a candidate species for negligible senescence. Interestingly, naked mole rats pose many challenges to molecular biogerontology theory. Their levels of oxidized lipids and proteins are remarkably high, without further age changes after maturation (Perez et al. 2008, Ungvari et al. 2008), which would not predict longevity in the usual model of critical oxidative load.

How variable is senescence?

Rates of senescence are highly variable both among and within species (Jones et al. 2014). The variance on species-level has been attributed to differences in e.g. body size, mating systems and reproductive schedules (Ricklefs 2010), as well as environment (Cayuela et al. 2019), and capabilities of escaping extrinsic hazard, e.g. flight (Austad and Fischer 1991, Healy et al. 2014), armored protection or weapons (Ricklefs 1998) sociality (Carey and Judge 2001, Buffenstein 2005, Keller and Jemielty 2006) and diet (e.g. Wasser and Sherman 2010). Trade-offs between reproduction and individual survival via self-maintenance are arranged differently across animal taxa with varying life-histories (Jones et al. 2008). A key factor of the rate of aging is assumed to be the level of exposition to hazardous environments (Ricklefs 1998, Williams et al. 2006). Species, experiencing high extrinsic mortality due to predation, pathogens or natural accidents, are expected to invest their energy into early reproduction as the possibility of survival to the next reproductive event may be low (Jones et al. 2008). This leads to a “fast pace of life” or the so called “r-selected” life-history (Pianka 1970, Austad 1997), probably resulting in fast growth rates and reduced investment in somatic maintenance after reproductive maturity, leading to rapid senescent declines after reaching sexual maturity. It has also been suggested that fast juvenile growth rates are associated with a lower degree of developmental ‘quality control’, and therefore connected with a higher probability of developmental error (Sibly and Calow 1986). Since smaller animals tend to be more prone to predation they are subsequently expected to follow this life-history strategy. Contrary, in longer-lived animals with a slow life-history ("K-selected" species), a higher investment in somatic maintenance would be expected as reproductive success over lifetime tends to accumulate over several reproductive events a higher lifespans and the potential for continuous fitness increases (Jones et al. 2008).

Remarkably, even though larger species tend to have longer life expectancies, numerous cases are showing that smaller individuals within a species live longer in captivity (e.g. mice, rats, horses, and dogs [Miller et al. 2002, Rollo 2002]). Subsequently, larger species tend to be longer-lived, but on intraspecific level, smaller individuals tend to live longer, at least under protected conditions. Extreme examples for intraspecific variation in longevity in the wild are eusocial insects (e.g. Keller and Jemielity 2006). For example, queen honeybees have an average lifespan of approx. one year, whereas female workers typically live for only several weeks (Huges and Reynolds 2005). Similarly, within the eusocial mole rat *Fukomys damarensis*, queens live considerably longer than non-reproductive females (Schmidt et al. 2013). Intraspecific differences of lifespan on population level can also be caused by variability in nutrition availability in different environments (e.g. Wolfe et al. 2004) or ambient temperatures (e.g. Mangel and Abrahams 2001). Usually, in ectotherms, lower ambient temperatures result in delayed maturity and increased lifespan (Gosden 1996). Moreover, caloric restriction can significantly influence an individual's lifespan by attenuating oxidative mitochondrial damage. Caloric restriction is a diet that reduces calorie intake without incurring malnutrition or a reduction in essential nutrients. Several animal species that were maintained under caloric restriction showed extended lifespans compared to their conspecifics, which were fed ad libidum (reviewed in Masoro 2005).

Within some taxa, certain species groups conspicuously stand out as being exceptionally long-lived. Within vertebrates, longitudinal data tend to be available mainly for rather large-bodied species exhibiting slow life-histories. Consequently, these taxa are best represented in studies of within-individual senescence (e.g. ungulates [Gaillard et al. 2008, Nussey et al. 2011], sea birds [Pardo et al. 2013], seals [Hindle et al. 2009], baboons [Altmann et al. 2010]). Still, a few studies have also been able to demonstrate senescence in natural populations of short-lived organisms, such as small fish (guppies [Reznick et al. 1990, Reznick et al. 2004]). Due to the predominant focus on species from the slow end of the pace-of-life continuum, considering their comparatively low risk of extrinsic mortality, it seems presumable that the observed patterns of senescence are biased towards seemingly preferred these habitats. Subsequently, due to their size and proximity to humans, mammals are the best-studied group. Besides a large diversity in average and maximum lifespan, we find many similarities in the aging process since it is postulated that the aging process of this taxon has, to some degree, a common origin (de Magalhaes and Toussaint 2002). Typically, mammals exhibited reproductive senescence (in females typically at mid-life), increased mortality with age, and evidence of functional decline with age (Cohen 2004). In general, eutherians are longer living than marsupials (e.g. Miller 1999). Comparing birds with mammals, several studies suggest that birds are the slower aging taxon (e.g. Holmes and Austad 1995, Holmes et al. 2001, Holmes and Ottinger 2003). Nonetheless, there are no verified reports of birds with negligible senescence. Since the longevities of some bird species approach those of humans, avian aging processes might actually be more similar to ours in some respects than those of the short-lived rodents typically used to model basic mammalian aging processes (Holmes and Ottinger 2003).

Reptiles tend to show a lower incidence and intensity of aging than most mammals (Finch 1990, de Magalhaes and Toussaint 2002). Some reptilian species exhibit signs of senescence comparable to that observed in mammals (Majhi et al. 2000, Jena et al. 2002, Olsson and Shine 2002). Several species, particularly chelonians, appear to feature negligible senescence and very long lifespans (Zug 1991). The record holder for longevity is Harriet, a Galapagos giant tortoise collected from the Galapagos islands a few years before Charles Darwin arrived, died in 2006 at the age of 175 years (Magelhaes and Costa 2009). In addition, crocodiles (e.g. Hutton 1986, Castanet 1994) and tuataras (Dawbin 1982) are known to be exceptionally long-living. By possessing shells (chelonians) and skin armor (crocodiles), both taxa show the so-called “protective phenotype” (reviewed in Schwartz and Bronikowski 2011), which predominantly shields them from environmentally-driven mortality. Besides, crocodiles show parental care (e.g. Tullberg et al. 2002) that highly increases juvenile survival probability (Garner 2016). Concerning tuataras, the low ambient temperatures and therefore reduced metabolic rates might favour their longevity. Although numerous species of squamate reptiles (lizards and snakes) exhibit long life-histories (Magelhaes and Costa 2009), we find several examples of short life-histories and even semelparity in this group (Dickmann 1999, Karsten et al. 2008, Rodrigos-Romero et al. 2011). Reptiles are an extremely diversified group, but regarding aging mechanisms, they are highly understudied. Recently, reptiles were found to have three times more variation in life-history traits compared to mammals (Babich Morrow et al. 2019). Moreover, they typically exhibit continued growth after their first reproduction, which distinguishes them from mammals and birds (Hariharan et al. 2016). Furthermore, a considerable increase in fecundity with age can occur in reptiles even in the oldest documented wild individuals (e.g. Sparkman et al. 2007, Warner et al. 2016). However, this does not exclude reproductive senescence at the end of the lifetime (Warner et al. 2016). Besides, several squamate species have tissue regenerative capacities that surpass those of mammals by far (e.g. Sun et al. 2018). Until now, there has been little consideration of how these regenerative capabilities impair the rate of aging in these species (Hoekstra et al. 2019). Therefore, it is highly possible that this group may feature various mechanisms to delay aging and age-related debilitation, making them an intriguing model to study aging. Lastly, comparisons among different taxa can yield insights into those mechanisms of aging that are fixed or constrained versus those that have evolved independently in different animal lineages.

Aging under natural versus protected conditions

Studies linking molecular alterations to senescence have mainly been focused in laboratory animals (e.g. Austad 1997). On the one hand, laboratory studies on the mechanisms of senescence profit from reduced heterogeneity among individuals and easier identification of senescent changes caused by the reduced mortality rates under protected conditions. However, previous selection for fertility may have also selected for short lifespans (e.g. laboratory mouse strains), generating biases in studies of senescence. In other words, it has been discussed that the life-extending gene variants found in these organisms may be simply restoring lifespan to what is usually found in the wild (Spencer and Promislow 2002). The fact that wild-derived mouse strains require a longer duration to reach sexual maturity and live significantly longer than common laboratory strains supports this view (Miller et al. 2002). An additional problem is that laboratory strains are regularly genetically homogeneous that offer more consistent results, but also implicate discrepancies between strains (Partridge and Gems 2007, de Magalhaes 2014). Thus, to evaluate the relative involvement of intrinsic and extrinsic factors shaping life-histories, comprising the onset and rate of senescence, it is essential to monitor senescence in natural populations. The main body of senescent studies are conducted under protective conditions Therefore, it is essential to evaluate how patterns observed in captivity are comparable to populations in nature to define how much of the decline observed in captivity depicts intrinsically biological versus behavioural variation, and the interactions of individual quality and the environment. Remarkable examples of a rapid increase in mortality rates following reproduction such as that documented in several salmon species and some small marsupial species (Lee et al. 1982, Oakwood et al. 2001) underpin the observation that senescence does occur in natural populations.

Besides its evolution-theoretic interest, knowledge about the basis of variance in senescence rates is required for human interventions of age-related disease and functional declines. Therefore, for entirely understanding the links between molecular self-maintenance, disease and mortality, and individual heterogeneity in these processes, it is vital to focus more senescence patterns in nature. In addition to intrinsic mortality by illness or physiological failures, morbid individuals may more likely succumb to mortality by extrinsic hazards, a process termed “selective disappearance” of lower quality individuals, (Bouwhuis et al. 2009, Hayward et al. 2013, Nussey et al. 2011, van de Pol and Verhulst 2006). Therefore, aging individuals may face an elevated mortality risk if their coping with extrinsic pressures is impaired by senescent declines in immunological, physical or cognitive functioning. If intrinsic qualities influence the lifespan of an individual, senescent declines would indeed be difficult to observe among populations in nature, as it may be difficult to conclude to what extent the observed deviation (particularly in cross-sectional data) in a given trait reflects the higher quality of the subset of animals alive. This issue is likely at the core of the earlier conclusions about an absence of a senescent decline in wild animals (Hayflick 2000, Williams 1957).

By the use of careful study designs, longitudinal data, and suitable methods of statistical inference, a number of studies have nevertheless been able to demonstrate senescence in the wild (Nussey et al. 2013). However, rates of senescence vary across traits (Nussey et al. 2009, Walker and Herndon 2010, Hayward et al. 2013) and senescent decline of physical function seems to be absent in some of the measured traits (e.g. Rueppell et al. 2007, Gonzalez et al. 2012). Due to the small number of studied taxa and the rarity of studies measuring senescence in multiple traits, it is still uncertain which aspects of the functioning of the organism are most affected and how environmental surroundings and life-history variation influence the traceability and rates of functional senescence. Nevertheless, the fact that different rates of senescence occur across the examined traits suggests that selective pressures to maintain functioning are stronger for some features than others. However, little attention has been paid to this possibility (Burger and Promislow 2006, Bouwhuis et al. 2012). The influence of extrinsic mortality on senescence has been studied using experimental manipulations and comparison of life-history characteristics in different natural environments (Reznick et al. 1990, Reznick et al. 2004). A comparison of wild and captive populations could be used to complement these approaches (Bronikowski et al. 2002, Magalhaes et al. 2007, Lemaitre et al.

2013). However, this approach has thus far not been taken to assess how the environment shapes senescence.

Interestingly, and in contradiction to the results of Scharf et al. (2015) for squamates, captivity did not influence the recorded longevity within reptile species (Stark et al. 2018). Here, factors such as stress, poor keeping conditions, and disease are suggested to cause premature deaths in captivity, offsetting the positive effects of reducing predation and starvation (Robinson et al. 2015). Thus, average and maximum lifespan in several reptile species might be highly underestimated. In total, comparative life-history studies of reptiles in the wild and captivity are scares. This is probably because reptiles are often difficult to monitor in the wild and infrequently kept in laboratories in higher numbers, which is probably due to significant logistical, financial and ethical limitations (McDiarmid et al. 2012). Therefore, future work is needed to clarify differences in lifespan in captive and wild reptile populations.

Sex-specific patterns of senescence and mortality

Differences in life-history and rates of aging also arise on an intraspecific level (Stearns 1992). Among vertebrates, one of the main differences is the frequently documented sex-bias in longevity. Within mammals, females tend to be the longer living sex (Bonduriansky et al. 2008), whereas, in birds, the opposite is often, but not generally true. However, contrary to mammals, large-scale comparisons of the sex gap in aging patterns are still lacking in birds (Liker and Székely 2005). These differences among mammals and birds are partially attributed to the heterogamety in male mammals and female birds (e.g. Pipoly et al. 2015). Finally, intersexual differences in lifespan and senescence have been mainly addressed to the divergent strength of sexual selection on both sexes (Promislow et al. 1992, Liker and Székely 2005, Clutton-Brock and Isvaran 2007). Especially in species with polygamous mating systems, males achieve higher lifetime fitness by investing severely in reproduction during the peak reproductive age (Clutton-Brock and Isvaran 2007). For females, the fitness gains are more evenly distributed over successive reproductive events over their lifetime (Clutton-Brock and Isvaran 2007). Following Bateman’s principle of sex-specific reproductive variance (Bateman 1948), mammalian females (or the sex that benefits from a prolonged reproductive lifespan) should live longer (Bonduriansky et al. 2008). In general, female vertebrates have greater immune responses than males (e.g. Terres et al. 1968), and it has been assumed that this is a consequence of the immunosuppressive effect of testosterone (Alexander and Stimson 1988, Schuurs and

Verheul 1990). However, detailed studies of sex differences in immune functioning imply that humoral immune responses are higher in females rather than suppressed in males (Olsen and Kovacs 1996). Moreover, testosterone may also favour behaviours, such as aggression and dispersal, increasing the likelihood of external mortality risks (e.g. Zuk and McKean 1996, Roberts et al. 2001).

Semelparity

The term semelparity was shaped by the evolutionary biologist Lamont Cole (1954) and originates from the Latin words semel 'once, a single time' and pario 'to beget'. This differs from iteroparity seeing that iteroparous organisms spread their reproductive investments across multiple occasions during their lifetime (Bossert 1970, Benton and Grant 1999). Semelparity is also known as "big bang" reproduction, as the single reproductive event is usually large as well as deadly (Ricklefs and Miller 1999). It has been frequently demonstrated that semelparous species produce more offspring during their single reproductive event than closely related iteroparous species in any one of theirs (Charnov and Schaffer 1973). Within the animal kingdom, semelparity is frequently found in invertebrates. It is widespread among insects, including several taxa of lepidopterans, hemipterans, ephemeropterans etc. Ultimately, their fragile wings that become more tumbled over time might explain the relatively short life of most adult insect stages. This life-history strategy also occurs in several arachnids (e.g. Schneider and Lubin 1997), and numerous species of cephalopods (Rocha et al. 2001).

Among vertebrates, a classic example of semelparity are anadromous salmon species (Hendry et al. 2004, Morbey et al. 2005), which live for several years in the ocean before swimming to the freshwater rivers of its birth, spawning, and dying. The transition between oceanic water to freshwater and steep elevation changes in rivers could explain the evolution of semelparity because it would be extremely demanding to return to the ocean. Highly elevated glucocorticoid levels mediate the post-spawning death of the semelparous salmon species by causing tissue degeneration, suppressing of the immune system, and impairing various homeostatic mechanisms (Dickhoff et al. 1989). One of the main factors accelerating rapid senescence is that they do not feed during reproduction resulting in extremely reduced body weight (Morbey et al. 2005). Moreover, certain annual African fish species from the genus *Nothobranchius*, which are adapted to life in temporal water bodies, are semelparous and do not commonly live more than 12 weeks (Valdesalici and Cellerino 2003). Additional examples for this life-history strategy in bony fish are the smelt *Thaleichthys pacificus* (Clarke et al. 2007) and male capelin *Mallotus villosus* (Huse 1998).

However, this phenomenon is rarely found in vertebrates other than bony fish. Within amphibians, strict semelparity has never been documented, though a few species, such as the Northern cricket frog *Acris crepitans* exhibit an almost population-wide turnover within a year (Lehtinien and MacDonald 2011). In reptiles only a few lizards such as the Labord's chameleon of southwestern Madagascar (Karsten et al. 2008), bunchgrass lizards *Sceloporus bicanthalis* of the highlands of Mexico (Rodríguez-Romero et al. 2011) as well as some Australian agamid species of the genus *Ctenophorus* (e.g. Henle 1991, Dickman et al. 1999) exhibit an annual life-history. However, within these lizards, occasionally, a few individuals survive until the next breeding season. Among mammals, semelparity seems to be predominantly restricted to a few didelphid and dasyurid marsupials (e.g. Braithwaite et al. 1979, Ooakwood et al. 2001, Lopes and Leiner 2008). Apart from marsupials, semelparity has been detected in a rodent species, the desert woodrat *Neotoma lepida* from the Death Valley, California (Smith and Charnov 2001). Within birds, annual species have never been documented and lifespan of birds is typically 2 – 3 fold longer than that of mammals of comparable size (Austad 1997, Austad 2011). Lower aging rates in birds (e.g. Travin and Feniouk 2016), frequent biparental care (Cockburn 2006), and the ability to escape hazards by flight (Austad and Fischer 1991) might be the key factors behind this phenomenon.

The evolution for semelparity in both sexes has occurred several times in invertebrates and bony fish. Among tetrapods it occurs in in a few reptile species (e.g. Karsten et al. 2008). However, it is seldom found in mammals because maternal care is obligatory due to internal fertilization and incubation of offspring as well as subsequent nursing, requiring high maternal survival rates after fertilization and offspring weaning. Moreover, female mammals have relatively low reproductive rate compared to invertebrates or bony fish because they invest much energy in maternal care. However, male reproductive rate is much less constrained in mammals because only females bear young. Males that die after one mating season may still produce a high number of offspring by investing all their energy in intense mating with numerous females (Fischer et al. 2013). Natural selection might have allowed semelparity to evolve in the Dasyuridae and Didelphidae because of certain ecological preconditions. Females ancestral to these groups may have shortened their mating period to coincide with peak prey abundance. Because this window is so small, these females exhibit a reproduction pattern where the estrous of all females occurs simultaneously. Therefore, selection would favour aggressive male behaviour due to increased intersexual competition for accessing females. As mating period is so short, it is more advantageous for males to locate all their energy on mating, even more so if it is improbable to survive to the next reproductive season (Braithwaite et al. 1979). Within males, high levels of corticosteroids are sustained over long periods triggering immune and inflammatory system failure and gastrointestinal hemorrhage, which ultimately leads to death (Oakwood et al. 2001). However, in the relatively large marsupial *Dasyurus hallucatus*, a lack of elevated cortisol levels during mating periods indicates no current universal explanation for the mechanism behind increased male mortality in Dasyuridae. Post-reproductive senescence has also been suggested as an explanation (Bradley 1997). In fact, most semelparous tetrapods live in habitats characterized by high environmental unpredictability (e.g. Henle 1991, Oakwood et al. 2001, Smith and Charnov 2001, Karsten et al. 2008).

Study species: The Labord's chameleon *Furcifer labordi* (Grandidier, 1872)

Of the approx. 210 described chameleon species, 90 species are endemic to Madagascar (Glaw 2015, Prötzel et al. 2017, 2018). Malagasy chameleons comprise the four genera *Calumma*, *Furcifer, Brookesia* and *Palleon*. The genus *Furcifer* is a monophyletic group (Tolley et al. 2013), actually presently containing 24 species (Sentis et al. 2018). All species of the genus show distinct sexual dimorphism and males are generally larger than females. Species of the genus are mainly located in western arid regions, whereas some species occur in rainforests. Typical features of *Furcifer* are a high and rounded casque (especially males), as well as the absence of occipital lobes (Glaw and Vences 2007). Within the genus, *F. labordi* is a comparatively small species. Males have a snout-vent length (SVL) of 80 – 120 mm and exhibit a large cranial casque, a well-defined rostral appendage, and a continuous dorsal crest containing more than 35 gular cones and up to 2 mm high. Ground colouration is a light green with a white lateral stripe. Females have an average SVL of 70 – 80 mm and sometimes offer a poorly developed dorsal crest situated in the nuchal region. Ground colouration is green with blue and violet blotches on the flanks and dorsally orientated bright orange markings and a red spot above the insertion of the arms (Glaw and Vences 2007). They inhabit the western and southwestern seasonal dry forests of Madagascar. They seem to be forest-associated and avoiding degraded forests and scrubland (Soazandry et al. 2010).

Furcifer labordi was suspected to occur on the peninsula Katsepy near Mahajanga and Soalala in northwestern Madagascar (Glaw and Vences 2007). However, within the recent study of Sentis et al. (2018), these northern populations were assigned to the recently resurrected species *F. voeltzkowi* (Boettger 1892), which was formerly treated as a synonym. Both species look very similar, but males of *F. voeltzkowi* have a smaller rostral appendage and heterogeneous scalation on the lateral continuous white band, whereas *F. labordi* has a constant homogeneous scalation and a discontinuous lateral band (Sentis et al. 2018).

Furcifer labordi, in particular is interesting to study senescence, as it is the shortest living tetrapod species and therefore provides an insight into aging mechanisms in short-lived vertebrates and especially reptiles, which are an understudied group according to senescence. Furthermore, their relatively small body size facilitates their husbandry in single cages for excluding extrinsic risks of mortality for comparative studies. Additionally, because this species has longer living sympatric congeners, this chameleon assemblage constitutes a promising model for comparative investigations of the ecological and physiological determinants of longevity and senescence in wild tetrapods.

Study aims and approaches

In the previous sections, I have illustrated the state of the art in studies of senescence in wild populations and identified several gaps. In particular, I have communicated a necessity for further examining patterns of senescence in multiple traits in nature. In light of these gaps, the main aim of this thesis was to disentangle exogenous and endogenous factors that shape the extremely short life-history in an annual chameleon.

The specific questions I defined to answer were:

1. How variable is the lifespan of *F. labordi*? In particular, I investigated environmentally-dependent variability in lifespan: a) between populations (Kirindy vs. Ranobe); b) differences in lifespan within a population due to environmental variation between years in Kirindy; c) differences in sex-specific mortality, d) differences between wild and captive specimens, which were excluded from extrinsic factors of mortality.

2. To what extent does the life-history of *F. labordi* differ with the one of the two sympatric *Furcifer* species (*F.* cf. *nicosiai* and *F. oustaleti*) concerning population densities, survival, - growth - and reproduction rates?

3. Does parasite burden have an effect on the lifespan of *F. labordi*.?

4. To which extent do age-related changes in molecular mechanisms such as chronic physiological stress and telomere shortening contribute to the short lifetime of *F. labordi?*

To answer these questions, I examined the population dynamics of a wild population of *F. labordi* in the Kirindy forest via capture-mark-recapture study. I measured age trajectories three different components of health: 1) parasite burden, 2) H/L profiles that indirectly reflect baseline glucocorticoid hormone levels, and 5) telomere shortening. These indicators were selected because they reflect different aspects of general health and can significantly affect fitness and survival of wild individuals. All collected data of health parameters from the wild population were compared with the sympatric and longer living *F.* cf. *nicosiai* as well as captive specimens of *F. labordi*. I collected data within three field seasons (November 2013 –- mid July 2014, January – mid July 2015, October – December 2015). Blood samples that were used to measure H/L profiles and telomere shortening were only collected in adult individuals. During my absence, the caged individuals were fed and watered, but no data except the date of death were collected.

Chapter 2: Highly variable lifespan in an annual reptile, Labord's chameleon (*Furcifer labordi*)

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Keywords: semelparity, lifespan, aging, reptiles, Madagascar

Eckhardt, F., Kappeler, P.M., Kraus, C. (2017). Highly variable lifespan in an annual reptile, Labord's chameleon (*Furcifer labordi*). Scientific Reports, 7(1), 11397. https://doi.org/10.1038/s41598-017-11701-3

Abstract

Among tetrapods, the current record holder for shortest lifespan is Labord's chameleon, *Furcifer labordi*. These reptiles from the arid southwest of Madagascar have a reported lifespan of 4 – 5 months during the annual rainy season and spend the majority of their life (8 – 9 months) as a developing embryo. This semelparous, annual life-history is unique among tetrapods, but only one population (Ranobe) in the southernmost distribution range has been studied. We therefore investigated the potential for environmentally-dependent variability in lifespan in a population in Kirindy Forest, which has a much longer warm rainy season. While no adults were found after March in Ranobe, the disappearance of adults was delayed by several months in Kirindy. Our data also revealed sex-biased mortality, suggesting that females have a longevity advantage. Furthermore, we found that, after an unusually long previous rainy season, one female was capable of surviving until a second breeding season. Keeping *F. labordi* in cages under ambient conditions demonstrated that also males can survive until the next season of activity under these conditions. Our study therefore revealed considerable variability in the extreme life-history of this tetrapod that is linked to variation in ecological factors.

Introduction

The fast-slow continuum is the dominant axis of life-history variation in tetrapods. Fast-living species are characterized by fast growth, high reproductive rates, high aging rates and short lifespans, compared to their slow-living counterparts (Dunham and Miles 1985, Saether 1998, Jones 2008). Among tetrapods, the most extreme example for short lifespan is provided by Labord's chameleon, *Furcifer labordi*. During their study in the arid southwest of Madagascar in Ranobe, Karsten et al. (2008) reported synchronous hatching of *F. labordi* with the onset of the annual rainy season in November. Here, early life of this chameleon is characterized by fast growth, resulting in sexual maturity at less than two months of age. After mating, senescent decline becomes apparent, and by the end of the rainy season in March, a population wide die-off of both sexes occurs. Thus, with an incubation period of 8 – 9 month, *F. labordi* spend the majority of their lifetime as a developing embryo in the egg, probably as an adaption to the highly seasonal climate. High adult mortality combined with relatively high juvenile survival might have ultimately selected for this semelparous, annual life-history (Charlesworth 1994, Charnov and Schaffer 1973).

Semelparity, the strategy to invest in only one mating event, is rare among tetrapods, including a few small-sized marsupial species from the families Didelphidae and Dasyuridae. However, in these marsupials the die-off following the mating season is restricted to males, while some females survive to breed a second time (Lee et al. 1982). Strong prey seasonality leading to a short breeding season has been proposed to explain obligate male semelparity in these marsupials (Fisher et al. 2013). Interestingly, males that were captured before the mating season and prevented from competing for mates survived for more than two years (Bradley et al. 1980, Schmidt et al. 2006). Less extreme cases of semelparity among marsupials have also been described. Here, facultative male die-off in the wild is restricted to some populations and/or only observed in some years and linked to variable resource availability due to variable climatic conditions (Dickman and Braithwaite 1992, Wolfe et al. 2004).

Furcifer labordi has so far only been studied in the southernmost and driest part of its distribution range (Karsten et al. 2008). We therefore conducted a field study of a population in Kirindy Forest, which is situated near the northern distribution range and characterized by a longer annual rainy season, to investigate potential intraspecific variation in lifespan. Since Madagascar is known for its high unpredictability in inter-annual rainfall (Dewar and Richard 2007), we also focused on differences in lifespan due to environmental variation between years. Additionally, we examined differences in sex-specific mortality because mortality varies by sex in some semelparous marsupials (Fisher et al. 2013). To characterize intrinsic rates of aging, we excluded extrinsic factors of mortality due to predation, costs of reproduction, fighting as well as water and food shortage by keeping some individuals of both sexes in field cages.

Materials and Methods

Study site: Kirindy Forest is a dry deciduous forest, in Western Madagascar (44°39′E, 20°03′S, 30 - 60 m above sea level). Climate is characterized by a hot rainy season (November – March, with on average 900 mm annual precipitation), followed by a cool dry season from (April–October) (Kappeler and Fichtel 2012). Sampling took place over three field seasons: November 18, 2013 – July 9, 2014, January 11 – July 15, 2015, and October 12, 2015 – December 17, 2015.

Capture-mark-recapture: *Furcifer labordi* were located at night using flashlights. They often roost on distal branches at a height of 0.5 – 3 m, and were therefore relatively easy to detect. Chameleons were either captured by hand or removed from higher branches using a stick to which they were encouraged to grip. We sampled alternating along two transects of 3 km length each. We had a recurring order of 10 sampling nights with a break of four nights within each field season. Each capture location was marked by a flag and a GPS recording was taken. Animals were taken to the research camp and handled the following morning. They were sexed and their age categorized (hatchling, juveniles and adults), and their snout-vent-length (SVL) was measured. Hatchlings were identified by the little opening of the abdominal wall due to the former connection with the yolk sack, which is visible during the first days after hatching. Growing individuals were assigned juvenile status until they were sexually mature. Female chameleons were identified as adult when the colourful mating patterns appear and males by the presence of a distinct hemipenis bulge and a hard, ossified rostral appendage. Chameleons were individually marked by visual implant elastomers (VIE; Northwest Marine Technology Inc., Shaw Island, WA). Hatchlings and small juveniles were individually marked by nail polish on the toes. Animals were released at their point of capture the next day. We measured juvenile growth rates and estimated adult sex ratios. To determine growth rate, we calculated daily growth rate from juveniles between their first capture and subsequent re-capture. We compared sex ratios monthly throughout the reproductive season using a χ^2-test.

Experimental housing

We collected juveniles in early January at around two months of age in the forest outside the transect system to exclude any influence on the study population. In total, 20 males and 20 females were housed separately without visual contact in cylindrical outdoor enclosures made of nylon screen (90 cm height, 60 cm diameter). The enclosures were equipped with branches and artificial plants. They were placed in a large outdoor cage in the forest, close to the research camp. Thus, they experienced largely the same temperature and daylight conditions as their conspecifics in the wild. Chameleons received a standardized amount of insect food, depending on age and size to match growth and final size of free-ranging animals. Specifically, chameleons were fed five times per week with two crickets, grasshoppers or butterflies. Water was offered daily with a spray flask. We used the Kaplan – Meier estimator (Bland and Douglas 1998) to assess survival probability of both sexes in captivity.

Ethics statement

All applicable international, national, and institutional guidelines for the capture and keeping of animals were followed. Research protocols, capture procedures, and keeping of chameleons were approved and permitted by the Ministry for the Environment, Water and Forests of Madagascar, MINEEF.

Results

Capture-mark-recapture study

Hatchlings were mainly found between mid-October and early December. Recaptures of marked individuals allowed us to estimate average daily juvenile growth rates for males (1.37%, n = 13, 0.76 ± 0.48 mm; mean ± SD) and females (1.18%, n = 12, 0.55 ± 0.27 mm; mean ± SD). We first found adult males in early January and adult females in late January. Average snout-ventral length (SVL) for adult males was 100.3 ± 8.62 mm (n = 344) and for females 73.3 ± 3.7 mm (n = 500). Gravid females appeared from the end of January onwards. Later in the reproductive season, we found that some females could reproduce more than once (n = 3, 4.6% of all re-captured adult females). We assumed a gestation period of maximally four weeks and recaptured these females at least five weeks after we noticed that they were gravid. Additionally, later in the reproductive season several gravid females (n = 48) showed abrasion on their wrist. These marks most likely resulted from excavation for the deposition of a previous clutch. While the sex ratio in juveniles was almost even, the adult sex ratio became highly female-biased across the reproductive season in 2014 (Feb to May-Jul $\chi^2 = 39.2$, df = 3, $P < 0.001$) and 2015 (Feb to Jun-Jul: $\chi^2 = 48.2$, df = 4, $P < 0.001$). In the season 2013/14, no males were found after May 27, while single females were detected until July 1 (Fig. 2.1A). In contrast, in the reproductive season 2015, males were detected until June 9, and several females were active until the end of the field season in mid-July, when some were still in good physical condition and even gravid (Fig 2.1B). In total, recapture rates were rather low (98 out of 881 captures; 11.12%) in the season 2013/14 and (33 out of 439 captures, 7.52 %) in the second season 2015 and (2 of 142 captures, 1.41%) in the third field season. At the beginning of the third field season, we encountered an adult female originating from the previous active season on October 29. This animal was in good physical condition (Fig. 2.1B). For further observation, we kept her in captivity. In December, she was put together with an adult male, which had been kept alive in captive field conditions, resulting in immediate mating. This female survived until March 2016, a presumed lifespan of 16 months.

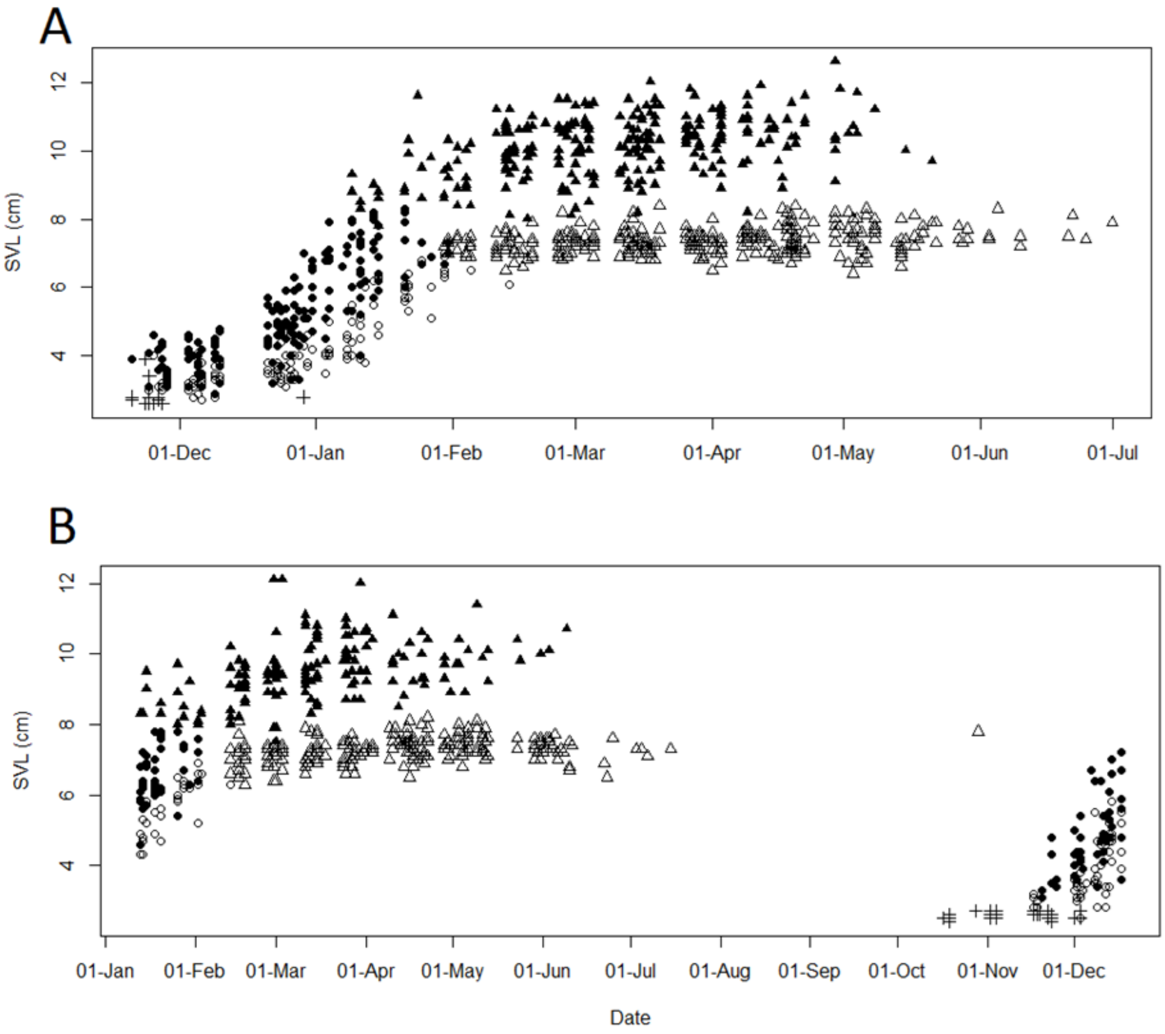

Fig. 2.1. Growth curves of *F. labordi* in Kirindy Forest. Depicted is SVL against date: unsexed hatchlings (+); males (filled symbols); females (open symbols); juveniles (circles); and adults (triangles). (A) Data of field season from November 19, 2013 – July 9, 2014 (n = 881), (B) Data of field seasons from January 11 – July 2015 (n = 439), and October 12 – December 17, 2015 (n = 142).

Experimental field enclosures – Keeping *F. labordi* in protected field conditions

We found no significant differences in survival probability between captive males and females (logrank-test: χ^2 =0.3, df = 1, P = 0.59). Median survival time for females was 9.5 months and for males 8.2 months. Maximum lifespan for females was 11.5 months and for males 16 months.

Two males and two females escaped from damaged cages during a cyclone. Moreover, three females died because of egg binding (Fig. 2.2).

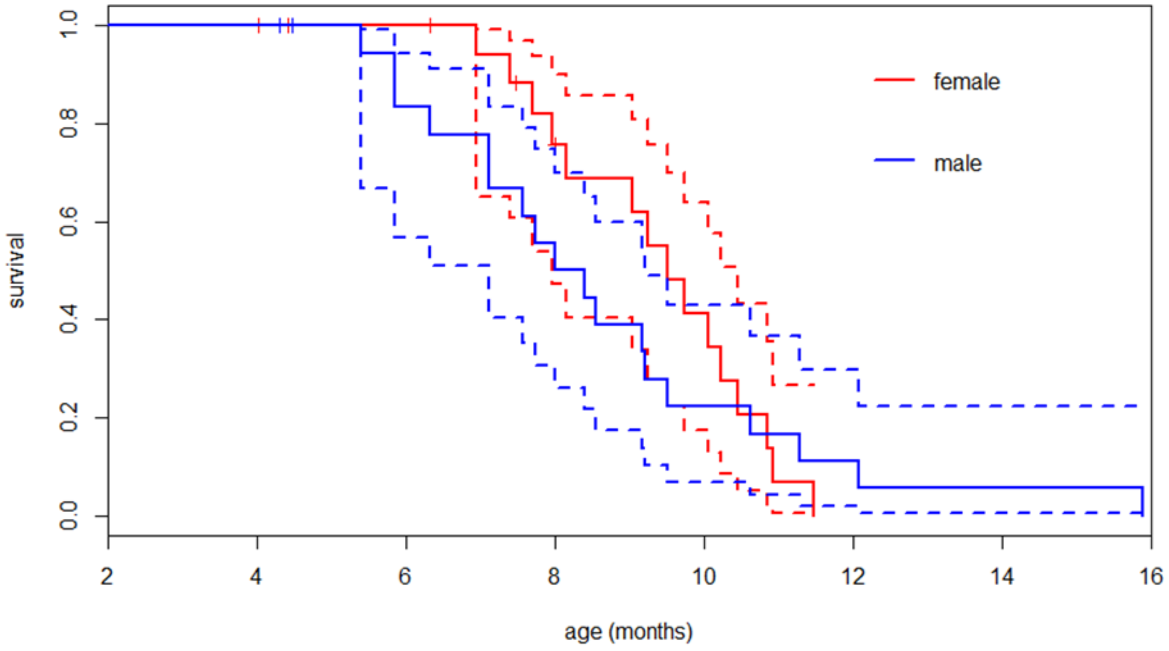

Fig. 2.2: Kaplan-Meier survival curves of *F. labordi* in captivity. Continuous lines indicate probability of survival of males (blue) and females (red). Dashed lines represent corresponding 95% confidence interval. Small vertical bars show censored events due to escape or death by egg-binding.

Discussion

Our study revealed considerable variability in the life-history of *F. labordi*, suggesting that their survival in nature is to some degree responsive to ecological conditions. During an average rainy season in 2013/14, lifespan ranged from 6 – 9 months and was therefore longer compared to longevity reported for the southern population. Both populations also differed in several aspects of their life-history, with all stages of life being more extended at Kirindy. Karsten et al. (2008) reported synchronous hatching and rapid daily juvenile growth rates of 1.86% in SVL in both sexes reaching maturity at less than 60 days. Hatchlings emerged around four weeks earlier at Kirindy and we observed slower juvenile growth rates, resulting in first sightings of adult males after 79 and adult females after 105 days, counted from the detection of first hatchlings. Karsten et al. (2008) hypothesized that females produce only one clutch in their lifetime. We recaptured females that were gravid at least twice, suggesting that females in Kirindy may lay more than one clutch and, hence, are not strictly semelparous. Furthermore, both populations show a considerable difference in male adult body size (SVL, Kirindy: 100.3 ± 8.62 mm, n = 344; Ranobe: 87.3 ± 1.3 mm, n = 99, (Karsten et al. 2009b). All these differences might be linked to higher annual precipitation levels and longer vegetation periods at Kirindy (900 mm) compared to Ranobe (420 mm) (Karsten et al. 2008).

We reviewed climate data for Kirindy (2005 – 2015) and found that rainfall during the second field season was, compared to the long-term average, exceptionally high, resulting in an extended vegetation period. Consequently, members of both sexes were detected about 2 weeks longer (minimum estimate for females), and one marked female was even found during the following active season. Thus, in rare cases, a very small proportion of adult females may survive the dry season under favourable environmental conditions and they may even enter the next reproductive season.

Our data revealed a remarkable difference in sex-specific mortality, indicating that females have a longevity advantage somewhat similar to semelparous marsupials (Lee et al. 1982). The obligate male die-off after reproduction in nature is likely related to intense intra-sexual competition (Karsten et al. 2009a, 2009b), as many adult males showed wounds or scars, especially at the head. Karsten et al. (Karsten et al. 2009b) reported potentially receptive females being very selective in terms of mate choice, compared to a larger perennial species. Intense intra-sexual selection might have led to physically intense male encounters with increasingly females-biased adult sex ratios. Additionally, in several other chameleon species, mate guarding was observed, and some males did not feed for several days (Cuarando 2001). We detected numerous males roosting close to females, which is suggestive of mate guarding in this species as well.

Keeping *F. labordi* in captive field conditions revealed that both sexes could outlive their conspecifics in the wild, showing that senescence can be delayed. Moreover, no significant differences in sex-specific mortality were found under these sheltered conditions. However, two males showed extended survival (12 and 16 months) compared to free-living conspecifics. These observations resemble that of some male semelparous marsupials, where lifespan was dramatically expanded by capturing them before the mating season (Bradley et al. 1980). Moreover, one of the *F. labordi* males was able to mate at the age of 13 months. Hence, lifespan might strongly depend on external causes of mortality that were excluded in captivity.

Despite the remarkable plasticity in lifespan of *F. labordi*, the accelerated life-history remains unique and several aspects favouring such a short lifespan remain unknown. Because *F. labordi* has perennial sympatric congeners, this chameleon assemblage constitutes a promising model for comparative investigations of the ecological and physiological determinants of longevity and senescence in wild tetrapods (Karsten et al. 2008).

Acknowledgements

We thank the Commission Tripartite de Direction des Eaux et Forêts, and CNFEREF Morondava for authorization and support for this study. We are thankful for support in the field to Camilla Chenni, Hauke Thiesler, Wanda von Bremen and the team of field assistants in Kirindy Forest.

Author contributions statement

F. E., P. K., and C. K. designed research and wrote the paper, F. E. conducted research, F. E. and C. K. analyzed data. All authors reviewed the manuscript.

Additional information

We have no competing financial interest.

Chapter 3. Life-histories, demographies and population dynamics of three sympatric chameleon species (*Furcifer* spp.) from western Madagascar

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Key words: *Furcifer labordi,* chameleon communities, lifespan, ageing, life-history

Eckhardt, F., Kraus, C., Kappeler, P.M. (2019). Life histories, demographies and population dynamicsof three sympatric chameleon species (*Furcifer* spp.) from western Madagascar. Amphibia-Reptilia, 40(1), 41-54. https://doi.org/10.1163/15685381-20181039

Abstract

The life-histories and population dynamics of chameleons remain poorly known, most likely due to practical challenges related to their cryptic nature. However, several studies have indicated that some of these reptiles have unusually brief life-histories. Specifically, one Madagascan chameleon (*Furcifer labordi*) was found to have an annual life cycle characterized by population-wide survival of the austral winter in the egg stage; a unique life-history among tetrapods. In this study, we compare the life-history of *F. labordi* with two locally sympatric congeners (*F.* cf. *nicosiai* and *F. oustaleti*) in Kirindy forest, western Madagascar, to determine how these species adjust their life-histories to a highly seasonal and unpredictable climate. We found differences in lifespan, timing of hatching, growth rates, survival, reproductive rates, adult body size, and roosting heights among all three species. Moreover, two species exhibited relatively short lifespans: 6 – 9 months in *F. labordi* and 16 – 18 months in *F.* cf. *nicosiai*. In contrast, *F. oustaleti* is perennial and large-sized juveniles and adults aestivate during the dry season, but survival rates of adults seemed relatively low. Strikingly, the annual cohort of *F. labordi* was already adult when hatchlings of *F. oustaleti* and subsequently *F.* cf. *nicosiai* emerged. Our study suggests the co-existence of three different life-histories with seasonal adjustment that might be related to the partitioning of overall food availability and contributes valuable life-history data on enigmatic chameleon species.

Introduction

Life-history theory predicts that a high risk of extrinsic adult mortality selects for fast growth, early maturation, high investment in reproduction and short lifespans, whereas high adult survival probabilities favour slow growth rates, delayed maturation, low reproduction output and long lifespans (Stearns 1992). Reptiles contain both, the longest-lived tetrapod species (tortoises [Patnaik 1994, De Magalhaes and Costa 2009]), as well as the shortest-lived one: Labord’s chameleon, *Furcifer labordi*. These chameleons from the deciduous dry forests in western Madagascar have a lifespan of only 4 – 9 months (Karsten et al. 2008, Eckhardt et al. 2017), probably as an adaptation to the biphasic and highly variable annual climate. At the beginning of the local rainy season in November, hatchlings emerge that grow rapidly, achieving sexual maturation after 2 – 3 months. Hereafter, a mating season characterized by intense combat among males and egg deposition of females ensues. With the beginning of the dry season in April, both sexes begin to disappear rapidly. As temperature drops towards the dry season, eggs are presumably in torpor for several months, while embryonic development probably continues from August on until a new cohort of hatchlings appears with the onset of the next rainy season (Karsten et al. 2008).

Interestingly, there seems to be considerable intraspecific variability in lifespan of this species related to variation in ecological factors, such as precipitation and food availability, indicating that some life-history traits exhibit regional variability in response to ecological variation. While in the southernmost and driest part of their distribution range (Ranobe Forest, ca. 30 km north of Toliara), both sexes disappear synchronously at 4 – 5 months of age (Karsten et al. 2008), lifespan near the northern distribution range (Kirindy Forest, ca. 60 km northeast of Morondava) is prolonged in both sexes to 6 – 9 months (Eckhardt et al. 2017). Males from Kirindy were also significantly larger than their conspecifics from Ranobe (Eckhardt et al. 2017). Moreover, following an unusually long rainy season, one female at Kirindy survived until the next breeding season, and some caged individuals of both sexes survived the entire austral winter (Eckhardt et al. 2017).

While no other chameleon was yet reported to have such a short lifespan, little is known about the nature of life-histories of most other chameleon species. The few existing studies indicate that chameleons as a group are characterized by relatively fast life-histories. A study of Andreone et al. (2005) revealed that panther chameleons (*F. pardalis)* show rapid growth rates and rarely live longer than one year. Furthermore, a study of Dwarf chameleons (*Bradypodion pumilum)* documented annual survival of about 5% (Tolley et al. 2010). Moreover, a study of mortality rates of captive reptiles indicated that chameleons were the group in which probability of death within one year after purchasing was highest (Robinson et al. 2015). On the other hand, a recent study on one of the largest chameleon species Parson's chameleon (*Calumma parsonii)* revealed that some specimens survived up to 9 years in nature (Tessa et al. 2017). Therefore, additional studies are indicated to unravel the factors favouring the evolution of life-histories in this lizard family.

Labord's chameleon occurs sympatrically with other *Furcifer* species, making this lineage particularly well suited for comparative studies of interspecific life-history variation and its ecological determinants. In relation to other members of their genus, *F. labordi* is relatively small-sized exhibiting a mean snout-vent length (SVL) of 100.3 ± 8.52 mm for males and 73.3 ± 3.7 mm for females), *F.* cf. *nicosiai* a medium-sized (mean SVL 136.4 ± 13.6 mm for males, 102 ± 5.2 mm for females) and *F. oustaleti* a large-sized (mean SVL: 273.5 ± 7.5 mm for males, 170.2 ± 9.6 mm for females) species. While *F. labordi* and *F.* cf. *nicosiai* are restricted to the remaining intact forests within their distribution range in western Madagascar, *F. oustaleti* is also relatively common in disturbed areas and has a vast distribution (Glaw and Vences 2007). Regarding the nomenclature of *F.* cf. *nicosiai*, the study population in Kirindy Forest is similar to *F. nicosiai* described from PN Tsingy de Bemaraha (Jesu et al. 1999). However, Randrianantoandro et al. (2010) mentioned some morphological differences between both populations and named the population occurring in the Menabe Central region *F.* cf. *nicosiai*, which we maintain here.

The specific aim of this study was to compare the life cycles of these three locally sympatric *Furcifer* species, focusing on population densities, survival, growth and reproductive rates. Moreover, we examined interspecific variation in roosting heights, which might be indicative of potential spatial niche segregation. Additionally, as the climate of Madagascar is characterized by highly unpredictable rainfall patterns from year-to-year (Dewar and Richard 2007), we also investigated inter-annual variation within species to compare intraspecific plasticity in life-histories. As chameleon populations might heavily depend on environmental factors such as rainfall, primary production and therefore insect occurrence, we correlated our findings to fluctuation of green vegetation cover. Here, we used the Normalized Difference Vegetation Index NDVI (Myneni et al. 1995) to determine the relationship between vegetation alteration and chameleon population dynamics.

Materials and Methods

Study site

Kirindy Forest is located in the region of Menabe Central, Western Madagascar, *ca.* 60 km northeast of Morondava (44°39′E, 20°03′S, 30 – 60 m asl). It is one of the largest remaining Malagasy dry deciduous forest fragments that has historically undergone selective logging, mainly for *Dalbergia* spp. (Zinner et al., 2014). The local climate is characterized by a hot rainy season between November and April, followed by a cool dry season from May to October (Kappeler and Fichtel 2012).

Study species

Furcifer labordi is a medium-sized and sexually highly dimorphic chameleon from the western and southwestern regions of Madagascar. Males have a snout-vent length (SVL) of approx. 100 mm and exhibit a large cranial casque, a well-defined rostral appendage and a continuous dorsal crest. Ground colouration is a light green with a white lateral stripe. Females have an average SVL of 73 mm and offer a poorly developed dorsal crest situated in the nuchal region. Ground colouration is green with blue and violet blotches on the flanks and dorsally orientated bright orange markings (Glaw and Vences 2007).

Furcifer* cf. *nicosiai is a relatively large and seems to be associated with intact dry forests (Jesu, et al. 1999, Glaw and Vences 2007). Males have a SVL of approx. 136 (this study; see below) mm and a continuous dorsal crest extending on the tail. Females have a SVL of approx. 102 mm (see below) and a poorly developed short dorsal crest emerging from the nuchal region. Ground colouration of both sexes is a pale brown with diffused brown marbling and a white stripe, which is more dominant in males. During the mating season, males exhibit a bright yellow latero-ventrally orientated colouration. The body of the females appears in a reddish colouration accompanied by bluish cheeks. The population in the Kirindy Forest is similar to *F. nicosiai* that was described from the PN Tsingy de Bemaraha (Jesu et al. 1999), but morphology indicates some differences between both populations (Randrianantoandro et al. 2010). We therefore refer to the individuals from the population in Kirindy as *F.* cf. *nicosiai* to indicate that this taxon in need of further taxonomic investigation.

Furcifer oustaleti is one of the largest chameleon species growing to 273 mm in SVL in males and widespread throughout Madagascar (Glaw and Vences 2007). Males possess a high casque and a notable continuous dorsal crest. Ground colouration is a grayish brown with a reddish throat. Females have a SVL of approx. 170 mm and exhibit a smaller continuous dorsal crest and ground colouration is more variable.

Vegetation cover: Normalized Difference Vegetation Index (NDVI)

The NDVI has been reported to correlate strongly with above-ground net primary productivity (Myneni et al. 1995; see also http://daacmodis.ornl.gov). The index is obtained from the reflection rate of red light RED and near infrared light NIR [NDVI = (NIR – RED)/(NIR + RED)], in which NIR and RED are the amounts of near infrared light and red light that is reflected by the vegetation and recorded by the satellite's sensor. The formula results from the fact that chlorophyll strongly absorbs RED whereat NIR is re-emitted. To compare seasons with each other, we measured the proportion of vegetation cover from the beginning of the rainy season (Nov – Dec), from the peak until the end of the rainy season (Jan – Apr) and during the dry season (May – Oct) from November 2013 until December 2015. For analysis of the NDVI, we selected a square in the center of our study site.

Capture-mark-recapture study

Chameleons were located at night using LED flashlights. The capture location was marked and GPS data were taken. We sampled alternating along two transects of 3 km length each. We had a perseverative order of 10 sampling nights with a pause of four nights in each field season. Animals were transported to the nearby research station in cloth bags and handled the following morning. They were sexed, age categorized (hatchling, juvenile, adult), and their snout vent length (SVL) was measured. Hatchlings were identified due to a little opening of the abdominal wall caused by former connection with the yolk sack. In all three species, growing individuals were classified as juveniles until sexual maturity, which was inferred from the appearance of a colourful mating pattern in females and the presence of a distinct hemipenis bag in males. Animals were individually marked by visual implant elastomers (VIE; Northwest Marine Technology Inc., Shaw Island, WA) (MacNeil et al. 2011). Hatchlings and small juveniles were individually marked with nail polish on the toes. All chameleons were released at their point of capture within 12 h. Sampling took place over three field seasons: November 19, 2013 – July 8, 2014, and January 11, 2015 – July 15, 2015, and October 12, 2015 – December 17, 2015. *Furcifer oustaleti* were only sampled in the second and third field season.

Growth rates

We calculated the growth in snout-vent length (SVL) for each species per sex and month. We took the estimated point of hatching and calculated average SVL of hatchlings as a starting point for subsequent calculations. To assess intraspecific differences in growth and SVL between years, we used a *t* - test to compare specimens captured in March, as sample size was highest in this month for adults of *F. labordi* and *F.* cf. *nicosiai*. Moreover, we showed the percentage of SVL difference from 2014 and 2015 in *F. labordi* from January until May and *F.* cf. *nicosiai* from January until April, when the amount of specimen was sufficient to compile an average SVL (≥ 5 individuals/sex).

Survival probability (recapture rates)

To estimate survival probabilities, we calculated the percentage and frequency of recaptured chameleons with regard to species, sex and age. Here, we treated *F. labordi* as one cohort and distinguished between juveniles and adults in the two other species. The cohort entering the rainy season was considered as adult. Individuals that hatched during the rainy season were considered as juveniles.

Reproductive rates

The abdomen of each collected adult female was palpated for the presence of eggs in the abdominal cavity. We calculated the percentage of gravid females per month during the entire reproductive period to estimate reproductive rates.

Roosting heights

For each detected specimen, we recorded roosting height to the nearest 0.1 m with a telescopic pole. We compared roosting heights among adults of all three species with ANOVA. When comparing roosting heights within species between different years, we used a *t* - test. Here, we compared roosting heights between the years 2014 and 2015 that were measured from February until May to determine inter-annual variation within species that might be related to differences in rainfall patterns.

Results

Normalized Difference Vegetation Index (NDVI)

The average vegetation cover in the beginning of the rainy season in 2013/14 (Nov – Dec) showed a proportion of 0.586 ± 0.045 SD, followed by 0.8265 ± 0.06 SD (Jan – Mar) and 0.6349 ± 0.1063 SD in the dry season (May – Oct). The average vegetation cover at the beginning of the rainy season 2014/15 (Nov – Dec) was 0.5147 ± 0.038 SD, followed by 0.8507 ± 0.008 SD (Jan – Apr) and 0.7123 ± 0.078 SD in the dry season (Apr – Oct). Comparing seasons among each other revealed that the beginning of the rainy season in 2013/14 was characterized by more vegetation cover than in 2014/15. However, the following rainy and dry season in 2015 showed remarkably more vegetation than in 2014, following heavier rainfalls. Reviewing average vegetation cover from 2006 until 2015, we calculated an average value of

0.6415 ± 0.1272 SD for the beginning of the rainy season, 0.8382 ± 0.048 SD for the rainy season and 0.6395± 0.095 SD for the dry season. Comparing the field season with the average vegetation cover with the ten-year average, we found that the beginning of the rainy seasons showed relatively little vegetation cover in 2013/14 and especially in 2014/15. The rainy season in 2014 showed marginally lower, and in 2015 marginally higher proportion of vegetation cover. The dry season in 2014 was rather average, whereas the dry season in 2015 showed remarkably higher vegetation cover (Tab. 1). The high vegetation cover in this dry season was followed by an above average vegetation cover in the beginning of the subsequent rainy season.

Tabel 1. Averaged NDVI for the onset of the rainy season (Nov – Dec), rainy season (Jan - Apr), and dry season (May – Oct).

Year	onset rainy season	rainy season	dry season
2013/14 (Nov – Oct)	0.586 ± 0.045	0.8265 ± 0.06	0.6349 ± 0.1063
2014/15 (Nov – Oct)	0.5147 ± 0.038	0.8507 ± 0.008	0.7123 ± 0.078
2006 – 2015	0.6415 ± 0.1272	0.8382 ± 0.048	0.6395 ± 0.095

Age, population structure and life-histories

Furcifer labordi

We detected first hatchlings in mid-October, at the beginning of the rainy season. This cohort grew up and reached maturity by February. After the mating season, males disappeared in late May, whereas the last females were found at the beginning of July. However, after an unusually long rainy season (2015) both sexes survived considerably longer. For more detailed information, see Eckhardt et al. (2017) and Fig. 3.1.

Furcifer cf. *nicosiai*

At the beginning of the rainy season in November 2013, we found a cohort of juveniles that had hatched during the previous active season. We encountered first adult males around mid-January and the first adult females at the beginning of February. After this date, all collected individuals from this cohort were sexually mature. Hatchlings were found around mid-February. In the season 2013/14, the last adult male was encountered on June 10, and the last adult female at the end of June. In the second season in 2015, however, adult males and females were encountered until the end of the field season in mid-July. At this point, some adults seemed to be in good physical conditions, whereas others showed visible signs of senescence. During the third field season in October 2015, we detected a cohort of juveniles as well as two adult females that had overwintered from the previous dry season. One of these females was marked in the previous season and subsequently recaptured several times in good physical condition (Fig. 3.1).

Furcifer oustaleti

In the second field season 2015, we encountered a cohort of large juveniles originating from the previous rainy season that were close to maturation. Additionally, we detected adult individuals that hatched at least in 2013. In mid-January, we observed hatchlings. Afterwards, we detected exclusively adults and young juveniles that grew up rapidly. During the final field season, we found both large juveniles that hatched in the previous rainy season and adults (Fig.3.1).

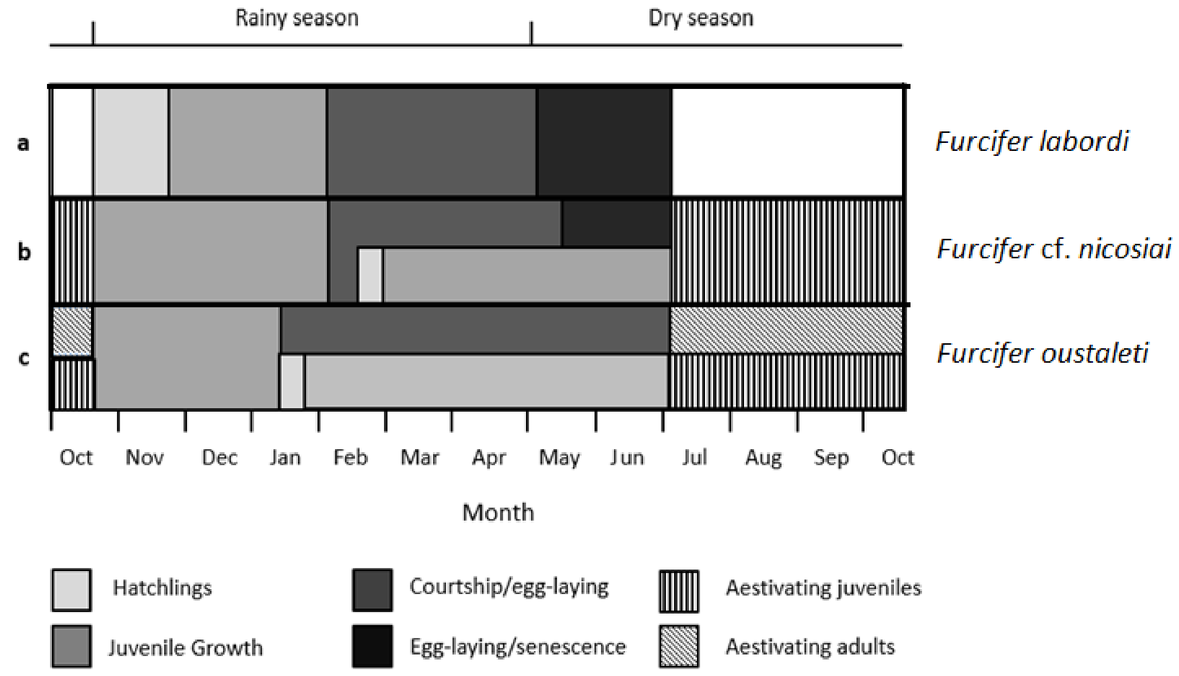

Fig. 3.1. Averaged life-histories of the three *Furcifer* species in Kirindy Forest. Upper row **a** shows the life-history of the annual *F. labordi*. Hatching takes place at the beginning of the rainy season, followed by rapid growth and maturation and subsequently population wide die-off. Middle row **b** displays the life cycle of *F.* cf. *nicosiai*. With the beginning of the rainy season, a cohort of previously aestivating juveniles restarts growing until maturation in February. Adults disappear after the reproductive season. Juveniles hatch in mid-February and grow until the beginning of dry season with subsequent aestivation. Lower row **c** indicates the life cycle of *F. oustaleti*. Large juveniles grow fast to maturation and adults enter the rainy season. A new cohort of hatchlings emerges in mid-January that grows until the onset of the dry season.

Species abundances

In the second field season, *F. labordi* were the most frequently found chameleons until May, followed by a decline towards the dry season. Adult *F.* cf. *nicosiai* represented the second most abundant category of animals captured between January and March, with a subsequent decline in detection towards the onset of the dry season. From January (month of hatching) onwards, the number of detected juvenile *F. oustaleti* continuously increased. We found similar pattern for juvenile *F.* cf. *nicosiai* from February onwards (month of hatching). The number of recorded adult *F. oustaleti* was low throughout the season (Fig. 3.2).

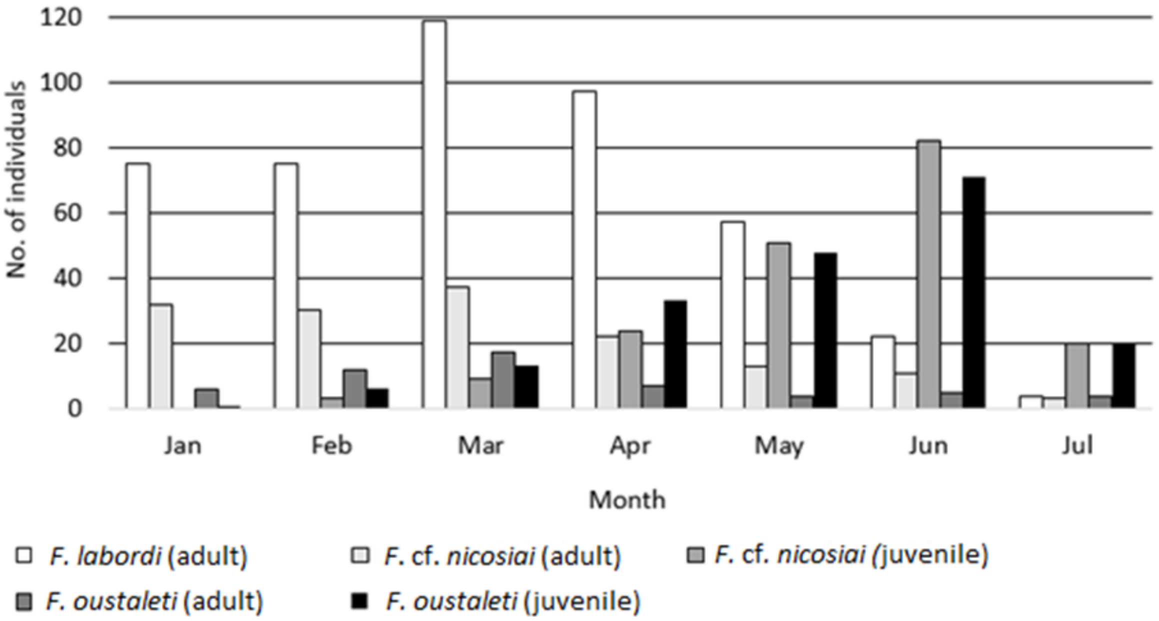

Fig. 3.2. Abundance of *Furcifer labordi* and adult and juvenile *F.* cf. *nicosiai* as well as adult and juvenile *F. oustaleti* from January to July 2015.

Survival probability (rate of recaptures)

The lowest rate of recaptures was found for *F. labordi*. Comparing adult *F.* cf. *nicosiai* with *F. oustaleti*, we found about twice as many individuals in the former species, whereas the probability to re-capture an adult *F. oustaleti* several times was much higher. Juvenile *F. oustaleti* had the highest percentage of repeated recaptures (see Fig. 3.3).

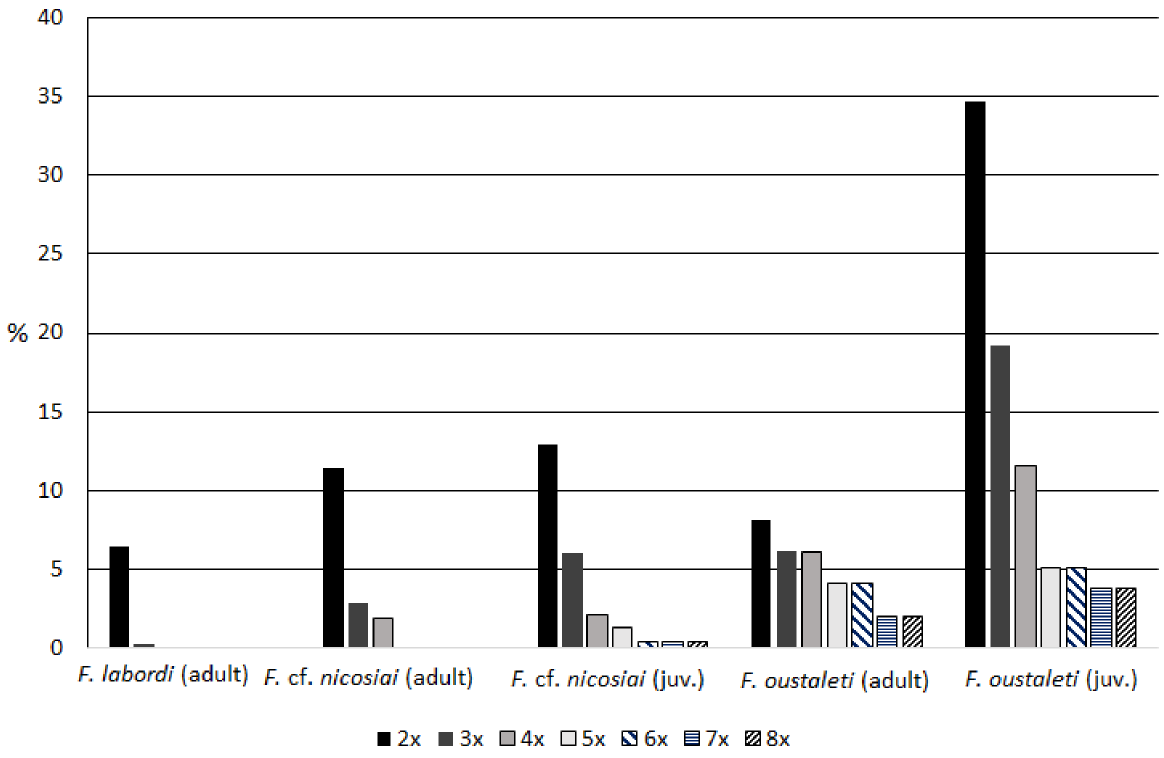

Fig. 3.3. Percentage of chameleons that were recaptured at least twice, or multiple times in the second field season between January and July 2015. Sample size was 439 for *Furcifer labordi*, 123 for adult *F.* cf. *nicosiai*, 205 for juvenile *F.* cf. *nicosiai*, 40 for adult *F. oustaleti* and 178 for juvenile *F. oustaleti.*

Growth rates

Furcifer labordi

We found first hatchlings at mid-October and considered this as the hatching date to calculate subsequent growth rates. We measured an average SVL for *F. labordi* hatchlings of 2.56 ± 0.09 cm ($n = 20$). Even after maturation, growth seemed to continue in both sexes (Fig. 3.4). From the point of hatching (mid-October) until the beginning of the dry season (May) males gained 316% in average SVL and females gained 190.6% in average SVL. In 2014, average adult SVL was significantly larger in both males ($t = 3.89$, $P < 0.001$) and females ($t = 2.41$, $P < 0.05$) compared to 2015 (Fig. 3.5).

Furcifer cf. *nicosiai*

In the first and second field season, we detected hatchlings in mid-February. Their average SVL was 3.08 cm ± 0.09 cm (*n* = 4). In 2014, juvenile males gained 113% in average SVL and females gained 67.2% in average SVL until the onset of the dry season. In 2015, males average SVL increased by 107.8% and females average SVL increased by 60.4% of SVL until the onset of the dry season. As in *F. labordi* in 2014, average SVL was significantly larger in adult males ($t = 3.51$, $P < 0.001$) and females ($t = 3.74$, $P = < 0.001$) compared to 2015 (Fig. 3.5). In both seasons, we found remarkably more juvenile males (*n* = 319, 71.8%) than females (*n* = 125, 28.2%). In average, males gained 342% and females 231% in SVL from the size of a hatchling until adult body size.

Furcifer oustaleti

In mid-January 2015, we found two hatchlings with an average SVL of 2.5 cm ± 0.14 cm. Both sexes grew rapidly, gaining 510% of average SVL in males and 424% of average SVL in females until the onset of the dry season. Within this cohort, we found more females (*n* = 104, 58.4%) than males (*n* = 74, 41.6%) (Fig. 3.4). In total, males gained in average 992% in SVL and females 580% from the size of a hatchling until adult body size.

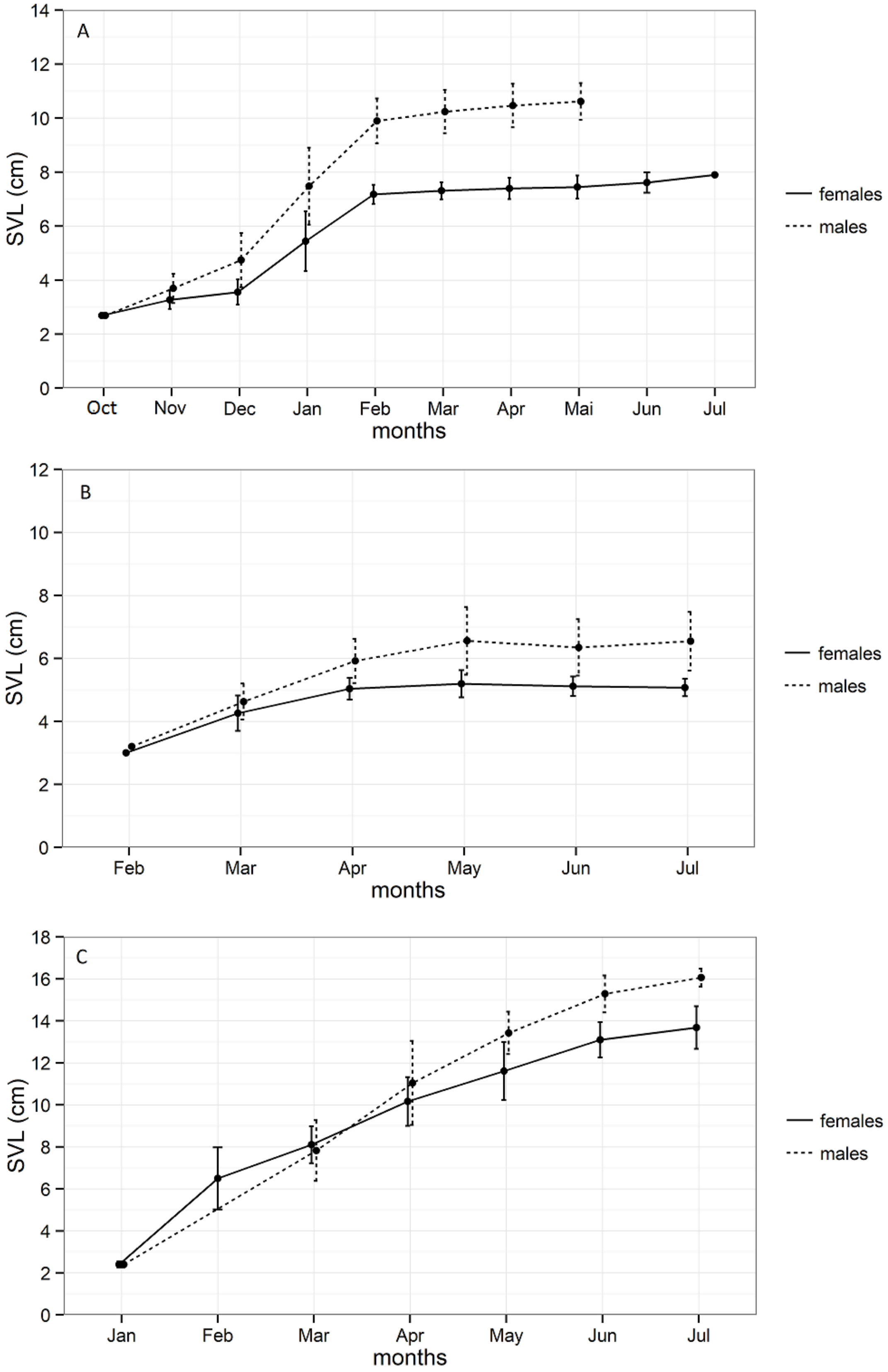

Fig. 3.4. Growth curves of (A) *F. labordi* in 2013/14, (B) *F.* cf. *nicosiai* in 2014 (C) *F. oustaleti* in 2015, Error bars indicate standard deviations.

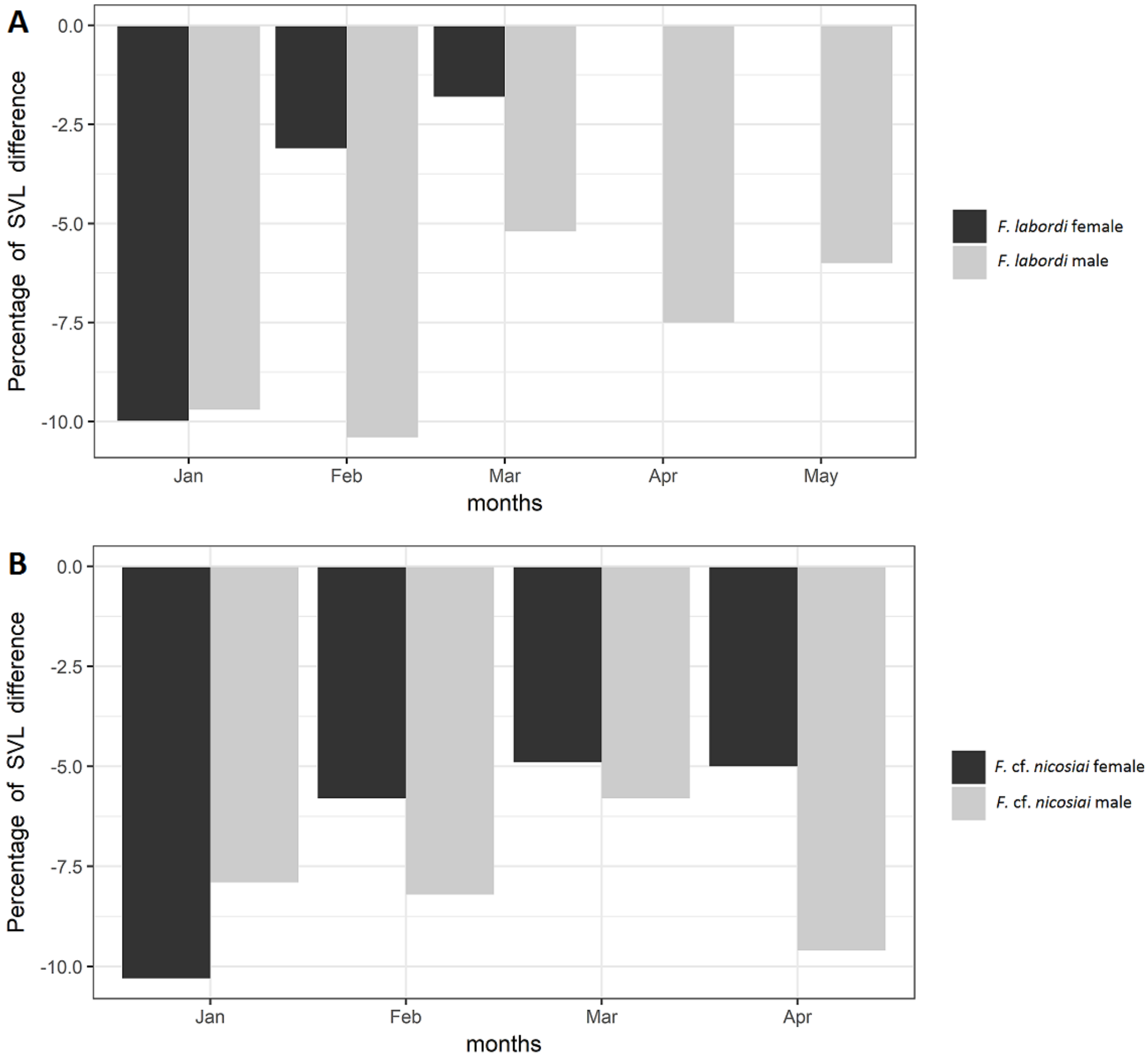

Fig. 3.5. Percentage of SVL difference of (A) *F. labordi* and (B) *F.* cf. *nicosiai* between 2014 and 2015. Zero line reflects the average SVL per month in 2014.

Reproductive rates

Furcifer labordi

In the reproductive season 2014, we first detected gravid females at the end of January. From this date on, we regularly found gravid females, and their percentage increased steadily (Tab. 2), whereas their total number decreased from the end of April onwards. During the reproductive season 2015, we found first gravid females at the beginning of February. Again, the percentage of gravid females increased towards the end of the reproductive season, and the total number of females decreased from the end of April onwards (Tab. 2). In total, we found four dead gravid females containing 6 – 8 eggs in their bodies. Eggs of one female containing 7 eggs had a mean length of 1.24 ± 0.03 cm and weight of 0.375 ± 0.01 g. Another clutch of 8 eggs showed a mean length of 1.16 ± 0.01 cm and a weight of 0.313 ± 0.01 g.

Furcifer* cf. *nicosiai

In the reproductive season 2014, we found two gravid individuals (11.8%) among 17 adult females. One gravid female was found in mid-February and the other one in mid-March. During the reproductive season 2015, we detected 41 females of which none seemed to be gravid. Subsequently, within the reproductive time of this species we found 58 adult females, of which two were definitely gravid (3.45%).

Furcifer oustaleti

During the reproductive season in 2015, we encountered 12 adult females of which not a single one was detected as gravid.

Table 2. Total number of detected *Furcifer labordi* females per month and number of gravid females during reproduction time in 2014 and 2015.

month/year	**tot. no. of females**	**no. of gravid females**	**% gravid females**
Jan 2014	55	1	1.81
Feb 2014	63	17	26.98
Mar 2014	88	27	30.68
Apr 2014	98	52	53.06
May 2014	54	34	62.96
Jun 2014	8	6	75
Jul 2014	1	1	100
Total 2014	**367**	**138**	**37.6**
Jan 2015	25	0	0
Feb 2015	37	6	16.21
Mar 2015	54	12	22.22
Apr 2015	50	22	44
May 2015	45	25	55,56
Jun 2015	20	15	75
Jul 2015	3	3	100
Total 2015	**234**	**83**	**35.47**

Roosting heights

When evaluating roosting heights of all three species (reproductive season 2015, Feb – May), we found a highly significant difference in roosting heights among the three species (ANOVA, $F_{2, 428} = 80.15$, $P < 0.001$). Here, *F. labordi* showed an average roosting height of 1.28 ± 1.13 m. *Furifer* cf. *nicosiai* exhibited a roosting height of 1.93 ± 1.1 m. The roosting site of *F. oustaleti* averaged 3.68 ± 1.51 m. During the reproductive season 2014 (Feb – May) we found that the average roosting height of *F. labordi* was significantly lower compared to adult *F.* cf. *nicosiai* ($t = -5.98$, $P < 0.01$). Both, *F. labordi* showed heigher roosting heights $t = 8.07$, $P < 0.001$) in 2014 compared to 2015 as well as *F.* cf. *nicosiai* ($t = 4.54$, $P < 0.01$) (Fig. 3.6).

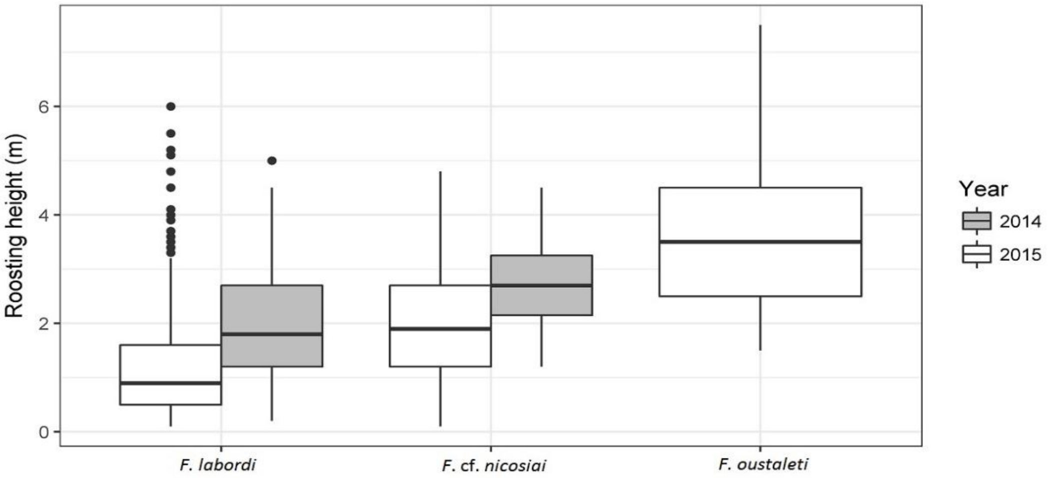

Fig. 3.6. Boxplots showing roosting heights of adult specimens of *F. labordi*, *F.* cf. *nicosiai* in the reproductive season in 2014 and *F. labordi*, *F.* cf. *nicosiai* and *F. oustaleti* in the reproductive season 2015.

Discussion

Age, population structure and life-histories

Our study revealed considerable differences in life-history characteristics across three locally sympatric *Furcifer* species. We detected differences in the period of hatching, species abundance, survival probability, growth, reproduction and roosting heights. Hatchlings of *F. labordi* appear from mid-October onwards, and this cohort is already adult by the time hatchlings of *F. oustaleti* and *F.* cf. *nicosiai* appear. As juveniles of all three species are of similar size, the temporal shift in species-specific occurrence of hatching suggests resource portioning as an underlying factor. Since the increase in ambient temperature is considered to initiate development of diapausing embryos in chameleons (Andrews and Donoghue 2004; Andrews et al. 2008), the three species might have different temperatures terminating diapause. Moreover, we found different lifespans that correlated positively with body size [see also Speakman 2005] in combination with male-biased early mortality, which might be related to sex-specific costs of reproduction (Cuadrado 2001, Gehring et al. 2008). Finally, we documented some variability in the lifespan of *F. labordi* and *F.* cf. *nicosiai*, suggesting that their survival is related to the availability of suitable prey, water and habitat.

Compared to other lizards, chameleons seem to have remarkably shorter lifespans. Published reports of life-histories of chameleons in nature are rare, but available studies suggest that this family displays short lifespans in nature (Andreone et al. 2005, Karsten et al. 2008, Tolley et al. 2010). These findings also indicate that the reported high mortality rates of chameleons in captivity (Robinson et al. 2016) are due to their naturally fast life-histories.

An important variable of the life-history is age at maturity, which in lizards is generally positively correlated with body size (Meiri et al. 2012). However, several medium-sized Madagascan chameleons, such as *F. campani*, *F. lateralis* and *F. antimena*, are known to reach maturity within one season (Raselimanana and Rakotomalala 2003), suggesting a short lifespan in these species as well. Moreover, compared to many other similar-sized lizard taxa, chameleons have the largest clutches and are among the most productive ones (Meiri et al. 2012), indicative of fast life-histories. In addition, large clutch volumes and high relative clutch mass have predominantly been documented in reptiles with cryptic behaviour, where the probability of escaping predators does not depend on rapid movements (Vitt and Price 1982).

The short documented life cycles in chameleons might be due to their inability to store sufficient fat reserves to endure energy-demanding periods such as the mating season or aestivation. As most chameleons have a laterally compressed body and depend on their ability to hide behind branches, the storage of fat reserves might be disadvantageous in terms of predation avoidance. In contrast to many other lizards, chameleons do not store fat in their tail (Pond 1978). Additionally, chameleons rely on camouflage, but are the slowest of all lizards (Abu-Ghalyun, 1988), and when they are detected by a predator they have only a small probability of escaping. Ultimately, the reduced possibility of survival after detection of a predator and inability to store fat to endure strenuous periods might have selected for fast live cycles. The restricted time and probability of reproduction may also have driven the often mentioned high aggression in males (e.g. Nečas 1999, Karsten et al. 2009a) probably resulting in intense intra-sexual selection in this group. Especially males offer large casques that are a good indicator of bite force as males bearing higher heads probably have more jaw musculature, and in some species, casque height is positively correlated with fighting outcome (Stuart-Fox et al. 2006).

However, one of the largest chameleon species Parson's chameleon *Calumma parsonii* takes 3 to 5 years to reach maturity (Brady and Griffiths 1999). A recent study of this species based on skeletochronology of preserved specimens found that the highest minimum age was nine years for one male and eight years for a female, affirming a considerably longer lifespan (Tessa et al. 2017). This species occurs in rainforests where environmental conditions are more stable, which might explain the slower life-history compared to the ones reported here. Given the lack of long-term studies, further examinations are necessary to evaluate differences in life-histories from chameleons originating from seasonal or more stable habitats.

Species abundances

We found that seasonality had an influence on species composition. In the second field season, we compared the abundances of *F. labordi*, *F.* cf. *nicosiai* and *F. oustaleti* directly. The cohort of adult *F. labordi* was the most abundant one until May, while the number of adult *F.* cf. *nicosiai* was relatively low, followed by adult *F. oustaleti*. Nevertheless, it is possible that the number of adult *F. oustaleti* was underestimated since they chose the highest roosting places and might therefore sometimes not have been detected. Regarding adult cohorts in the rainy season, our findings support those of Randrianantoandro et al. (2010), who examined chameleon populations in western dry forests of Madagascar. We add to this information by showing that there are pronounced seasonal fluctuations in species abundance and the timing of age cohorts.

From their point of hatching, the detection of juveniles *F. oustaleti* and *F.* cf. *nicosiai* increased substantially. From June onwards, juveniles of *F.* cf. *nicosiai* followed by *F. oustaleti* were the most abundant cohort. The fact that only a few hatchlings were found in both species is probably due to the fact that the forest is highly foliated during this period, hampering detection. Hatchlings of *F. labordi* were found in November, when the forest is not yet abundantly covered with vegetation.

Survial rates

The probability and frequency of recaptures was lowest in the cohort of *F. labordi.* Here, besides intrinsic mortality risks with age, the declining detection over time may be due to external sources of mortality, such as predation and starvation. The probability to capture an adult *F.* cf. *nicosiai* for a second time was higher compared to *F. oustaleti,* but the probability for repeated recapture was higher in *F. oustaleti,* indicating some survival advantages in this cohort. Highest number of recaptures were for juvenile *F. oustaleti,* followed by juvenile *F.* cf. *nicosiai,* suggesting that the survival probability until the following reproductive season is relatively high. The patterns of survival as a function of body size and age at maturation coincide with those of lizards documented by Shine and Carnov (1992).

Growth rates

Furcifer labordi exhibited fast growth, *F. oustaleti* very fast growth and *F.* cf. *nicosiai* relatively slow growth. As an annual species, *F. labordi* invests all available energy into growth and reproduction in one season. *Furcifer oustaleti* hatches around the peak of the rainy season when insect food is abundant, enabling fast growth rates. At the onset of the dry season, juveniles are large-sized, which might provide an advantage with respect to predation. Since the cohort of *F.* cf. *nicosiai* hatches later in the rainy season, the period when insects are abundant is shortened, and probably results in slower growth. Conversely, their small size might protect these juveniles from detection of predators during the dry season. At least in *F. labordi* and *F.* cf. *nicosiai* we were able to show gradual growth after maturation. As fecundity in most female reptiles often increases with increasing SVL (Meiri et al. 2012), continuous growth might provide an advantage for further clutches.

Interestingly, adult *F. labordi* and *F.* cf. *nicosiai* had smaller average SVL in the reproductive season in 2015 compared to 2014. The smaller SVL might be explained by the lower vegetation cover at the onset of the rainy season in 2014/15. Additionally, heavy rainfalls and heavy cyclones in 2015 might have interfered with foraging. These observations indicate that growth rates and adult SVL in these species might depend strongly on environmental factors. Diaz-Paniagua et al. (2002) found similar pattern in females of the common chameleon (*Chamaeleo chamaeleon*), which exhibited smaller SVL in drier years. In contrast to our findings, Karsten et al. (2008) reported ceasing growth in adults with the onset of the reproductive season, and even negative growth was found in a small set of marked-recaptured males. Reptiles as a group are known for indeterminate growth after maturation (Halliday and

Verrell 1988). The stagnating or even negative growth might be explained by the reduced availability of resources, inducing these specimens to invest more energy into fast reproduction.

Reproduction rates

As the smallest of our study species, *F. labordi* has the smallest clutch size (6 – 8 eggs). Although, we have no information about the clutch size of *F.* cf. *nicosiai*, we estimate that females of this species to lay 10 – 30 eggs, as known from similar-sized *F. pardalis* females (Müller et al. 2016, Gehring and Althaus 2017). *Furcifer oustaleti* was recorded to have an average clutch size of 42 eggs (n = 24) (Smith et al. 2016). These findings are in accordance with those reported by Meiri et al. (2012), who found that lizard species with larger mean adult female SVL tend to have larger clutch sizes. Interestingly, though adults of all three species differ significantly in SVL, hatchlings were similar-sized. In *F. pardalis*, Dierenfeld et al. (2002) mentioned that SVL from neonates range from 25 – 30 mm. As documented in our study, larger chameleon species need longer to reach maturity, but survival probability over time is comparatively low, which might drive their investment in a larger numbers of eggs, rather than larger offspring.

Roosting heights

We found interspecific differences in roosting heights among all three species that might be related to habitat and food partitioning. Our findings resembled those of a previous study in western Madagascar (Randrianantoandro et al. 2010). Roosting site selection by chameleons remains poorly investigated but is probably related to the avoidance of nocturnal predators (e.g. snakes), as well as foraging opportunities the next day. Additional data on behaviour and feeding biology are necessary to develop a better understanding of whether differences in the roosting heights are related to diurnal resource partitioning.

Interestingly, the roosting heights of *F. labordi* and *F.* cf. *nicosiai* differed between 2014 and 2015. Both species seemed to prefer lower roosting heights in 2015, perhaps due to extremely heavy rainfall. Nevertheless, a denser foliage during the latter rainy season might have had a negative influence on the detection of individuals at higher roosting places. Some authors suggested that chameleons shift roost sites depending on weather conditions, such as rainfall, wind and cold temperatures, resulting in roosting sites on inner branches or under leaf cover (Raselimanana and Rakotomalala 2003). However, roosting site selection was found to be connected to perch diameter and thus likely related to hand and foot size (e.g. Razafimahatratra et al. 2008), so that the vertical distribution of the present study species might be size-dependent. Moreover, Akani et al. (2001) found that roosting and foraging sites differ strongly because chameleons moved from roosting to foraging sites during the day. Therefore, roosting sites might not always reflect foraging sites.

Acknowledgements

We thank the CAFF/CORE of the Direction Général des Eaux et Forêts, and CNFEREF Morondava for authorization and support for this study. We are thankful for support in the field to Camilla Cenni, Hauke Thiesler, Wanda von Bremen and the team of field assistants in Kirindy Forest. We thank Mark Scherz and three anonymous referees for helpful comments on this paper.

Chapter 4: Parasite burden in a short-lived chameleon, *Furcifer labordi*

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Eckhardt, F., Strube, C., Mathes, K.A., Mutschmann, F., Thiesler, H., Kraus, C., Kappeler, P.M. (2019). Parasite burden in a short-lived chameleon, *Furcifer labordi*. International Journal for Parasitology: Parasites and Wildlife, 10, 231-240. https://doi.org/10.1016/j.ijppaw.2019.09.010

Abstract

Life-history theory predicts that species with shorter lifespan should show higher investments into growth and reproduction at the expense of immune defenses. Labord's chameleon (*Furcifer labordi*) is the tetrapod with the shortest known lifespan. To investigate to which extent immunosenescence influences the die-off of these chameleons when they are only about 6 months old, we examined the gastrointestinal, - blood - and ectoparasite burden in *F. labordi* in Kirindy Forest (western Madagascar) and compared them with sympatric and longer living *F.* cf. *nicosiai*. Moreover, we included data from wild *F. labordi* that were singly housed under ambient conditions with daily food and water supply. Gastrointestinal parasite prevalence of wild *F. labordi* increased dramatically during the last 3 months of their lives, which include the reproductive period. *Furcifer* cf. *nicosiai* was found to have a belated increase in gastrointestinal parasites compared to *F. labordi*. In *F.* cf. *nicosiai* higher prevalence of blood parasites were found, which probably result from the longer exposure to the arthropod intermediate host. Both species showed infestations with ectoparasites, which peaked in the rainy season but disappeared towards the dry season. Male *F. labordi* showed a significantly higher prevalence of gastrointestinal - and ectoparasites and higher intensities of coccidians and ectoparasites than females. Males of *F.* cf. *nicosiai* exhibited higher prevalence of blood- and ectoparasites, as well as higher intensities in ectoparasites. Caged individuals of both sexes showed delayed senescence, reduced parasite burden and lived longer than their wild conspecifics. Overall, the increase in the prevalence in gastrointestinal - and blood parasites towards the disappearance of the wild population of *F. labordi* indicates that this species invests comparatively less energy in efficient immune system function, supporting the prediction of life-history theory.

Keywords: parasites, chameleons, immunosenescence, aging, *Furcifer*

Introduction

During aging, the accumulation of molecular and cellular damage is thought to lead to functional decline, resulting in compromised health and, finally, death (Kirkwood, 2005). According to "disposable soma theory", natural selection evaluates how much an organism invests into growth and reproduction *versus* self-maintenance and repair, and hence, determines the rate of aging and lifespan (Kirkwood 1977, Kirkwood 1979). Regarding immunity, "fast-living” species are supposed to rely more on low-cost nonspecific and inflammatory immune defenses, whereas “slow-living” species exhibit rather cost-intensive stronger specific and especially antibody-mediated immunity, which is required as defense against parasites, for instance (Lee 2006). Besides these trade- offs, within vertebrates the functioning of the immune system changes over time, from the development of adaptive immunity at birth to the deterioration of the system at old age (Albright and Albright 1994, Malaguanera et al. 2001, Humphreys and Grencis 2002, Hayward 2013). The latter process is known as immunosenescence, which is characterized by a down-regulation of type Th2 immunity, which is involved in parasite resistance (Malaguanera et al. 2001). For example, tissue destruction is often caused by parasites and Th2 cell mediated immunity evolved as an adaptive tissue repair mechanism that quickly heals the wounds they inflict (Allen and Wynn 2011).

Because non-invasive measurements of immunocompetence in free-ranging animals are challenging, parasite burden is often used as a surrogate index of general health (Zuk 2002, Hämäläinen et al. 2015). Especially gastrointestinal parasites can be monitored with marginally invasive means via fecal egg counts. While many parasites induce only moderate clinical symptoms, they may provoke considerable energetic costs due to immune defense investment required to countervail the effects of infection (Zuk 1996, Marcogliese and Pietrock 2011). These costs are intensified by reduced health due to poor nutrition Marcogliese and Pietrock, 2011) or other stressors (Zuk 1996), resulting in a trade-off between parasite resistance and reproductive performance (Helle et al. 2004, Mills et al. 2010) and an accelerated rate of immunosenescence (Hudson et al. 1992). Furthermore, parasite infections induce additional costs, including enhanced risks of predation (Temple 1987, Graham 2008) and further infections by additional parasites (Petney and Andrews 1998, Cox 2001, Bordes and Morand 2009, Johnson and Buler 2010), resulting in a more than linear increase of the associated costs (Ezeamama et al. 2008).

Host sex is one of the important determinants of the immune function profile (Alexander and Stimson 1988). Among mammals, a male bias in parasite infection rates is common (Moore and Wilson 2002). Ultimately, sex differences in immune responses are thought to originate from sex-specific life-history strategies, where males benefit from investing into reproductive effort during their prime reproductive age, whereas female fitness is generally improved by a longer reproductive lifespan due to their higher investment in each offspring. These conditions can favour greater female investment into health maintenance, which ought to prolong their lifespan, whereas males are more likely to invest in competitive success (Williams 1957, Trivers 1972, Clutten-Brock and Isvaran 2007), resulting in comparatively accelerated immunosenescence. For example, when male greater kudu *Tragelaphus strepsiceros* reach adulthood and begin to rut successfully, they compete so intensely in the annual rut that they commonly either die from exhaustion or are killed by predators (Owen-Smith 1993).

Labord's chameleon *Furcifer labordi* from the highly seasonal deciduous dry forests in western and southwestern Madagascar has a post-hatching lifespan of only 4-9 months (Karsten et al. 2008, Eckhardt et al. 2017). This extreme life-history makes this species an interesting model to study potential mechanisms of accelerated senescence, especially because longer-lived sympatric congeners are available for comparative studies. During their short lives, this species undergoes hatching, juvenile growth, maturation and courtship followed by death of both sexes early during the annual dry season (Karsten et al. 2008, Eckhardt et al. 2017). Females tend to enjoy a slight longevity advantage, whereas no significant intersexual differences in lifespan were found in caged individuals that were kept under ambient conditions (Eckhardt et al. 2017). With such a fast life-history, chronic physiological stress might proximately contribute to immune suppression, which in turn facilitates parasite infections and ultimately leads to death. These mechanisms have been demonstrated in semelparous marsupials (Bradley et al. 1980, Lee et al. 1982, Dickman and Braithwaite 1992), where males in wild populations died considerably earlier compared to females, whereas captive males outlived their wild conspecifics.

To investigate to which extent changes in parasite burden across the lifespan contribute to the early die-off in *F. labordi*, we examined their gastrointestinal-, blood- and ectoparasite burden. To this end, we determined the prevalence of ectoparasites as well as gastrointestinal parasite reproductive stages (e.g. eggs, oocysts), and blood parasites (e.g. microfilariae) encountered in fecal and blood samples obtained from *F. labordi* throughout their life. Our study included two comparisons; one between wild *F. labordi* and their sympatric and longer-lived congener *F.* cf. *nicosiai*, and one with *F. labordi* kept in single cages under ambient conditions, which buffered them substantially from physiological stress and to some degree from parasite infection. We predicted an increase in parasite loads towards the end of the reproductive season in wild *F. labordi* and a reduced increase in *F.* cf. *nicosiai*. Furthermore, as age-related changes in immunocompetence should be delayed in the longer-lived females, we predicted female *F. labordi* to exhibit a lower and comparatively slower increase in parasite load than males. Additionally, caged *F. labordi*, which were safeguarded against extrinsic mortality, the costs of reproduction and starvation, were expected to exhibit slower rates of aging, and hence reduced parasite infection, compared to their wild conspecifics.

Materials and Methods

Study site, study species and capture-mark-recapture

The study was conducted at Kirindy Forest (44°39′E, 20°03′S, 30–60 m asl), one of the largest remaining tracts of dry deciduous forests in central western Madagascar. The local climate is characterized by a hot rainy season from November until March and a cool dry season from April until October. The forest is relatively dense and has undergone selective logging (Kappeler and Fichtel 2012).

Furcifer labordi is a medium-sized and sexually highly dimorphic chameleon from the western and southwestern regions of Madagascar (Glaw and Vences 2007). Males have a body size of approx. 100 mm, and females have a body size of 73 mm. *Furcifer* cf. *nicosiai* is a relatively larger species, also sexually dimorphic, and appears to be associated with intact dry forests (Jesu et al. 1999, Glaw and Vences 2007). Males reach a body size of 136 mm and females 102 mm (Eckhardt et al. 2019a). Concerning ecological studies in the Kirindy forest, both species differ significantly in point of hatching, growth rates and roosting heights, which might suggest some interspecific niche segregation (Eckhardt et al. 2019a).

Chameleons were located at night using flashlights. The roost perch of each detected chameleon was marked with flagging tape. Collected animals were placed in a cloth bag and handled the following morning. Snout-vent length and body mass were recorded, as well as age and sex. Animals were released at their point of capture within 12 h. Sampling took place over three field seasons: November 2013-July 2014, and January 2015-July 2015, and October 2015-December 2015.

Experimental housing

We collected a total of 20 male and 20 female juveniles of *F. labordi* in early January, at approximately two months of age. On January 2014 as well as 2015, each 10 males and 10 females were collected. They were kept individually without visual contact in cylindrical outdoor enclosures (90 cm height, 60 cm diameter) made of nylon screen. The enclosures were equipped with branches and artificial plants. In order to experience the same temperature fluctuations and daylight conditions as their wild conspecifics, caged animals were positioned in a large outdoor cage in the forest. Chameleons received a standardized amount of food (crickets, grasshoppers or butterflies), adjusted to their age and size to match growth and final size of the wild population. Water was offered daily with a spray flask. We used the Kaplan – Meier estimator to assess the survival probability of both sexes in captivity.

Analyses of gastrointestinal parasites

Fecal samples were collected opportunistically from cloth bags or during animal handling and stored in 70% ethanol. Parasite identification was based on size, shape and internal structure of eggs, oocysts and larval stages. We determined prevalence, intensity of egg and/or oocyst shedding and morphospecies richness of helminth and protozoan parasites. Egg shedding intensity was estimated using fecal egg counts (FEC/g feces) with a modified McMaster flotation egg counting technique (Sloss et al. 1994), a method commonly employed to estimate shedding intensity in wild populations of lizards (Hallas and Bull 2006, Fenner et al. 2011). Fecal samples that weighed less than 0.1g, were directly dispersed with a toothpick in a counting chamber, subsequently diluted with water and examined. Although FECs are a generally used method to study parasite infections, the method has been criticized for its potential inaccuracy, as parasite egg shedding rates fluctuates over time and a sample may not always contain the eggs of a parasite that is present in the host (Hallas and Bull 2006). However, as coccidians are considered as harmful protozoans (Modry et al. 2000, Schneller et al. 2008), the number of their spores (oocysts) reflects the degree of intestinal cells that are infected with macrogametes. Therefore, we evaluated the number of oocytes in the fecal samples.

Analyses of blood parasites

We quantified the prevalence of blood parasites such as microfilaria. Blood-sucking arthropods serve as intermediate hosts and infective microfilarial stages are transmitted to other reptiles (Mancianti et al. 2000). False-negative results are rare because both the adults and the microfilariae of the members of the family Onchocercidae are long-lived, and several species often produce significant microfilaremia (Szell et al. 2001). For detection of filariid infections, we used blood smears (Irizarry-Rovira et al. 2002). Here, a drop of blood was taken by lateral puncture of the caudal vein and placed on a microscope glass slide and distributed applying a second slid. After air-drying, blood smears were processed with a rapid differential haematology staining, using the Diff-Quik staining solution system (Medion Diagnostics AG, Düdingen, Switzerland). Samples were analyzed for prevalence of blood parasites applying a brightfield microscope (Zeiss Primo Star) and 100-fold or 400-fold magnification. No samples were taken from individuals that weighted less than 5 g or females that were very close to oviposition.

Analysis of ectoparasites

The body of each chameleon was inspected with a magnifying lens to identify and count the number of ectoparasites.

Statistical analyses

Generalized linear mixed effects (GLMM) models for longitudinal data were used to model the parasite data. As fixed factors, we added month (age), sex and species, while ID was included as a random factor for recaptured individuals. We conducted models including both species and models including *F. labordi* only. To test effects on prevalence, we used a binominal distribution and for intensity, we used a Poisson distribution (e.g. Peterson and Lello 2003, Verbeke and Molenberghs 2005). For all models, we compared the respective full model with the null model by using a likelihood ratio test. We also checked for model stability by determining Variance Inflation Factors (VIF) for a standard linear model excluding the random effects. In addition, we visually inspected normality and homoscedasticity with residual plots. For model analysis, we used the package lme4 (Bates et al. 2016). All data analysis was conducted in R (R-Code Team 2017). To test for interspecific differences according to multiple infections with different endoparasite taxa, we conducted a two-proportion Z – test.

Results

We observed first hatchlings of *F. labordi* in mid-October, at the onset of the rainy season. This cohort grew up and reached maturity not later than February. Towards the end of the mating season, males disappeared in late May, whereas females were found until the beginning of July. Thus, the lifespan of animals in this population ranged from six to nine months. However, after a remarkably long rainy season, males and females survived considerably longer; one adult female even survived until the next breeding season (Eckhardt et al. 2017). In November, we found a cohort of juvenile *F.* cf. *nicosiai* that had hatched during the previous active season. These juveniles grew up to adult size by February, i.e. more slowly than juvenile *F. labordi* (see also Eckhardt et al. 2019a). Hatchlings were detected around mid-February. Adult males were encountered until mid-June, and adult females until the end of June. After this date, we only detected small juveniles that ceased growing. However, after an unusually long rainy season adult males and females were found for longer and we found two adult females that overwintered the previous dry season. Concerning the 40 caged individuals of *F. labordi*, we found no significant differences in survival probability between males and females. Median lifespan for females was 9.5 months and for males 8.2 months. Maximum lifespan for females was 11.5 months and for males 16 months (see also Eckhardt et al. 2017).

In the coproscopic analyses, we identified one protozoan morphotype that was assigned to the Coccidians (*Isospora* sp.). We also detected two egg morphotypes that were assigned to the Cestoda (Cyclophyllidae, *Oochoristica* sp.) and Nematoda belonging to the family Heterakidae (*Spinicauda* sp.). Additionally, we found Oxyurids in five samples and Ascarids (*Hexametra* sp.) in two samples. Due to their rare occurrence and the possibility that the latter two nematode taxa were parasites of prey species, we exclude them from our analysis.

Between hatching in mid-October until December, we did not detect any infestation in the fecal samples of *F. labordi*. However, from January onwards, the prevalence of gastrointestinal parasites in this species increased significantly from 12.3% in January until 57.1% in June ($z = -8.539$; $P < 0.001$, Fig.4.1A and Tab. 4A). Moreover, adult males showed a significantly higher prevalence compared to females ($z = 4.432$; $P < 0.001$, see Fig. 4.1A and Tab. 4). In *F.* cf. *nicosiai*, we found a low prevalence (6.8%, $n = 207$) of gastrointestinal parasites in fecal samples of hatchlings and juveniles that were sampled between mid-February and mid-July. From January onwards, we also detected an increase of the prevalence of gastrointestinal parasites; in this species from 14.3 to 60% in May. When comparing gastrointestinal parasite prevalence among adult *F. labordi* and *F.* cf. *nicosiai*, we found that prevalence was higher in the latter species ($z = -9.211$; $P < 0.001$, see Fig. 4.1B and Tab. 4B). In contrast to *F. labordi*, the prevalence of gastrointestinal parasite infection was lower in male *F.* cf. *nicosiai*. Regarding the shedding intensity of *Isospora* sp., we detected an average number of oocysts per g feces (OPG)/month in *F. labordi* ranging between 2600 and 73747. The highest number of oocysts was detected in May. Comparing both species, we did not find any significant differences. However, in male *F. labordi*, we detected a highly significant increase of oocyte number in May (Tab. 5)

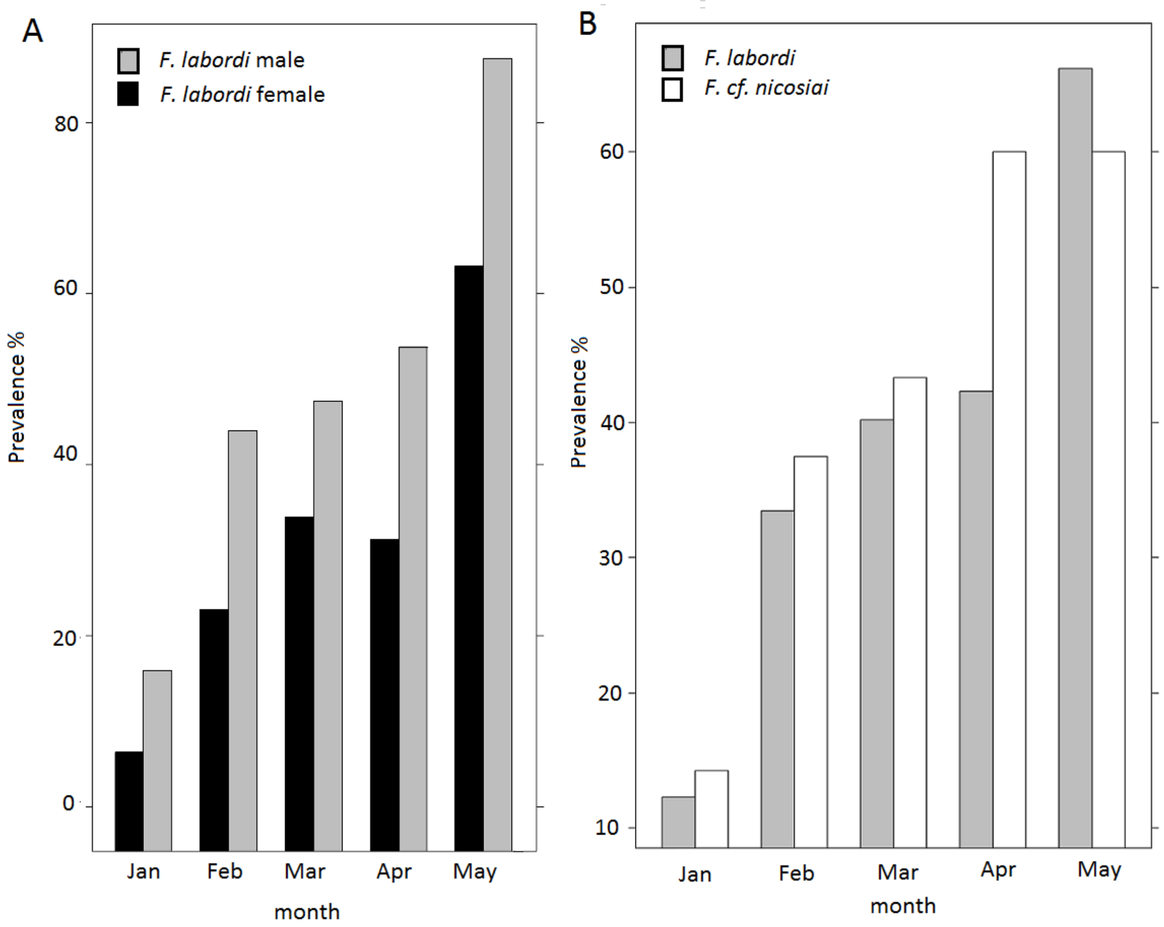

Fig.4.1: Prevalence in percentage of gastrointestinal parasitic infection A) in *F. labordi* males and females, B) *F. labordi* and *F.* cf. *nicosiai* (adult stages).

Tab. 3: Number of fecal samples collected per species (*F. labordi* and *F.* cf. *nicosiai*) and sex (*F. labordi*) per month.

Species/sex	Jan	Feb	Mar	Apr	May	Jun
F. labordi	204	183	224	164	64	8
F. cf. *nicosiai*	27	24	30	17	10	9
F. labordi male	126	92	114	41	8	0
F. labordi female	78	99	128	133	56	8

Concerning the taxonomic composition of gastrointestinal parasite taxa in the fecal samples of *F. labordi*, *Isospora* sp. (Eimeriidae, Coccidia) were most common and present in 31.7% of all fecal samples. *Oocherisitica* sp. (Cyclophyllidae, Cestoda) (12%) and *Spinicauda* sp. (Heterakidae, Ascaridida) (8.5%) had lower prevalences. In *F.* cf. *nicosiai*, we found a similar parasite composition, with 22% *Isospora* ssp., 18.3% *Oocheristica* sp. and 10.3% *Spinicauda* sp. prevalence (Fig. 4.2).

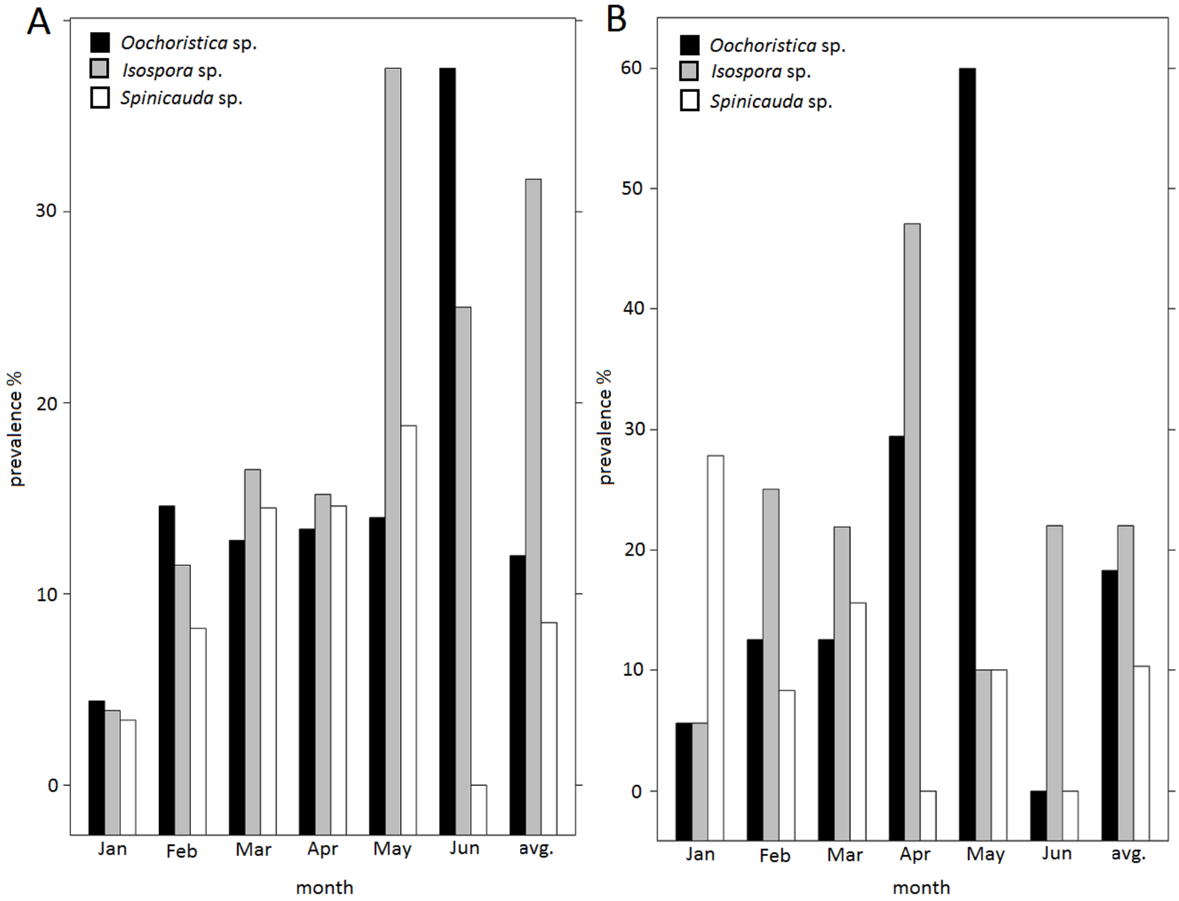

Fig. 4.2: Composition of gastrointestinal parasite taxa in the fecal samples of A) adult *F. labordi* and B) adult *F.* cf. *nicosiai* from January to June and in total.

Regarding multiple infections, the number of gastrointestinal parasite taxa ranged from 1 to 3 in infected samples. Of the 381 infected samples, 7.9% contained two gastrointestinal parasite taxa. Three morphotypes where only found in 0.5% of the infected samples. Double infection rates for males and females were 12.1% and 8.1%, respectively. Triple infections where only found in 1.3% of female samples. As the rate of multiple infections was rather low, no clear dynamics with age could be observed. Of the 30 double infections, 53.3% contained *Spinicauda* sp. and *Oocheristica* sp., 30% contained *Isospora* sp. and *Ooceristica* sp., and 16.7% contained *Isospora* sp. and *Spinicauda* sp.. In 118 infected samples of *F.* cf. *nicosiai*, we found 7 double infections (5.9%). The two-proportion Z-test revealed no significant differences of the rate of double infections between both species ($\chi^2 = 0.252$, df =1, P = 0.615)

Among the caged chameleons, two males exhibited coccidiosis from February onwards and subsequently died at an age of approximately five months. The number of oocytes/g in their samples fluctuated between 2 and 86750, with an average increase towards the end of their lives. In parallel with the increase in oocytes, their body mass decreased dramatically. Additionally, we found that two males and two females were infected with *Spinicauda* sp..

The analysis of blood samples revealed that both species were infected by the nematode species *Foleyella* aff. *furcata*. Parasites of this genus have a limited geographic distribution and have been found only in the lizard family Agamidae and Chamaeleonidae (Barlett 1986). *Foleyella* spp. are long-lived and viviparous (Brygoo 1963). Adults are relatively large and predominantly inhabit muscle or skin whereas their progeny, the so-called microfilaria circulate in the blood of the host (Fenner et al. 2011). The prepatent period takes about six months (Szell et al. 2001). In *F. labordi*, the prevalence of filarial infection increased from the beginning of the reproductive season (Fig. 4.3). Within *F.* cf. *nicosiai*, incipiently the prevalence of blood parasites decreased from January until March, but hereafter increased remarkably from April towards June. The interspecific comparison revealed that *F.* cf. *nicosiai* was more frequently infected by *Foleyella* aff. *furcata* than *F. labordi* ($z = 2.187$; $P < 0.05$). Concerning intersexual differences of prevalence in *F.* cf. *nicosiai*, we found that males showed higher significant prevalence of filarial infection. ($z = 2.34$; $P < 0.05$, see Fig. 4.3 and Tab. 4C). Among the chameleons in the cages, we detected filarial infection in one male.

Tab. 4: Number of blood samples collected per species per month.

Species	Jan	Feb	Mar	Apr	May	Jun
F. labordi	6	109	94	59	44	13
F. cf. nicosiai	3	15	32	30	13	11

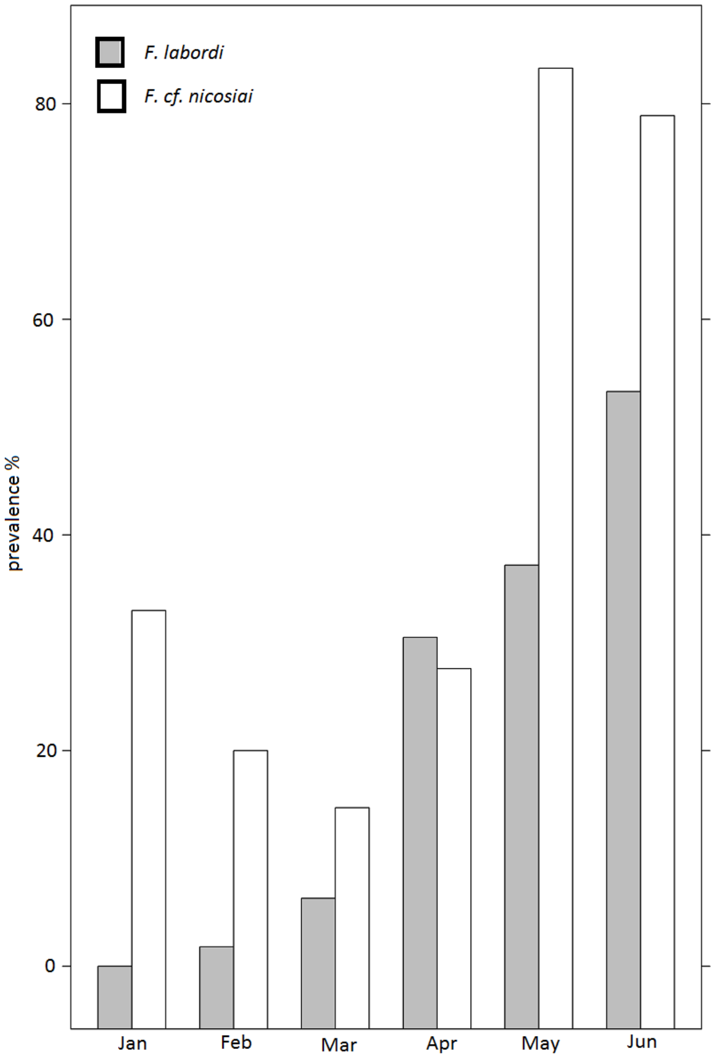

Fig. 4.3: Prevalence of filarial infection in *F. labordi* and *F.* cf. *nicosiai*.

We identified acarians of the family Trombiculidae (trombiculids) as ectoparasites in both *Furcifer* species, which were mostly located in the axillary pits. Acarian were detected from January onward. Their prevalence peaked in February and March and they were no longer detectable in June. Comparing both species, we found that a higher prevalence of acarians in *F.* cf. *nicosiai* (Fig. 4.4). Moreover, we found that the number of trombiculids was significantly higher in *F.* cf. *nicosiai* compared to *F. labordi*. Males of both species exhibited a higher prevalence and intensity of these ectoparasites than females ($z = 5.617$; $P < 0.001$). In *F. labordi*, males showed a prevalence of 66.7% ($n = 486$) and carried 16.6 ± 15.1 mites, whereas 48.5% of the females were infested with an average of 8.0 ± 8.1 mites. In *F.* cf. *nicosiai*, 82.3% of all examined males ($n = 96$) were infested and showed 31 ± 27 mites, whereas 57.6% of females (n = 92) were infested and carried on average 12 ± 12.7 mites. Regarding intensity of ascarian infestation, we did not detect significant interspecific differences, though specimens of *F.* cf. *nicosiai* tended to have more mites compared to *F. labordi* (see Fig. 4.5, Tab. 6). Similar to the prevalence, the intensity increased at the peak of the rainy season and decreased towards the rainy season (see Fig. 4.5, Tab. 6). Among the caged animals, we rarely detected acarians and therefore excluded them from any statistical analysis.

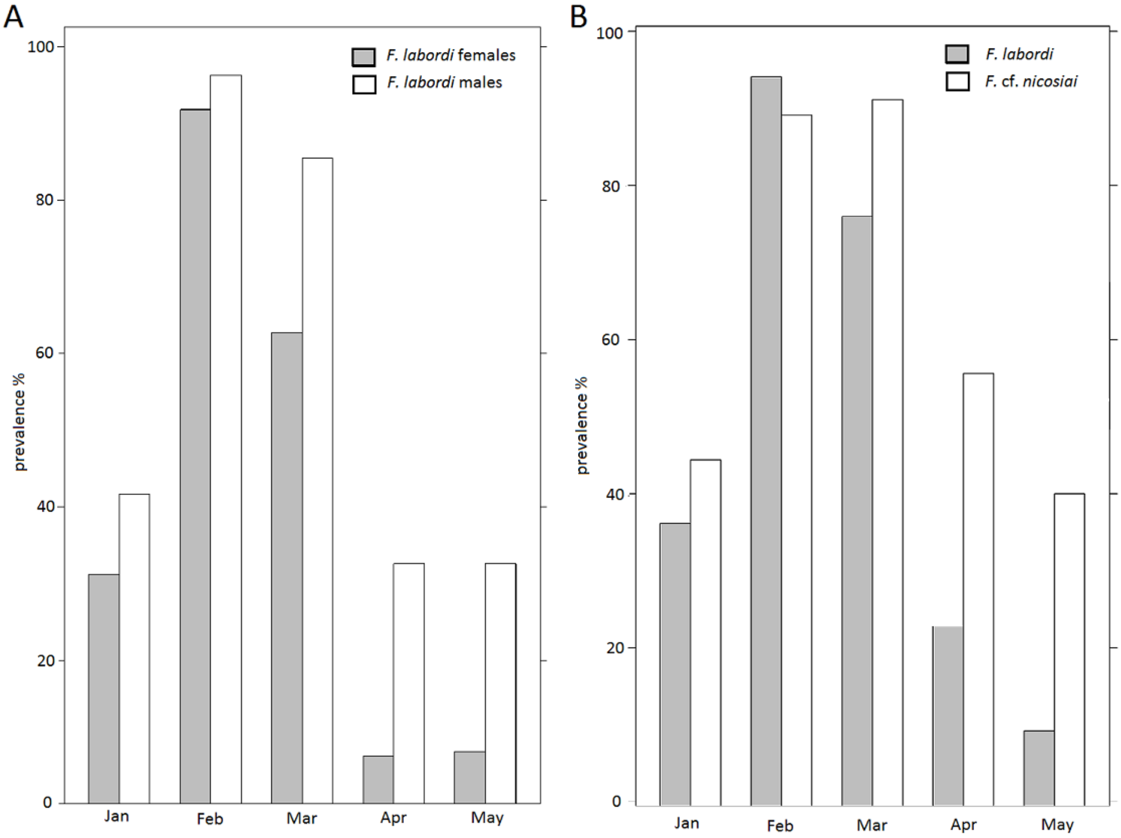

Fig. 4.4: Prevalence of mite infestation in A) *F. labordi* males and females, B) *F. labordi* and *F.* cf. *nicosiai.*

Tab. 5: Number of individuals per species/sex and month that were inspected for ectoparasites.

Species/sex	Jan	Feb	Mar	Apr	May
F. labordi	207	205	308	246	119
F. cf. nicosiai	52	37	55	36	17
F. labordi male	127	108	166	86	20
F. labordi female	80	97	142	160	99

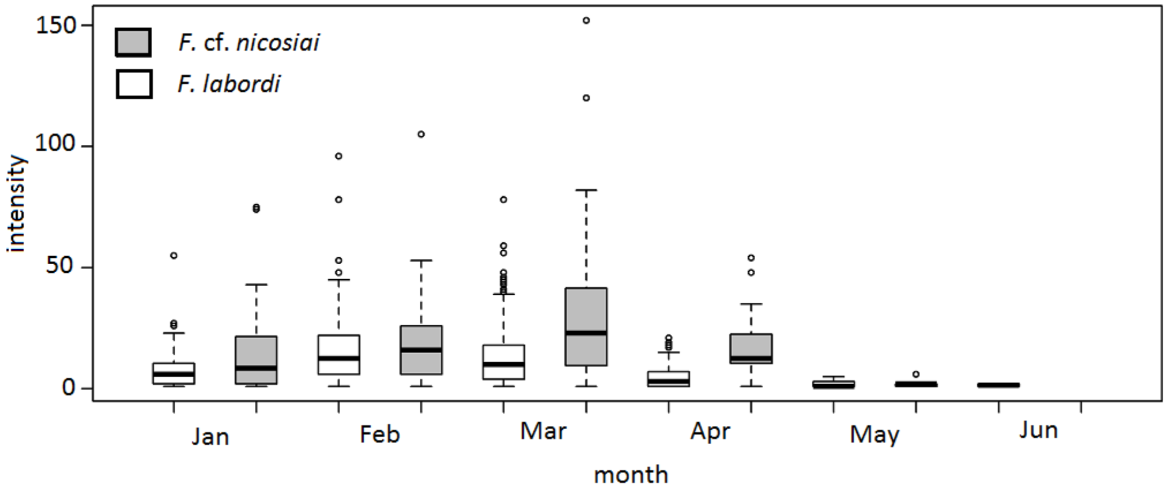

Fig. 4.5: Intensity of mite infestation in adult *F. labordi* and *F.* cf. *nicosiai*.

Discussion

Furcifer labordi in nature

As predicted, among wild living specimens of *F. labordi*, we detected a massive increase of gastrointestinal parasites related to prevalence and mixed infections over the reproductive period towards the dry season. A similar pattern of decline in immune function was reported for feral Soay sheep *Ovis aries* (Hayward et al. 2009). In contrast, Hämäläinen et al. (2015) found that parasite prevalence and morphotype richness decreased with increasing age in a small and relatively short-lived primate species (*Microcebus murinus*), indicating acquired immunity by older specimens. Besides, studies focusing on parasite infection with regard to aging in the wild are lacking. However, the detected increase of prevalence of gastrointestinal parasites according to time is probably linked to a decrease in immune functioning in *F. labordi*. We also found that males, but not females, of *F. labordi* showed a remarkable increase of oocyst shedding towards the end of the reproductive season. Concerning the prevalence of coccidian oocysts in the fecal samples, Modry et al. (2000) found that 32.5% of 83 examined chameleon specimens from east Africa were infected with coccidians, which is similar to our findings in both species. Additionally, in a coproscopic study on chameleons in captivity, Biallas (2013) found that *Isospora* was regularly detected (21.7%). Accordingly, coccidians in general seem to be frequent gastrointestinal parasites in both, wild living and caged chameleons. *Isospora* sp. was the most commonly detected gastrointestinal parasite taxon in our study, but we may not have detected all kinds of gastrointestinal parasites, such as trematode eggs, that are too heavy to float during the flotation process in saturated NaCl solution. In their study, Morsey et al. (2012) found that 26.1% of 115 specimens of the common chameleon, *Chamaeleo chamaeleon,* were naturally infected with the digenetic trematode *Postorchigenes* sp. and 32.1% with *Malagashitrema* sp.. Thus, the number of gastrointestinal parasite taxa and their prevalence might be underestimated in our study. Moreover, as the samples were stored in ethanol, a detection of intestinal flagellates and ciliates was not possible. Although these groups are mostly considered as commensals, under physiological stress, they can have a severe effect on the host (Schneller et al. 2008).

Regarding blood parasites, we detected filarias belonging to the genus *Foleyella*. Here, we detected an increase of prevalence towards the dry season, which might have a severe influence on the individual's health. However, little is known about the clinical signs of foleyellosis in chameleons. Higher mortality rates were noted in *Foleyella*-infected chameleons than in uninfected animals, which were transported from a tropical to a temperate zone (Brygoo 1963). In their study, Maia et al. (2014) report a relatively high incidence of filarial infections in the Malagasy chameleon genus *Furcifer*, which we reported as well.

Concerning ectoparasites, we first detected acarian infestation in January, when humidity was relatively high. Their prevalence was highest in February and March, but decreased towards the dry season, eventually decreasing to zero. Thus, their detection seems to be highly associated with the rainy season, when chameleons are still in good physical conditions. In their study of the impact of tick load on the fitness of their lizard hosts, Bull and Burzokott (1993) did not find any influence on the longevity of the sleepy lizard *Tiliqua rugosa*. In addition, we found that trombiculids were obviously restricted to axillary situated so called "mite pockets".

At first sight, mite pockets are paradoxical structures as they seem to provide an optimal environment for the mites, giving protection from solar radiation, high temperatures, desiccation, and offering easily penetrated skin. Here, the most likely hypothesis for their function is that they reduce damaging effects of mite infestations (Arnold 1986). Mites are probably attracted to the pockets because they provide ideal conditions, whereas in return these invaginations appear to ameliorate much of their potential damage. These pockets have a large internal area of exposed skin compared with their volume, which enables large numbers of mites to be concentrated in places where they do not interfere with general cutaneous function. In particular, the epidermis is resilient and recovers rapidly after a mite has fed. The shape of the pocket enables large numbers of lymphoid cells to be concentrated around the feeding mites and it is probable that these cells reduce the effects of antigens and any pathogens introduced by the feeding mites, as well as contributing to their diet. Thus, due to the peak of the infestation in the rainy season and evolutionary adaptation to these parasites, we do not consider ascarian infestation as a decisive factor that is influenced by immunosenescence. However, these parasites cause blood loss and are potential vectors for pathogens that can have negative impacts on the health of the host (Schneller et al. 2008).

Interspecific comparison of *F. labordi* and *F.* cf. *nicosiai*

We found higher prevalence of gastrointestinal parasites in *F.* cf. *nicosiai*, but we found the first infections in *F. labordi* approx. 2 – 3 months after hatching. Among juvenile *F.* cf. *nicosiai*, which hatched around mid-February, we rarely detected any gastrointestinal parasites until the dry season in June. The delayed occurrence of gastrointestinal parasite infection in *F.* cf. *nicosiai* might be caused by a higher energy investment in the immune system and especially in parasite defense. In contrast to *F. labordi*, juveniles of this species exhibit rather slow growth rates, later sexual maturity and higher rates of recaptures and therefore potentially higher probability of survival (Eckhardt et al. 2019a) that might enable them to invest comparatively more energy into immune defense. Besides slow growth rates, juveniles probably digest less food insects and are therefore less prone to gastrointestinal parasites that are transferred by this route. Especially tapeworms that require reptiles as definite host use invertebrates as intermediate host. Furthermore, insects, such as flies can function as vectors to allocate parasite eggs to the next host (Schneller et al. 2008). However, the probability of infection might not be equal during the sampling period and might be an additional factor for the later detection of gastrointestinal parasites in *F.* cf. *nicosiai*. Regarding the comparison of the adults of both species per month, we found that *F.* cf. *nicosiai* exhibited a higher prevalence of gastrointestinal parasites apart from May. We suspect that the longer cumulative exposure might have an influence on this observation. The higher prevalence of gastrointestinal parasites in *F. labordi* in May might be attributed to the relatively small amount of fecal samples of *F.* cf. *nicosiai* (n = 10) compared to *F. labordi* (n = 64).

With respect to multiple infections, we observed no significant interspecific differences. Although, triple infections were only found in *F. labordi*. *Furcifer* cf. *nicosiai*, as the longer living species probably has a comparatively longer exposure to potential infections, might have developed some resistance against these pathogens. However, when entering the mating season, the prevalence of gastrointestinal and blood parasites increased in *F.* cf. *nicosiai* as well.

Concerning the intensity of coccidian oocyst shedding, we did not detect interspecific differences. However, as *F*. cf. *nicosiai* is the larger species, similar intensities of coccidian infection probably have milder effects on the individual's body condition.

Within the samples of both species, we found a very low prevalence of oxyurids, which is in accordance to the findings of Lutzmann (2007), who examined fecal samples of several wild living chameleon species from Masoala, Madagascar. Contrary to our findings, these parasites were frequently detected in specimens that were kept in captivity (Biallas 2013). Probably, in a terrarium, where the home range is very restricted, oxyurid density can increase rapidly due to their direct life cycle and resistant eggs.

In *F*. cf. *nicosiai,* we found a higher prevalence of filariid infection, which could be in turn explained by the comparatively longer exposition to blood-sucking arthropods such as *Culex* and *Aedes* due to their comparatively longer lifespan. Moreover, adult specimen of *F.* cf. *nicosiai* are considerably larger than adults of *F. labordi* and might therefore be easier to detect for mosquitos. As the prepatent period takes approx. 6 months (Szell et al. 2001), due to its shorter lifespan *F. labordi* is less prone to be adversely affected by foleyellosis. Subsequently, this species rather irregularly functions as primary host for *Foleyella* aff. *furcata*. Contrary to *F. labordi,* we found that the comparatively high prevalence in January decreased towards March in *F*. cf. *nicosiai,* but hereafter rises towards June. Initially, this observation might be explained by the small amount of blood samples (n = 3) from *F.* cf. *nicosiai* in January. However, as sample size is respectively higher in the following months, this might indicate some immune defense mechanisms against the parasite, which changes to immunosenescence towards thebeginning of the dry season. Additionally, the life cycle of *Foleyella* might also have an influence of the observed pattern. As adult stages are known to predominantly inhabit skin or muscle tissue, an infection with this parasite might not have always been detected.

We found that the prevalence and intensity of mites was higher in *F.* cf. *nicosiai,* which could be caused by their larger average body size and subsequently easier detection for mites. Moreover, regarding the differences in intensity, mite pockets are larger in *F.* cf. *nicosiai* and might therefore offer more space for these ectoparasites.

Concerning interspecific comparison, niche differentiation may in turn result in differences in the exposure to parasites. In our previous study (Eckhardt et al. 2019a), we observed that adults of *F.* cf. *nicosiai* showed significant higher roosting sites, which might reflect differences in habitat use of both species. Here, the composition of food insects (vectors for gastrointestinal parasites), mosquitos (vectors for blood parasites) and mites might be unequal.

In total, detailed studies investigating parasite burden and in connection with their life-history and seasonality in reptiles are lacking (Zimmerman et al. 2010). However, a comparative study in mammals revealed weak relationships between parasite species richness and longevity (Cooper et al. 2012). These authors found a significant negative relationship between longevity and parasite species richness for ungulates, but not for carnivores or primates, indicating no general pattern of parasite richness according to life-history in vertebrates. In contrast to our expectations, we found higher prevalences of gastrointestinal-, blood - and ectoparasites in adult *F.* cf. *nicosiai* compared to adult *F. labordi*. As *F.* cf. *nicosiai* is the longer living and larger species, these observations could be caused by differences of cumulative exposure, as well as body size. Here, it is difficult to disentangle, which factors or interplay of factors influence these pattern. However, the fact that juveniles of *F.* cf. *nicosiai* show comparatively low infection rates until their maturation that takes approx. 11 months, suggests some immune defense mechanisms in juveniles compared to *F. labordi*. Although, following maturation this species seems to be affected by serious parasite infections, indicating that this age cohort reallocates their energy investment from self-maintenance to reproduction. Moreover, the accelerated growth rates that we observed after the aestivation (Eckhardt et al. 2019), which involves higher food requirements might additionally influence the raise in gastrointestinal parasites.

Intersexual comparison

In *F. labordi*, males showed a significantly higher prevalence of gastrointestinal parasites and additionally higher intensity in coccidian infestation than females. Similarly, a study of the small marsupial *Antechinus stuartii* found that males, which are the significantly shorter living sex, had remarkably higher prevalence of gastrointestinal parasites at the end of the mating season compared to females (Beveridge and Barker 1976). Additional studies in lizards (Uller and Olsson 2003) found that males are more susceptible to parasite infection, which was attributed to the immune-suppressive effects of testosterone, at least during the reproductive period. Among wild vertebrates, the prevalence and intensity of parasitic infections is also generally higher in males than females (Klein 2000). Here, sex differences in exposure as well as susceptibility to parasites probably contribute to sex-based differences in the intensity and prevalence of parasites. For example, males are more likely to engage in behaviours, such as aggression and dispersal, increasing the likelihood of contact with parasites (Zuk and McKean

1996, Roberts et al. 2001). Males also often are larger than conspecific females, which may make them more obvious targets for parasites (Zuk and McKean 1996). Despite differences in the likelihood of exposure, several studies illustrate that harsh intrasexual combats and chronic physiological stress leads to increased susceptibility to infections, which is in accordance with the earlier die-off of in males (Eckhardt et al. 2017). In their review examining the immunocompetence handicap hypothesis, Roberts et al. (2004) suggest that there is at best weak evidence that testosterone directly influences immune function of males.

In contrast to our predictions, we did not find any sex difference in *F. labordi* with respect to filarial infections. As observed in both species, the microfilaria prevalence increased with season, and males of *F.* cf. *nicosiai* showed a higher prevalence than females, perhaps because of their larger body size and/or higher susceptibility.

Moreover, in both species, we detected sex differences in the intensity of acarian infestation, with males housing considerably more mites. This pattern could be caused by the differences in body size and therefore higher amount of blood, which enables larger males to host more mites without higher losses of blood compared to their female conspecifics. Higher intensities of mites in male lizards were also found in a study of Cox and Alder (2007), where males on average carried twice as many mites compared to females. Interestingly, castration reduced mite parasitism to levels comparable to that of females and treatment of castrated males with exogenous testosterone elevated mite counts to levels characteristic of intact males.

***Furcifer labordi* in captivity**

When comparing caged individuals with their wild conspecifics, we found that the prevalence of gastrointestinal parasite infection was significantly higher in the latter one. Although we tried to shield captive specimens from infection, they probably took up parasites from the crickets, lepidopterans and orthopterans that were fed to them. These insects might have transferred parasitic eggs or oocysts. Since the caged chameleons were collected from the forest at the age of approximately two months, they might have also taken up parasite stages before. The two males that were infected with coccidians showed obvious senescent declines. Compared to the median survival time of caged males, (8.2 months, Eckhardt et al. 2017), these specimens showed a remarkably shorter lifespan. In contrast to their wild living conspecifics, we did not find significant intersexual differences in survival within the caged animals. Similar results were obtained in mouse lemurs *M. murinus*, where survival in the wild was strongly female-biased (Kraus et al. 2008, Languille et al. 2012), whereas longevity in captivity was slightly male-biased (Perret 1997). These findings support the previously mentioned suggestion of

Roberts et al. (2004) that testosterone alone is unlikely to be responsible for accelerated senescence and die off in males in the wild. In total, as the caged chameleons were shielded from predation, starvation, desiccation, and at least partly from infections, it is not possible to pinpoint the factors facilitating their longer survival in captivity. To identify to which extant the presence or absence of parasites influence the lifespan of caged animals, an experimental manipulation of parasite burden could give insight into the direct effect of parasite infection.

Conclusions

Our study provides rare information about the age-related patterns of health in the shortest living tetrapod species in the wild and suggest that *F. labordi* rather relies on nonspecific and inflammatory immune defenses than on acquired immunity. Moreover, as vertebrates obligatorily develop an adaptive immune system, we assume a downregulation of the acquired immunity with age. As the prevalence in parasites increased comparatively later in *F.* cf. *nicosiai*, we suspect that this species invests more energy in the development of an adaptive immune system until maturation. However, adults of both species seem to suffer from parasite infections. The parasite burden observed in fecal and blood samples revealed the combined outcome of several factors: the parasites encountered by the host, when the parasite matured and reproduced within the host, and how effective the host’s immune system was in preventing or eliminating the infection. While it is difficult to disentangle these factors, we found males having higher parasite infection rates than females, and the older animals to suffer from the higher parasite burden, suggesting a downregulation of the acquired immunity in both species. Finally, the conduction of cage experiments including both species and sexes with a controlled manipulation of parasite burden could clarify the direct influence of the effect of parasites on the lifespan of both species.

Acknowledgements

We thank the CAFF/CORE of the Direction Général des Eaux et Forêts, and CNFEREF Morondava for authorization and support for this study. We are thankful for support in the field to Camilla Cenni, Wanda von Bremen and the team of field assistants in Kirindy Forest.

Tab. 6. Parameter estimates from the generalized linear mixed model for the prevalences of A) gastrointestinal parasites in *F. labordi* B) gastrointestinal parasites of both species, C) blood parasites in both species, D) ectoparasites in both species

A) Prevalence: gastrointestinal parasites *F. labordi*							
Parameter estimates					**Likelihood ratio test**		
Fixed effects	**Est.**	**SE**	**z**	**P**	χ^2	**df**	**P**
Intercept	-2.7265	0.3193	-8.539	**<0.001**	110.75	6	**<0.001**
Sex (male)	0.8484	0.1914	4.432	**<0.001**			
Feb	1.5103	0.3007	5.023	**<0.001**			
Mar	1.8755	0.2986	6.280	**<0.001**			
Apr	2.1040	0.3263	6.448	**<0.001**			
May	3.3748	0.4497	7.505	**<0.001**			
Jun	2.7370	0.8500	3.220	**<0.01**			
B) Prevalence: gastrointestinal parasites *F. labordi* and *F.* cf. *nicosiai*							
Parameter estimates					**Likelihood ratio test**		
Fixed effects	**Est.**	**SE**	**z**	**P**	χ^2	**df**	**P**
Intercept	-2.6471	0.2874	-9.211	**<0.001**	129.93	8	**<0.001**
F. cf. *nicosiai*	1.0532	0.3197	3.295	**<0.001**			
Sex (male)	0.8159	0.1832	4.453	**<0.001**			
Feb	1.4755	0.2726	5.413	**<0.001**			
Mar	1.8089	0.2689	6.727	**<0.001**			
Apr	2.0870	0.2974	7.018	**<0.001**			
May	3.2360	0.4002	8.085	**<0.001**			
Jun	2.0317	0.6291	3.230	**<0.01**			
F. cf. *nicosiai* (male)	-2.0865	0.5175	-4.032	**<0.001**			
C) Prevalence: blood parasites *F. labordi* and *F.* cf. *nicosiai*							
Parameter estimates					**Likelihood ratio test**		
Fixed effects	**Est.**	**SE**	**z**	**P**	χ^2	**df**	**P**
Intercept	-2.7623	0.7641	-3.615	**<0.001**	90.78	6	**<0.001**
F. cf. *nicosiai*	0.7187	0.3287	2.187	**<0.05**			
Sex (male)	0.7567	0.3233	2.340	**<0.05**			
Feb	-1.7501	1.0150	-1.724	0.08468			
Mar	-0.3750	0.7466	-0.502	0.61550			
Apr	1.1826	0.7179	1.647	0.09950			
May	2.3222	0.7508	3.093	**<0.01**			
D) Prevalence: ectoparasites *F. labordi* and *F.* cf. *nicosiai*							
Parameter estimates					**Likelihood ratio test**		
Fixed effects	**Est.**	**SE**	**z**	**p**	χ^2	**df**	**P**
Intercept	-1.3332	0.2198	-6.066	**<0.001**	607.48	7	**<0.001**
Sex (male)	0.9934	0.1769	5.617	**<0.001**			
F. cf. *nicosiai*	0.8087	0.2213	3.654	**<0.001**			
Feb	3.6267	0.4200	8.634	**<0.001**			
Mar	2.1469	0.3087	6.955	**<0.001**			
Apr	-0.3134	0.2158	-1.452	0.14649			
May	-1.3439	0.3434	-3.914	**<0.001**			
Jun	-2.7206	0.7833	-3.473	**<0.001**			

Tab. 7: Parameter estimates from the generalized linear mixed model for shedding intensity of coccidian oocysts of *F. labordi* and *F.* cf. *nicosiai*

Intensity: *Isospora* sp. of *F. labordi* and *F.* cf. *nicosiai*								
Parameter estimates						**Likelihood ratio test**		
Fixed effects	Est.	SE	df	t-value	p	χ^2	df	P
Intercept	17358.7	20290.8	192.0	0.855	0.393	29.39	8	**<0.001**
Mar	-13592.0	24402.5	189.9	-0.557	0.578			
Apr	-2188.9	25262.6	197.2	-0.087	0.931			
May	21675.3	25424.7	195.0	0.853	0.395			
Sex (male)	-14996.7	29319.3	192.0	-0.511	0.610			
F. cf. *nicosiai*	-10617.4	19035.0	192.1	-0.558	0.578			
Mar (male)	14460.4	37649.4	191.1	0.384	0.701			
Apr (male)	5659.0	43324.8	193.6	0.131	0.896			
May (male)	388477.9	84369.7	192.2	4.604	**<0.001**			

Tab. 8: Parameter estimates from the generalized linear mixed model for intensity of ectoparasites of *F. labordi* and *F.* cf. *nicosiai*

Intensity: ectoparasite infestation of *F. labordi* and *F.* cf. *nicosiai*							
Parameter estimates					**Likelihood ratio test**		
Fixed effects	**Est.**	**SE**	**t-value**	**P**	χ^2	**df**	**P**
Intercept	1.775	0.139	12.808	< **0.001**	290.43	15	**<0.001**
F. cf. *nicosiai*	0.402	0.205	1.959	0.0501			
Feb	0.179	0.144	1.248	0.212			
Mar	-0.119	0.161	-0.739	0.46			
Apr	-1.107	0.228	-4.861	< **0.001**			
May	-1.508	0.398	-3.789	< **0.001**			
Jun	-1.547	0.834	-1.854	0.064			
Sex (male)	0.076	0.175	0.436	0.663			
F. cf. *nicosiai* Feb	-0.232	0.247	-0.939	0.348			
F. cf. *nicosiai* Mar	0.333	0.241	1.380	0.167			
F. cf. *nicosiai* Apr	0.899	0.294	3.061	< **0.01**			
F. cf. *nicosiai* May	-0.286	0.627	-0.456	0.648			
Male Feb	0.675	0.193	3.506	< **0.001**			

Chapter 5: Stress-related changes in leukocyte profiles and telomere shortening in the shortest-lived tetrapod, *Furcifer labordi*

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Eckhardt, F., Pauliny, A., Rollings, N., Mutschmann, F., Olsson, M., Kraus, C., Kappeler, P. M. (2020). Stress-related changes in leukocyte profiles and telomere shortening in the shortest-lived tetrapod, *Furcifer labordi*. BMC Evolutionary Biology, accepted.

Abstract

Life-history theory predicts that, during the lifespan of an organism, resources are allocated to either growth, somatic maintenance or reproduction. Resource allocation trade-offs determine the evolution and ecology of different life-history strategies and define an organisms' position along a fast-slow continuum in interspecific comparisons. Labord's chameleon (*Furcifer labordi*) from the seasonal dry forests of Madagascar is the tetrapod species with the shortest reported lifespan (4 – 9 months). Previous investigations revealed that their lifespan is to some degree dependent on environmental factors, such as the amount of rainfall and the length of the vegetation period. However, the intrinsic mechanisms shaping such a fast life-history remain unknown. Environmental stressors are known to increase the secretion of glucocorticoids in other vertebrates, which, in turn, can shorten telomeres via oxidative stress. To investigate to what extent age-related changes in these molecular and cellular mechanisms contribute to the relatively short lifetime of *F. labordi*, we assessed the effects of stressors indirectly via leukocyte profiles (H/L ratio) and quantified relative telomere length from blood samples in a wild population in Kirindy Forest. We compared our findings with the sympatric but longer-lived sister species *F.* cf. *nicosiai,* which exhibit the same timing annual of reproductive events, and with wild-caught *F. labordi* that were singly housed under ambient conditions. We found that H/L ratios were consistently higher in wild *F. labordi* compared to *F.* cf. *nicosiai*. Moreover, *F. labordi* already exhibited relatively short telomeres during the mating season when they were 3 – 4 months old, and telomeres further shortened during their post-reproductive lives. At the beginning of their active season, telomere length was relatively longer in *F.* cf. *nicosiai*, but undergoing rapid shortening towards the southern winter, when both species gradually die off. Captive *F. labordi* showed comparatively longer lifespans and lower H/L ratios than their wild counterparts. We suggest that environmental stress and the corresponding accelerated telomere attrition have profound effects on the lifespan of *F. labordi* in the wild, suggesting physiological mechanisms proximately contributing to their relatively early senescence and mortality.

Key words: *Furcifer labordi,* life-history, telomeres, H/L ratio, body condition

Introduction

Life-history theory is based on the premise that during the lifetime of an individual, energy and resources are allocated to either growth, somatic maintenance, or reproduction (Stearns 1976, Stearns 1992, Stearns 2000). For example, resources, which are invested into fast growth and early reproduction, cannot be used for somatic maintenance, which may lead to shorter lifespans. The trade-offs between traits shape life-history strategies and the distribution of species along a fast–slow continuum of life-history speeds (Sæther and Bakke 2000, Sæther et al. 2004, Salguero-Gómez et al. 2016). In spite of the supposed significance of extrinsic factors in shaping life-histories, aging research is still largely biased towards captive animals living under standardized, optimal conditions (e.g. Languille et al. 2012). In the wild, studies of senescence have largely focused on long-lived animals that face relatively low levels of extrinsic mortality (e.g. sea turtles [Plot et al. 2012], birds [Barrett et al. 2013], Soay sheep [Fairlie et al. 2016]). However, studies focusing on age-related changes in short-lived species in the wild are rare. Hence, studies of wild populations with high extrinsic mortality are essential for testing hypotheses on the evolution of lifespan and senescence.

Oxidative stress and its damage to macromolecules is one of the most cited causes of aging (Sohal and Weindruch 1996). The oxidative damage is a byproduct of aerobic respiration (Sena and Chandel 2012) and intensified by chronic stress conditions characterized by a persistent release of glucocorticoids (GCs) in vertebrates (Salman et al. 2010). Physiological stress is an important mediator in the trade-off between survival and reproduction (Boonstra et al. 2001, Ricklefs and Wikelski 2002). GCs are released in response to a wide range of stressful stimuli (e.g. Romero et al. 2000), and several of their effects parallel those observed during aging, suggesting that chronic stress has a potential to accelerate the aging process (Sapolsky et al. 1987, Velthuis et al. 2013).

The immunosuppressive effects of chronic GC elevation and their consequences for morbidity and mortality have been studied intensively (Sapolsky et al. 2000, Dahabar 2002). Alterations in key immunological parameters during chronic stress parallel those during normal immunosenescence to a large degree (Bauer 2008). These hormones are important regulators of carbohydrate, lipid, and protein metabolism (Dallman 1993), and several earlier studies linked poor body condition to elevated GC concentrations (e.g. Wingfield and Romero 2001). The direct measurement of baseline GC levels in wildlife via blood plasma can be challenging as stress hormones can rise immediately following capture (Romero and Reed 2005). However, leukocyte profiles are a suitable tool to indirectly assess stress levels as these hormones increase the number of heterophils and decrease the number of lymphocytes. Leukocyte responses to stress take about 12 h to several days in ectotherms (reviewed in Davis et al. 2008). Heterophils are the primary phagocytic leukocyte, which proliferate in circulation in response to infections, inflammation and stress (Jain 1993, Campbell 1995, Rupley 1997, Harmon 1998, Thrall 2004). Lymphocytes are involved in a variety of immunological functions such as the production of immunoglobulin and modulation of immune defense (Campbell 1996).

At the cellular level, telomere length (TL) and shortening are thought to be significant proximate contributors to the aging process. Telomeres are short, tandem-repeated sequences of DNA found at the ends of linear eukaryotic chromosomes, whose sequence (TTAGGG) is highly conserved among vertebrates (Meyne and Radliff 1989). Telomeres function in stabilizing chromosomal end integrity (Prowse and Greider 1995), inhibiting aberrant fusions and rearrangements that occur on broken chromosomes (McClintcock 1941), and aiding in the completion of duplication (Watson 1972). During each cell cycle, telomeric repeats are lost because DNA polymerase is unable to completely replicate the 3′end of linear DNA (Watson 1972).

There is great variation among species in age-specific TL (Gomes et al. 2010). Sexual differences in TL and attrition have been suggested to contribute to sex-specific disease and mortality patterns in humans (Stindl 2004, Eskes and Haanen 2007), where women typically have longer telomeres and are longer-lived (e.g., Benetos et al. 2001). Telomerase, the enzyme that countervails telomere shortening was found to be active in stem cells, gametes and most cancer cells, but normally absent from or at very low levels in most somatic cells (Tanaka et al. 2005). However, some studies in reptiles suggested that telomerase may not be turned off in adult somatic cells (Dantzer and Fletchter 2015). Besides cell division dependent telomere shortening, elevated levels of corticosterone can further affect TL via increased oxidative damage by reactive oxygen species (ROS) (Agostinho et al. 2010, Constantini et al. 2011). Elevated GCs, particularly during long-term physiological or psychological stress, have been linked to increased oxidative stress and concomitant telomere shortening and reduced telomerase activity (Constantini et al. 2011). As the nucleobase guanine is a major oxidation target for ROS, the (TTAGGG) repeats are particularly exposed to oxidative damage (Wang et al. 2010).

Telomeres may also act as sentinels of the general level of DNA damage in a given cell. High levels of telomere damage would be indicative of high levels of damage to the coding sequences. Thus, telomeres could offer a mechanism to ensure that cells with high levels of

DNA damage soon terminate division (von Zglinicki 2003). Overall, demanding life-history stages and harsh environmental conditions seem to be linked to a rapid rate of telomere degradation, and there is also a clear connection between physiological stress and telomere attrition in humans, laboratory rodents and wild vertebrates (Eple et al. 2004, Kartschal et al. 2007, Haussmann and Marchetto 2010, Shaley et al. 2013, Bateson 2016). This evidence suggests that telomere dynamics could be closely related to stress in wild vertebrates (reviewed in Angelier et al. 2018), and Houben et al. (2008) emphasized that telomeres are a promising biomarker for chronic oxidative stress.

Labord's chameleon (*Furcifer labordi*) from the seasonal deciduous dry forests in western and southwestern Madagascar has a lifespan of only 4 - 9 months (Karsten et al. 2008, Eckhardt et al. 2017). This extreme life-history makes this species an interesting model for studying potential mechanisms of accelerated senescence, especially because longer-lived sympatric congeners are available for comparative studies. During their short lives, these chameleons undergo hatching at the beginning of the wet season in November, subsequent fast juvenile growth, maturation and courtship followed by death of both sexes towards the beginning of the dry season in May (Karsten et al. 2008, Eckhardt et al. 2017). Wild females tend to live slightly longer, whereas no sex difference in lifespan was found in caged individuals kept under ambient conditions (Eckhardt et al. 2017). Fast growth rates, high reproductive rates and intense mating competition might proximately contribute to increased stress levels and telomere shortening, which in turn may facilitate the decrease of physiological functioning, ultimately leading to death (e.g., Braithwaite and Lee 1979, Oakwood et al. 2001).

To investigate whether the ratio of heterophils and lymphocytes (H/L ratio) and telomere shortening are associated with the early die-off in *F. labordi* in the wild, we determined their telomere dynamics and leukocyte profiles as an indict measure of physiological stress. Our study included two comparisons; one between wild *F. labordi* and their sympatric and longer-lived congener *F.* cf. *nicosiai*, and one with *F. labordi* kept in single cages under ambient conditions, shielding them substantially from environmental stressors, like hunger or predation risk. We predicted an increase in H/L ratios as well as rapidly shortening telomeres in post-reproductive wild *F. labordi* as well as lower H/L ratios and decelerated telomere attrition in *F.* cf. *nicosiai*. Furthermore, as age-related changes should be delayed in the longer-lived females of both species, we predicted females to exhibit comparatively slower rate of senescence than males. Finally, caged *F. labordi*, which were shielded from extrinsic mortality and from a substantial part of the costs of reproduction and starvation, were expected to exhibit slower correlates of aging compared to their wild conspecifics.

Material and Methods

Study site and study species

This study was conducted in Kirindy Forest, which is located in the region of Menabe Central, Western Madagascar, ca. 60 km northeast of Morondava (44°39′E, 20°03′S, 30 - 60 m asl). It is one of the largest remaining Malagasy dry deciduous forest fragments. The local climate is characterized by a hot rainy season between November and April, followed by a cool dry season from May to October (Kappeler and Fichtel 2012). Kirindy Forest is located near the northern end of the range of *Furcifer labordi*, a medium-sized and sexually highly dimorphic chameleon from the western and southwestern regions of Madagascar (Glaw and Vences, 2007). *Furcifer* cf. *nicosiai* is a relatively larger species, also sexually dimorphic (Eckhardt et al. 2019a), and appears to be limited to intact dry forests (Jesu et al. 1999, Glaw and Vences 2007).

Capture-mark-recapture study

Chameleons were located at night using LED flashlights. The capture location was marked and GPS data were taken. We sampled alternating along two transects of 3 km length each. Animals were transported to the nearby research station in cloth bags and handled the following morning. They were sexed, age categorized (hatchling, juvenile, adult), and their snout vent length (SVL) was measured. Animals were individually marked by visual implant elastomers (VIE; Northwest Marine Technology Inc., Shaw Island, WA) (MacNeil et al. 2011). Hatchlings and small juveniles were individually marked with nail polish on the toes. All chameleons were released at their point of capture within 12 h. Sampling took place over three field seasons: November 19, 2013 – July 8, 2014, and January 11, 2015 – July 15, 2015, and October 12, 2015 – December 17, 2015.

Experimental housing

We collected a total of 20 male and 20 female juveniles of *F. labordi* in early January, at approximately two months of age, and kept them individually without visual contact in cylindrical nylon cages (90 cm height, 60 cm diameter) inside a large outdoor forest enclosure. Chameleons received a standardized amount of insects, adjusted to their age and size to match growth and final size of the wild population. Water was offered daily with a spray flask. Between February and June, the caged animals were handled biweekly to obtain blood samples. Only captive animals were sampled repeatedly.

Leukocyte profiles

To measure leukocyte profiles, a drop of blood was taken by lateral puncture of the caudal vein. No blood was taken from females that were obviously gravid. The drop of blood was placed on a microscope slide and distributed as a blood smear. After air-drying, blood smears were processed with a rapid differential haematology staining utilizing the Diff-Quik staining solution system (Medion Diagnostics AG, Düdingen, Switzerland). We determined the ratio of heterophils to lymphocytes (H/L ratio) in at least 200 cells per slide, using the 100 x oil objective. For identification, the general description of reptilian blood cells and terminology was used (Origgi 2007, Tamukai et al. 2011). Counting of the leukocytes started at the most distal edge of the feather end of the smear and proceeded one field of view at a time, across the entire smear in an 'S' fashion. Only fields of view with >15 erythrocytes in a monolayer were considered (Davis and Maerz 2008). All cell counting was conducted by FE. Blood samples were taken from February onwards until mid-July in 2014 and 2015. In total, 319 samples from wild *F. labordi*, 103 samples of *F.* cf. *nicosiai* and 278 samples from 40 captive animals were analysed.

Telomere length (TL)

Blood samples for telomere analysis were taken between March and mid-July in 2015. In total, 66 blood samples of *F. labordi* and 39 of *F.* cf. *nicosiai* were obtained. Captive animals were excluded from this analysis because not enough blood samples were available.

For the determination of TL, we took approx. 5-10 µl blood from the caudal vein after lateral puncture with a capillary and transferred it into a 1.5 ml tube containing 0.5 ml SET buffer. Samples were directly frozen at -20°C. To avoid melting during transportation, samples were stored in a compressor cooling box. Telomere length was measured using real-time quantitative PCR (qPCR) using SensiMix SYBR No- ROX Kit (Bioline, Sydney, NSW, Australia) and a Rotor-gene 6000 thermocycler (Qiagen, Chadstone, VIC, Australia) according to published protocols (Rollings et al. 2017a, Rollings et al. 2017b) using techniques developed by (Plot et al. 2012) with the 18S ribosomal RNA (18S) gene as the non-variable copy number reference gene. The telomere primers used were Telb1 (5′-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT- 3′) and Telb2 (5′-

GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT- 3′, (Plot et al. 2012). The 18S gene (92 bp amplicon in Anolis) was selected as the reference gene as it had previously been validated in reptiles (Plot et al. 2012, Rollings et al. 2017b, Rollings et al. 2019). The primer sequences used were 18S- F (5′- GAGGTGAAATTCTTGGACCGG- 3′) and 18S- R (5′- CGAACCTCCGACTTTCGTTCT- 3′). Reactions were run in triplicate for each sample, with each run containing either Telb or 18S primers. Amplifications were carried out in a Rotor-Gene 6000 thermocycler (Qiagen, Australia) using an initial Taq activation step at 95°C for 10 min and a total of 40 cycles of 95°C for 15 s, 60°C for 15 s, and 72°C for 15 s. Each reaction had a final volume of 20 µl with 10 ng of DNA. A melt curve 412 was generated after each run over the temperature range of 60 to 95°C to ensure that there was no nonspecific product amplification. All of the DNA samples for a given individual were included in the same run. No- template control reactions were run in triplicate for each primer set during every qPCR run to ensure that there was no contamination.

Statistical analyses

Linear models (LM) were used to examine the influence of leukocyte profiles on TL in wild *F. labordi* and *F.* cf. *nicosiai*. As fixed factors, we added month (age), sex and species. For captive *F. labordi*, we used linear mixed models (LMM). As fixed factors, we added month (age), and sex, while ID was included as a random factor for recaptured samples. For all models, we compared the respective full model with the null model by using a likelihood ratio test. In addition, we visually inspected normality and homoscedasticity with residual plots. For model analysis, we used the package lme4 (Bates et al. 2016). All data analysis was conducted in R (R-Code Team 2017). To check for correlation between H/L profile and TL, we calculated the Pearson correlation coefficient.

Declarations

Ethics approval and consent to participate

All work conducted in Madagascar was done with the authorization by the Commission Ad hoc Faune et Flore / Commité d'Orientation sur la Recherche Environnementale (CAFF/CORE) of the Direction Général des Eaux et Forêts, and the Centre National de Formation, d'Etudes et de Recherche en Environnement et Forestier **(**CNFEREF) Morondava, Madagascar.

Results

Concerning white blood cell counts, we found heterophils, lymphocytes, azurophils and basophils. In both species, heterophils were the most abundant leukocyte type, followed by lymphocytes and azurophils. Heterophiles exhibited a spherical shape with an eccentric mostly lobed nucleus containing clumpy basophilic purplish chromatin. Most lymphocytes contained a large nucleus with coarse chromatin, leaving only a small visible band of cytoplasma around it. Basophils were only found sporadically. On average, the H/L ratio of F. labordi (2.45 ± 0.97 SD, n = 319) was significantly higher compared to that of F. cf. nicosiai (1.51 ± 0.47 SD, n = 103, t = - 9.921, p <0.001). Moreover, we detected an increase of the H/L ratio in both species between February and May (Fig. 5.1, Tab. 9), reflecting the cessation of mating activities. In captive specimen, we found an average H/L profile of (1.42 ± 0.14 SD, n = 40) and no significant sex differences (Tab. 5.2). As in their wild conspecifics, the H/L ratio of captive chameleons increased significantly from February until June (Tab. 10).

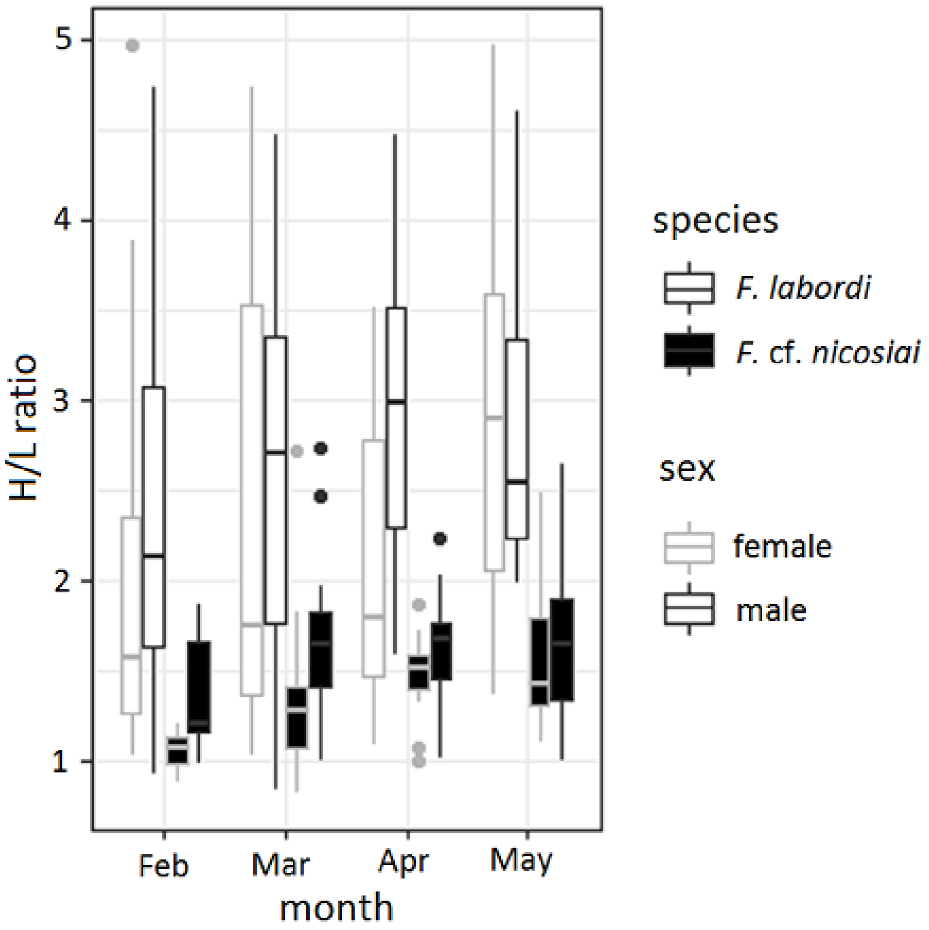

Fig. 5.1: H/L ratio of adult wild specimen of *F. labordi* and *F.* cf. *nicosiai*. Boxplots depict H/L ratio per species and sex from February until May.

During our sampling period, we did not detect any significant sex and age related changes in TL in *F. labordi*. Average TL was significantly longer in *F.* cf. *nicosiai* (t = 6.438, p <0.001). Furthermore, TL of *F.* cf. *nicosiai* was comparatively long in March (1.87 ± 0.77 SD, n = 14) and decreased dramatically until May (1.14 ± 0.33 SD, n = 10, t = -2.686, p <0.01). Moreover, TL of *F.* cf. *nicosiai* males was significantly shorter compared to females (t = -2.67, p <0.01, df = 38). For statistical analyses (Tab. 11), the months June and July were excluded due to small sample sizes (but June is included in Fig. 5.2), and we found a negative correlation between the H/L ratio and TL in *F. labordi* (*r* =-0.556, df = 65, p < 0.01) and in *F.* cf. *nicosiai* (*r* = -0.687, df = 38, p < 0.01; see Fig. 5.3).

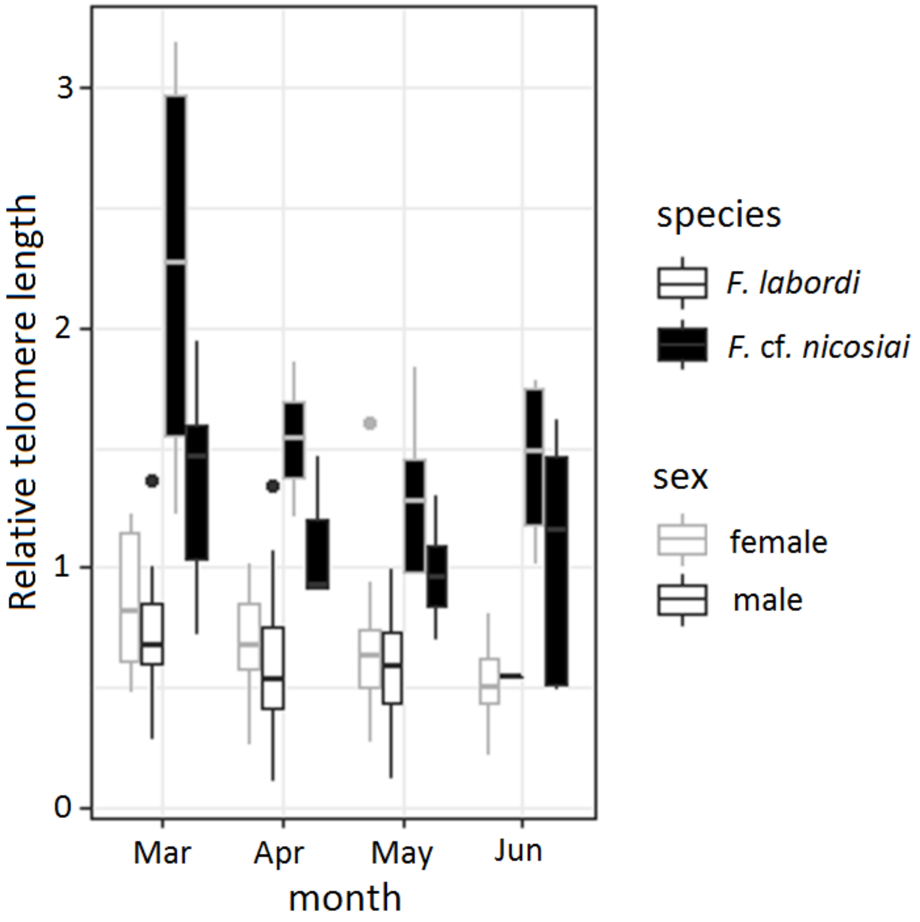

Fig. 5.2: Relative telomere length of adult wild specimen of *F. labordi* and *F.* cf. *nicosiai*. Boxplots depict relative TL per species and sex from March until June.

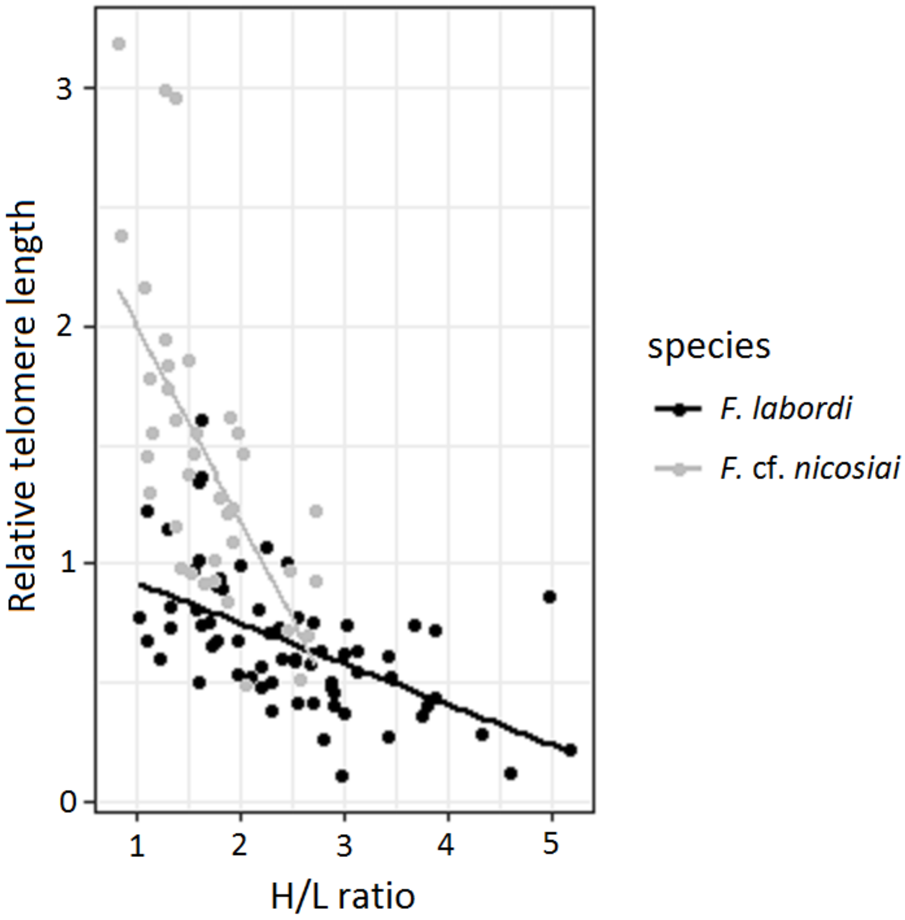

Fig. 5.3: Association of H/L ratio and TL in *F. labordi* (n = 66) and *F*. cf. *nicosiai.* (n = 39).

Discussion

Our study revealed that H/L ratios were consistently higher in wild *F. labordi* compared to *F*. cf. *nicosiai*, indicating higher stress levels in the shorter-lived species. *Furcifer labordi* already exhibited relatively short telomeres when they were 3 – 4 months old. TL was initially comparatively longer in *F*. cf. *nicosiai*, but undergoing rapid shortening after the mating season. In this species, we also detected intersexual differences in H/L ratio and TL, with shorter living males exhibiting higher H/L ratios and shorter telomeres. Interestingly, heterophils were the most common leucocyte type in both wild and captive chameleons. Captive *F. labordi* exhibited comparatively longer lifespans and lower H/L profiles than their wild conspecifics. In planning this study, we assumed that the captive chameleons would be buffered from some environmental stressors, like starvation, desiccation and predation risk. Our data therefore indicate that relatively long-lived wild *F. labordi* individuals were, on average, more stressed and lived shorter lives than their captive conspecifics, indicating a link between stress and longevity.

Baseline stress levels and leukocyte profiles

Investigations in other reptile species indicated large differences between hematology values of different species as well as intraspecific variation as a function of season and sex (Campbell and Ellis 2007, Strik et al. 2007). In their study of blood chemistry and hematology in captive panther chameleons (*Furcifer pardalis*), Laube et al. (2016) found that lymphocytes were the predominant leucocyte type in both summer and winter. In contrast, Cuadrado et al. (2002) reported that heterophils were the most frequently found leucocyte type in dystoic and healthy post-reproductive females of the common chameleon (*Chamaeleo chamaeleon*). The H/L ratio from that study (2.24) resembled the values reported here for *F. labordi* (2.45). More recently, Eshar et al. (2018) found that heterophils were the most abundant leukocytes type in wild common chameleons. As part of their study of leukocyte profiles of an iguanid species, Davis et al. (2011) reviewed several studies of white blood cell profiles of iguanids and other lizard species. They extracted data on the relative numbers of all cell types (mean percentages) and categorized the studies based on whether lizards were from captivity or the wild. They showed that all wild animals had higher H/L ratios than the captive conspecifics. In fact, the relative abundance of lymphocytes and heterophils was completely opposite in both groups, with lymphocytes being the most abundant leukocyte type in captive lizards and heterophils being most common one in wild specimens. Thus, either wild lizards naturally have higher baseline stress levels (and thus higher H/L ratios) than captive ones, or trapping of wild animals induced stress-related alterations in the animals' leukocyte profiles, a notion also supported by the elevated H/L ratios of the captive *F. labordi* in our study.

During a stress response, GC secretion increases partly to mobilize more metabolic energy to deal with the stressor. While this stress response provides obvious short-term benefits, chronic elevation of GCs is harmful (Sapolsky et al. 2000, Romero and Wikelski 2001, Romero and Wikelski 2002, Mc Ewen 2004,). In the present study, we observed stress-related changes in leukocyte profiles in both chameleon species, which may contribute to their rapid senescence after the reproductive season. Captive *F. labordi* showed comparatively lower, but in relation to other captive lizards, elevated H/L ratios (Davis et al. 2011), indicating that they perceived these captive conditions as mildly stressful, but that they were also buffered from major environmental stressors. It is possible that the brief biweekly handling to obtain blood samples might have contributed to the perceived stress level of caged individuals, but this manipulation did most likely not impact the measurements of H/L ratios because such effects were found only after 12 h in other species (Davis et al. 2008).

Any interpretation of the potential physiological effects of variable H/L ratios should take into account that a review published after our field work found inconsistent relationships between GC profiles and leukocyte profiles across studies (Davis and Maney 2018). In gopher tortoises, *Gopherus polyphemus,* both GC levels and leucocyte profiles changed across seasons, but the changes were not correlated (Goessling et al. 2016). Moreover, in two studies of garter snakes, *Thamnophis sirtalis*, conducted by the same research group, but on different populations and in different years, one study revealed a positive correlation between GC levels and H/L ratio (Sparkman and Bronikowski 2014), whereas the other did not (Gangloff et al. 2017). Furthermore, the interpretation of leukocyte dynamics relies on baseline data for the taxon of interest (Davis and Maney 2018). Reports about leukocyte profiles in chameleons in the wild (Cuarando et al. 2002, Eshar et al. 2018) and captivity (Laube et al. 2016) are rare and based on relatively small sample sizes. Our study therefore contributes valuable comparative data based on large samples of two wild chameleon species, but future studies may want to assess stress levels more directly, e.g. by measuring GC levels from fecal samples.

Telomere dynamics

Telomere dynamics differed between the two chameleon species. Telomeres were relatively longer in *F.* cf. *nicosiai,* but shortened rapidly with the disappearance of the adult cohort. In contrast, the telomeres of *F. labordi* were relatively short, but a deterioration over time was not detectable. The first three months in the life of *F. labordi* are characterized by fast growth rates, whereas juvenile *F.* cf. *nicosiai* show much slower growth and reach maturity at an age of 11 – 12 months (Eckhardt et al. 2019a). The lifespan of *F.* cf. *nicosiai* is longer, but both species mate at the same time and die off afterwards. A study of wild jackdaws (*Corvus monedula*) revealed that long telomeres shorten more rapidly than short ones, regardless of the individual’s age (Salomons et al. 2009). Additionally, telomere degradation was highest in humans with long telomeres (Nordfjäll et al. 2009). These studies suggest that mechanisms for telomere maintenance exist in vivo, which potentially protect the shortest telomeres from further attrition and might explain why we could not detect any significant TL reduction in *F. labordi*. It would therefore seem interesting to also examine telomerase activity in these species. In ectothermic vertebrates, the expression of telomerase is frequently found in somatic tissues and is thought to be due to the indeterminate growth (Gomes et al. 2010). Thus, regulation by this enzyme might enable *F. labordi* to maintain its TL up to a certain level.

Whether TL is a universal predictor of longevity is still up for debate. Whittemore et al. (2019) found that the telomere shortening rate, but not the initial telomere length alone, is a powerful predictor of life span in several bird and mammal species. These results support the notion that critical telomere shortening and the consequent onset of telomeric DNA damage and cellular senescence are a general determinant of species life span. In humans, telomere attrition is also more rapid in the first decade of life, stabilizes in adulthood and is followed by a gradual loss at old age (Frenck et al. 1998). We could not study telomere dynamics because of low recapture rates and a lack of data on juveniles, but a relatively large male juvenile *F.* cf. *nicosiai* was sampled at approx. 4 months of age and showed a rTL of 3.44, which was the highest measured in this species. In contrast, TL of hatchling pythons (*Lisais fuscus*) was significantly shorter than that of older snakes, increasing during their first year of life and subsequently decreasing with age (Ujvari et al. 2009). Similar curvilinear telomere dynamics were found in frilled-necked lizards (*Chlamydosaurus kingii*) (Ujvari et al. 2017).

In *F.* cf. *nicosiai*, we also observed sexual dimorphism in telomere length across the sampling period, with females having longer average telomeres. The associated longer female survival may be adaptive as the maturation of eggs after insemination takes several weeks, and female chameleons are capable of producing additional clutches from stored sperm (Tolley et al. 2014, FE pers. observation). In several other species, including sand lizards (*Lacerta agilis*) (Olsson et al. 2011), Medaka fish (Gopolakrishnan et al. 2013) and humans (Gomes et al. 2010), females also live longer and have longer telomeres. The actual mechanisms contributing to sex-specific telomere patterns are unknown, however. Previous work on humans suggested that the difference in TL stems from larger body mass in men compared to women (Seluanov et al. 2007), leading to the assumption that larger bodies require more tissue growth and cell division. However, female sand lizards are larger than males (Gullberg et al. 1997) and have longer telomeres. Gopalakrishnan et al. (2013) postulated that estrogen is a key factor contributing to the decelerated telomere shortening in female Medaka fish, but corresponding data from other species are lacking. Thus, telomere attrition probably depends on multiple factors that remain to be identified.

Nowadays, telomere attrition is widely recognized as one of the hallmarks of aging (e.g. Lopez-Otin et al. 2013), and telomeric assessments are widely used in evolutionary biology as biomarkers of somatic integrity. However, limited attention has been paid to addressing the fundamental question raised by these relationships: Which role do telomeres play in shaping the evolution of life-history trade-offs and senescence (Young 2018)? While it is broadly accepted that telomere degradation can have causal effects on cell fates, the extent to which it contributes to age-related declines on organismal level is less clear. A proximate causal role for telomeres would more possibly reflect an adaptive strategy, born out of telomere maintenance costs and/or a function for telomere attrition (e.g. in counteracting cancer), the relative importance of which is currently unclear. Nevertheless, it is frequently mentioned that telomere length as a predictor of overall health could instead reflect it acting as a non-causal biomarker of accumulated damage to other biological structures that themselves have causal deleterious effects on the organismal performance (e.g. Simons 2015). While it is mechanistically conceivable that telomere dynamics are one proximate cause of current-future trade-offs and senescence, whether telomeres play a significant proximate causal role relative to alternative mechanisms, such as oxidative damage to other biological structures, is currently uncertain (Young 2018). Finally, advances in understanding of the selection pressures that might have shaped a proximate causal role for telomeres according to life-history trade-offs have the potential to shed light on the nature of the evolutionary restrictions at play in life-history evolution and help explain the form of the current-future trade-offs and ageing trajectories (Young 2018).

Stress-related leukocyte profiles and telomere shortening

In both species, we found a negative correlation between average H/L ratio and TL. Chronical stress has potentially negative consequence through an increase in oxidative damage (Agostinho et al. 2010, Constantini et al. 2011) and ultimately telomere shortening (Wang et al. 2010). Oxidative stress also dramatically decreases telomerase activity (Borrás et al. 2004, Kurz et al. 2004). Therefore, oxidative stress not only accelerates telomere shortening by direct damage to telomeres, but also by inhibiting telomere restoration as well. Even though we are well aware of the correlational nature of our study, we suggest that physiological stress negatively affected TL in our two study species. Although our findings and additional studies suggest a strong association between stress and telomere shortening (Haussmann et al. 2011, Quirici et al. 2016), we cannot discard other mechanisms that could affect TL, like alterations of early growth rates (e.g. Schultner et al. 2014). More direct future studies should acknowledge that the link between stress response and telomere degradation is probably not straightforward and depends on the benefits and costs of activating an emergency life-history state that is species- and context-dependent.

At an ultimate level, rates of extrinsic mortality are thought to determine where a species falls on the slow-fast continuum, with high rates of extrinsic mortality selecting for fast life-histories (Williams 1957). The results of our previous capture-mark-recapture study (Eckhardt et al. 2019a) also suggest that extrinsic mortality rates in both chameleon species are presumably high in adults. Williams also postulated that juvenile mortality has no influence on the evolution of senescence; predicting that senescence should be associated with extrinsic mortality rates (Williams 1957). However, formal, mathematical theory (Abrams 1993, Caswell 2007, Wensink et al. 2017) showed that this particular prediction is wrong. Accordingly, selection leading to senescence does not directly depend on survival to old age, but rather on the shape of the stable age distribution. The aim of evolutionary theories of aging is to clarify why organismal fitness mechanisms decline with age. Moorad et al. (Moorad et al. 2019) therefore proposed to investigate the actual phenomenon of aging, not its proxies. More theory and careful physiological measurements from many species under many different environmental conditions are therefore required to further illuminate factors that shape life-histories. Remarkably, Reznick and colleagues (2004) even found that guppies (*Poecilia reticulata*) derived from natural populations with high levels of predation live the longest in the laboratory. This study demonstrates that our understanding of the evolution of senescence will profit from modeling numerous aging parameters, traits other than age at death as well as the causes of mortality.

Although there are many examples of negative correlations between lifespan and the apparent extrinsic risk of death faced by organisms, this risk is more often deduced than measured. In our study species, besides extrinsic mortality at old age, several factors might impact the short lifespan of this species. High juvenile mortality in *F. labordi* might lead to the extremely high investment in reproduction that in turn facilitates the pronounced stress response and relatively short telomeres. As physiological stress also has a strong influence on immune responses (Padgett and Glaser 2003), the increasing gastrointestinal - and blood parasite burden observed in both species in the wild towards the dry season (Eckhardt et al. 2019b) might reflect an unavoidable consequence of this adaptation. This notion about the physiological processes contributing to such a short life span in *F. labordi* is also supported by a maximum lifespan in caged individuals of 16 months, indicating that their lifetime is indeed bounded by molecular and cellular mechanisms of aging.

Conclusions

The results of our study provide rare information about leukocyte profiles and telomere dynamics in relation to senescence and mortality patterns of two chameleon species in the wild. The results of this study suggest that the presumably energetically demanding reproductive season in the short-lived species contributes to environmental stress ensued by increased oxidative damage and subsequent accelerated telomere shortening. To fully understand telomere dynamics and their relation to stress-related measures (H/L profiles) in these species, repeated samples from wild specimens and samples from younger life stages are necessary, however.

Tab. 9: Parameters of the LM on the influence on H/L ratio

Fixed Effects	Estimate	SE	t-value	P	F	df	P
(Intercept)	1.974	0.095	20.766	**<0.001**	25.64	400	**<0.001**
March	0.325	0.105	3.093	**<0.01**			
April	0.374	0.119	3.140	**<0.01**			
May	0.735	0.139	5.366	**<0.001**			
Sex: male	0.356	0.087	4.102	**<0.001**			
Species: *F.* cf. *nicosiai*	-1.022	0.103	-9.921	**<0.001**			

Tab. 10: Parameters of the LMM on H/L ratio in captive *F. labordi*

Fixed effects	Estimate	SE	df	t-value	P	χ^2	df	P
(Intercept)	1.185647	0.056175	163.3084	21.10641	**<0.001**	33.75	5	**<0.001**
sex: male	-0.00934	0.041367	33.16293	-0.22582	0.822726			
March	0.222828	0.075443	239.0659	2.95359	**<0.01**			
April	0.192228	0.079707	238.8112	2.411677	**<0.05**			
May	0.39695	0,072926	243.9702	5.443204	**<0.001**			
June	0.295445	0.059703	175.842	4.948552	**<0.001**			

Tab. 11: Parameters of the LM on the telomere length of *F. labordi* and *F.* cf. *nicosiai*

Fixed effects	Estimate	SE	t-value	P	F	df	P
Intercept	0.8249	0.1162	7.100	**<0.001**	25.67	80	**<0.001**
Species *F.* cf. *nicosiai*	1.3070	0.1660	7.871	**<0.001**			
April	-0.1001	0.1291	-0.775	0.441			
May	-0.1343	0.1229	-1.092	0.278			
sex male	-0.1015	0.1055	-0.962	0.339			
species F. cf. nicosiai April	-0.3844	0.2378	-1.616	0.11			
species *F.* cf. *nicosiai* May	-0.5464	0.2001	-2.731	**<0.01**			
Species *F.* cf. nicosiai sex male	-0.5176	0.1773	-2.919	**<0.01**			

Chapter 6: General Discussion

The aim of this thesis was to disentangle the extrinsic and intrinsic mechanisms shaping the short life-history of the annual chameleon *F. labordi*. I aimed to shed light on the ultimate and proximate factors by examining a wild population of *F. labordi* and compared my findings to captive specimens. Moreover, I related my findings with the sympatric and longer living *F.* cf. *nicosiai*. Besides the assessment of differences in life-histories, the interspecific comparison was applied to investigate if changes might be age-dependent or seasonally influenced. In the previous chapters, I have presented original research on the internal and environmental mechanisms influencing the lifespan of *F. labordi* and the congeneric species *F.* cf. *nicosiai*. In this chapter, I will discuss the external drivers of the disappearance of *F. labordi* in the wild by comparing two populations from different habitats and vegetation periods as well as the results from the experimental housing of this species. Additionally, I will consider intersexual variations in life-history and lifespan. Moreover, I will contrast the wild living *F. labordi* with the caged ones regarding lifespan and discuss the differences. Subsequently, I will summarize the interspecific differences in life-history concerning the comparison with the congeneric species. In this context, I will discuss the factors favouring the evolution of relatively fast life-histories found in chameleons in general. Furthermore, I will outline the examined intrinsic factors driving senescence in *F. labordi* and consider their interplay. Finally, I will propose future research suggestions to better understand the causes and consequences of aging in the study species and reptiles as a group.

Intraspecific, interannual and intersexual differences in lifespan in *F. labordi* in the wild

I found considerable variability in the life-history of *F. labordi* between the population in the southern and drier habitat (Ranobe) and northern and wetter part of their distribution range (Kirindy) that was attributed to more extensive precipitation (**Chapter 2**). In addition, both populations differ considerably in different aspects. In Kirindy, males were considerably larger compared to the population in Ranobe. Moreover, females in Kirindy seemed to be capable of producing at least two clutches and are therefore not strictly semelparous, whereas females in Ranobe seemed to be restricted to a single clutch (Karsten et al. 2008). In addition, probably due to the longer active season, animals in Kirindy required more time to reach sexual maturity.

In total, the observed differences between both populations can be ascribed to ecological differences in their habitat, suggesting that the life-history is to some degree variable and not fully programmed. Furthermore, the slower growth rates that I observed in Kirindy probably lead to a comparatively decelerated shortening of the telomeres and might in turn facilitate the longer lifespan is this population. Interestingly, Karsten et al. (2008) reported that they found several obviously weak individuals and unmutilated dead bodies in the wild. I rarely observed dead weak animals or dead bodies in the wild. Only one male was so weak that he fell down from a branch. Considering the high differences in sample size of adult individuals of both populations, animals from Ranobe seem to suffer from high senescent declines, whereas geriatric animals were rarely found in Kirindy. These observations indicate that *F. labordi* in Kirindy may suffer from substantially higher predation rates concealing apparent senescent declines. This could be attributed to the different types of habitats both populations live in. Ranobe is a spiny forest with a sand substrate, and vegetation that usually does not exceed 3 m in height except for occasional trees that may reach up to 10 m in height (Vose et al. 1992). In contrast, whereas the Kirindy forest is characterized by rather dense vegetation and in average higher trees (Zinner et al. 2014). Therefore, the number of predators such as snakes and birds might be considerably higher as this forest might inhabit more species or specimens that feed on chameleons. Here, predators might directly eliminate individuals with slight senescent declines. Moreover, Karsten et al. (2008) also observed negative growth in some adult individuals towards the dry season, whereas specimens in Kirindy showed indeterminate growth until their disappearance. Interestingly, also chameleon species composition differs between both habitats. While *F. labordi* lives sympatrically with *F. verrucosus* in Rabobe (Karsten et al. 2008). The latter species has never been observed within the Kirindy forest, but frequently in degraded forest fragments and scrubland nearby villages in the region. Here, *F. verrucosus* is living sympatrically with the smaller sized *F. viridis*, which seems to have an annual cycle as well (pers. observation).

Furthermore, I found that the lifespan of *F. labordi* could vary significantly between years depending on the rainy season. After an unusually long previous rainy season in 2015, both sexes survived considerably longer and one female was capable of surviving until a second breeding season. Thus, in rare cases, a very small proportion of adult females may survive the dry season under favourable environmental conditions, and they may even enter the next reproductive season. These results indicate that the lifespan of this species highly depends on precipitation pattern and vegetation period. The climate of Madagascar is highly variable (Dewar and Richard 2007). In response to high climate fluctuations, many mammals of

Madagascar differ from relatives in more stable environments in that the Malagasy species exhibit more extreme versions of both ‘‘short-lived’’ and ‘‘long-lived’’ life-histories (Dewar and Richard 2007).

The short life-history of *F. labordi* seemingly has a strong influence on intra- and intersexual selection. Karsten et al. (2009b) showed that potentially receptive females were selective according to the male’s body size and size corrected rostral width. Males used their rostral appendages during courtship encounters in which they tried to persuade resistant females to permit copulation. Here, broader rostral appendages may be beneficial if females use the amount of pressure applied by the male as a possible indication for male physical performance in assessing suitable mates. Concerning intrasexual selection, game theory predicts that more size-matched rivals should have more intense physical encounters (Earley and Dugatkin 2002). Indeed, Karsten and colleagues (2009a) found that during male-male contests, the more naturally size-matched *F. labordi* had much more physically intense encounters than *F. verrucosus* where males were less equally sized. Interestingly, during male-male combats, the shape of the rostral appendage does not seem to influence the outcome of the fight. Within males, intrasexual selection seems to be restricted to body size and casque height. Therefore, males should invest highly in growth. Growth rates in males are extreme as males can potentially increase body size from hatchling to adult by 300-400% in two to three months (Karsten et al. 2009a, Eckhardt et al. 2019a). The strong allocation to growth by males in many species might result in decreased somatic maintenance and earlier respectively faster aging (Kirkwood and Rose 1991), which increases the magnitude of the sex gap in aging in favouring females. The hormonal regulation associated with growing and maintenance of secondary sexual traits as found in male *F. labordi* is likely to have far-reaching deleterious effects on their adult survival. Furthermore, the extensive male allocation to specific sexual traits (e.g., large body mass or conspicuous ornaments [e.g. Weckerly 1998, Emlen 2008]) is assumed as energetically costly (Kotiaho 2001). As predicted by recent evolutionary theories of aging (e.g., disposable soma theory, [Kirkwood and Rose 1991]), the stronger reproductive expenses by males during the onset of reproduction might be costly in terms of aging (Lemaitre et al. 2015). For example, in most sexually dimorphic and polygynous mammal species, males grow faster and larger than females because attaining a large body rapidly can be advantageous to increase access to mating opportunities (Dmitriew 2011). On the other hand, rapid growth is associated with many physiological costs (e.g. increased oxidative damage and faster speed of telomere attrition (Geiger et al. 2012), steeper rate of body mass decline with increasing age, (Douhard et al. 2017), possibly resulting in higher mortality rates in adult males (Metcalfe and

Monanghan 2003). In addition, testosterone that controls the development of many sexual traits in males is likely to have a negative effect on some aspects of biological performance, such as immunocompetence (Foo et al. 2017).

The costs of sexual selection and sexual dimorphism has long been considered the best explanation for the observed sex gap in senescence. Recent data suggests that sex chromosomes may play an important role in shaping the sex gape. This is mostly true in species, such as monogamous birds, with no or very little sexual dimorphism, which display some sex gap in aging and longevity. Consequently, it is currently unclear what the underlying mechanisms are. Within their review on studies of wild tetrapod species, Pipoly et al. (2015) found that taxa with female heterogamety had a significantly more male biased adult sex ratio compared to taxa with male heterogamety. Mammals are fixed for the XX/XY type with male heterogamety, whereas birds are fixed for the ZZ/ZW type with female heterogamety. Among reptiles and amphibians both patterns occur. The type of genetic sex determination explains up to 36% of variance in adult sex ratio in reptiles, and 76.5% of species exhibiting the ZZ/ZW type showed male biased adult sex ratio whereas it was 24.2% in the species with the XX/XY type (Pipoly et al. 2015). Recently, the chameleon genus *Furcifer* was found to have female heterogamety (Ravatsos et al. 2015). Controversially to the assumption of male bias in adult survival, I detected a female bias in wild *F. labordi* as well as *F.* cf. *nicosiai*, indicating that several additional factors in these species might lead to the observed pattern.

Lifespan of *F. labordi* in captivity

By keeping specimens in captivity, I found that both sexes lived comparatively longer compared to their wild conspecifics (**Chapter 2**). Again, these findings indicate that the short lifespan in the species is not fully programmed and depends on several extrinsic factors of mortality such as predation, food, water availability, encounter rate with parasites, and exhaustion from reproduction. Interestingly, in captivity, I found no significant difference in longevity between both sexes. This leads to the assumption that faster growth and higher testosterone levels alone might not be solely responsible for the faster die-off in males in the wild. Similar results were found in mouse lemurs *Microcebus murinus*, where survival in the wild was strongly female-biased (Kraus et al. 2008, Languille et al. 2012), whereas males in captivity exhibited longer lifespans (Perret 1997). Probably, male behaviour that is influenced by testosterone leads to the shorter lifespan we observed in nature. However, I did not measure testosterone levels and captive males might have considerably lower levels as they were shielded from intraspecific encounters. A comparative study in captivity using normal and castrated males may disentangle the direct effect of testosterone on the lifespan. Nevertheless, captive males might have comparatively lower testosterone levels as they were visually shielded from male as well as female conspecifics. Anecdotal reports from some lizard species in zoos indicate that the visual absence of other males lowers their display behaviour towards females (e.g. Petr Kodym, pers. comm.). Therefore, the influence of lower testosterone levels on the extended lifespan of males in captivity cannot be excluded. Roaming and fighting for females probably involves an increased risk of predation and pathogen encounters in the wild. In general, *F. labordi* males tended to be much more aggressive and exhibited fatal injuries resulting from intrasexual competition compared to the larger and longer living *Furcifer* species in Kirindy (pers. observation). Similarly, Karsten and colleagues (2009b) found that *F. labordi* showed higher aggression and had higher graded antagonism scores per trial during male-male encounters than did the larger sized *F. verrucosus.* These observations imply that male *F. labordi* are high in testosterone levels most likely resulting in increased risky behaviours. Ultimately, their low probability of survival and relatively high densities during mating season might have led to an increased enhancement of testosterone.

By indirectly examining stress hormone levels via H/L ratio, captive specimens showed lower H/L ratios (**Chapter 5**) probably resulting from lower physiological stress compared to the wild living ones (Eckhardt et al. 2020). Moreover, the extenuated encounter rate with parasites might have additionally contributed to their longer lifespan in captivity (**Chapter 4**). Overall, as several factors of extrinsic mortality were excluded in captivity, I can only speculate which factors are essentially responsible for the shorter life expectancy in nature. Finally, the conduction of cage experiments with a controlled manipulation of single factors such as visibility of conspecifics or parasite burden could clarify the direct influence of these effects on the lifespan of this species.

Interspecific comparison and life-histories of chameleons

While comparing the life-histories of *F. labordi* with these of the *F.* cf. *nicosiai* and *F. oustaleti* (**Chapter 3**), I found that all three species differed in hatching time, growth rates, body size at maturity, recapture rates and roosting heights suggesting some interspecific niche segregation among the species. Long-term ecological studies on chameleon communities are scarce. Although Karsten et al. (2008) indicates synchronous hatching of *F. labordi* and *F. verrucosus* in Ranobe, a detailed look at the data shows that the cohort hatchlings in *F. verrucosus* emerges after the one of *F. labordi*. Therefore, interspecific time shifts in the period of hatching and other ecological determinants might occur more often within sympatric chameleon species. Giving the lack of ecological studies within this lizard family, additional research is necessary to unravel the factors regulating the coexistence of sympatric chameleon species. Notably, the study of Luiselli (2007) revealed that chameleon communities are not randomly organized, but instead show precise deterministic patterns. In lowland rainforest, chameleon communities are arranged deterministically along the food niche resource axis, but not along the habitat niche resource axis. The opposite holds for chameleon communities inhabiting montane rainforests. As chameleons inhabit various habitats (e.g. dry forests, heathers etc.), additional research is required to understand the composition of chameleon communities in detail. However, my findings indicate, that species in the Kirindy forest might be assembled both, along the food niche and habitat niche resource axis.

Interestingly, I found that *F.* cf. *nicosiai* and *F. oustaleti* exhibited unexpectedly short lifespans as well. Only *F. oustaleti*, the largest of all three species, and one of the world's largest chameleons, seemed to be at least temporary perennial. Publications on life-histories of chameleons in nature are rare, but available studies suggest that chameleons show rather short lifespans in nature (Andreone et al. 2005, Karsten et al. 2008, Tolley et al. 2010). In general, compared to other lizards of comparable size, chameleons seem to have remarkably shorter lifespans. Also in captivity, chameleons show relatively short lifespans and high mortality rates that is probably due to their naturally fast life-histories (Robinson et al. 2016). An important variable of the life-history is age at maturity, which in lizards is generally positively correlated with body size (Meiri et al. 2012). However, such as *F. labordi*, several medium-sized Malagasy chameleons from the genus *Furcifer* (e.g. *F. lateralis*) are known to reach maturity within one season (Raselimanana and Rakotomalala 2003), suggesting a short lifespan in these species as well. Within captivity, the maximum documented lifespan of *F. lateralis* was approx. five years, while their lifespan in captivity typically ranges from two to three years (Gehring 2008). Moreover, indicative of fast life-histories, compared to many other similar-sized lizard taxa, chameleons have the largest clutch sizes and are among the most productive ones (Meiri et al. 2012). Interestingly, large clutch volumes and high relative clutch mass have mainly been documented in reptiles with cryptic behaviour, where the probability of escaping predators does not depend on rapid movements (Vitt and Price 1982).

The short documented life-histories in chameleons might be partially due to their inability to store enough fat reserves to endure energy-demanding periods such as the mating season or aestivation. As most chameleons have a laterally compressed body and depend on their ability to hide behind branches, the storage of fat reserves might be disadvantageous in terms of predation avoidance. In contrast to many other lizards, chameleons are not able to store fat in their tails (Pond 1978). Since chameleons highly rely on camouflage, but are the slowest of all lizards (Abu-Ghalyun 1988), their probability of escaping after the detection of a predator is relatively low. Their specialization for grasping is, thought to exhibit a trade-off with running ability. Ultimately, the inability to store fat to endure strenuous periods combined with the reduced possibility of survival after being detected by a predator might have selected for fast live cycles. The restricted time and probability of reproduction may also have driven the often mentioned high aggression in males (e.g. Nečas 1999, Karsten et al. 2009a), probably resulting in intense intrasexual selection in this group. Especially males offer large casques that are a good indicator of bite force as individual bearing higher heads probably have more jaw musculature, and in some species, casque height is positively correlated with fighting outcome (Stuart-Fox et al. 2006). Furthermore, chameleons possess an acrodont dentation. In squamates, this kind of dentation is found in chameleons and agamid lizards. Acrodont teeth are superficially attached to the tooth-bearing bone and teeth are worn down with age. With the exception of chameleons, most acrodontic species also have a number of conical pleurodont (teeth that are replaced throughout life) in the rostral-most portion of the upper and lower jaws (e.g. Cooper et al. 1970). Tooth erosion is a frequent hallmark of aging among different organisms, particularly in mammals (Finch 1990). The fact that chameleons have no replaceable but easily wear off teeth shows again that they are physically not adapted for long lifespans. Actually, especially males of all three *Furcifer* species from Kirindy showed worn teeth towards the end of the mating season (personal observation).

According to their study of ecological drivers of longevity in squamates and tuataras, Scharf et al. (2015) documented that besides age at maturity, hatchling size is positively correlated with longevity. The three *Furcifer* species in Kirindy exhibited similar body size (2.5 - 3.0 cm) (**Chapter 3**), which is comparatively small compared to similar-sized lizards, predicting short life-histories in this species. They also found that carnivorous species were shorter living than omnivorous or herbivorous species of similar size. These findings are explained by the assumption that carnivorous animals may be faster growing, become injured while pursuing prey, and are more likely to acquire parasites through their diet. According to the aging theory, this leads to reduced longevity in carnivorous species (Huges and Raynolds 2005). Chameleons are predominantly feeding on invertebrates and rarely digest plant matter (Tolley and Herrel 2013), which in turn may contribute to their short life-histories. In total, their high morphological specialization to the cryptic and prominently vegetation but not shelter associated live style as well as the obvious specialization to feed on mobile food via the projectile tongue have ultimately selected for fast live histories. Thus, their short lifespans leading to high investment in growth, reproduction, and low investment in self-maintenance, as for instance, seen in their incapability to replace lost or wear off teeth.

Chameleons are a young clade of lizards, dating back to the onset of the Cenozoic, whereas most other lizards are much older (Tolley and Herrel 2013). Despite their young age, there are more than 210 described chameleon species and when considering the age of this clade, the number of species is relatively high compared to other lizard families. It seems that the diversification of this family is linked to their invasion of the arboreal habitats some 45 million years ago (Herrel and Tolley 2013). Lastly, their specialization might have driven a high species diversity that is in turn accompanied with fast life-histories. Actually, their short lifespans presumably facilitated species divergence due to shorter generation times. In addition, probably because of their life-history, several chameleon species demonstrated to be successful invasive species when escaping into suitable environments (e.g. Chiaverano et al. 2014, Smith et al. 2016).

Conversely, a recent study in one of the largest chameleon species, *Calumma parsonii* based on skeletochronology of preserved specimens revealed that the highest minimum age was nine years for a male and eight years for a female, affirming a considerably longer lifespan (Tessa et al. 2017). This species takes 3 to 5 years to reach maturity (Brady and Griffiths 1999) and occurs in rainforests where environmental conditions are comparatively stable, which might explain the slower life-history compared to the ones reported here. Besides *C. parsonii*, some other relatively large rainforest dwelling congeners (e.g. *C. globifer*, *C. ambreense, and C. oushaugnessyi*) exist that might have comparable longer lifespans in nature. Life-history studies, including small-sized genera such as *Brookesia* are absent. Regarding my personal observation in the Kirindy forest, *Bookesia brygooi* exhibited high fluctuations in population densities concerning differences in rainy seasons. From the onset of the dry season, no specimens were observed. The small amount of eggs per clutch (2 - 5) and their relatively short incubation period (Glaw and Vences 2007) indicates that there are overlapping generations, at least within their active period. However, concerning the lack of long-term studies in chameleons, further examinations are necessary to evaluate differences in life-histories originating from seasonal or more stable habitats. In general, high temperatures should lead to high metabolic rates, and therefore to a fast rate of living that could shorten lifespan (Sohal

1986) predicting that montane species might be longer living. Interestingly, on the African mainland but not Madagascar, viviparity has evolved in several chameleon clades (e.g. Huges and Blackburn 2019), and most viviparous species are associated with high elevation grasslands and heathers. It would be interesting to investigate if reproduction biology has an influence on the life-history and lifespan in chameleons. In summary, most chameleon species seem to be rather short living and the notorious rapid death of captivity probably represent the natural adult lifespan. Consequently, a new assessment may be necessary concerning the viability of chameleon conservation and breeding programs. Especially chameleons with annual life-history traits linked to particular climate events such as the onset of the rainy season may suffer from unexpected extreme weather conditions caused by climate change.

Intrinsic mechanisms of senescence in *F. labordi*

High extrinsic mortality rates are known to select for early reproduction, placing animals under additional physiological stress that can, in turn, increase intrinsic mortality (Quinlan 2010). Glucocorticoids are known to suppress maturation, differentiation, and proliferation of all kinds of immune cells (Sternberg 2006), and even activate apoptosis in immature T and B cells and mature T cells (Sapolsky et al. 2000). This is measurable both in the decreasing number of some classes of immune cells, especially the T - and B - cells of the adaptive immune system. On the other hand, proinflammmatory cytokines are increasingly produced. This has led to the word “inflammaging”, pronouncing the close connection between aging and inflammatory processes. Reasons for the low number and performance of B - and T lymphocytes are likely to be caused by the proliferative senescence of stem cell production in the bone marrow due to telomere shortening. These changes in the immune system increase the susceptibility to infections (Rensing and Rippe 2013). Among the T-lymphocytes, the T-helper cells from the type Th2 are involved in parasite resistance (Malaguanera et al. 2001). Tissue destruction is often caused by parasites and Th2 cell-mediated immunity evolved as an adaptive mechanisms of tissue repair that quickly heals wounds they cause (Allen and Wynn 2011). In *F. labordi* and *F.* cf. *nicosiai* I found that the prevalence of gastrointestinal- and blood parasites highly increased towards the end of the reproductive season, indicating a down-regulation of the adaptive immune system in both species. The examination of fecal samples indicates that *F.* cf. *nicosiai* invests into an adaptive immune system and therefore self-maintenance in the younger stages. In wild *F. labordi* and *F.* cf. *nicosiai,* I observed continuously elevated H/L ratios that most likely contribute to the increasing gastrointestinal - and blood parasite burden. Overall, the results of

Chapter 4 and **Chapter 5** indicate that elevated GC levels of wild *F. labordi* and *F.* cf. *nicosiai* may lead to accelerated telomere shortening and thereby a downregulation of the immune system (Eckhardt et al. 2020). However, the parasite burden observed in fecal and blood samples revealed the combined outcome of several factors: the parasites encountered by the host, when the parasite matured and reproduced within the host, and how effective the host's immune system was in preventing or eradicating the infection. While it is problematic to disentangle these factors, I found males having higher parasite infection rates than females. Moreover, older animals suffered from higher parasite burden, suggesting a downregulation of the acquired immunity in both species. Finally, the conduction of cage experiments, including both species and sexes with a controlled manipulation of parasite burden, could clarify the direct influence of the effect of parasites on the lifespan of both species.

Cellular proliferation is a key factor of an effective adaptive immune system. Thus, the role of telomeres in leukocytes, especially in lymphocytes, is of great interest (Weng 2008). Short telomeres of leukocytes have also been associated with several immune-related diseases (Andrews et al. 2010). The relatively short TL in *F. labordi* and the rapidly decreasing TL in *F.* cf. *nicosiai* might be connected with the increased burden of gastrointestinal - and blood parasites that I found towards the dry season in both species. In turn, the presence of pathogens was linked to accelerated telomere loss in mice (Ilmonen et al. 2008) and birds (Ashgar et al. 2015), which might also lead to a shortened lifespan in the two wild living chameleon species. Especially the presence of blood parasites might negatively affect the TL of both chameleon species as it was detected in birds (Ashgar et al. 2015). In particular, the prevalence of filarial infection was high in *F.* cf. *nicosiai*, which may have contributed to the accelerated telomere loss in this species.

Both in the wild and captivity, the accumulation of molecular and cellular damage would be expected to lead to physiological and physical deterioration at old age. However, the level of deterioration may be influenced by factors such as resource availability and lifelong trade-offs among growth, reproduction and somatic maintenance. In the comparative study, *F. labordi* in captivity was significantly longer living than the wild conspecifics. However, the fact that the maximum age of a captive individual was 16 months indicates that intrinsic mechanisms play an essential role in the lifespan of *F. labordi*. Captive specimens showed comparably lower H/L ratios and were, for the most part, shielded from the risk of parasitic infections. The documented average and maximum lifespan of the captive individuals (**Chapter 2**) suggests that besides elevated GC-levels and parasite infections, additional factors are jointly responsible for the short life expectancy in this species.

The vertebrate organism is highly complex, and no physiological parameter acts in isolation. Hence, measuring several different aspects of senescence can lead to a better understanding of organismal senescence (Burger and Promislow 2006, Nussey et al. 2008). One of the main problems in developing a coherent aging theory is unraveling causes from effects. Just because two processes parallel each other, we cannot postulate a causal relation in any direction. Therefore, it is challenging to predict which, if any, mechanistic theory of aging is correct. One approach to infer the impact on aging of the pathways described here is using a system-level methodology. By disturbing each component of a pathway under study and integrating the observed effects, it is likely to distinguish causes from effects and formulate new hypotheses (de Magalhaes and Toussaint 2004).

For *F. labordi* and chameleons in general, further insights of senescence could be gained via the study of e.g. body composition, sex hormone levels and further health indicators. Longitudinal studies of multiple indicators of physiological and physical functioning are required to resolve the significance and interplay of the different parameters. To determine which traits best influence the aging phenotype, further comparison and connections between the different components of health also warrant further studies. For example, parasite burden is a widely employed indicator of general health. While parasites may cause disease, it is also likely that bacterial or viral infections that could not be measured within this thesis are more direct mediators of morbidity and mortality than parasites alone. Especially in wild living males of *F. labordi*, the number of facial wounds was increasing towards the end of the reproductive season and often resulted in eye infections. Moreover, future studies would benefit from direct investigation of immune function, including markers representing the functioning of the different parameters of the immune system. Additionally, GCs were measured indirectly by the H/L profiles of blood samples. The increase in heterophils is known to be connected with permanent physiological stress and might also be influenced by other factors such as infections and inflammations (Jain 1993, Campbell 1995, Rupley 1997, Thrall 2004). Therefore, a direct measurement of GCs could be advantageous to obtain, but difficulties according to direct measurements of baseline stress levels were already discussed in **Chapter 5.** Moreover, oxidative stress that is one of the most cited causes of aging (Sohal et al. 1996) would be an essential factor to quantify. In addition, testosterone levels, which may have sex-specific immunosuppressive effects, were interesting to examine regarding senescence in this species.

In summary, there is a lot of potential work to shed light on the mechanisms behind the short lifespan of *F. labordi*. However, the analysis of most parameters requires blood samples and sometimes relatively large quantities. Specifically, in *F. labordi* the removal of blood was often difficult due to their small body size. Therefore, larger and additionally fast living chameleon species might be more suitable for these studies. Including larger chameleon species also gives the advantage that samples could be taken from younger stages, giving additional information about factors such as telomere dynamics during lifetime. Concerning standardized housing under laboratory conditions for aging studies, researchers should consider that most chameleon species have to be housed in single cages, have special requirements according to light, temperature, food and water supply and equipment of the enclosure. Thus, longitudinal studies in the laboratory will be time-consuming and costly.

Primarily, the congeneric and larger species *F.* cf. *nicosiai* was selected to compare aging mechanisms and examine if physiological alterations and parasite burden might be age intdependent or seasonally influenced. During my study period, this species appeared to be longer living, but their reproduction period is restricted to the rainy season, followed by senescent declines as well. In this species, slowly growing juveniles are the longest-lasting phase (approx. 10 months).

Potential of aging research in reptiles

Regarding vertebrates, most studies focusing on the relationship between lifespan and the related factors have been conducted on mammals and birds (e.g. Holmes et al. 2001, Wilkinson and South 2002, Brunet-Rossinni and Austad 2004, Speakman 2005, Turbill et al. 2011, Healy et al. 2014). This is probably due to the fact that the ecology and life-history of many reptiles makes them difficult to monitor in the wild (McDiarmid et al. 2012). Moreover, their husbandry is partially laborious and expensive. Therefore, reptiles are underutilized models in studying the evolution and mechanisms of senescence (Bronikowski 2008). Their special physiology, indeterminate growth, and often increasing female fecundity with proceeding age should motivate the investigation of how life-history at the organismal level, physiology at the mechanistic level, and natural selection at the evolutionary timescale define lifespan in this diverse taxonomic group.

Finch (1990) suggested that reptiles tend to show lower incidence and intensity of senescence than most mammals. However, some species show signs of aging comparable to what is documented in mammals (Majhi et al. 2000, Jena et al. 2002; Olsson and Shine 2002). Recently, reptiles were found to have three times more variation in life-history traits in comparison to mammals (Babich Morrow et al. 2019), indicating broad taxonomic divergence in aging phenotypes. Furthermore, a recent study of longevity in reptiles indicates that body mass explained little of the variance in reptile longevity, which is controversial to the findings in mammals (Stark et al. 2019). They explain this result by the assumption that reptiles as ectotherms are less affected by metabolic byproducts due to their lower metabolic rates. (e.g. Bronikowski and Vleck 2010). Intriguingly, several squamate species have tissue regenerative capacities that surpass those of mammals by far (e.g. Sun et al. 2018). Until now, there has been little consideration of how these regenerative capabilities impair the rate of aging in these species (Hoekstra et al. 2019). Therefore, it is highly possible that reptiles may feature various mechanisms to delay aging and age-related debilitation, making them an interesting model to study aging. Overall, comparisons among different animal taxa can yield insights into those mechanisms of aging that are fixed versus those that have evolved independently in different taxonomic lineages.

Interesting examples about divergence of aging mechanisms in this group are pythons *Lisais fuscus* (Ujvari and Madson 2009) and frilled-necked lizards *Clamydosaurus kingii* (Ujvari 2017), which were found to have curvilinear telomere dynamics. Furthermore, research of the European freshwater turtles *Emys orbicularis* did not exhibit reduction in TL during aging, which implies a control in TL by the expression of the telomerase in somatic stem cells of this species (Girondot and Garcia 1999). Moreover, in *Alligator mississippiensis*, an inverse relationship has been detected between TL and body length (Scott et al. 2006).

Chameleons are an interesting group to study mechanisms that are related with fast aging in reptiles. As mentioned in previous chapters, this lizard family is a rather fast-living taxon. A great advantage of these animals for studies in the wild is that population density is at least seasonally often very high, and they are easy to capture during night. However, regular repeated measurements in the wild might be difficult to achieve, as the recapture rate is often rather low. Furthermore, especially in smaller species and age stages obtaining adequate blood samples might be challenging.

Semelparity in reptiles has almost exclusively been detected in chameleons (e.g. Karsten et al. 2008, Eckhardt et al. 2017), agamids (Henle 1991, Dickman et al. 1999), and iguanid lizards (Rodríguez-Romero et al. 2011), which all assemble the squamate infraorder Iguania (Forst et al. 2001). Apart from this taxonomic group only tendencys for semelparity were observed in populations of the the viviparous asp viper *Vipera aspis* (Bonnet 2011). Therefore, semelparity in reptiles, respectively annual life-histories might be confined towards the infraorder Iguania. Typically, species belonging to this infraorder show high sexual dimorphism that is often accompanied with male ornamentations (e.g. Ord and Stuard-Fox 2006). Within this group, sexual dimorphism is highest compared to the remaining reptile taxa (Vitt and Caldwell 2013). Up to my knowledge, sexual dimorphism that is driven by sexual selection has never been considered in analysis about longevity in reptiles (e.g. Scharf et al. 2015, Stark et al. 2019), but should be included in future analysis. However, besides several short living species, several large-bodied species are known to reach lifespans of more than 20 years (e.g. rock iguanas *Cyclura* spp., Magelhaes and Costa 2009).

Besides the examination of aging mechanisms, the measurement of stress hormones, oxidative stress and telomere dynamics can additionally offer valuable clues for species conservation. For example, GC levels were found to predict survival probabilities of animals in the wild (e.g. Romero and Wikelski 2001). Furthermore, once the relationship between TL and the age has been well determined for a given species, it is possible to use blood samples to estimate the age of animals in nature (Haussmann and Vleck 2002). This might enable researchers to monitor populations regarding age structure relatively time-efficient.

Conclusion

In this thesis, I have provided rare information about extrinsic and intrinsic mechanisms shaping the life-history of the shortest living tetrapod species. I have demonstrated that the lifespan of this species is highly connected to environmental factors and, therefore, not exclusively inevitable. Via a comparative approach, I could provide rare data of the comparability of lifespan of both sexes of *F. labordi* in the wild and captivity. The results indicate that males as the shorter living sex in the wild, are capable of reaching comparable lifespans as captive females, when exogenous factors of mortality are excluded. Therefore, faster growth rates and higher testosterone levels in males that are often suspected to lead to intersexual differences in lifespan might play a subordinate role in this species. In this connection, the data of **Chapter 4** and **Chapter 5** suggest that physiological stress also has a strong influence on immune responses. The increasing gastrointestinal - and blood parasite burden observed in *F. labordi* in the wild towards the dry season might reflect an unavoidable consequence of their adaptation. The influence of physiological processes contributing to such a short lifespan in *F. labordi* is also supported by a maximum lifespan in caged individuals of 16 months, indicating that their lifetime is indeed bounded by molecular and cellular mechanisms of aging.

I discovered interspecific differences in several aspects of the three sympatric *Furcifer* species with seasonal adjustment that might be related to the partitioning of overall food availability and contributes valuable life-history data on enigmatic chameleon species. Additionally, I showed that the lifespan of *F. labordi* and *F.* cf. *nicosiai* usually ends after the reproductive season. Moreover, *F. oustaleti* as one of the largest chameleon species in the world, showed unregular survival until a second reproductive season. As discussed in the section "Interspecific comparison and life-histories in chameleons", the lizard family Chamaeleonidae seems to be the taxon exhibiting the fastest life-histories in reptiles. I have outlined the characteristics of chameleons such as the inability of fat storage and low escape probability after the detection of a predator that might favour fast life-histories. In this connection, I have discussed the advantages and disadvanages of this life-history. On the one hand, chameleons are a young but relatively species-rich clade. Their short generation times and numerous offspring might have facilitated species divergence. Additionally, probably because of their life-histories, several chameleon species are successful invasive species when escaping into suitable environment. On the other hand, short life-histories accompanied with investment in only one mating season might be disadvantageous in relation to harsh environmental inconsistancies. In case of repeated missing regional rainfalls that might arise more frequently in the nearer future due to climate change, annual chameleon species are in danger of extinction. Consequently, chameleon conservation and breeding programs should consider potentially short lifespans.

Overall, in my thesis I contributed valuable data about life-histories of chameleons in the wild. I have discussed the most likely explanations for my findings. The results of this thesis broaden the current knowledge of life-histories and aging in reptiles. Moreover, chameleons that rarely find consideration for studies regarding life-history and ecology became in this connection a better-studied group.

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Acknowledgement

First, I have to thank Peter Kappeler and Cornelia Kraus for giving me the opportunity to work on this interesting topic. I am deeply grateful for all your guidance, advices and freedom I felt during the project. I am obliged to the Ministère de l'Environment et des Eaux et Fôrets, MINEEF, Direction des Eaux et Forêts of Madagascar and CNFEREF Morondava, for authorizing my work in Kirindy. I am very thankful to the Kirindy field station team, including all the field assistants and cooks. In particular, I am deeply appreciative to Bruno Tsiverimana for taking care for the caged chameleons when I was not in the field. In this regard, I am much obliged that I was a part of the field station and becoming acquainted with parts of the Malagasy culture, giving the worthy opportunity to see issues from other perspectives. Especially, I want to thank Leon Razafimanantsoa for running everything smoothly during my stay in the field station and Rodin Rasoloarison for organizing the permits for my work. I am also indebted to the interns Wanda von Bremen and Camilla Cenni for supporting me in data collection.

The sample analyses for the thesis were done in collaboration with several people: I am grateful to Prof. Dr. Christina Strube, Dr. Karina Mathes and the Department of Parasitology from the University of Veterinary Medicine Hannover. I am thankful to Dr. Frank Mutschmann, who regrettably deceased in 2018. I admire his huge contribution of knowledge in reptile medicine. I am thankful to Prof. Dr. Mats Olsson and Dr. Angela Pauliny from the University of Gothenburg for supporting the telomere analysis. Moreover, I want to thank the “Deutsche Gesellschaft für Herpetologie und Terrarienkunde” for partly financing the analysis of the telomeres via the Wilhelm-Peters Fonds.

During my PhD, I met several terrific people who enriched and often still enrich my life. First, I want to thank Bako for the cheering of the ups and support during the downs during my last years. I have to thank Flavia, Josue, Tilman, Andrea, Franzi, Anna, Luca, Wanda, Jan, Finja, Camilla, Julia, Sylvio, Charlotte and numerous other people relieving long field seasons in Kirindy. Many thanks to my parents, sisters and friends for staying in touch during my long absences and for supporting my choices. I am thankful for every bit of encouragement from all of you. In particular, I have to thank my family for supporting my deep passion in herpetology from my childhood onwards and subsequently tolerating various terrariums in our house. I am grateful to Daniel Kahlen who gave me the opportunity to work in a reptile zoo during my adolescence and thereby learning a lot about this animal group. Furthermore, I am glad for long and helpful discussions about chameleon biology with Dr. Sebastian Gehring and Dr. Nicola Lutzmann.

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Curriculum Vitae

Publications

Publications related to the PhD-thesis

Eckhardt, F., Pauliny, A., Rollings, N., Mutschmann, F., Olsson, M., Kraus, C., Kappeler, P. M. (2020). Stress-related changes in leukocyte profiles and telomere shortening in the shortest-lived tetrapod, *Furcifer labordi*. BMC Evolutionary Biology, - *accepted.*

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Declaration

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

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