

Thermal limits of reptiles

Ecological and environmental constraints on the thermal biology of Malagasy lizards

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Summary

Ectotherms are particularly affected by fluctuations and changes in environmental conditions because their body temperature largely depends on heat exchange with the environment. However, a constant body temperature has the advantage that physiological body functions and performance can be optimized at this temperature. Ectotherms evolved sophisticated mechanisms to either maintain a rather constant body temperature (behavioural plasticity) or to compensate temperature fluctuations through shifts in the thermal reaction norm of physiological processes (physiological plasticity). However, under natural conditions, body temperature is also affected by multiple ecological factors that constrain the thermal scope of an animal.

This dissertation examines the question how ecological requirements and environmental constraints affect the thermoregulation of lizards. I investigated the activity temperature, activity time, thermoregulatory behaviour and the energy budget of three syntopic lizard species (*Oplurus quadrimaculatus*, *O. saxicola* and *Zonosaurus laticaudatus*) from southeast Madagascar. The study took place in the Andohahela National Park, which comprises a unique and steep environmental gradient in temperature and precipitation. Habitats change within a distance of less than 10 km from cool and humid rainforest to hot and dry spiny forest, with moderately tempered gallery and transitional forest in between. The proximity of these extremes and the fact that my study species occur all along the gradient provide an ideal natural setup to investigate the effects of different thermal environments on the thermoregulation of lizards.

In the first chapter, I used temperature loggers to analyse skin temperature patterns of *O. quadrimaculatus* in differing habitats along the gradient. The results show a precisely regulated skin temperature during activity along the entire gradient despite marked differences in the thermal environment. However, cooler and shadier conditions in the rainforest reduced the activity time of individuals by 35%. Additionally, I measured the field resting metabolic rate using open flow respirometry to quantify daytime field resting costs (maintenance costs of non-fasting animals) of the lizards, which were reduced by 28% in the cooler habitat. Hence, in the rainforest, these lizards have less time available for foraging but they also require less food to cover their basic energetic costs.

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Nevertheless, the cool rather than the hot environment seems to present a thermal limitation in this species, because it also matches its distribution limit.

In the second chapter, behavioural observations and temperature measurements of *O. saxicola* in the hot spiny forest and the moderately tempered gallery forest reveal how the activity of this species is adapted to different thermal environments. Individuals from the spiny forest show a bimodal distribution of activity with the highest peak in the morning, reduced activity during midday and again increased activity in the afternoon. In contrast, individuals in the gallery forest show highest activity during midday and activity in the afternoon is constrained by a lack of direct sunlight. Nevertheless, the mean activity body temperature of *O. saxicola* did not differ between habitats which argues strongly against a change in the temperature set-point and thus against physiological adjustments. This species rather relies on behavioural thermoregulation and compensates thermal differences mainly through shuttling between microclimates and posture changes.

The third chapter compares skin temperature patterns over the course of the day in *O. quadrimaculatus* and *Z. laticaudatus*. While *O. quadrimaculatus* maintains a permanently high activity temperature during the day, *Z. laticaudatus* shows a different but distinct temperature pattern. After elevated skin temperature in the morning during basking, the skin temperature decreases and remains low for the rest of the day during foraging in shady leaf litter and crevices. Despite the opportunity to reheat in the sun, which would improve its performance, *Z. laticaudatus* remained cool. The elevated skin temperature in the morning most likely promotes physiological processes, such as digestion, detoxification, and immune response. The lower skin temperature thereafter nevertheless allows sufficient performance to forage on snails, hissing cockroaches, millipedes and carrion. While *O. quadrimaculatus* forages at high body temperature and can therefore satisfy physiological requirements during normal activity, *Z. laticaudatus* is forced into a trade-off between foraging in cooler microclimates and the need to accelerate physiological processes through elevated body temperature. This dilemma is solved by a temporal and spatial separation of daily activities that is so far unknown in diurnal lizards. This pattern allows for an increase in foraging time and the lizards can exploit food sources further away from the basking site.

Overall, this study shows that behavioural thermoregulation is the major compensatory mechanism for environmental differences in heliothermic lizards under natural

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conditions. The degree to which the thermal niche is affected by ecological constraints and habitat use varies between species. *Oplurus* spp. for example, are relatively unaffected by ecological factors because they are able to maintain permanently stable body temperatures through simultaneous foraging and thermoregulation. In contrast, if a species is not able to satisfy ecological and physiological needs simultaneously, because the temperature of the foraging sites is too low, it faces a dilemma. *Zonosaurus laticaudatus* solves this trade-off with an unexpected but reasonable split of the day instead of constant shuttling.

The results of my study are important to understand thermal strategies under natural conditions. This interdisciplinary approach with physiological measures in an ecological context highlights the importance of thermal reality of lizards in the wild. The combination of ecological constraints, thermal strategies and compensatory mechanisms has been largely overlooked because it requires studying animals in their natural environment under natural conditions. It might, however, be exactly this interplay that decides about the future distribution of species and their resilience to habitat modification and climate warming.

Zusammenfassung

Ektotherme Tiere sind besonders stark von Umweltveränderungen und Fluktuationen der Umgebungstemperatur betroffen, da ihre Körpertemperatur fast ausschließlich durch Wärmeaustausch mit der Umgebung reguliert wird. Da physiologische Prozesse und somit die Leistungsfähigkeit allerdings für eine bestimmte Temperatur optimiert sind, ist eine konstante Körpertemperatur klar von Vorteil. Deshalb nutzen viele ektotherme Tiere hochentwickelte Mechanismen (Thermoregulationsverhalten und/oder physiologische Anpassungen), um Veränderungen in der Umgebungstemperatur zu kompensieren bzw., um die spezifische Körpertemperatur im optimalen Bereich für die Leistungsfähigkeit zu halten. Allerdings wird die Körpertemperatur auch durch ökologische Faktoren beeinflusst, was den verfügbaren Temperaturbereich teilweise deutlich einschränkt.

In dieser Dissertation untersuche ich die Frage, auf welche Weise ökologische und abiotische Faktoren die Thermoregulation von Reptilien beeinflussen. Hierfür habe ich den Effekt von unterschiedlichen Habitatterperaturen auf die Aktivitätstemperatur, die Aktivitätszeiten, das Thermoregulationsverhalten und das Energiebudget von drei syntopen Echsenarten (*Oplurus quadrimaculatus*, *O. saxicola* und *Zonosaurus laticaudatus*) aus Südostmadagaskar untersucht. Das Untersuchungsgebiet im Andohahela Nationalpark bietet einen einzigartigen, natürlichen Umweltgradienten bei dem sich auf einer Distanz von weniger als 10 km ein Wechsel vom kühlen, immergrünen Regenwald, über den moderat temperierten Übergangs- und Galeriewald, bis hin zum heißen, trockenen Dornenwald vollzieht. Durch die räumliche Nähe dieser Extreme und die Tatsache, dass die untersuchten Echsenarten entlang des gesamten Gradienten vorkommen, bietet Andohahela ideale Voraussetzungen, um den Einfluss unterschiedlicher Habitate auf die Thermoregulation von Reptilien unter natürlichen Bedingungen zu untersuchen.

Im ersten Kapitel habe ich Hauttemperaturmuster analysiert, die von auf den Tieren befestigten, temperatursensitiven Datenloggern aufgezeichnet wurden. *Oplurus quadrimaculatus* thermoreguliert während der Aktivitätszeit, unabhängig von der Habitatterperatur, sehr präzise und konnte dabei durchgehend eine hohe Hauttemperatur aufrechterhalten. Allerdings gab es im kühlen, schattigeren Regenwald starke Einschränkungen in der täglichen Aktivitätszeit von etwa 35% im Gegensatz zum

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Dornenwald. Gleichzeitig haben respiratorische Messungen und der daraus berechnete Energiebedarf gezeigt, dass die energetischen Kosten um etwa 28% geringer waren. Den Tieren steht demnach in kühleren Habitaten zwar weniger Zeit für die Nahrungssuche zur Verfügung, sie benötigen allerdings auch weniger Nahrung um den niedrigeren Energiebedarf zu decken. In diesem Fall limitiert nicht das warme Habitat (Dornenwald), sondern das kühle Regenwald-Habitat die Tiere, das gleichzeitig auch die Verbreitungsgrenze dieser Art darstellt.

Im zweiten Kapitel zeige ich anhand von Verhaltensbeobachtungen und Körpertemperaturmessungen an *O. saxicola* im heißen Dornenwald und im gemäßigten Galeriewald, dass die Echsen ihre Aktivitätszeiten den thermischen Gegebenheiten der Habitate anpassen. Die Individuen im Dornenwald zeigten eine bimodale Verteilung der Aktivitätszeiten, mit dem Aktivitätshöhepunkt am Morgen, gefolgt von einem Mittagstief und einer wieder erhöhten Aktivität am Nachmittag. Die Individuen im Galeriewald hingegen zeigten die höchste Aktivität während der Mittagszeit und sind nachmittags durch mangelnde direkte Sonneneinstrahlung stark eingeschränkt. Der Wechsel zwischen verschiedenen Mikroklimata, aber auch die Veränderung der Körperhaltung sind die wichtigsten Thermoregulationsmechanismen bei dieser Art. Die mittlere Körpertemperatur während der Aktivitätszeit zeigte dabei keine Unterschiede zwischen den Populationen, was darauf hindeutet, dass der Sollwert für die Körpertemperatur unverändert ist und es dahingehend keine physiologischen Anpassungen an die Habitate gibt. Insgesamt ist es *O. saxicola* möglich, durch eine Verschiebung der Aktivitätszeiten und durch die Anpassung der Wechsel zwischen Mikroklimata, Habitatunterschiede auszugleichen.

Im dritten Kapitel vergleiche ich die Hauttemperaturmuster von *O. quadrimaculatus* und *Z. laticaudatus* im Tagesverlauf. Während *O. quadrimaculatus* eine konstant hohe Aktivitätstemperatur hält, zeigt *Z. laticaudatus* einen abweichenden, aber ebenfalls sehr ausgeprägten Hauttemperaturverlauf. Nach einer erhöhten Temperatur während des Sonnens am Morgen sinkt die Hauttemperatur während der Nahrungssuche in der schattigen Laubstreu und in Felsspalten deutlich ab und verbleibt für den Rest des Tages auf diesem deutlich niedrigeren Niveau. Trotz der Möglichkeit, sich wieder in der Sonne aufzuheizen, was eine höhere Leistungsfähigkeit für die Nahrungssuche bedeuten würde, nutzen die Echsen dies nicht. Die erhöhte Körpertemperatur dient vermutlich dazu,

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physiologische Prozesse, wie Verdauung, Entgiftung oder Immunreaktionen, zu beschleunigen. Die niedrigere Körpertemperatur ermöglicht offensichtlich ausreichende Beweglichkeit bei der Nahrungssuche nach Schnecken, Fauchschaalen, Tausendfüßern und Aas. *Oplurus quadrimaculatus* jagt ohnehin bei einer hohen Körpertemperatur, was gleichzeitig auch die physiologischen Bedürfnisse befriedigt. Hingegen muss *Z. laticaudatus* auf der einen Seite physiologische Prozesse durch aktive Thermoregulation in der Sonne verbessern, auf der anderen Seite findet die Echse seine Nahrung in kühleren Mikrohabitaten. Dieses Dilemma wird durch eine räumliche und zeitliche Trennung dieser Verhaltensweisen gelöst. Eine solch klare Zweiteilung der Hauttemperatur im Tagesverlauf, trotz der Möglichkeit sich wieder aufzuheizen, war bisher nicht bekannt. Durch dieses Muster steht den Echsen mehr Zeit für die Futtersuche zur Verfügung und sie können Nahrungsquellen nutzen, die weiter von den Sonnenplätzen entfernt sind.

Insgesamt zeigen meine Ergebnisse, dass Echsen unter natürlichen Bedingungen allein durch Verhaltensanpassungen große Temperaturunterschiede ausgleichen können. Zudem ist das Ausmaß, mit dem die thermische Nische durch ökologische Einschränkungen und die Habitatnutzung beeinflusst wird, artabhängig. Die *Oplurus*-Arten, zum Beispiel, werden weniger durch ökologische Faktoren beeinflusst, da sie den ganzen Tag über präzise thermoregulieren und gleichzeitig ihre Nahrung suchen. Wenn sich physiologische und ökologische Bedürfnisse aber unterscheiden, kommt es zu einem Konflikt, der, wie im Fall von *Z. laticaudatus*, auf unerwartete, aber durchaus sinnvolle Weise, durch eine zeitliche und räumliche Aufteilung gelöst werden kann.

Die Ergebnisse meiner Dissertation sind wichtig, um die Kompensationsmöglichkeiten ektothermer Tiere unter natürlichen Bedingungen zu verstehen. Meine interdisziplinäre Studie bringt physiologische Messungen in einen ökologischen Kontext, um die thermische Realität aufzuzeigen, denen die Tiere in der freien Wildbahn ausgesetzt sind. Die Kombination von ökologischen Einschränkungen, thermischen Strategien und Kompensationsmöglichkeiten wurde bisher wenig untersucht, da solche Feldstudien sehr arbeits- und zeitaufwändig sind. Nichtsdestotrotz ist es genau dieses Zusammenspiel verschiedener ökologischer und abiotischer Faktoren, das über die zukünftige Verbreitung von Arten und deren Widerstandsfähigkeit gegen Habitatveränderungen und Klimawandel entscheidet.

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Changes in environmental conditions and fluctuations in ambient temperature (T_a) have various and complex effects on the behaviour, ecology and physiology of species (Lillywhite 2016). However, animals must maintain a balanced energy budget over time with a certain body temperature (T_b) that is optimal for physiological functioning. T_b affects biochemical processes, energy expenditure and locomotion performance of an organism (Schmidt-Nielsen 1997; Heldmaier *et al.* 2013) and fluctuations in T_b disturb the efficiency of these functions (Huey 1982). Ectotherms are especially affected by environmental changes as their T_b depends on heat transfer with the environment (Little and Seebacher 2016) and precision in thermoregulation is a key for optimal performance (Bennett and Ruben 1979; Clarke and Pörtner 2010). Hence, in changing environments compensatory mechanisms are needed to control T_b or to adjust biochemical processes in order to maintain balanced energy budgets.

Ectotherms with relatively high T_b generally outperform species with lower T_b , which is also known as the “hotter-is-better” hypothesis (Angilletta *et al.* 2010). However, in contrast to endotherms, few ectotherms produce a significant magnitude of body heat. So far, only a few fish species, such as tuna, lamnid sharks (Carey and Teal 1968; Carey *et al.* 1971) and the deep sea opah (Wegner *et al.* 2015), are known to attain permanently higher T_b than the ambient water temperature. Furthermore, pythons and tegu lizards show temporary endothermy through shivering thermogenesis but only during reproduction (Harlow and Grigg 1984; Tattersall *et al.* 2016). Most ectotherms thus depend on their thermal environment for achieving suitable T_b . Gaining body heat is not always the major concern for species though. Quite the contrary, the challenge for animals in hot environments is staying cool because if T_a exceeds the critical maximum T_b , animals face overheating and death (Kearney *et al.* 2009).

A high T_b is generally beneficial for physiological performance but the stability of T_b (avoiding T_b that would be too cold and too hot) is even more important because physiological function peaks at a certain T_b (Huey and Slatkin 1976). This is less challenging in large ectotherms, for example in the Komodo dragon *Varanus komodoensis*, because high body mass and a small surface-to-volume ratio facilitate rather stable T_b through thermal inertia (McNab and Auffenberg 1976; Harlow *et al.* 2010).

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In contrast, the greater surface to volume ratio in smaller reptile species results in increased heat conduction and faster heating and cooling rates, and thus a limited potential to store attained body heat (Stevenson 1985).

The concept that ectotherms are able to control their T_b and that temperature is an essential factor in reptile ecology and physiology has been developed in several pioneering studies. Mosauer (1936) experimentally showed that the desert lizard *Uma notata* does not tolerate high sand temperatures of more than 55°C, even though it inhabits this harsh environment. Cowles and Bogert (1944) showed in a comprehensive ecophysiological study that “cold-blooded” organisms do not just passively take on the temperature of their environment. Instead, they have sophisticated behavioural mechanisms that allow them to precisely regulate their T_b within a wide range of ambient conditions. Colbert *et al.* (1946) studied the heat tolerance of the American alligator *Alligator mississippiensis* and revealed the important effect of posture and body orientation on thermoregulation. Bogert (1949) demonstrated that behavioural thermoregulation of spiny lizards (*Sceloporus* spp.) can be surprisingly precise with T_b fluctuations of less than 3°C during activity. Norris (1953) recognized that T_b , T_a and habitat structure are important factors for a detailed description of the ecology of the desert iguana *Dipsosaurus dorsalis*. Finally, Huey and Slatkin (1976) and Huey and Stevenson (1979) integrated thermal physiology and ecology of ectotherms. By reviewing analytical methods of describing and comparing aspects of performance, they created a hypothetical performance curve of ectotherms that is still used to describe temperature related body functions until today (Fig. 0.1).

This performance curve is essential for an understanding of the thermal reaction norm. No matter which physiological trait is selected (e.g. endurance, sprint speed, hearing, or digestion), all show similar performance patterns and this may even be transferable to endotherms (Huey and Kingsolver 1989; Boyles *et al.* 2011). The lower critical T_b is the lowest T_b tolerated by an ectotherm before death. Performance then increases with increasing T_b until it reaches the maximum (thermal optimum, T_{opt}). The curve has a negative skew and drops sharply towards the upper critical T_b . For this reason, the preferred body temperature (T_{pref} ; selected T_b by an undisturbed ectotherm in an artificial temperature gradient; Huey and Kingsolver 1989) and activity T_b in the wild are often

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below but closely related to T_{opt} to maintain a high performance level with an additional safety margin to the lethal critical maximum (Huey *et al.* 2012).

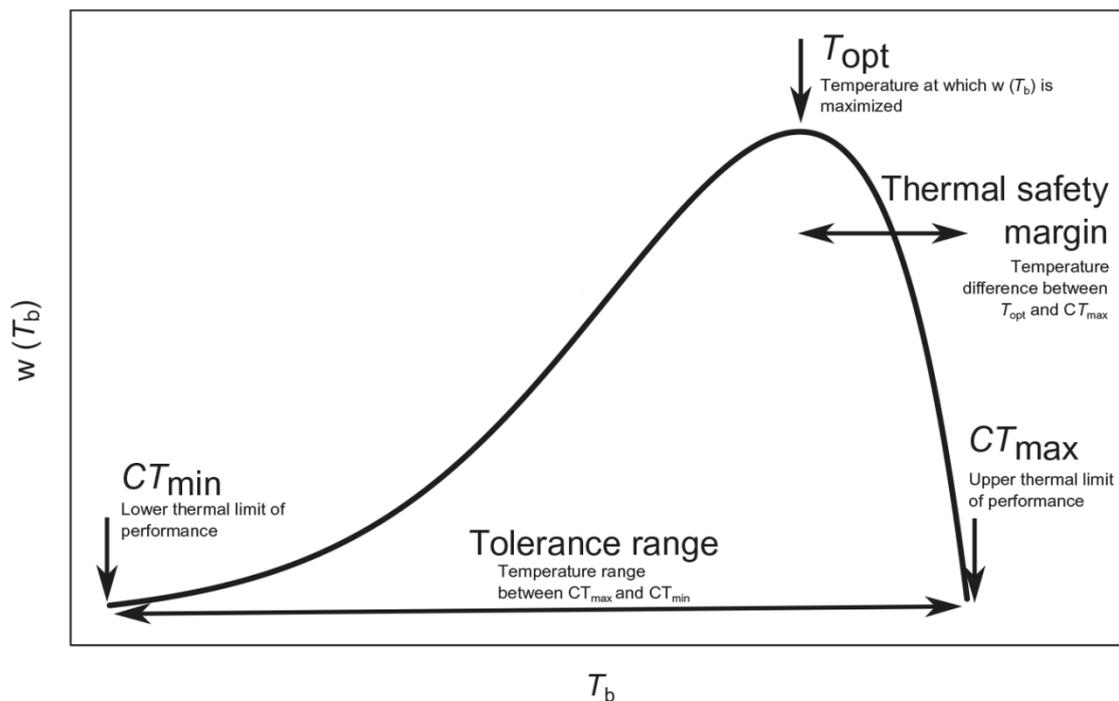


Figure 0.1: Hypothetical performance curve of an ectotherm as a function of body temperature (modified from Sinclair *et al.* 2016). $w(T_b)$: relative fitness/performance.

The fact that lizards are able to achieve T_b that deviate from T_a shows that T_a itself does not adequately describe the thermal reality for these animals. The operative environmental temperature range (T_e ; theoretically obtainable T_b range for a species in a given habitat) is therefore used to describe the thermal scope of an animal across microhabitats rather than a single record of T_a (Bakken *et al.* 1985; Bakken 1992; Dzialowski 2005). Lizards are able to use a range of environmental temperatures depending on their capacity of behavioural thermoregulation. The magnitude of the T_e range depends on the thermal heterogeneity of the environment, temporal and spatial habitat use of the species and its body mass, shape and colouration (Bakken and Angilletta 2014; Sears and Angilletta 2015). Hence, T_b can be stable despite large environmental differences and even if T_a fluctuates, T_e can still provide a constantly suitable range of temperatures.

If T_b deviates from T_{opt} or it is outside the thermal reaction norm, animals have two options to retrieve optimal performance. Either they actively select sites with optimal

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thermal conditions (behavioural thermoregulation) or the optimal range of the thermal reaction norm shifts to match the current T_b (physiological acclimatization) (Glanville and Seebacher 2006). These two strategies have fundamentally different approaches but they are not necessarily mutually exclusive.

The great advantage of behavioural thermoregulation is that responses are quick, flexible, and, depending on the animals' ability and the environment, highly efficient (Munoz *et al.* 2014). The disadvantage is that behavioural thermoregulation can only occur within the thermal scope of T_e . Besides adjustments of activity time, posture, orientation to the sun, shuttling and panting are the most common behavioural traits that are used by terrestrial ectotherms for thermoregulation (Grant and Dunham 1988; McConnachie *et al.* 2009; Gifford *et al.* 2012). Physiological adjustments are more inert but they optimize biochemical processes if changes in temperature are short term relative to the animal's life-span (Angilletta *et al.* 2002). Based on changes in regulatory enzymes, a shift of the thermal reaction norm allows enhanced performance at otherwise suboptimal T_b . This strategy is used by many reptiles to compensate seasonal changes in the environment but short-term changes of ambient conditions, e.g. severe weather events over several weeks, may also provoke physiological adjustments (Seebacher 2005). Under natural conditions, behavioural and physiological plasticity often act jointly. The plastic modification of T_{pref} , for example, has been proposed to reduce costs for thermoregulation and contributes to buffer unsuitable conditions (Gvozdik 2012) and a seasonal change of T_{pref} in newts does indeed increase their time window for thermoregulation (Hadamova and Gvozdik 2011). Ectotherms that select lower T_b show changes in behaviour (T_b selection) accompanied by changes in the thermal reaction norm (lower set-point of T_b). Hence, thermoregulatory behaviour and physiological acclimatization do not represent mutually exclusive thermal responses. They have coevolved, in at least some species, to help offset potential costs associated with each strategy (Little and Seebacher 2016).

At the same time, behaviour and physiology are often directly affected by biotic and abiotic influences (Clusella-Trullas and Chown 2014). Although many lizards aim to achieve T_{opt} in the wild, their actual activity T_b often deviates from the expected one (Huey *et al.* 1989). Predation pressure (Herczeg *et al.* 2008), nutritional state and food quantity (Gatten 1974; Gienger *et al.* 2013), nocturnal activity (Huey *et al.* 1989), competition (Downes and Shine 1998), reproductive state (Harlow and Grigg 1984) and

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the lack of suitable microclimates (Huey and Slatkin 1976; Stelatelli *et al.* 2013) force animals into trade-offs between physiological and ecological demands. For this reason, results from semi-natural field experiments and observations from free-ranging animals can differ significantly (Clusella-Trullas and Chown 2014). For example, T_{pref} of two populations of the New Zealand common gecko *Hoplodactylus maculatus* differed in field acclimatized individuals but not after acclimation in the laboratory (Tocher 1992). The legless lizard *Lialis burtonis* shows post-feeding thermophily in the laboratory but not in semi-natural enclosures (Wall and Shine 2008) and the arboreal banded snake *Hoplocephalus stephensii* selects lower T_b in the wild than it does in an artificial temperature gradient (Fitzgerald *et al.* 2003). However, these differences between the realized and the fundamental thermal niche are important because these data are often used for the prediction of animals' future distribution.

Mechanistic models on the (local) extinction of species in several taxa are often based on physiological tolerances measured under controlled laboratory conditions with limited reference to the realized ecological niche to predict the effect of environmental change (Sinervo *et al.* 2010; Böhm *et al.* 2016). On the one hand, lizards are constrained in their T_b selection by these multiple ecological factors. On the other hand, lizards may have more options for thermoregulation (e.g. burrowing, cooling in water) than laboratory studies would reveal and hence, more potential to buffer environmental fluctuations than expected (Wall and Shine 2008). However, different species are affected in different ways and empirical studies on behavioural and physiological compensation under natural conditions are needed to understand the magnitude of species resilience (Basson and Clusella-Trullas 2015; Pacifici *et al.* 2015).

Madagascar, as one of the world's hottest biodiversity hotspots, is known for its high species richness and the high level of endemism but also for a high level of degradation (Myers *et al.* 2000; Ganzhorn *et al.* 2001; Harper *et al.* 2007). Additionally, the climate is hypervariable, unpredictable and the predicted temperature increase affects particularly the dry southern part of the island (Dewar and Richard 2007; Tadross *et al.* 2008; Hannah *et al.* 2008). In the southeast, the Anosy Mountains act as a rain barrier for humid winds from the Indian Ocean and create a steep environmental gradient on the western flank. While the mountains consist of evergreen rainforest, less than 10 km further west spiny forest is the predominant vegetation form that continues all the way to the southwest.

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Precipitation decreases from more than 2,400 mm/year to 400 mm/year and mean T_a increases approximately 8°C within this short distance (Goodman 1999; Rakotondranary *et al.* 2011). This gradient has led to high habitat diversity on a small geographic scale (Fig. 0.2). The extremes (spiny and rain forest) are connected by patches of moderately tempered transitional forest (Fig. 0.3). While rain forest and spiny forest are protected in the Andohahela National Park, the transitional forest lies between the protected areas and is rather degraded and fragmented. Within the spiny forest parcel, stretches of gallery forest can be found along rivers. This forest type is cooled by the river and also moderately tempered, similar to the transitional forest. The gallery forest is a very heterogeneous habitat with large, evergreen trees and, in parts, large rocks or sandy spots and open water is available year-round. The gallery forest is often not wider than 100 m but it is an important refuge for animals within the hot and arid landscape (Mares and Ernest 1995). The proximity of these habitats with differing thermal conditions and the fact that these habitats share many of their reptile species (Theisinger 2009) provide an excellent setup for the investigation of compensatory thermoregulation mechanisms.



Figure 0.2: Photographs of study sites in the Andohahela National Park: A) spiny forest; B) gallery forest; C) transitional forest; D) rain forest (photos by Ole Theisinger).

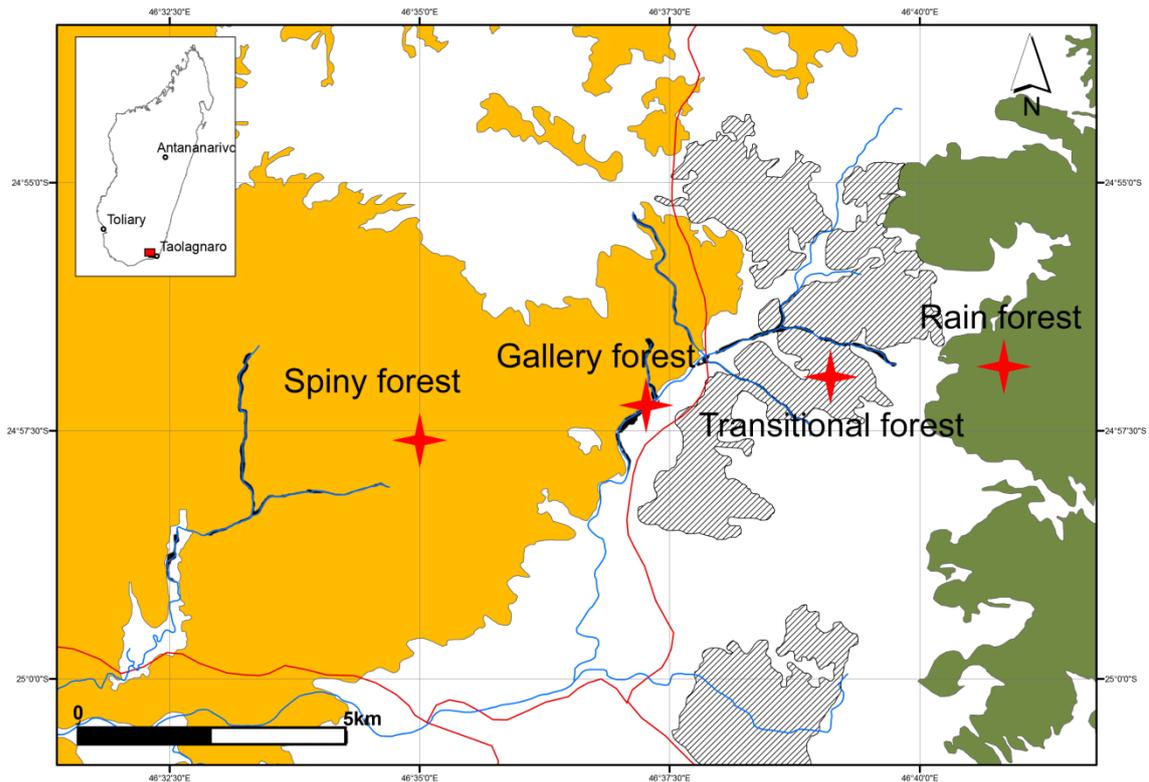


Figure 0.3: Map of our study sites. Differently coloured areas show different vegetation formations. Yellow: spiny forest; black (along rivers): gallery forest; shaded: transitional forest; green: rain forest; white: savannah. Red asterisks show our study sites (modified from Rakotondranary *et al.* 2011).

The overall goal for this dissertation was to investigate lizards' behavioural and physiological capacity to compensate for differences in the thermal environment and to assess the effect of foraging activity on their thermal ecology. Furthermore, we aimed to evaluate environmental and ecological effects on the energy budgets of lizards. We used three lizard species as model organisms (*Oplurus quadrimaculatus*, *O. saxicola* and *Zonosaurus quadrimaculatus*; Fig. 0.4) which occur sympatrically all along the steep temperature and precipitation gradient in Andohahela (Glaw and Vences 2007). Field work was conducted between October 2010 and April 2012. All species are diurnal and occur in relatively high densities. Moreover, our study species, and particularly *O. saxicola* and *O. quadrimaculatus*, are highly philopatric even during their daily activity, which is of great advantage for a high recapture rate.



Figure 0.4: Study species: A) *Oplurus quadrimaculatus* with an attached temperature logger; B) *Oplurus saxicola* in “high” posture with minimal surface contact to the hot rock; C) measuring skin temperature of *Zonosaurus laticaudatus* by using an infrared thermometer (photos by Wiebke Berg and Ole Theisinger).

The dissertation consists of three research projects to answer the following questions:

1. The combination of behaviour and physiology for thermoregulation and a balanced energy budget is crucial to understand an animal’s potential to compensate environmental fluctuations and to persist in the face of climate change (Basson and Clusella-Trullas 2015). To investigate whether a decline in T_e along the environmental gradient in southeastern Madagascar is compensated through lowered activity T_b or through changes in activity time, we studied intraspecific differences in the daily skin temperature (T_{skin}) pattern (as proxy for T_b ; Berg *et al.* 2015) of *O. quadrimaculatus*. Additionally, we measured the field resting metabolic rate (field RMR) and used the temperature-field RMR regression to estimate field resting costs in free ranging individuals. A comparison of these

costs among habitats provides insight into possible energetic advantages of colder versus warmer environments.

- Does *O. quadrimaculatus* make use of physiological adjustments or are differences in the thermal environment solely compensated behaviourally?
- Do field resting costs of *O. quadrimaculatus* differ between the habitats?

2. Unfavourable T_b causes changes in behaviour in lizards, because they have to compensate or to avoid extreme environmental temperatures which often conflicts with normal activity (Sun *et al.* 2001). In hot habitats, this might result in bimodal activity patterns to avoid midday heat (Sinervo *et al.* 2010). In addition, adjustments in foraging time and thermoregulatory behaviour are necessary to compensate for constraints in activity time. We investigated the direct effect of environmental differences (hot spiny forest and moderate gallery forest) on overall activity, thermoregulatory behaviour and foraging over the course of the day in *O. saxicola* through focal observations, scan sampling and T_b measurements.

- Do these lizards show similar activity patterns in both habitats?
- Do these lizards show adjustments in mean T_b to the different thermal environments?
- Does microhabitat use and foraging behaviour differ between the habitats?

3. Ecological factors, such as foraging strategy, often differ between sympatric species to minimize interspecific competition. Trade-offs between physiological demands, such as a relatively constant T_b for optimal performance, and ecological constraints might result in spatial or temporal differences in microhabitat use between sympatric species, which can have a major effect on the thermal niche of the lizards (Clusella-Trullas and Chown 2014; Murray *et al.* 2016). We used an interspecific comparison to investigate the constraining effect of foraging ecology and microhabitat use on activity T_{skin} . We compared T_{skin} patterns of the sympatric lizards *O. quadrimaculatus* and *Z. laticaudatus*, which experience the same T_e conditions but differ in their foraging ecology (sit-and-wait forager, respectively, active forager).

- Does the foraging strategy affect the thermal niche of lizards?

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Table 1: Glossary

Glossary	
Body temperature (T_b)	Core temperature of a lizard. Measured with thermocouples inserted into the cloaca (approx. 1.5 cm).
Skin temperature (T_{skin})	Temperature measured on the back of lizards using either infrared thermometer or attached temperature logger. It can be used as proxy for T_b in our study species (Berg <i>et al.</i> 2015)
Ambient temperature (T_a)	Temperature of the environment measured in full shade in one meter height.
Operative environmental temperature (T_e)	Theoretically attainable T_b range for an ectotherm in a given habitat. Measured with a number of specifically designed copper models equipped with temperature loggers, distributed in the natural environment of a species (Bakken 1992).
Thermal optimum (T_{opt})	T_b at which performance is maximal for a certain physiological trait (Huey and Stevenson 1979).
Preferred body temperature (T_{pref})	Selected T_b of an ectotherm in an artificial temperature gradient without biotic disturbance (Huey and Stevenson 1979).
Lower critical T_b	Lowest T_b tolerated by an ectotherm associated with the loss of righting response and of locomotory functioning (Huey and Stevenson 1979).
Upper critical T_b	Highest T_b tolerated by an ectotherm associated with the loss of righting response and the onset of muscle spasms (Huey and Stevenson 1979).
Thermal tolerance	Range between lower critical T_b and upper critical T_b (Huey and Stevenson 1979).
Field resting metabolic rate (field RMR)	Metabolic rate of a resting, non-fasting animal measured across a range of temperatures.

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Chapter 1

Compensation of thermal constraints along a natural environmental gradient in a Malagasy iguanid lizard (*Oplurus quadrimaculatus*)

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Abstract

Physiological or behavioural adjustments are a prerequisite for ectotherms to cope with different thermal environments. One of the world's steepest environmental gradients in temperature and precipitation can be found in southeastern Madagascar. This unique gradient allowed us to study the compensation of thermal constraints in the heliothermic lizard *Oplurus quadrimaculatus* on a very small geographic scale. The lizard occurs from hot spiny forest to intermediate gallery and transitional forest to cooler rain forest and we investigated whether these habitat differences are compensated behaviourally or physiologically. To study activity skin temperature (as proxy for body temperature) and the activity time of lizards, we attached temperature loggers to individuals in three different habitats. In addition, we calculated field resting costs from field resting metabolic rate to compare energy expenditure along the environmental gradient. We found no variation in activity skin temperature, despite significant differences in operative environmental temperature among habitats. However, daily activity time and field resting costs were reduced by 35 % and 28 % in the cool rain forest compared to the hot spiny forest. Our study shows that *O. quadrimaculatus* relies on behavioural mechanisms rather than physiological adjustments to compensate thermal differences between habitats. Furthermore, its foraging activity in open, sun exposed habitats facilitates such a highly effective thermoregulation that cold operative temperature, not energetically expensive heat, presents a greater challenge for these lizards despite living in a hot environment.

Keywords

Behavioural compensation; energy expenditure; metabolic rate; operative environmental temperature; physiological adjustment; reptiles.

Introduction

Species' distribution limits are defined by various parameters (Guisan and Thuiller 2005). In ectotherms, the distribution of operative environmental temperature (T_e), i.e. the range of attainable body temperatures (T_b), is one of the key factors (Angilletta 2009; Huey *et al.* 2012; Sinervo *et al.* 2010). Even efficient and precise thermoregulation, as found in heliothermic lizards, still depends on T_e to provide suitable T_b (Bakken and Angilletta 2014). If T_e is too high, lizards must retreat to cool refuges to avoid overheating. If T_e is too low, the animals' performance is reduced. Sinervo *et al.* (2010), for example, predicted that Mexican lizards are prone to extinction because of increasing ambient temperature and prolonged hours of restriction (i.e. periods of forced inactivity due to unsuitably high ambient temperature). Tropical lizards are thought to show narrow thermal tolerances because they have adapted to relatively stable climates (Ghalambor *et al.* 2006; Huey *et al.* 2009; Janzen 1967). However, recent results contradict this assumption (Leal and Gunderson 2012). A variety of microclimates, altitudinal gradients, and pronounced seasonality provide a wide range of environmental conditions in the tropics (Dewar and Richard 2007) and thus might have led to higher thermal tolerances than assumed so far.

The prerequisite for ectotherms to occur in differing thermal environments are physiological or behavioural adjustments. Physiological adjustments comprise changes in physiological rates (Seebacher *et al.* 2015) or shifts in the thermal tolerance of a species (Gunderson and Stillman 2015). Furthermore, the preferred body temperature [T_{pref} ; the selected T_b of undisturbed ectotherms in an artificial temperature gradient (Huey and Kingsolver 1989)] and the physiological optimum can be lowered or increased in accordance with ambient conditions (Blouin-Demers *et al.* 2000; Clusella-Trullas and Chown 2014). An upshift of the T_b set-point as a response to higher ambient temperature, for example, increases the potential activity time and might reduce costs for thermoregulation in the face of climate change (Gvozdik 2012). Despite T_b selection

being a behavioural response, the driver for this adjustment are changes in the thermal reaction norm and hence of physiological nature (Little and Seebacher 2016). Lizards that are exposed to different temperatures over several weeks in the laboratory show acclimation of T_{pref} (Blumberg *et al.* 2002). This effect was also observed under natural conditions between seasons (Diaz *et al.* 2005; Seebacher and Grigg 1997) but there is no or only little evidence for adjustments of T_{pref} to different habitats and altitudes (Gvozdik and Castilla 2001; Van Damme *et al.* 1989). However, this apparent absence of physiological adjustments is not necessarily equivalent to an incapability of lizards to acclimatize.

Other mechanisms, such as changes in thermoregulatory behaviour and adjusted daily activity time, are known to be effective (Gvozdik 2002) and might be preferential to physiological acclimatization under natural circumstances (Gunderson and Stillman 2015). Thermal constraints through high ambient temperature and the effect of climate warming have often been studied (Deutsch *et al.* 2008; Huey *et al.* 2009; Kearney *et al.* 2009; Sinervo *et al.* 2010). Since metabolic rate increases exponentially with T_b , climate warming might lead to excessive energetic costs (Kearney 2013). These can be assessed by calculating maintenance costs, i.e. basic metabolic costs in fasting and resting ectotherms, using field T_b (or a proxy for field T_b) and the corresponding metabolic rate (Kearney and Porter 2004). However, low ambient temperature can also be challenging, especially for warm-adapted species (Grbac and Bauwens 2001; Van Damme *et al.* 1987). Lower T_b may be beneficial for reducing energy expenditure (Christian *et al.* 1996; Huey *et al.* 1989) but sprint speed, digestion, nutrient assimilation and other physiological functions may be negatively affected by suboptimal performance (Angilletta *et al.* 2002; Huey and Kingsolver 1989).

To study these compensatory mechanisms under natural conditions, we chose one of the world's steepest environmental gradients, which is found in southeastern Madagascar. The unique gradient in ambient temperature, humidity and rainfall, connects dry spiny forest and humid rain forest via transitional forest and gallery forest along rivers within less than 5 km (Goodman 1999). Due to this very small geographic scale, species could potentially move between all habitats within a lifetime or sooner, depending on mobility, and reptile species from the hot and dry spiny forest can also be found in the cooler rain forest (pers. obs.).

Duméril's Madagascar swift (*Oplurus quadrimaculatus*) is one of these species. It occurs in a wide range of habitats with strikingly different environmental conditions including the climatic extremes of hot, dry spiny forest and humid, cool rain forest. We analyzed daily skin temperature (T_{skin}) patterns (as a proxy for T_b ; Berg *et al.* 2015) in individuals across the entire hot to cold environmental gradient including all four habitat types. Our aim was to examine if these lizards compensate thermal differences through adjustments in activity T_{skin} or through differences in daily activity time. In addition, we measured field resting metabolic rate (field RMR) and compared individuals' field resting costs (maintenance costs of non-fasting lizards at daytime) to assess the effect of differing thermal environments on basic energy needs.

Methods

Study sites

Our study sites were located in the Andohahela National Park (24°57'S, 46°35'E) on the western slope of the Anosy Mountains in southeast Madagascar. Within our study area, the distance between the two most divergent habitats (spiny forest and rain forest) is less than 5 km. The spiny forest (150 – 160 m a.s.l.) is characterized by scant and xerophile vegetation such as the octopus tree (*Dideracea* spp.) and the evergreen rain forest (400 – 430 m a.s.l.) consists of large shady trees and dense understory. The transitional forest (280 – 380 m a.s.l.), as the name implies, comprises mixed vegetation with similar moderate environmental conditions as the gallery forest along rivers (130 – 140 m a.s.l.; Andriaharimalala *et al.* 2011).

Study species

Oplurus quadrimaculatus is a heliothermic iguana with a body mass of (mean \pm SD) 76.5 \pm 10.5 g (N = 310) and a snout-vent-length of 12.8 \pm 0.6 cm. It inhabits open rocky habitats and is a sit-and-wait forager that feeds mainly on flying insects. Its main distribution is the spiny forest of southern Madagascar but there are also populations at higher altitudes and more humid habitats (Glaw and Vences 2007). This species is highly philopatric with a small home range of sometimes less than 50 m². Hence, animals can easily be located for recapture.

Operative environmental temperature

We used specifically designed copper models (three models per habitat) that matched the lizards in shape and colour to characterize the thermal habitat of individual *O. quadrimaculatus* (Dzialowski 2005). We equipped these models with temperature loggers (Thermochron iButtons, model DS1921G; resolution $\pm 0.5^{\circ}\text{C}$; weight 3.3 g; Maxim Integrated Products Inc., San Jose, California, USA.; calibrated in a water bath before use). Calibration against live animals showed that filling the copper models with fine sand revealed the best correlation (Pearson correlation coefficient = 0.962; $p < 0.001$) with no differences in heating and cooling rates between copper models and lizards ($t_{33} = 0.857$; $p = 0.397$). After programming the loggers to record the core temperature of the model every five minutes, we distributed three models in each habitat (full sun, shade and crevice) in order to cover a representative T_e -range. Measurements were made for six consecutive days in each habitat on cloudless, sunny days to ensure similar abiotic conditions.

Skin temperature patterns

To measure daily T_{skin} patterns, we noosed 48 individuals from different sites across the designated habitats during the rainy season (Mid-October to Mid-March) of 2009/2010, 2010/2011 and 2011/2012. We assume a similar sex-ratio in each habitat because this species is highly philopatric with a very small home range and it occurs in pairs. However, the certain identification of sex was not always possible and thus sex is not taken into account. We glued calibrated temperature loggers on the animals' backs and released them at their points of capture (Fig. S1.1). To make sure that the weight of the device did not exceed the recommended 5 % of the animals' body weight (Lovegrove 2009), we only equipped adult individuals with a minimum body mass of 70 g with a temperature logger. We used superglue (UHU Sekundenkleber, UHU GmbH, Bühl, Germany) to attach the devices. The recording interval was set to five minutes and the temperature loggers were able to store up to 2084 data points, which led to a maximal measuring time of seven continuous days. We recaptured individuals after five to seven days to remove the loggers. We gained T_{skin} data from 13 individuals in the dry spiny forest, 25 individuals in the moderate gallery and transitional forest, and 10 individuals in the humid rain fores. T_{skin} is highly related to T_b and it can be used as a substitute in this species (Berg *et al.* 2015). In contrast to day T_{skin} , which includes the entire photoperiod

from 0600 hours until 1800 hours, activity T_{skin} is defined as the time-span from the first T_{skin} peak in the early morning after initial heating until a drop in T_{skin} indicating retreat to a crevice, which is clearly visible in distinct daily T_{skin} patterns (Fig. 1.1). As cloud cover and rain may lead to behavioural changes (Sun *et al.* 2001), we only used sunny, cloudless days in our analysis. We also installed temperature loggers on trees in 1 m height in full shade and protected from wind and rain in the same habitat to measure ambient temperature. These measurements served as reference for sudden weather changes.

Metabolic measurements

To measure the oxygen consumption and to calculate metabolic rate of *O. quadrimaculatus*, we used a portable open flow oxygen analyzer (OxBox, designed and constructed by T. Ruf and T. Paumann, FIWI, University of Veterinary Medicine Vienna, Austria) with fuel cell oxygen sensors (7OX-V CiTiceL; City Technology Ltd, Portsmouth, UK; accuracy < 0.02 vol. %). The device was calibrated using a gas mixing pump (type G27, Wölsthoff, Bochum, Germany) each time before and after the field season. We measured non-fasting, resting but awake animals and therefore refer to our measurements as field RMR and use these data to estimate energy budgets close to natural conditions (Niewiarowski & Waldschmidt, 1992; Levesque *et al.* 2016). We captured adult lizards (n = 35) in the morning between 0900 hours and 1200 hours, weighed and placed them in a plastic container of 1 l volume that served as metabolic chamber. We used a pull-mode respirometry setup and recorded sample air once per minute at an airflow of 40 l/h alternated with hourly six-minute samples from ambient reference air for baseline corrections. Sample and reference air was dried and cleaned through silica-scrubber before the measurement. Measurements lasted from early morning until 1600 hours. The temperature in the metabolic chamber was monitored with thermocouples to ensure smooth heating curves. Individuals showing signs of heat stress were immediately removed from the metabolic chamber. In addition, the lizards' T_{skin} was recorded via attached temperature loggers and ranged from 17.5°C to 42.5°C. We calculated VO_2 and corrected for CO_2 as recommended by Lighton (2008). Oxygen consumption ($\text{ml O}_2/(\text{g}\cdot\text{h})$) was then calculated with the following equation: field RMR = flow * Δ vol % O_2 * 10. Finally, oxygen consumption ($\text{ml O}_2/(\text{g}\cdot\text{h})$) was converted into energy expenditure ($1 \text{ ml O}_2/(\text{g}\cdot\text{h}) = 20.08 \text{ J}/(\text{g}\cdot\text{h})$). Field RMR values were then

assigned to the T_{skin} of the animal. We used 5-min-averages and manually excluded periods of animal activity. We obtained between 7 and 75 field RMR values per individual (25.9 ± 17.5 records/individual) for a total of 35 individuals distributed equally across habitats. After the measurement, the animals were weighed again and released at the original place of capture.

Daytime field resting costs

For the calculation of field resting costs ($\text{J}/(\text{g} \cdot 12\text{h})$) we first calculated the temperature-rate relationship of T_{skin} and field RMR using a linear mixed effects model (LMM). These models account for an unequal number of data points per individual and dependent data from the same individual. We used the `lme` function in the R package `nlme` (Pinheiro *et al.* 2015) and included T_b as covariate and individual ID as a random factor. We visually checked the model residuals with Q-Q plots and histograms to assess the model quality. All analyses were performed in R v3.2.1 (R Development Core Team 2015). Using the resulting equation, we then calculated corresponding field RMR values for each T_{skin} measurement of the daily T_{skin} pattern (measured in 5 min intervals) and summed these across the photoperiod to obtain field resting costs ($\text{J}/(\text{g} \cdot 12\text{h})$). Statistical analyses were conducted with IBM SPSS 21.0. We calculated an ANOVA to test for differences in activity T_{skin} , activity time and field resting costs. The number of measured days per individual varied between one and five days. We therefore used mean values per individual for a comparison among habitats. All means are given with standard deviation.

Results

Mean activity T_{skin} was 37.5 ± 0.8 °C ($n = 48$) and did not differ among habitats (Fig. 1.2; ANOVA: $F_{2;45} = 0.168$; $p = 0.846$) but the mean day T_{skin} during the photoperiod, which also includes hours of restriction due to constraints in T_e , decreased from spiny forest to rain forest (ANOVA: $F_{2;45} = 59.725$; $p < 0.001$). T_{skin} during the night (between 1800 hours and 0600 hours), when the lizards are inactive, also decreased from spiny to rain forest (ANOVA: $F_{2;45} = 47.427$; $p < 0.001$). Minimum and maximum T_e as well as T_e -range decreased along the gradient and the duration of temperatures in the activity T_{skin} range was shorter in the rain forest (Fig. 1.3). The fluctuating T_e measured in the sun in the rain forest was caused by shading from branches but sunny spots were constantly

available throughout the day and hence, T_e was constantly high between these peaks. Differences in daily activity time despite similar activity T_{skin} are shown using exemplary T_{skin} patterns from individuals across the environmental gradient (Fig. 1.1).

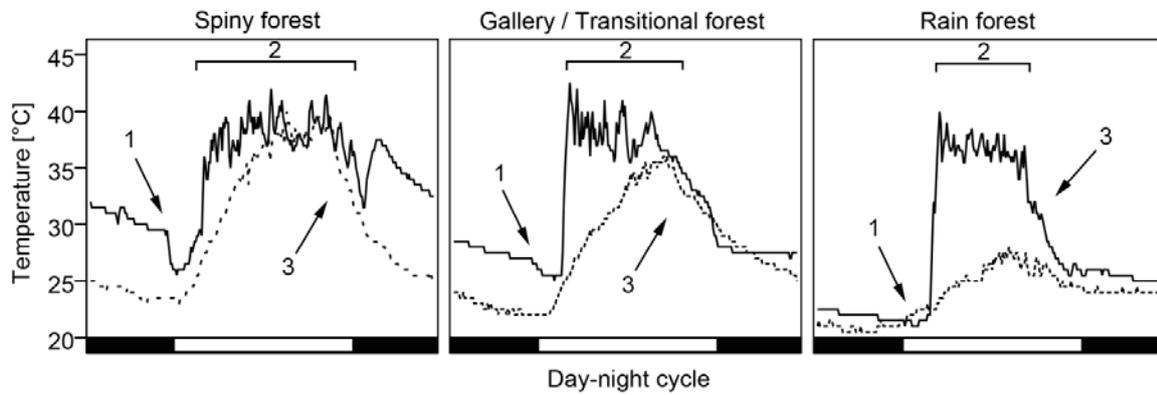


Figure 1.1: Daily skin temperature (T_{skin}) profiles of *Oplurus quadrimaculatus* across an environmental gradient in southeast Madagascar. The solid line shows T_{skin} of the lizard and the dashed line shows ambient temperature. The lizard 1) leaves its warm crevice and cools down to ambient temperature before heating up in the sun by basking, 2) is active, 3) cools down with ambient temperature and enters its crevice. Horizontal black bars indicate the scotophase.

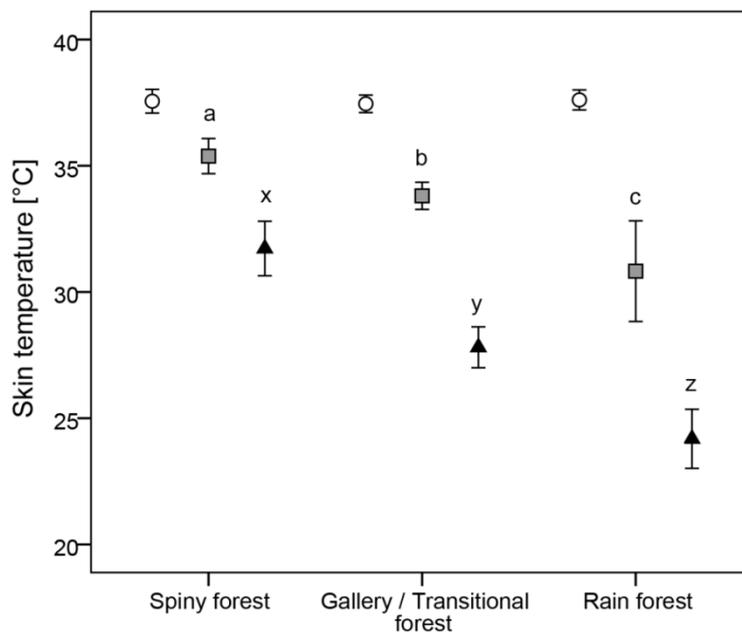


Figure 1.2: Mean activity skin temperature (T_{skin} ; open circles), mean day T_{skin} during the photopase (between 0600 hours and 1800 hours) including periods of inactivity (grey squares), and night T_{skin} (black triangles) of *Oplurus quadrimaculatus* in different habitats along an environmental gradient in southeast Madagascar. Error-bars show 95% confidence intervals and lowercase letters (a, b, c and x, y, z) indicate significant differences between habitats.

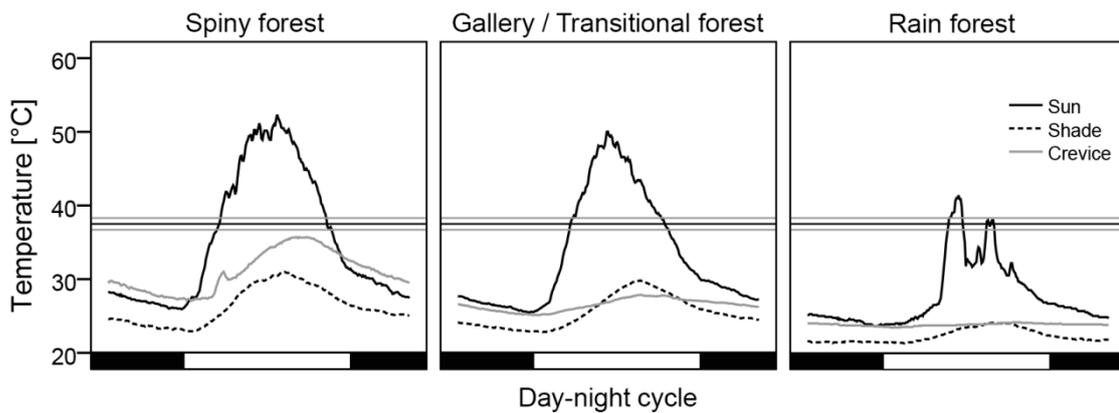


Figure 1.3: Mean operative environmental temperature of *Oplurus quadrimaculatus* in different habitats along an environmental gradient in southeast Madagascar measured with copper models during the same time period. The straight black line shows mean activity skin temperature in each habitat and straight grey lines are standard deviation. Horizontal black bars indicate the scotophase.

The daily activity time differed among habitats (ANOVA: $F_{2,45} = 8.582$; $p < 0.001$). We found a decline in activity time from the hot spiny forest through to the cooler rain forest (Fig. 1.4A). While lizards in the spiny forest were active for 464 ± 60 min per day ($n = 13$), individuals in the moderate gallery and transitional forest were active for 375 ± 103 min ($n = 25$), and individuals in the rain forest for 300 ± 108 min ($n = 10$). The duration of activity in the rain forest thus corresponds to only 65 % of the time spent active in the spiny forest.

The temperature-field RMR relationship is described by $\log(\text{field RMR}) = 0.09(T_{\text{skin}}) - 5.37$ (Fig. 1.5). We found differences in the daytime field resting costs of individuals among habitats (Fig. 1.4B; ANOVA: $F_{2,45} = 20.885$; $p < 0.001$). The highest field resting costs were found in the spiny forest (68.9 ± 6.4 J/(g*12h); $n = 13$) followed by the gallery and transitional forest (61.1 ± 5.5 J/(g*12h); $n = 25$) and the rain forest (49.8 ± 10.5 J/(g*12h); $n = 10$). This implies a reduction in field resting costs of 28 % from the spiny forest to the rain forest.

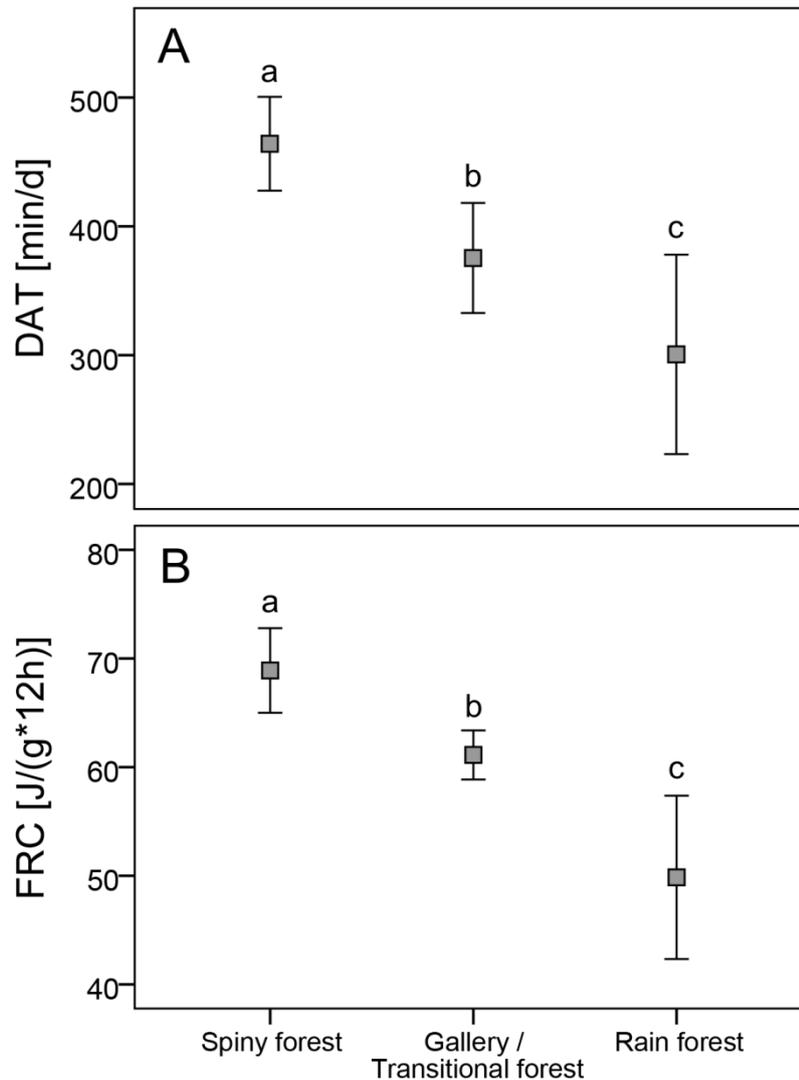


Figure 1.4: A) Mean daily activity time (DAT) and B) daytime field resting costs (FRC) of *Oplurus quadrimaculatus* in different habitats along an environmental gradient in southeast Madagascar. Error-bars show 95% confidence intervals and lowercase letters indicate statistical significance.

Discussion

Our data demonstrate that *O. quadrimaculatus* is a precise thermoregulator that maintains a high activity T_{skin} . While activity time differs significantly between habitats, this species shows no adjustments in activity T_{skin} between different thermal environments. During activity, T_b can be affected by several biotic and abiotic factors, such as food quantity, habitat structure and predation (Clusella-Trullas and Chown 2014). However, *O. quadrimaculatus* thermoregulates with high precision despite marked thermal differences between habitats and maintains a narrow range of activity T_{skin} throughout the day despite

a much wider range of T_e . It therefore seems reasonable to assume that the observed activity T_{skin} is closely related to the temperature set-point of these lizards and hence, that the thermal optimum does not vary along the environmental gradient. In accordance with other field studies, which failed to find adjustments of activity T_b or T_{pref} between different thermal environments (Brown and Griffin 2005; Gvozdik and Castilla 2001; Van Damme *et al.* 1989), this could imply that differences in the thermal environment are not sufficient for provoking physiological acclimatization because they are counterbalanced through behavioural thermoregulation. Plasticity of T_{pref} , either under laboratory conditions or seasonally in the natural environment, seems to occur only if the animals' T_b is constantly forced away from T_{pref} (Blumberg *et al.* 2002; Diaz *et al.* 2005; Seebacher and Grigg 1997), presumably because physiological acclimatization is also accompanied by energetic costs through increased rates of transcription of regulatory enzymes (Seebacher 2005). The precise thermoregulation to a narrow range of activity T_{skin} across habitats and the absence of metabolic responses along the gradient (Berg *et al.* in prep.) indicate that behavioural mechanisms are sufficient to compensate for thermal differences.

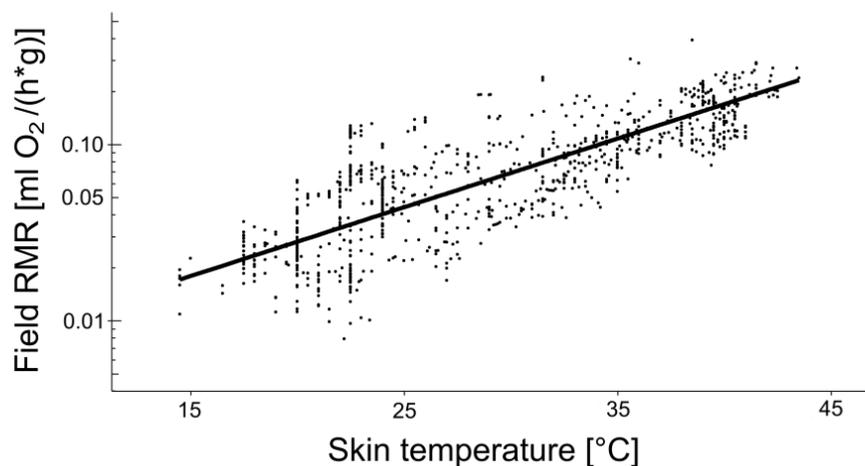


Figure 1.5: Temperature-field resting metabolic rate (field RMR) relationship for *Oplurus quadrimaculatus*. Data points represent repeated measurements from all individuals and the black line shows the fitted line of the linear mixed effects model ($\log(\text{field RMR}) = 0.09(T_{\text{skin}}) - 5.37$) that accounts for an unequal number of data points and repeated measurements.

Nevertheless, metabolic rate often shows acclimatization in species that are active year-round (Seebacher 2005). Seasonal metabolic acclimatization suggests that *O. quadrimaculatus* exhibits physiological compensation rather as a supplementary

mechanism if behavioural thermoregulation is limited. This acclimatization response is restricted to lower T_{skin} because increased rates at activity T_{skin} would result in overcompensation, which means that energy expenditure would be unnecessarily high. These data reveal that this species exhibits physiological acclimatization to compensate seasonal thermal challenges (Berg *et al.* in prep.) but physiological adjustments to the different habitats along the gradient are not necessary.

The daily activity time of *O. quadrimaculatus* declines from hot spiny forest to cooler rain forest by 35 %. Lizards in the rain forest can still reach their activity T_{skin} through basking but the window of suitable T_e is shorter and the overall activity time (between the first rise in T_e and the final decrease in T_e) is therefore reduced. Surprisingly, we did not find heat-induced hours of restriction in the hot spiny forest as has been described for numerous Mexican lizard species in similarly hot habitats (Sinervo *et al.* 2010). Considering that we only used T_{skin} patterns from cloudless, sunny days, one would expect that our measured T_e is higher than average but even in the hot spiny forest, the lizards did not seem to reach their upper thermal limits. This may be due to the heterogeneity of the habitat, which can be as important as mean ambient temperature for the animal's performance (Sears and Angilletta 2015). Despite being open rocky plateaus, the habitat provides sufficient spacial structure and shady spots to avoid overheating at increased but apparently not excessive costs for behavioural thermoregulation. Moreover, a constantly high T_{skin} seems to be important for *O. quadrimaculatus*: either lizards maintain a minimum T_{skin} of 36 °C or they retreat to crevices. No trade-off in the form of activity at lower T_{skin} has been observed, at least on sunny days. We assume that performance drops significantly at T_{skin} below 36 °C, making it difficult to perform quick targeted bursts to catch the preferred flying prey and reducing their ability to escape from predators (Cooper 2000). This indicates that the challenging factor for *O. quadrimaculatus* is not to keep cool but to stay hot, which is more difficult in the cool, shady rain forest. Hence, we only found thermal constraints at the cold end of this species' distribution range.

In our study, the field resting costs in the rain forest were 28 % lower than those in the hot spiny forest. Thus, field resting costs decline in a similar way to daily activity time, which was reduced by 35 % in the cooler habitat. We suggest two main reasons for this pattern. First, less time at high activity T_{skin} reduces the overall metabolic costs. Second, T_e , and

crevice temperature in particular, was lower in the rain forest, even during the photoperiod. Hence, when the lizards were thermoconform during hours of restriction (induced by low T_e), the field RMR was passively reduced. Our calculations are based on the effect of T_{skin} on mean field RMR and do not include costs of locomotion or possible seasonal acclimatization effects. One can argue that metabolic rate can be threefold higher during activity (Bennett and Nagy 1977). However *O. quadrimaculatus* is a sit-and-wait forager that performs only few targeted bursts for prey, so costs for physical activity adding to field RMR are relatively low. Field resting costs are thus likely to be close to the actual energy expenditure during the day. Since we did not measure standard metabolic rate during the night, we only calculated field resting costs during the photoperiod. However, the high mean night T_{skin} (31.7 ± 1.8 °C) in the spiny forest might be especially costly because at high temperatures even small increases in T_{skin} lead to a substantial increase in energy expenditure. Christian *et al.* (1996), for example, found that a decrease of 4 °C in night-time T_b resulted in a decrease of energetic costs of more than 30 % for the water monitor *Varanus mertensi*. Hence, lower night temperature in the rain forest might amplify the energy savings of *O. quadrimaculatus*.

Why is the cooler rain forest habitat more challenging if the lizards can save so much energy? Most striking is that the time suitable for activity is highly reduced, which includes feeding, mating, and territorial behaviour. Consequently, these lizards must feed and digest in a significantly shorter time period. The most challenging restriction is probably that low T_b also depresses biochemical processes that support digestion and energy assimilation. Even if the lizards find enough food within the limited time frame, digestion might suffer from low T_b as shown for the eastern fence lizard *Sceloporus undulatus* (Angilletta *et al.* 2002). This effect will be even more pronounced if one considers the higher number of cloudy and rainy days and cooler wind in the rain forest, where maximum T_e is below the threshold for activity and suboptimal for other physiological functions. In fact, while our study site in the rain forest is the eastern distributional limit of *O. quadrimaculatus*, the species occurs in even drier and hotter areas towards the southwest of Madagascar (Glaw and Vences 2007). Adult lizards have a wide spectrum of mechanisms to cope with different environmental temperatures but egg development and juvenile growth, which are especially sensitive to temperature, are largely understudied (Du and Shine 2015). Apart from potential movements within the egg (Du *et al.* 2011), the embryos' T_b depends largely on the environment. Cold

temperature lead to prolonged incubation periods, embryonic diapause and maybe reduced hatchling survival rate (Du and Shine 2015). Considering the distribution in the hot spiny forest and the high activity T_b of adult *O. quadrimaculatus*, the rain forest possibly acts as a population sink.

In summary, our study shows that heliothermic lizards strongly depend on behavioural mechanisms for coping with varying environmental conditions. Highly effective thermoregulatory behaviour and adjustments in activity time are the prevalent mechanisms and thus, low temperatures rather than hot environments seem to be challenging for these species. Cooler habitats may reduce the energy expenditure in warm adapted species but the consequences of decreased physiological functioning and reduced time for food intake remain unclear. Hence, heliothermic lizards may even profit - at least initially - from an enlarged distribution range caused by climate warming and by increased basking opportunities through forest degradation. We found that behavioural thermoregulation is sufficient to compensate extreme climatic variation and the potential of this mechanism may render open habitat species less vulnerable to climate change compared to species with limited capacity for behavioural adjustments.

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Supplementary material



Figure S1.1: Individually marked *Oplurus quadrimaculatus* in its natural habitat in southeastern Madagascar with attached temperature logger (photo by Wiebke Berg).

Author contribution

I hereby confirm that Ole Theisinger conceived, designed and performed the experiments, analysed the data and wrote the paper.

Hamburg,



Prof. Dr. Kathrin Dausmann

Chapter 2

Behavioural capacity of a heliothermic lizard (*Oplurus saxicola*) to compensate for differences in the thermal environment

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Abstract

Habitats with differing thermal environments require specific responses from ectotherms. This response can be based on changes in physiological processes or, more often, on behavioural adaptation to optimize the performance. Additionally, foraging constrains the thermal scope because it reduces time for thermoregulatory behaviour. Our aim was to investigate the capacity of behavioural and physiological plasticity of a lizard inhabiting a dry environment with a challenging thermal scope. We analyzed thermoregulatory behaviour, feeding behaviour and body temperature of the heliothermic lizard *Oplurus saxicola* in two different habitats: hot spiny forest and moderate gallery forest. The lizards maintained a high body temperature (37.7 ± 2.1 °C) throughout the day with no physiological adjustments to local conditions in the form of differing activity body temperature. In the gallery forest, most individuals were active around midday, whereas individuals in the spiny forest shifted their activity to the morning and the afternoon. Feeding activity patterns generally correlated with the overall activity in both habitats and shuttling was the main behavioural mechanism for thermoregulation. Our results show that adjusting behaviour and activity time is the primary mechanism used by heliothermic

lizards to compensate for environmental differences, while physiological plasticity is of secondary importance.

Keywords

Activity patterns; body temperature; Madagascar; physiological acclimatization

Introduction

Reptiles often occur in different habitats with varying thermal conditions, but they must attain a body temperature (T_b) that enables physiological processes and locomotion performance (Angilletta *et al.* 2002, Huey and Bennett 1987; Huey and Kingsolver 1989). Unless the species is entirely thermoconforming, each habitat requires specific thermoregulative behaviour (Adolph 1990; Zamaro-Camacho *et al.* 2015). The operative environmental temperature (T_e) (the theoretically attainable T_b for a species in a given habitat), the thermal quality (difference between T_e and the animals' selected T_b) and the structure of the habitat determine the costs of behavioural thermoregulation (Stellatelli *et al.* 2013; Hertz *et al.* 1993; Sears and Angilletta 2015; Huey and Kingsolver 1989). The ability to adapt to different thermal regimes varies between species and depends on the effectiveness of thermoregulatory behaviour (Hertz *et al.* 1993) or on the capacity for physiological adaptation and physiological plasticity (Gvozdik 2012). Species with eminent effectiveness in thermoregulatory behaviour often show limited physiological adaptation because of lower selective pressure on the thermal physiology (Buckley *et al.* 2015; Huey and Kingsolver 1993; Huey *et al.* 2003).

However, lizards are only able to act within the T_e range, and if the temperature is too high or too low, the animals must retreat (Kearney *et al.* 2009). This results in a reduction of time available for activities such as foraging, mating and territorial defense; if these restrictions are too severe, lower fitness and finally local extinction can also occur (Sinervo *et al.* 2010). Lizards that inhabit moderately tempered habitats usually show highest activity around midday (House *et al.* 1980), but increasing temperature due to climate warming may lead to a shift in activity time towards the early morning and late afternoon (Sinervo *et al.* 2010). Lizards in hot and arid habitats, for example, show bimodal patterns of activity with long hours of restriction during midday (Grant 1990).

Further warming might exceed the compensation capacity of the animal and reduce potential activity time to a level that limits survival (Sinervo *et al.* 2010; Huey *et al.* 2010).

Ecological factors also affect T_b and performance of lizards, perhaps in a more substantial manner than expected from the thermal environment itself (Blouin-Demers and Weatherhead 2002). Foraging strategy (Verwaijen and Van Damme 2007), competition (Downes and Shine 1998), predation (Herczeg *et al.* 2008), food quantity (Brown and Griffin 2005), reproductive state (Harlow and Grigg 1984) and infection (Bernheim *et al.* 1978) all significantly affect the thermal biology of reptiles. These complex interactions often interfere with physiological demands, making trade-offs unavoidable. This can lead to either the selection of an intermediate but suboptimal T_b (Angilletta *et al.* 2002), or a temporal split of the day to account for ecological and physiological demands (Theisinger *et al.* in review). Species with a high thermal sensitivity and a narrow activity T_b range tend to either operate at this T_b or retreat, because suboptimal T_b leads to decreased performance and possibly extinction (Cooper 2000; Huey and Kingsolver 1989).

Due to these constraints, increasing temperatures could lead to reduced survival and extinction of populations. Madagascar's reptiles are particularly threatened because the ambient temperature has already increased, and is expected to increase further during the next few decades (Hannah *et al.* 2008). Animals often cannot retreat to more suitable areas because their suitable habitat has often been degraded and fragmented by human activities (Harper *et al.* 2007; Kremen *et al.* 2008; Theisinger and Ratanarivo 2015). Thus, many species might face local extinction.

Our aim was to investigate the behavioural and physiological plasticity of a lizard that inhabits an environment with a challenging thermal scope. We analyzed T_b , foraging behaviour and thermoregulatory behaviour (shuttling and posture changes) of the heliothermic Marked Madagascar Swift *Oplurus saxicola* in two habitats (hot spiny forest and gallery forest along a river) with different ambient temperature regimes (Rakotondranary *et al.* 2011) and different habitat structures (Andriaharimalala *et al.* 2011). We examine whether different thermal environments constrained lizards' activity and thermoregulatory behaviour and whether these different environments affect foraging times. If temperature is limiting, we expect a pronounced bimodal activity pattern in the spiny forest population and one peak around midday in the gallery forest population with

a similar pattern for feeding activity. We also expect higher activity T_b in the hot spiny forest than in the moderate gallery forest.

Methods

Study site

We investigated activity patterns and thermoregulative behaviour of *O. saxicola* in the Andohahela National Park in southeastern Madagascar. The climate in this area is seasonal with a hot rainy season from November to April and a cool dry season from May to October (Tadross *et al.* 2008). Interannual variation is high, and the beginning and the end of the seasons differ between years (Dewar and Richard 2007). Our study took place between September and November 2011, when ambient temperature was already high but no rain had yet occurred. We studied populations in the hot spiny forest (24°57'40.55"S, 46°35'41.25"E) and the moderately tempered gallery forest along a river (24°57'9.43"S, 46°37'19.37"E). In November mean ambient temperature (T_a) was 31 °C in the spiny forest but 26 °C in the gallery forest. Hence, the mean difference in T_a between the two habitats is almost 5 °C at this time of the year (Rakotondranary *et al.* 2011). The habitat structure of the spiny forest is rather scarce whereas the habitat structure of the gallery forest is very heterogeneous with large, evergreen trees and varying substrate such as sand, rocks and grass (Andriaharimalala *et al.* 2011).

Study species

The Marked Madagascar Swift (*Oplurus saxicola*) is a small iguanid lizard (body mass \pm SD: 18.0 \pm 6.2 g; snout-vent length \pm SD: 8.3 \pm 0.9 cm; n = 184), endemic to southern Madagascar (Glaw and Vences 2007). With its flattened body shape, long digits and inconspicuous coloration, this lizard is exclusively adapted to a saxicolous lifestyle. *Oplurus saxicola* is heliothermic, occurs on large smooth rocks and feeds on small flying insects (sweat bees, flies, praying mantis and locusts) and occasionally on flowers. Its main predator is the Madagascar kestrel (*Falco newtoni*) (Glaw and Vences 2007).

Activity transects

To investigate the activity patterns of *O. saxicola*, we established one transect line (250 m x 10 m) in the gallery forest and one in the spiny forest, and surveyed both habitats alternately. For comparative reasons, all transect searches were conducted on days with comparable weather conditions (sunny, no or only light clouds and no wind) because the activity patterns of heliothermic reptiles change significantly with different weather conditions (Sun *et al.* 2001). We started the transect searches in the morning before first activity (0630 hours in the gallery forest and 0700 hours in the spiny forest), and continued until all lizards retreated again into their crevices (1700 hours in the gallery forest and 1800 hours in the spiny forest). Temporal differences between sites were caused by the terrain slope of the spiny forest site, where direct sunlight reached the ground approximately 20 min later, but lasted longer. We searched the same transect in 30-min intervals for 20 min alternately in opposite directions (22 times per day), resulting in 264 transect searches in six days for each habitat. An individual that was visible, independent of actual behaviour (e.g. running, resting, feeding or basking), was regarded as “active” because it is difficult to determine whether the lizard is indeed resting or waiting for prey. We recorded the number of active individuals of *O. saxicola* per transect walk, and their sun exposure (sun, half shade, shade, and cloud). Where possible, we noosed the lizard directly after recording behavioural data and inserted thermocouples approx. 1.5 cm into the cloaca to measure T_b (Greisinger GTH 175/Pt, resolution 0.1 °C, Regenstauf, Germany). We only used T_b from animals that were captured and measured within 30 seconds after first sight. Additionally, we measured T_a at the study sites using temperature loggers (Thermochron iButton DS1921G, Maxim Integrated Products, CA, USA).

Focal observations

To investigate the thermoregulative and feeding behaviour of the lizards, we conducted focal sampling observations. The focal observations took place in parallel to the activity transects by a second person on the same day in the same habitat. In contrast to other studies observing behaviour (e.g., Carter *et al.* 2012; Wasiolka *et al.* 2009), the natural behaviour of *O. saxicola* was not disturbed by our presence. We kept a distance of at least 3 m to the animal to avoid potential interactions. The focal sampling started with the first active individual in the morning and ended when all individuals had retreated into their

crevices. Each recording lasted 15 min and started in 30-min intervals with a new individual. A single individual was observed at most three times in total but not on the same day and, if observed on another day, at a different time of the day. Observations lasting less than 15 min (e.g. because the individual went out of sight) were excluded from the analysis. For each individual, we recorded the frequency of posture changes ('flat' on the ground with maximal surface contact, 'normal' in upright position with only tail and rear touching the ground and 'high' with spread legs and minimal surface contact; Fig. 2.1) and the shuttling frequency, defined as the number of movements from one microhabitat to another with different sun exposure (sun, half shade and shade). Temporary short term cloud cover was also taken into account, but we stopped the observations if significant weather changes occurred. To find out whether *O. saxicola* adapts its feeding strategy to different environments (Wasiolka *et al.* 2009), we recorded the foraging success (number of successful and unsuccessful feeding attempts) and prey size (small: sweat bees, flies; large: praying mantis, locusts).

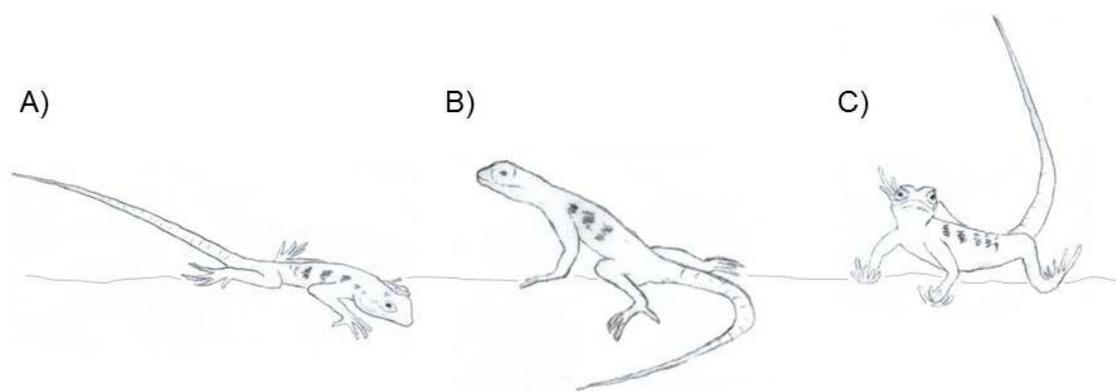


Figure 2.1: Body postures of *Oplurus saxicola*: A) flat on the ground with maximal surface contact; B) normal in upright position with tail and rear touching the ground; C) high with spread legs and minimal surface contact.

Operative environmental temperature

We used four specifically designed and colored copper models (SVL 8.5cm) to measure T_e (Dzialowski 2005, Bakken and Angilletta 2014). To calibrate the copper models, we equipped them with temperature loggers and placed them in a transparent plastic box next to a box with live lizards. The T_b of the live animal was measured simultaneously using thermocouples. During the measurements, both lizards and copper models were handled

to ensure similar heat conduction. By shuttling the boxes with animals and models between sun and shade, we were able to compare the cooling and heating rates, and the values are highly correlated ($R^2 = 0.964$; $p < 0.001$; $n = 80$). Hence, we used the copper models as T_e representative for *O. saxicola*. We then distributed five models for five consecutive days in each habitat to cover a representative T_e range.

Data analysis

Statistical analyses were conducted with IBM SPSS 21.0 and values are given in mean \pm standard deviation. Differences in T_a between habitats were tested with a paired t-test, and activity T_b of the two populations was compared using a two sample t-test. We categorized the time of the day in morning (from 0700 hours to 1030 hours), midday (from 1100 hours to 1430 hours) and afternoon (from 1500 hours to 1800 hours) to compare the different activities in the course of the day. We then used the one-way ANOVA to test for differences in overall activity, behaviour and feeding attempts.

Results

The spiny forest and the gallery forest showed distinct microclimatic differences. We found a significant difference in mean daily ambient temperature (spiny forest: 30.2 ± 3.5 °C, $n = 47$; gallery forest: 28.2 ± 3.4 °C, $n = 47$; $t_{92} = 2.734$; $p < 0.05$) and highly significant differences in maximum ambient temperature (spiny forest: 36.3 ± 4.6 °C, $n = 47$; gallery forest: 32.4 ± 4.1 °C, $n = 47$; $t_{92} = 4.375$; $p < 0.001$) and ambient temperature amplitude (spiny forest: 15.9 ± 5.1 °C, $n = 47$; gallery forest: 11.4 ± 4.4 °C, $n = 47$; $t_{92} = 4.588$; $p < 0.001$) during the activity time of the lizards (from 0600 hours to 1800 hours) (Fig. 2.2). Only minimum ambient temperature was similar in both habitats (spiny forest: 20.4 ± 3.0 °C, $n = 47$; gallery forest: 20.9 ± 3.6 °C, $n = 47$; $t_{92} = 0.839$; $p = 0.404$). Despite the significant difference in ambient temperature, the activity T_b of lizards was similar in both habitats (spiny forest: 37.6 ± 2.4 °C, $n = 61$; gallery forest: 37.8 ± 1.7 °C, $n = 49$; $t_{108} = 0.566$; $p = 0.573$) (Fig. 2.3).

T_e measurements showed less potential activity time for lizards in the gallery forest than in the spiny forest (Fig. 2.4). Whilst lizards in the spiny forest were able to reach their activity T_b for up to 9h 15min, the animals' activity in the gallery forest was restricted to 7h 30min. The lizards retreated earlier because direct sunlight was not available after

1600 hours and T_e did not allow for obtaining activity T_b . However, the lower end of T_e , measured in crevices during the hottest time of the day, was below mean activity T_b . Hence, during hours when activity was restricted in the spiny forest, the lizards had the opportunity to avoid heat stress by retreating to their crevices.

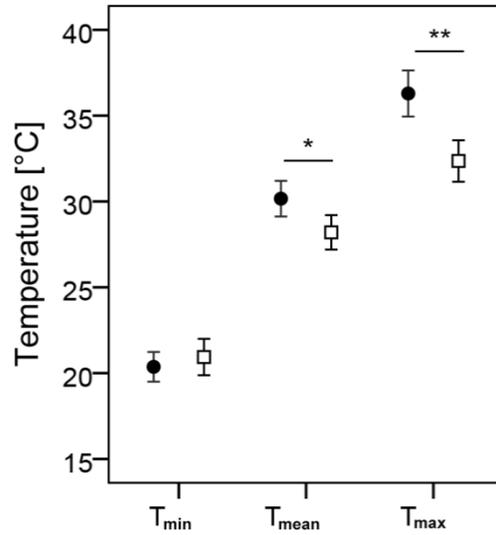


Figure 2.2: Minimum (T_{min}), mean (T_{mean}) and maximum (T_{max}) ambient temperature between 0600 hours and 1800 hours in the spiny forest (closed circles) and gallery forest (open squares). Asterisks indicate level of significance (* $p < 0.05$; ** $p < 0.001$).

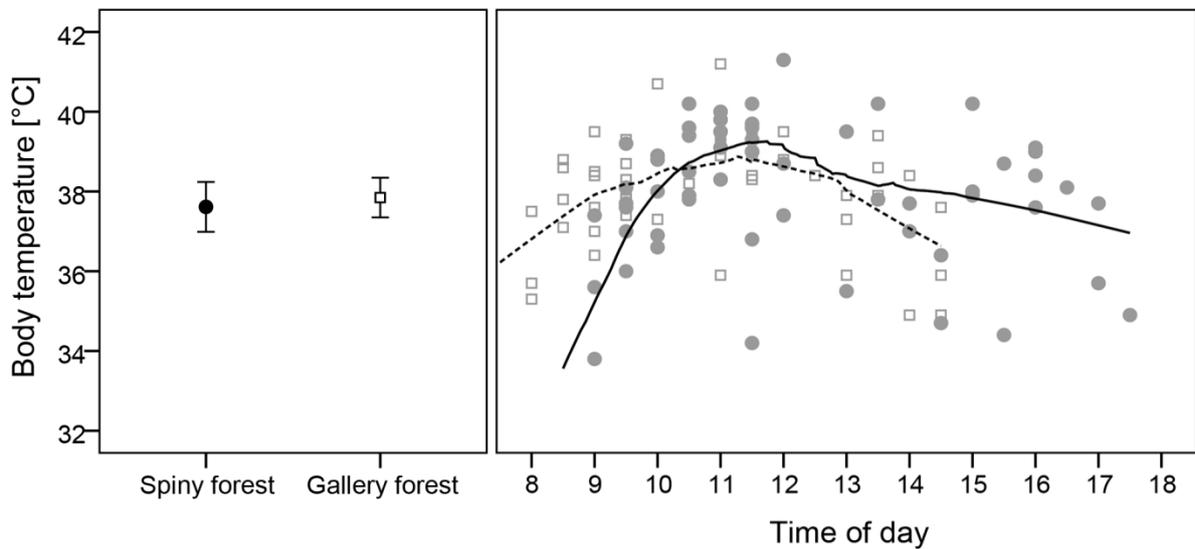


Figure 2.3: Mean body temperature during activity (left) and single body temperature data over the course of the day (right) of *Oplurus saxicola* in the spiny forest (closed circles) and gallery forest (open squares). Lines are loess curves for data from the spiny forest (solid line) and gallery forest (dotted line).

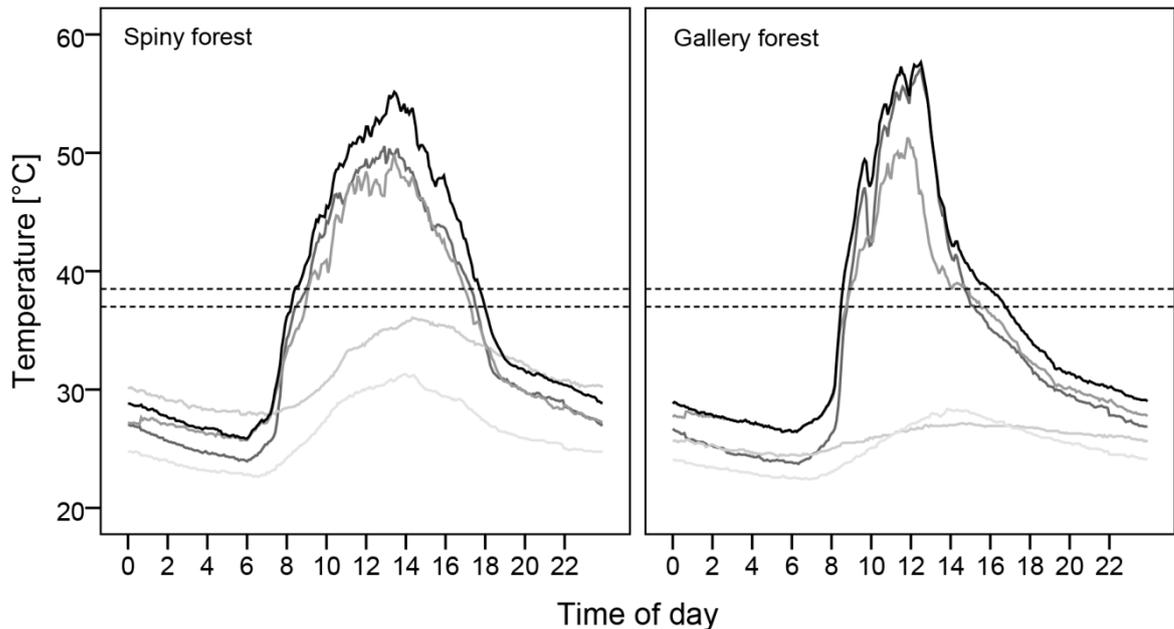


Figure 2.4: Operative environmental temperature of *Oplurus saxicola* in the spiny forest (left) and gallery forest (right). Each differently shaded line represents five-days-average temperatures of a copper model placed in different microhabitats including full sun, full shade and crevice. The dashed lines show the activity body temperature range.

We found distinct activity patterns of *O. saxicola* for each habitat that differed significantly from each other (Fig. 2.5A). In the spiny forest, the number of active individuals changed in the course of the day ($F_{2,63} = 9.160$; $p < 0.001$), with highest activity in the morning, significantly lower activity at midday ($p < 0.001$) and similarly low activity in the afternoon ($p = 0.717$). Lizards' activity in the gallery forest also varied in the course of the day ($F_{2,60} = 18.345$; $p < 0.001$), but patterns were different. The activity was also high in the morning but it reached a peak during midday ($p = 0.295$). We then observed a strong decrease in activity in the afternoon ($p < 0.001$). After 1700 hours, direct sunlight in the gallery forest decreased, restricting basking and heliothermic thermoregulation (Fig. 2.6). A few individuals still gained heat from the warm rocks, but most individuals retreated immediately into their crevices.

Shuttling behaviour changed significantly in the course of the day in both habitats (spiny forest: $F_{2,100} = 4.065$; $p < 0.05$; gallery forest: $F_{2,99} = 20.428$; $p < 0.001$) (Fig. 2.5B). In the spiny forest, the lizards showed a decrease in shuttling behaviour from morning to midday ($p < 0.05$) with no significant changes in the afternoon ($p = 0.245$). In contrast, there was a continuous decline in shuttling frequency in individuals from the gallery

forest from morning until the afternoon ($p < 0.001$). We found no difference between the habitats in mean shuttling frequency ($t_{203} = 0.364$; $p = 0.716$). Only the shuttling frequency in the afternoon was significantly lower in the gallery forest than in the spiny forest ($t_{47} = 3.976$; $p < 0.001$). The lizards did not show differences in posture change frequency in the course of the day (Fig. 2.5C), neither in the spiny forest ($F_{2,100} = 0.272$; $p = 0.763$) nor in the gallery forest ($F_{2,99} = 1.277$; $p = 0.284$), with a similar number of posture changes in both habitats ($t_{203} = 0.713$; $p = 0.477$).

Whilst the feeding behaviour of the spiny forest population did not differ in the course of the day ($F_{2,100} = 1.443$; $p = 0.241$), we found a significant decrease in the number of feeding attempts in the gallery forest ($F_{2,99} = 7.448$; $p < 0.001$) (Fig. 2.5D). In the morning, the mean number of successful feeding events did not differ between the habitats, but the number of failed feeding attempts was significantly higher in the gallery forest ($t_{101} = 1.998$; $p < 0.05$).

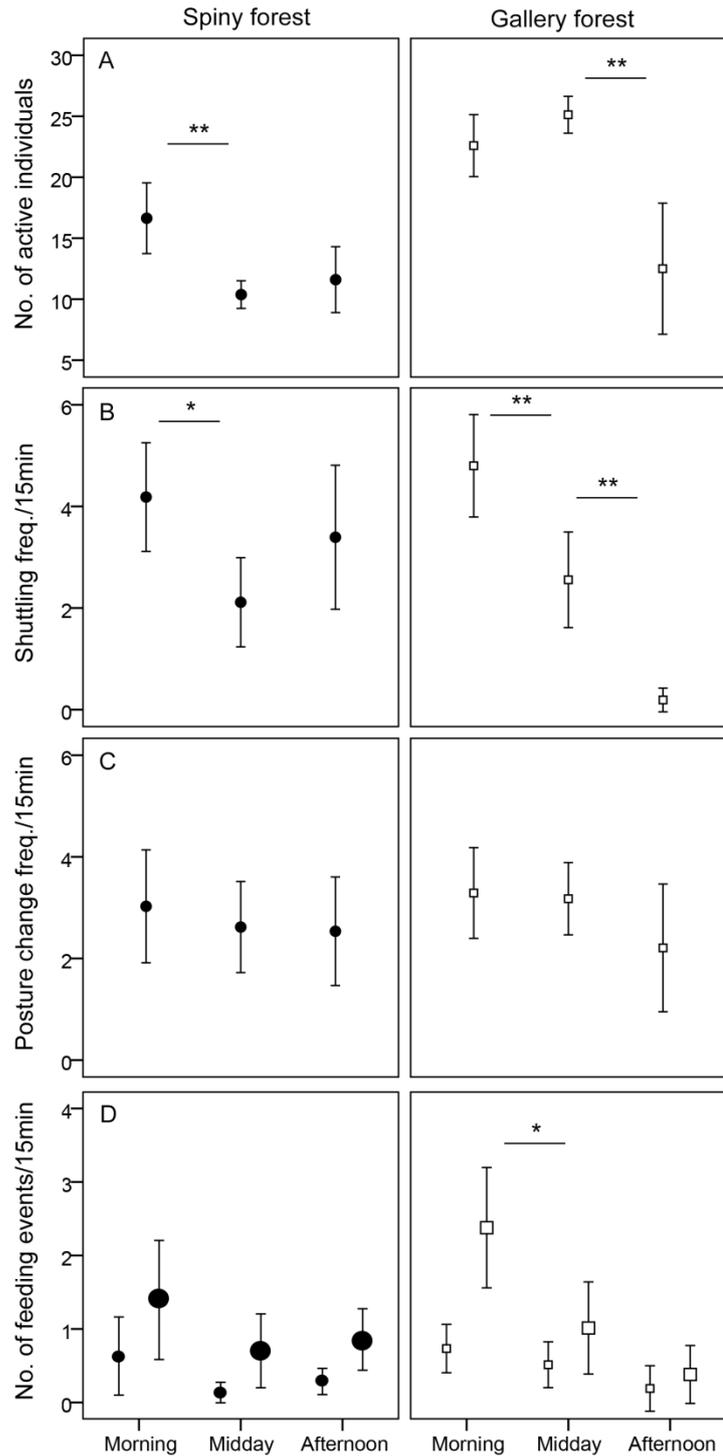


Figure 2.5: Different activities of *Oplurus saxicola* in the course of the day in the spiny forest and the gallery forest. A) Overall activity of individuals in each population, B) shuttling frequency between microhabitats, C) posture change frequency and D) the number of successful feeding events (small circles and squares) and the total number of feeding attempts (big circles and squares). Asterisks indicate level of significance (* $p < 0.05$; ** $p < 0.001$).

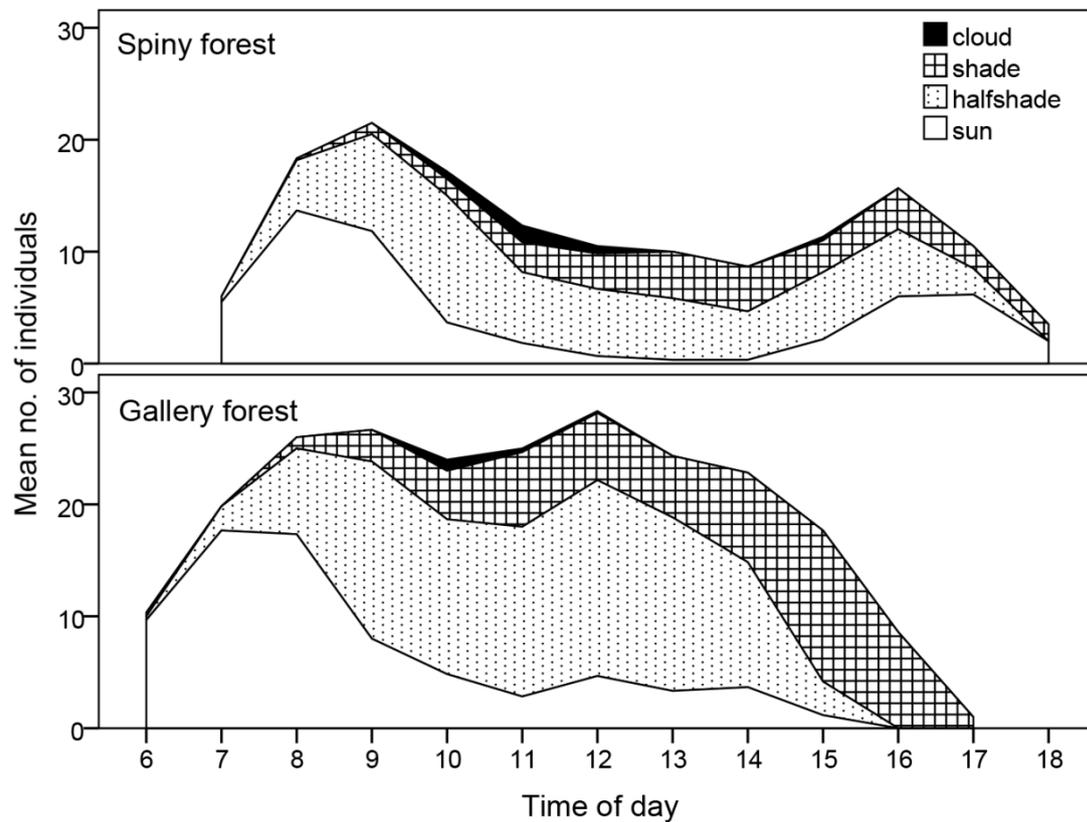


Figure 2.6: Overall activity and microhabitat use of *Oplurus saxicola* in two different habitats over the course of the day.

Discussion

Our study shows that the lizard *O. saxicola* occurs in habitats with different T_e without physiological adaptations reflected by adjustments of T_b . Behavioural thermoregulation is efficient and sufficient to compensate for environmental differences, and feeding behaviour is largely unaffected by T_e .

The patterns of overall activity correspond to our assumption that the population in the hotter spiny forest shows a bimodal distribution with more active individuals in the morning and in the late afternoon and hours of restriction during midday. However, the majority of individuals (63 %) were still active, indicating that these hours of restriction are not compulsory in *O. saxicola*. Either these individuals show a higher temperature tolerance that allows for regular activity (Leal and Gunderson 2012; Gvozdik 2012), or the retreated individuals simply did not find suitable microclimates to maintain activity T_b (Sears and Angilletta 2015). Since we did not observe higher activity T_b in individuals

active at midday (Fig. 2.3) nor in comparison with individuals from the gallery forest, a higher temperature tolerance seems unlikely. The spiny forest generally provides a broad T_e range, but vegetation is scarce (Andriaharimalala *et al.* 2011), and shade is essential for tropical ectotherms to stay cool during the hottest times of the day (Kearney *et al.* 2009). It might not be possible for every individual to have access to the whole T_e range because certain microclimates might show a patchy distribution. If only very hot or rather cool microclimates are available, the costs of shuttling behaviour outweigh the benefits of being active (Sears and Angilletta 2015). There might also be intraspecific competition for microhabitats with “preferred T_e ”, and some individuals could be forced to either select suboptimal microhabitats or retreat. The bimodal activity pattern of individuals in the spiny forest was not as pronounced as in the desert lizard *Sceloporus merriami*, which shows zero activity during midday (Grant 1990). However, the partial reduction in activity indicates that at least some individuals face their thermal limits at the hottest time of the day.

Theoretically, a lowered activity T_b in the gallery forest would be beneficial to compensate for thermal differences and to increase the potential daily activity time (Gvozdik 2012). However, we did not observe adjustments in activity T_b . The capacity for such physiological acclimatization is often observed after an artificial acclimation period, but rarely in the wild as a local adjustment to different habitats. Laboratory experiments have shown acclimation shifts in several physiological measures in ectotherms, such as performance, critical T_b and selected T_b (Hadamova and Gvozdik 2011; Blumberg *et al.* 2002; Seebacher 2005), but several field studies reveal that lizards and other ectotherms, adapt behaviourally if possible. A clade of *Anolis* lizards exhibits remarkably similar field T_b and heat tolerance, despite environmental differences of as much as 15°C in mean annual temperature (Muñoz *et al.* 2014). Zamora-Camacho *et al.* (2016) found behavioural adaptations of the lizard *Psammotromus algirus* to an altitudinal (temperature) gradient, but a marginal shift in selected T_b . Tadpoles of the European common frog *Rana temporaria* did not show physiological adaptation to different altitudes, even though the contrary was concluded from the authors, because the results for metabolic acclimatization were not consistent and rather random (Muir *et al.* 2014). Of two Indian anoles, only the open habitat species *Anolis roquet* shows behavioural thermoregulation, while the forest species *A. gundlachi*, with limited potential for behavioural compensation, shows physiological adjustments in its heat

tolerance along an altitudinal gradient (Hertz 1981). Heliothermic lizards with efficient behavioural thermoregulation are especially likely to exhibit less physiological plasticity (Buckley *et al.* 2015), showing that physiological acclimatization is also associated with costs (Seebacher 2005) and probably occurs only when behavioural thermoregulation is limited (Gunderson and Stillman 2015). In *O. saxicola* behavioural thermoregulation also seems to be sufficient to compensate for most of the observed thermal differences. It is still not clear whether lizards with proven acclimation capacity would resort to their physiological plasticity under natural conditions or if they only show behavioural adjustment for compensation. This would require further investigation and the extrapolation of laboratory results into the wild and vice versa.

Besides shuttling, changing posture is also an important mechanism for thermoregulation. Lizards can minimize (“high” posture) or maximize surface contact (“low” posture) to control heat transfer with the surface and to optimize body orientation to the sun (McConnachie *et al.* 2009). However, the frequency of posture changes is similar in both habitats and in the course of the day. This corroborates results from Stevenson (1985) who found that shuttling behaviour seems to be a preferred and more efficient compensatory mechanism than postural adjustments. *Oplurus saxicola* is a relatively small lizard species and heat conduction is probably faster than in its larger relatives (*O. cyclurus* and *O. quadrimaculatus*). Its flattened body shape promotes heat absorption through solar radiation and thigmothermic heat transfer from warm rocks. Simultaneously, the potential to store heat is reduced and physiological control mechanisms such as metabolic heat production or dermal blood flow regulation (Seebacher and Franklin 2005) probably have little effect on thermoregulation in this species. Other thermoregulatory behaviour such as increased breathing patterns (Tattersall *et al.* 2006), have not been observed. Thus, we found that the lizards are able to maintain a relatively high activity T_b within a narrow range throughout the day mainly by shuttling between microclimates.

The number of feeding attempts in the spiny forest correlates with the overall activity, but individuals from the gallery forest show highest feeding activity in the morning and not around midday, when most individuals are active. The overall feeding rate was relatively low (less than one attempt per focal observation), though the results may have been biased by a few individuals that were highly active, with several feeding attempts (up to

10 per focal observation). The lizards' prey in the spiny forest mainly consists of small sweat bees, small flies and occasionally, small praying mantis. This is similar in the gallery forest, but because of the proximity to open water, some larger prey species (e.g. dragonflies and butterflies) occur here. These prey items may have higher nutritional value, but they are harder to catch, which might be the reason for the higher number of unsuccessful feeding attempts compared to the spiny forest. However, the feeding activity does not seem to affect the T_b selection of the lizards, as T_b is similarly high in the course of the day and across habitats, promoting high locomotion performance throughout the day.

It remains questionable whether these compensatory mechanisms are sufficient to prevent this species from extinction in the face of climate warming. Predicted temperature increase through climate change will increase hours of restriction (Sinervo *et al.* 2010). Whilst fragmentation already hinders the animals from retreating to more suitable habitats (Kremen *et al.* 2008), ongoing degradation and the loss of habitat structure and heterogeneity in southern Madagascar will amplify this effect (Harper *et al.* 2007, Sears and Angilletta 2015). Although local extinction is most likely to occur, *O. saxicola* might have the capacity to compensate physiologically, but this might only become apparent after behavioural mechanisms are exhausted.

Conclusion

Oplurus saxicola is a lizard with efficient thermoregulatory behaviour, even in an environment with a challenging thermal scope. The ability to maintain a stable T_b by compensating behaviourally for most thermal fluctuations is important to persist in changing environments, be it seasonal or anthropogenic changes. Physiological plasticity was not observed, but this does not necessarily exclude the capacity. However, as long as behaviour is sufficient to cope with different conditions, costs for physiological acclimatization are dispensable (Gunderson and Stillman 2015).

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Chapter 2

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Author contribution

I hereby confirm that Ole Theisinger conceived, designed and performed the experiments, analysed the data and wrote the paper.

Hamburg,



Prof. Dr. Kathrin Dausmann

Chapter 3

Ecological constraints in the thermal biology of heliothermic lizards

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Abstract

Reptiles must attain a body temperature that facilitates physiological processes and body functions, with different functions sometimes having different thermal optima. At the same time, ecological factors have constraining effects on the attainable body temperature. Besides food quantity, competition and predation, foraging significantly affects the thermal niche of lizards because it often claims a vast majority of an animal's daily activity time. We studied the effect of foraging mode on the thermal ecology of two sympatric Malagasy lizard species, a sit-and-wait forager (*Oplurus quadrimaculatus*) and an active forager (*Zonosaurus laticaudatus*), in their natural environment where both species experience the same thermal conditions. Using attached temperature loggers, we measured skin temperature (T_{skin}) over the course of several days and analyzed mean T_{skin} and T_{skin} pattern. The sit-and-wait forager maintains constantly high T_{skin} (37.5 ± 1.2 °C) throughout the day. This probably promotes performance to catch flying prey and, at the same time, satisfies physiological demands. The active forager shows a split pattern with elevated T_{skin} in the morning (33.6 ± 1.3 °C) followed by lower T_{skin} (31.7 ± 2.0 °C) during foraging in leaf litter and crevices. This shows that foraging performance is sufficient at low temperature but elevated temperature is nonetheless necessary to promote other physiological body functions. Our field study shows that ecological life-history traits, such as foraging strategy, can have a significant effect on the thermal niche of lizards.

Keywords

Foraging strategy, Madagascar, temperature pattern, tropical reptiles

Introduction

Many lizards are able to thermoregulate behaviourally and select a specific body temperature (T_b) that is optimal for physiological processes (Huey and Bennett 1987; Angilletta 2009; Gvozdik 2012). However, T_b selection is constrained by several factors. First of all, the thermal environment determines the range of theoretically attainable T_b in a given habitat (operative environmental temperature (T_e), Huey and Kingsolver 1989). The accessibility of microclimates and the thermal heterogeneity of the habitat furthermore influences the thermoregulatory performance and the costs for thermoregulation (Blouin-Demers and Weatherhead 2002; Sears and Angilletta 2015; Sears *et al.* 2016).

In addition to these environmental parameters, free-ranging animals are also constrained by multiple ecological factors (Kearney *et al.* 2009; Angilletta 2009). Although high thermal heterogeneity reduces the costs for thermoregulation, free-ranging animals are additionally constrained by multiple ecological factors (Kearney *et al.* 2009; Angilletta 2009). These include, for example, food quantity (Gatten 1974; Brown and Griffin 2005; Gienger *et al.* 2013), spacial structure of the habitat (Stellatelli *et al.* 2013; Sears and Angilletta 2015), predation pressure (Herczeg *et al.* 2008) and bacterial infection (Bernheim *et al.* 1978). A strong correlation has also been found between the thermal ecology of lizards and their foraging strategy. Pianka (1966) described two foraging modes in lizards, the sit-and-wait forager and the active forager but discussions are ongoing whether these are only extreme forms of a continuum (Perry 1999; Cooper 2005). Sit-and-wait foragers are generally active at lower T_b , while an active foraging strategy is associated with higher T_b to promote locomotion performance (Verwaijen and Van Damme 2007). Differing foraging strategies can thus result in a spatial and temporal niche separation, which then leads to differing thermal niches (Murray *et al.* 2016).

Finally, ecological limitations arise from interspecific competition as sympatric species are expected to show some degree of resource partitioning as a basic principle for coexistence (Basset 1995; Downes and Shine 1998). Extreme examples for temporal

niche separation are nocturnal geckos in comparison with their diurnal relatives. Nocturnality permits access to new food sources and reduces predation pressure but trade-offs in thermal physiology are inevitable. Both, nocturnal and diurnal geckos show similar thermal optima for sprint performance in experiments, but nocturnal geckos experience significantly lower T_b in the field during activity and thus suboptimal performance (Autumn *et al.* 1994; Huey *et al.* 1989). However, periods of high T_b seem to be a physiological necessity to promote temperature-sensitive physiological processes, such as digestion (Angilletta *et al.* 2002), which nocturnal geckos achieve through ‘protected basking’ (i.e. basking under tree bark to avoid predation, with indirect solar heat gain) during daytime (Huey *et al.* 1989).

Oplurus quadrimaculatus and *Zonosaurus laticaudatus* are two Malagasy lizards with overlapping distribution ranges in southeastern Madagascar, where they occur syntopically on rocky plateaus throughout a variety of habitats. Both species are diurnal and active year-round with a similar body mass but they differ in their foraging ecology and preferred prey items. Our aim was to assess whether foraging requirements and activity patterns affect the thermal niche and contribute to the syntopic existence of these lizards. Using external temperature loggers, we investigated the daily skin temperature (T_{skin}) pattern (as proxy for T_b ; Berg *et al.* 2015) of both species under natural conditions to examine possible differences in activity T_{skin} which would indicate a constraining effect of foraging ecology.

Methods

Study site

Our study site was located in the Andohahela National Park (24°57' S, 46°35' E) on the western slope of the Anosy Mountains in southeast Madagascar. Ambient temperature shows high daily fluctuations from 18 °C to 45 °C during the rainy season and 12 °C to 38 °C during the dry season (Rakotondranary *et al.* 2011). We studied lizards from the hot spiny forest with its scant and xerophile vegetation, from the moderately tempered gallery forest along rivers that consists of large, evergreen tamarinds and mango trees and from the similarly tempered transitional forest that connects spiny forest and evergreen rain forest.

Study species

We studied T_{skin} profiles of two heliothermic lizard species that occur in different but overlapping geographic ranges and that show different foraging strategies. *Oplurus quadrimaculatus* (BM \pm SD: 76.5 g \pm 10.5 g; SVL \pm SD: 12.8 cm \pm 0.6 cm; N = 310; pers. obs.) is a sit-and-wait forager, feeding on flying insects. It is obligate saxicolous, occurring in open, rocky habitats. It is mainly distributed in the hot and dry southern part of Madagascar with sometimes high abundances. *Zonosaurus laticaudatus* (BM \pm SD: 113.5 g \pm 15.9 g; SVL \pm SD: 15.3 cm \pm 0.78 cm; N = 191; pers. obs.) is rather opportunistic concerning its diet and has been described as generalist. It is an active forager, showing a high flexibility in its diet including insects as well as frogs (Glos 2004), fruits, flowers (Urbani and Bels 1994), small lizards, snails, cockroaches, millipedes and carrion (pers. obs.). *Zonosaurus laticaudatus* has a broad geographic distribution across the western portion of the island and in the southeast where it overlaps with the occurrence of *O. quadrimaculatus* (Glaw and Vences 2007). It can be found on open rocks but it searches for prey in leaf litter and crevices. Both species are diurnal and have been observed to be active from sunrise until sunset. Especially in the morning, they can often be encountered together, basking on the same rock (Fig. 3.1).



Figure 3.1: An adult *Oplurus quadrimaculatus* (top) and a subadult *Zonosaurus laticaudatus* are sharing a basking spot on a rock in southeast Madagascar.

Operative environmental temperature

We used specifically designed and coloured copper models that were equipped with temperature loggers (Thermochron iButton model DS1921G; resolution ± 0.5 °C; Maxim Integrated Products Inc., San Jose, California, USA) to measure T_e (Dzialowski 2005). Temperature loggers were calibrated using a water bath with a gradual temperature increase from 15 °C to 45 °C. To verify that our models mirror the lizards' T_b , we compared the core temperature of the copper models to T_b and T_{skin} of live lizards under semi natural conditions in a pre-study. The temperature loggers recorded the core temperature of the models in 5-min-intervals and the lizards' T_b was measured simultaneously by both, rectally inserted thermocouples (Greisinger GTH 175-PT; resolution 0.1 °C; GREISINGER electronic GmbH, Regenstauf, Germany) and attached temperature loggers. We placed the corresponding copper models and the lizards in a transparent plastic box. Both lizards and copper models were handled every five minutes. By shuttling the boxes with animals and models between sun and shade, we were able to compare the cooling and heating rates using regression analysis. Core temperature of the models was highly correlated with T_b and T_{skin} of the lizards (*Z. laticaudatus*: Pearson correlation coefficient = 0.918; $p < 0.001$; *O. quadrimaculatus*: Pearson correlation coefficient = 0.962; $p < 0.001$) with no differences between paired measurements (*Z. laticaudatus*: $t(63) = 1.477$; $p = 0.145$; *O. quadrimaculatus*: $t(33) = 0.857$; $p = 0.397$). We placed three models per species for six consecutive days with comparable weather conditions (cloudless, sunny and no wind) in a crevice, shade and full sun to obtain a representative T_e range, again with loggers recording temperature every five minutes.

Temperature profiles

Calibration tests by Berg *et al.* (2015) showed that T_{skin} can be used as a proxy for T_b in small-sized lizards and specifically in our study species. To record T_{skin} over the course of the day, we noosed adult lizards with a minimum body mass of 70 g for *O. quadrimaculatus* ($n = 48$) and 90 g for *Z. laticaudatus* ($n = 25$) and attached temperature loggers to their backs using superglue (UHU Sekundenkleber, UHU GmbH, Bühl, Germany). The weight of the temperature loggers was 3.3 g and thus did not exceed the recommended 5 % of the lizards' body weight (Lovegrove 2009). We then released the individuals at the original place of capture within three minutes. After two to seven days we recaptured the lizards and removed the devices. In cases where the temperature logger

had fallen off the animal before recapture, we checked the data for distinct temperature patterns that indicate the time of loss and only used data before the logger had dropped off (Fig. S1). If no clear time of loss could be identified, the complete data was dismissed. We also recorded ambient temperature with temperature loggers (Thermochron iButton model DS1921G) that were attached to a tree in 1 m height as reference for sudden weather changes because activity patterns change significantly with changing weather conditions (Sun *et al.* 2001). For comparable and maximal activity of the lizards, we only used data from cloudless sunny days for the analysis. Activity T_{skin} was defined as the time span from initial basking in the morning after exiting the crevice until retreat into the crevice in the evening. For the comparison of daily activity time (averaged across days for each individual) and the mean activity T_{skin} , we used a two sample t-test.

Results

Operative environmental temperature

We found no differences between the species in mean $T_{\text{e min}}$ (*O. quadrimaculatus*: 23.1 ± 2.1 °C; *Z. laticaudatus*: 22.6 ± 2.5 °C), mean $T_{\text{e max}}$ (*O. quadrimaculatus*: 40.7 ± 11.3 °C; *Z. laticaudatus*: 41.2 ± 12.3 °C) and the mean daily amplitude of T_{e} (*O. quadrimaculatus*: 17.6 ± 10.0 °C; *Z. laticaudatus*: 18.5 ± 10.7 °C) (Fig. 3.2). Hence, despite differences in body size, both lizard species experience similar thermal conditions in the same habitat.

Temperature profiles and behaviour

Both species showed distinct patterns in their daily activity T_{skin} profiles (Fig. 3). *Oplurus quadrimaculatus* maintained a precisely regulated mean activity T_{skin} of 37.5 ± 1.2 °C ($n = 48$) with only little fluctuations. The lizards exploited the daily time period that is available for activity at constantly high T_{b} . They became active with the first sunlight in the morning and instantly basked in the sun. For the major portion of the day, *O. quadrimaculatus* remained motionless but alerted on a rock. This motionlessness was only interrupted by occasional shuttling between microhabitats and irregular feeding attempts (quick targeted bursts), which occurred whenever flying insects came close to the lizard. At sunset, these lizards sat directly in front of their crevices and retreated when ambient temperature dropped.

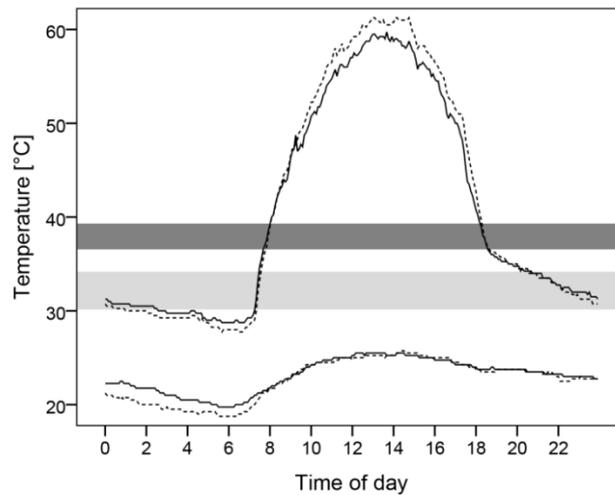


Figure 3.2: Operative environmental temperature (T_e) ranges of *Oplurus quadrimaculatus* (solid line) and *Zonosaurus laticaudatus* (dashed line) over the course of the day. Upper and lower lines show maximum T_e and minimum T_e . Grey bars indicate the central 50% of the activity skin temperature range (dark grey: *O. quadrimaculatus*; light grey: *Z. laticaudatus*).

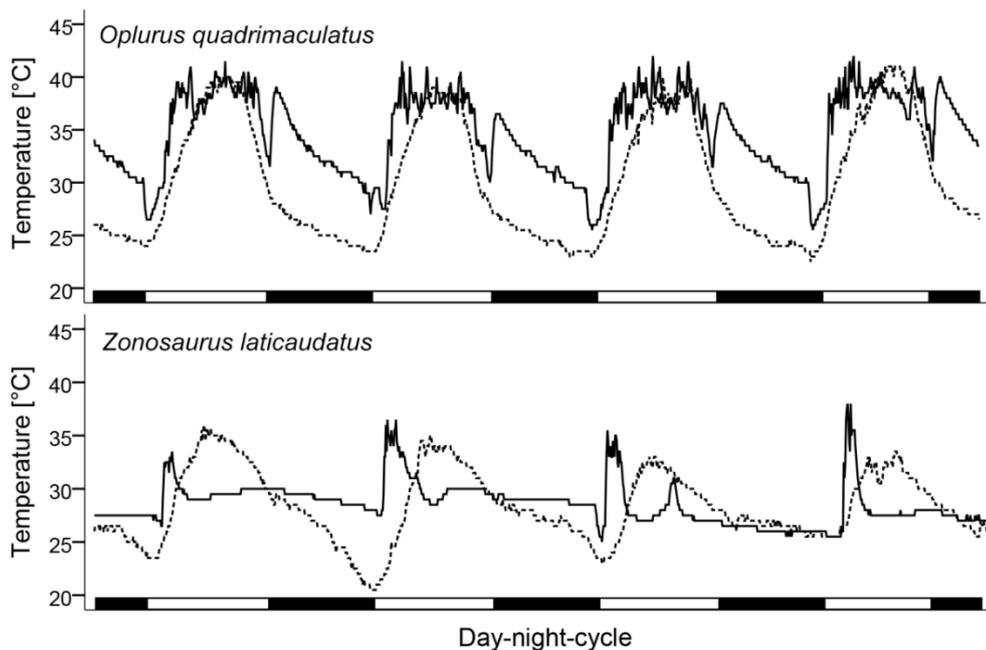


Figure 3.3: Typical skin temperature profiles (solid line) of A) *Oplurus quadrimaculatus* and B) *Zonosaurus laticaudatus* and ambient temperature (dashed line) on sunny, cloudless days with a broad operative environmental temperature range. Both species leave their warm crevices after sunrise and cool down with ambient temperature before they heat up through basking in the sun. *Oplurus quadrimaculatus* is active at high skin temperature until it cools down with ambient temperature at sunset and reheats when entering the warm crevice. *Zonosaurus laticaudatus* is active in shady leaf litter and crevices. It cools down directly after a short period of basking in the morning.

Zonosaurus laticaudatus showed significantly lower activity T_{skin} (32.2 ± 1.9 °C, $n = 25$; $t_{71} = 23.800$; $p < 0.001$). The lizard showed a divided daily T_{skin} pattern with elevated T_{skin} in the morning until 0900 hours (33.6 ± 1.3 °C) and lower T_{skin} (31.7 ± 2.0 °C) thereafter (Fig. 4). These lizards basked on open rocks in the morning, often directly next to *O. quadrimaculatus*, with some shuttling behaviour between sun and shade for approximately two hours. After basking, the lizards were actively foraging on open rocks and in crevices, showing a sniffing-like behaviour. With increasing ambient temperature, the animals continued foraging in the bordering forest where they were observed chewing on snails, small lizards, cockroaches and millipedes. Faecal samples even revealed tenrec spikes which were most likely consumed as carrion. *Zonosaurus laticaudatus* was active until sunset and individuals usually retreated to the same crevice that was used the night before.

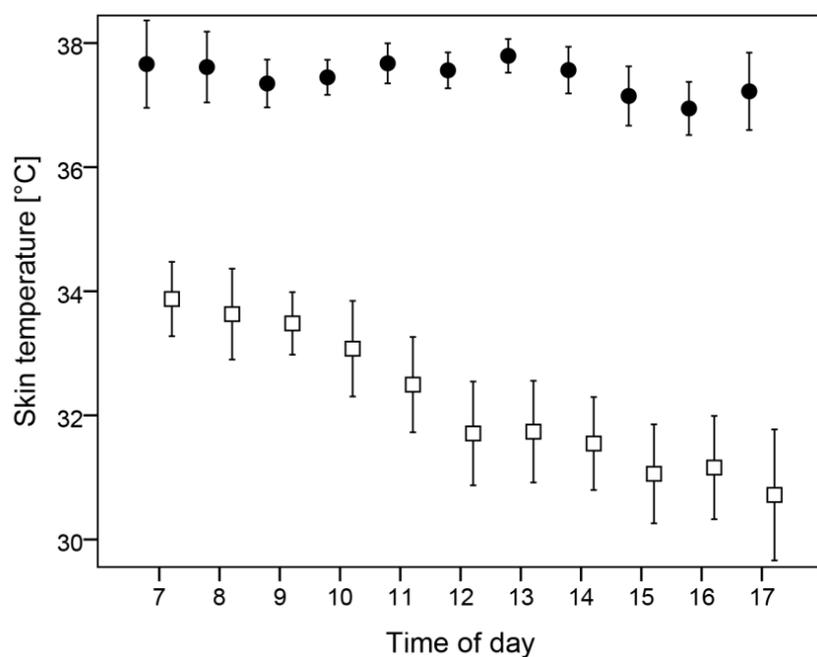


Figure 3.4: Mean skin temperature of *Oplurus quadrimaculatus* ($n = 48$; filled circles) and *Zonosaurus laticaudatus* ($n = 25$; open squares) over the course of the day.

The activity T_{skin} range experienced by *Z. laticaudatus* was broader (central 50% between 30 °C and 34 °C) and the frequency distribution curve of activity T_{skin} showed positive skewness, whereas activity T_{skin} of *O. quadrimaculatus* showed a narrower range (central 50% between 36 °C and 39 °C) and was equally distributed (Fig. 5). T_{skin} during the night (between 1800 hours and 0600 hours) did not differ between the species (*O.*

quadrifasciatus: 28.0 ± 3.1 °C, $n = 48$; *Z. laticaudatus*: 27.0 ± 1.2 °C, $n = 25$; $t_{71} = 1.625$; $p = 0.109$).

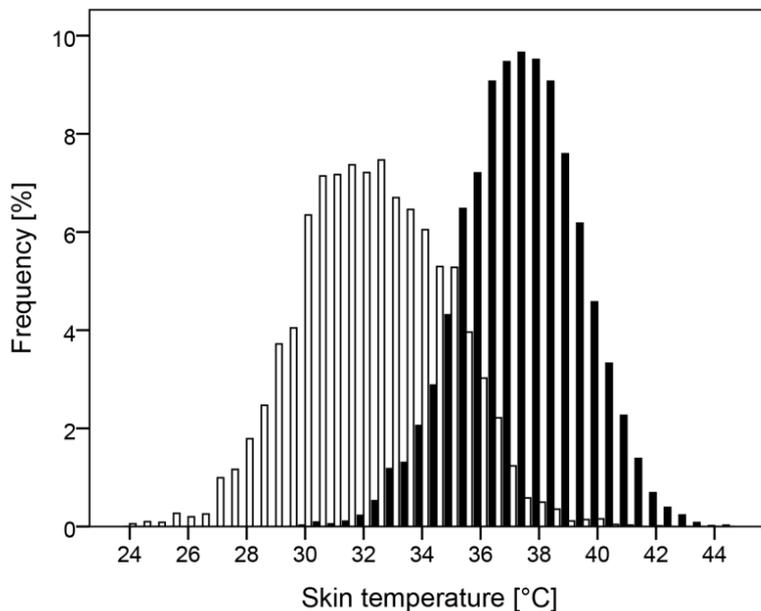


Figure 3.5: Histogram of the relative frequency of activity skin temperature for *Oplurus quadrifasciatus* (black bars) and *Zonosaurus laticaudatus* (white bars).

Discussion

Our field study on the thermal ecology of two sympatric lizards reveals different but distinct T_{skin} patterns for each species. Since T_e is similar for both species, these differences are not induced by constraints from the thermal environment. Instead, we propose that differences in foraging strategy and microhabitat use lead to differing thermal niches (Blouin-Demers and Weatherhead 2002; Murray *et al.* 2016).

Oplurus quadrifasciatus maintains high T_{skin} throughout the day. This correlates with a sun exposed sit-and-wait foraging strategy, which forces this lizard to cope with high environmental temperatures. However, the consistency across individuals and their precision in thermoregulatory behaviour indicate that this high T_{skin} is indeed selected by the lizards. This assumption is further supported by the heterogeneity of the habitat with plenty of shady microsites and the shuttling of lizards between sun and shade whilst waiting for prey. The high activity T_{skin} of *O. quadrifasciatus* corresponds to thermal preferences in other species of the same genera. The sympatric and likewise saxicolous

lizard *O. saxicola* attains a mean field T_b of 37.6 °C (Theisinger *et al.* in review) and the arboreal species *O. cuiveri* and *O. cyclurus* show mean field T_b of 36.2 °C and 38.5 °C, respectively (Randriamahazo 1998). This corroborates the observation that thermal preferences are rather conservative within taxonomic families, and even more within genera, due to phylogenetic inertia (Clusella-Trullas and Chown 2014).

A great advantage of high T_b is that it permits a high level of physiological performance, as biochemical processes strongly depend on temperature (Angilletta 2009; Angilletta *et al.* 2010). The prey of *O. quadrimaculatus* mainly consists of flying insects that require quick reactions. Hence, high T_b is particularly beneficial in this species. Assuming that locomotion, i.e. sprint speed and endurance, is less sensitive to T_b than other body functions (Angilletta *et al.*, 2002), it might be senses like vision, hearing (Huey and Kingsolver 1989) and nerve conduction velocity (Rosenberg 1978) that must be kept on an optimal level to allow for quick and precise bursts.

Oplurus quadrimaculatus indeed shows an overall high thermal sensitivity because changes in T_{skin} lead to immediate changes in behaviour. At a T_{skin} of approximately 36 °C, which is less than two degrees below their mean activity T_{skin} , we observed that the lizards' escape responses changed significantly. The flight distance of the lizards increased, they stayed closer to their crevices and showed higher alertness. At temperatures below this threshold, the animals retreated into their crevices at the slightest disturbance. Temperature-dependent changes in behaviour are not unusual in reptiles. Similar responses have been observed in the keeled earless lizard *Holbrookia propinqua* (Cooper 2000) and changes to rather aggressive behaviour at cooler T_b have been reported in the agamid lizard *Trapelus pallida* (Herrel *et al.* 2007). In *O. quadrimaculatus*, this change in behaviour could indicate that reaction time is not sufficient at lower temperatures, necessitating an increased safety zone and early escape behaviour to compensate the lack of performance.

Overall, the preference for high T_b in *O. quadrimaculatus* supports activity in open habitats and this may be particularly beneficial for its future distribution. As the predicted climate warming is often accompanied by ongoing degradation (Hannah 2008; Tadross *et al.* 2008), this thermophile lizard may even profit from enlarged distribution possibilities.

Zonosaurus laticaudatus shows a very different but also distinct T_{skin} pattern over the course of the day. After initial basking, the lizard maintains an elevated T_{skin} for approximately two hours in the morning followed by active foraging activity at lower T_{skin} . We observed this pattern repeatedly in almost all individuals across different habitats. This lizard searches for prey in shady leaf litter and crevices which provide only low operative temperatures. While it is not certain that the elevated T_{skin} in the morning reflects the animals' thermal preference, the lower T_{skin} thereafter is most likely to be suboptimal for physiological functions. It is, nevertheless, sufficient for foraging. This indicates that *Z. laticaudatus* either forages with suboptimal locomotory function or that the performance breadth for locomotion is broad enough to ensure high performance even at low temperature.

However, other physiological processes are more sensitive to T_b (Sinclair *et al.* 2016). The thermal sensitivity of digestion, for example, has been experimentally shown to be higher than the thermal sensitivity for sprint speed and endurance in the eastern fence lizard *Sceloporus undulatus* (Angilletta *et al.* 2002). This sensitivity is also reflected in higher selected T_b after feeding or lower selected T_b after food deprivation in most reptile species (e.g. *Pseudemys scripta* and *Terrapene ornate* in Gatten 1974; *Anolis carolinensis* in Brown and Griffin 2005; *Heloderma suspectum* in Gienger *et al.* 2013; but see *Sceloporus jarrovi* in Schuler *et al.* 2011). The high T_{skin} of *Z. laticaudatus* in the morning may therefore promote rather sensitive body functions. In addition to the acceleration of digestion and nutrient assimilation, higher T_b facilitates immune responses (Bernheim *et al.* 1978) and accelerates detoxification (Talent 2005). The latter would be of particular importance for *Z. laticaudatus*, as some of its prey items, such as millipedes and carrion, are known to contain indigestive and toxic substances (Janzen 1977; Eisner *et al.* 1978).

We assume that basking takes place in the morning before feeding because the lizards achieve a T_{skin} that promotes thermally sensitive physiological processes and facilitates foraging activity whilst crevice and leaf litter temperature is still low. Moreover, the probability of cloud cover increases over the course of the day (pers. obs.). If elevated T_b is essential to enable digestion, nutrient assimilation and other physiological processes, the chance for the lizards to attain high T_{skin} is higher in the morning than after feeding in

the afternoon. Although the T_{skin} patterns in this analysis were solely from sunny days and T_e would allow reheating to the initial T_b later in the day, we observed this only rarely.

Similarly to *Z. laticaudatus*, the Middle American lizard *Ameiva festiva* forages in the forest but also basks at clearings and forest edges. This lizard constantly shuttles between forest and clearing (foraging and basking) in a 20-min-cycle to achieve higher T_b for foraging (Van Berkum *et al.* 1986). However, the daily T_{skin} pattern of *Z. laticaudatus* demonstrates that the trade-off between physiological demands (high T_b , e.g. for digestion) and ecological constraints (foraging in cooler microclimates) does not necessarily result in constant shuttling or an intermediate T_{skin} but can also be met through a clear temporal division of T_{skin} over the course of the day. The major advantage of this strategy is increased time for foraging as time-consuming and predation-prone shuttling can be omitted and food sources away from basking sites can be exploited. However, we are unaware of a similar daily temporal split of activity T_b in other species with no thermal restrictions from the environment.

Based on the distinct temporal split between high T_{skin} in the morning and low T_{skin} thereafter, one would expect a bimodal distribution in the T_{skin} frequencies for *Z. laticaudatus*. This is not the case because the lizards' T_{skin} during foraging is not actively regulated but rather depends on particular microhabitat conditions. Hence, T_b varies during activity and might overlap with the initial heating period. However, the positive skewness in the frequency distribution of *Z. laticaudatus* still indicates this partial thermoconformity. If a lizard thermoregulates precisely throughout the day, the T_{skin} frequency is narrow and evenly distributed as in *O. quadrimaculatus*.

Zonosaurus laticaudatus is well adapted to shade based activity, and cloudy and rainy days probably do not affect its foraging activity to a great extent. In contrast, *O. quadrimaculatus* relies solely on solar radiation to attain T_b that allow for optimal foraging performance. Cloudy and rainy days severely constrain the activity in this species and these weather conditions are more frequent during the rainy season in the austral summer. Hence, although it is the warmer season, the rainy season would constrain activity more than the cooler but sunnier dry season.

In summary, different foraging strategies in *O. quadrimaculatus* and *Z. laticaudatus* entail differing demands on locomotion performance and habitat use. The resulting differences

in their thermal niches reflect extensive resource partitioning between these lizard species and facilitate their syntopic occurrence. Our study species, which exhibit almost identical thermal conditions from the environment, show significantly different but distinct daily patterns in T_{skin} . While the sit-and-wait forager behaviourally controls its T_{skin} precisely throughout the day, the active forager faces a thermal dilemma to satisfy either physiological or ecological demands. Instead of facing the trade-off by operating at intermediate T_b or through frequent shuttling, this lizard shows a clear temporal division of activities and daily T_{skin} pattern. This has so far been unknown for a lizard with basically no T_e restrictions. Similar ecological constraints might remain undetected under laboratory conditions (Wall and Shine 2008) but they are crucial to understand the biogeographic distribution, thermal requirements and potential threats of lizards. We therefore emphasize the need to integrate ecological field observations into physiological studies to draw a complete picture on physiological demands and restrictions.

Acknowledgements

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Supplementary material

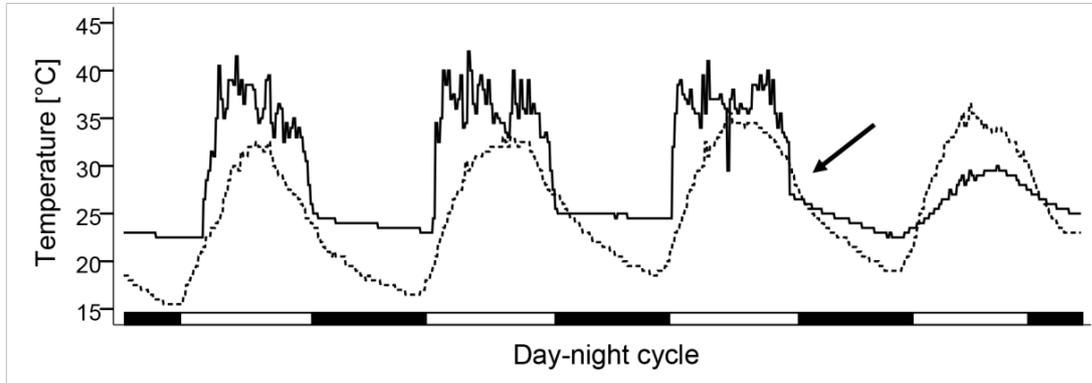


Figure S3.1: Skin temperature profile (solid line) of *Oplurus quadrimaculatus*. The dashed line shows ambient temperature. The arrow indicates the clearly discernible moment when the temperature logger detached from the lizard.

Author contribution

I hereby confirm that Ole Theisinger conceived, designed and performed the experiments, analysed the data and wrote the paper.

Hamburg,

Two blue ink signatures are shown side-by-side. The signature on the left is a cursive 'Ole' with a looped end. The signature on the right is a more complex cursive signature, likely 'Theisinger', with multiple loops and a long horizontal tail. Both signatures are written in blue ink on a light background.

Prof. Dr. Kathrin Dausmann

Discussion

This thesis investigates thermal and ecological constraints in Malagasy lizards and how these influence the animals' potential to compensate environmental variation across habitats. This work combines field observations with physiological measurements to elucidate the behaviour-physiology nexus and the interplay between physiological demands and ecological constraints (Basson and Clusella-Trullas 2015).

Thermal conditions at the study site

Many lizard species occurring on the western flank of the Anosy Mountains in southeastern Madagascar have to cope with strikingly different conditions along one of the steepest environmental gradients in the world from hot and dry spiny forest to cool and humid rain forest (Goodman 1999). The spiny forest is the hottest study site with scant vegetation and little structural heterogeneity, which also leads to a higher T_e amplitude between day and night temperatures. The T_e range is broad and allows for the longest activity period along the gradient but high temperatures during midday are challenging and increase locomotion costs for shuttling. Unless lizards forage in the shade and in crevices, as seen in *Z. laticaudatus* (Chap. 3), they must retreat to avoid overheating, as shown in the scan sampling of *O. saxicola* (Chap. 2). The gallery forest is moderately tempered with higher humidity, due to its proximity to the river. This buffers daily fluctuations in T_e and leads to a T_e amplitude that is slightly lower than in the spiny forest but sufficient for activity. The habitat structure is rather heterogeneous with large trees, sun exposed spots, bushes and driftwood accumulations. Here, the prerequisite for thermoregulation during midday is better as a variety of microclimates is available, which might reduce associated locomotion costs (Sears *et al.* 2016). However, cooler T_e constrain the potential activity time in the morning and in late afternoon. Conditions in the transitional forest are comparable with the gallery forest. Lower T_e in the morning and in the afternoon constrain the activity of lizards rather than high T_e during midday (Fig. 1). The most striking constraints are found in the rain forest. The activity frame, as provided by the T_e range, is significantly shorter than in all other habitats and the daily T_e amplitude is lower (Chap. 1). In addition, days with cloud cover occur more frequently, which amplifies constraints in potential activity time (Sun *et al.* 2001). The environmental

Discussion

conditions along the gradient thus differ markedly between habitats. T_e is not just lower in the rain forest but the available time window for activity is shorter.

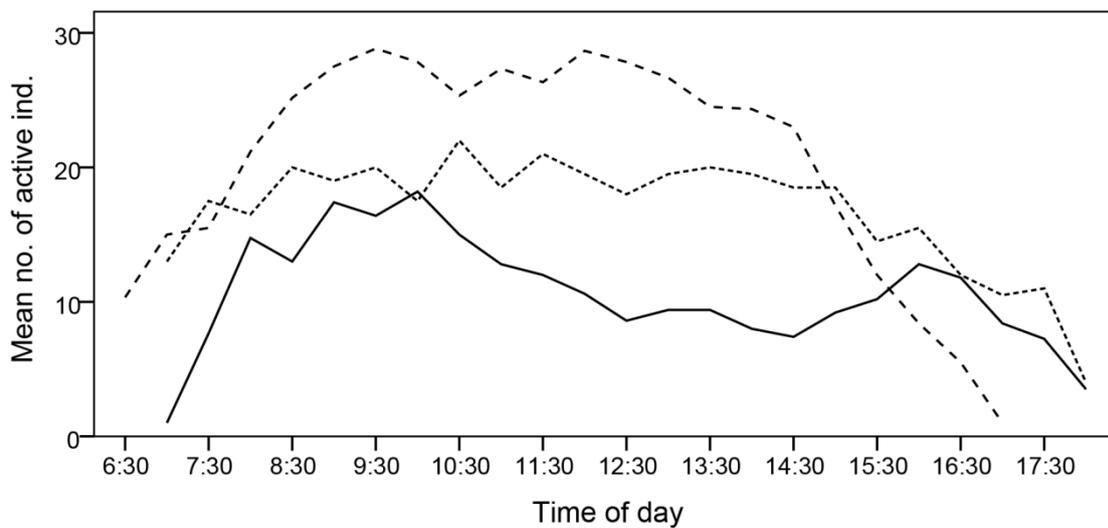


Figure 4.1: Mean number of active individuals of *Oplurus saxicola* over the course of the day in different habitats. Solid line: spiny forest; dashed line: gallery forest; dotted line: transitional forest.

Precision and accuracy of thermoregulation in Oplurus spp.

Chapters one and two concern the constraining effect of different thermal environments on the activity time and activity T_{skin} of *O. quadrimaculatus* and *O. saxicola* and physiological mechanisms that might contribute to thermal compensation.

Both species show a high accuracy in behavioural thermoregulation but the precision is higher in *O. quadrimaculatus*. *Oplurus saxicola* shows a slight bell-curved progression of T_{skin} over the course of the day with higher values at midday, whilst T_{skin} of *O. quadrimaculatus* is rather precisely regulated. Our study species compensate environmental differences solely through adjusted shuttling behaviour and changes in body posture, which are generally considered to be the main thermoregulatory mechanisms in heliothermic lizards (Van Berkum *et al.* 1986; McConnachie *et al.* 2009; Sears and Angilletta 2015; Sears *et al.* 2016). These lizards are able to cope with environmental conditions all along this natural gradient (except that *O. saxicola* does not occur in the rain forest). Our study species are active from sunrise, when they initially bask in the sun, until sunset, when they retreat. The precision in thermoregulation of *O. quadrimaculatus* and *O. saxicola* is not unusual for lizards. Especially heliothermic species from arid regions are able to maintain a very stable T_b throughout the day,

although hours of restriction during midday may occur (Huey *et al.* 2010; Kearney 2013; Sinervo *et al.* 2010). The desert lizard *Sceloporus merriami*, for example, is able to maintain a stable field T_b of 32.2 ± 2.1 °C with little fluctuation (Grant and Dunham 1988) but only if T_e does not constrain thermoregulation (Grant 1990). The African striped skink *Trachylepis striata* holds a T_b of 33.9 ± 1.9 °C but precision degrades with adverse weather conditions (Patterson 1992).

Coexistence of Oplurus spp.: similarities and differences

The mean activity T_{skin} is similar in both species (*O. quadrimaculatus*: 37.5 ± 0.8 °C, $n = 51$; *O. saxicola*: 37.7 ± 2.1 °C, $n = 110$). These similar thermal preferences are likely to be based on four factors: (1) Both species have a similar body shape (flattened body, flat and broad head and long legs and digits for a saxicolous lifestyle). (2) *Oplurus quadrimaculatus* and *O. saxicola* are closely related. It has been shown that thermal preferences and thermal tolerances are rather conservative within taxonomic families, and even more within genera, due to phylogenetic inertia (Clusella-Trullas and Chown 2014). This conservatism among related species has also been shown in a global analysis of the thermal tolerance breadth of lizards in combination with evolutionary history (Grigg and Buckley 2013). (3) These species occur syntopically in Andohahela and have extensive overlaps in their geographic distribution range and thus they have to cope with similar environmental conditions (Glaw and Vences 2007). (4) Both are similar in their ecology; they are obligate saxicolous, highly philopatric and their foraging strategy is similar (sit-and-wait forager) with a focus on flying prey.

However, the precision in thermoregulation over the course of the day differs between these species and the main factor that may contribute to this difference is different body masses. It is more challenging for a 20g-lizard to regulate precisely than for an 80g-lizard because heat conduction is faster in smaller animals (Stevenson 1985). The body mass of *O. quadrimaculatus* is almost four times higher, which leads to slower heat loss and less costly thermoregulatory behaviour (McNab and Auffenberg 1976). This difference might also be crucial for the basic principle for coexistence because it leads to a specialization on differently sized prey and hence partitioning of food resources (Basset 1995). Additionally, *O. quadrimaculatus* is more resistant to cooler temperature. While suitable habitats in the spiny, gallery and transitional forest provide optimal conditions for both species, where they are always encountered together, *O. saxicola* is absent in the

rainforest (Theisinger 2009). The habitat surface in the rainforest is similar to the other habitats (large rocks, open areas for basking, numerous crevices for retreat), so we assume that either T_e is too low, with a low thermal heterogeneity in the habitat (i.e. few sunny spots for basking) which leads to an unsustainably short activity time (Sears *et al.* 2016), or there is a lack of suitable prey species for *O. saxicola*. This species was observed to mainly feed on small, flying insects and particularly sweat bees (Chap. 3) but this prey is scarce in cooler habitats (personal obs.). Although *O. quadrimaculatus* is also specialized on flying prey, its larger body size allows for catching larger prey species and promotes slower heat exchange which might reduce costs for thermoregulation in this habitat. Hence, *O. quadrimaculatus* is able to cope with cooler habitat conditions that are unsuitable for *O. saxicola*.

Thermal restrictions and energy budgets

Reduced activity time, no matter for which kind of activity, is the first constraint that lizards are facing when compensatory mechanisms fail (Angilletta 2009; Sinervo *et al.* 2010). It should, however, be noted that low temperature can similarly constrain an animal's activity time as high temperature (Huey and Stevenson 1979; Huey *et al.* 2010). Case studies about behavioural adjustments across altitudinal gradients show that potential activity time decreases with increasing altitude and decreasing temperature (*Zootoca vivipara* in Gvozdik 2002; *Sceloporus graciosus* in Sears 2005). On the other hand, a comparative study across Mexico about the extinction risk of *Scleroporos* lizards shows that high daily maximum T_a increases hours of restriction and leads to local extinction (Sinervo *et al.* 2010). In chapter one, restrictions in daily activity time induced by low temperature are shown for *O. quadrimaculatus* across the environmental gradient. In the cool rainforest habitat, which at the same time marks this species' distribution limit, activity time is reduced by 35% compared to activity time that is available in the hot spiny forest. Simultaneously, these lizards save a significant amount of field resting costs (28%), which is caused by a shorter activity time and lower T_{skin} during inactivity during the day and thus reduced field RMR. In contrast, heat-induced hours of restriction and higher night temperatures have a more severe effect on lizards' energy budgets (Christian *et al.* 1996). Even if activity time is reduced in the same manner, lizards would have a significantly higher energy expenditure. The lizards face a higher shade and crevice temperature and higher T_{skin} which passively increases energetic costs through a

higher MR. For this reason, Kearney (2013) identified increasing shade temperature as the driving factor for recent extinctions rather than increasing hours of restriction and maximum T_a (Sinervo *et al.* 2010). It is a truism that lizards with higher T_{skin} have a higher energy expenditure. Despite the reduced costs for locomotion during hours of restriction, high T_a therefore has a more severe effect on the energy budget than constraints through lower environmental temperature. Although it is a common view that daily maximum T_a has the most striking effect on the biology of lizards because it directly constraints potential activity time (Sinervo *et al.* 2010), it is reasonable to assume that times of inactivity, when animals are passively exposed to higher temperature, can affect the energy budgets of the lizards in a more severe way and much earlier, even before activity time is affected.

The value of a shift in T_{pref}

A possible mechanism to extend the activity time and to balance energy budgets are physiological adjustments accompanied by a plastic shift in T_b selection (Gvozdik 2012; Hadamova and Gvozdik 2011). The adjustment of the thermal reaction norm of physiological processes and thus a shift of T_{opt} facilitates a similar performance at higher or lower T_b (Angilletta 2009; Little and Seebacher 2016). However, differences in activity T_b do not necessarily reflect a physiological shift of T_{opt} because activity T_b can also be affected by multiple ecological factors. Higher predation pressure, for example, leads to differing microhabitat selection activity patterns and reduced accuracy in thermoregulation, as shown in the European common lizard *Zootoca vivipara* (Herczeg *et al.* 2008). Nevertheless, as shown in the comparison of activity T_b (and activity T_{skin}) of *O. quadrimaculatus* and *O. saxicola* in chapters one and two, we did not observe shifts in activity T_b and both species thermoregulate with high precision. This indicates a similar temperature set-point across habitats and thus no differences in ecological constraints as well as no adjustment in the thermal reaction norm.

Physiological plasticity is a costly process as it requires changes in enzymatic reactions. It can take from days up to several weeks until physiological changes are measurable (Seebacher 2005). Hence, the benefits from such protracted processes are highest if environmental changes are long-lasting and directed. Nevertheless, the gain of activity time through shifts in T_b selection of *O. quadrimaculatus* and *O. saxicola* would be relatively low. The T_e range is broad but the temperature increase in the morning and

decrease in the afternoon is steep in all habitats. Mean activity T_b would have to decrease by 8 – 10°C to increase the potential activity time by a significant amount. Acclimation experiments on crested newts *Triturus dobrogicus* under extreme temperatures revealed shifts of more than 6°C in T_{pref} (Gvozdik *et al.* 2007) but such magnitude in acclimatization is very unlikely under natural conditions. Usually, acclimation and acclimatization experiments do not provoke shifts in T_{pref} of more than 3°C and mostly between 1°C and 2°C (Christian and Bedford 1995; Blumberg *et al.* 2002; Hadamova and Gvozdik 2011).

Thermal biology of Zonosaurus laticaudatus

Chapter three concerns the interspecific comparison of T_{skin} patterns between sympatric lizards (*O. quadrimaculatus* and *Z. laticaudatus*) and ecological trade-offs that constrain the thermal scope of these species.

In contrast to the highly specialized *O. quadrimaculatus*, *Z. laticaudatus* is opportunistic in all respects. It feeds on a great variety of prey species such as frogs, insects and other invertebrates and also on carrion and fruits with no apparent food preference (Fig. 4.2). Its thermal ecology also reflects this opportunistic behaviour. Whilst these lizards regularly show thermoregulatory basking behaviour in the morning, T_{skin} depends on the surrounding substrate thereafter. Its mean activity T_{skin} is significantly lower than in the *Oplurus* spp.. However, the precise and accurate temperature regulation in the morning during basking, with little fluctuation in T_{skin} , indicates that *Z. laticaudatus* actively selects this T_{skin} for a reason. Many physiological processes show high thermal sensitivity (Angilletta *et al.* 2002) and ectotherms often select higher T_b to promote the performance of these processes. Even nocturnal species select protected but warmer sites during inactivity at daytime despite higher energetic costs through higher T_b (Huey *et al.* 1989a). Hence, there are vital body functions and processes other than foraging activity which must be facilitated through increased T_b .

The large geographic distribution range of *Z. laticaudatus* (Glaw and Vences 2007) could suggest that this species is able to deal with a wide range of climatic conditions because large distribution ranges generally indicate a broad thermal tolerance (Bozinovic 2011). However, the thermal conditions at foraging sites in this species (in crevices and leaf litter) are buffered and climatically more stable than temperature on open rocks. Hence,

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thigmothermy or even thermoconformity during foraging does not necessarily indicate a broad thermal tolerance but rather a low thermal sensitivity in locomotor function (Angilletta *et al.* 2002). The wide range and the negative skew of the frequency distribution of activity T_{skin} in *Z. laticaudatus* also shows that the precision and accuracy in thermoregulation is indeed higher in the *Oplurus* species, which is solely heliothermic.



Figure 4.2: Prey items of *Zonosaurus laticaudatus*. Left: *Z. laticaudatus* is feeding on a hissing cockroach. Right: a millipede partially eaten by *Z. laticaudatus*.

Vulnerability to environmental change of Zonosaurus laticaudatus

Non-thermoregulating lizards are believed to have a higher extinction risk than heliothermic species because their compensatory capacity depends solely on physiological mechanisms (Sinervo *et al.* 2010). Regarding *Z. laticaudatus*, the foraging period would increase its vulnerability to climate warming despite its proven ability for behavioural thermoregulation because it requires certain characteristics regarding its environment. Although this lizard is known to occur in habitats with strong anthropogenic impact (D’Cruze 2006), the habitat should be well-structured with basking opportunities and shady sites for foraging. The high level of degradation in southern Madagascar (Harper *et al.* 2007; Kremen *et al.* 2008) and large areas that are only covered with savannah might be the reason why the small eastern population is not connected with the population from western Madagascar (Glaw and Vences 2007). However, it is not known whether the populations got separated by human activities or by drastic climatic events

that happened frequently in Madagascar's natural history (Wilmé *et al.* 2006; Vences *et al.* 2009).

Metabolic acclimatization

Considering the broad range of T_e and the precision of behavioural thermoregulation in our study species, it seems reasonable to argue that the absence of shifts in activity T_{skin} indicates an absence of shifts in thermal preferences. This would support the hypothesis by Janzen (1967) that tropical ectotherms, in contrast to temperate species, have limited capacity for physiological acclimatization. However, the term “tropical” has often been equalized with a constantly warm climate, which is not always the case, and Madagascar in particular exhibits strong daily and seasonal temperature fluctuations (Dewar and Richard 2007).

Nevertheless, the capacity for physiological compensation in tropical lizards is still given as shown by metabolic adjustments in *O. quadrimaculatus* and *Z. laticaudatus* (Berg *et al.* in review). Whilst *O. quadrimaculatus* shows seasonal acclimatization but no adjustments to different thermal environments, *Z. laticaudatus* shows exactly the contrary pattern with adjustments to the habitat but no seasonal acclimatization. In combination with the daily T_{skin} patterns, these findings lead to three conclusions for the thermal ecology of lizards. First, as long as behavioural adjustments allow for an optimal operating temperature, there is no need for physiological compensation. The activity T_{skin} of our study species is not affected environmental variation because the animals are able to control T_{skin} during hours of unconstrained activity. Hence, behavioural plasticity is indeed the primary mechanism to buffer environmental changes (Chap. 1 and 2). Conversely, physiological acclimatization occurs when these lizards are not able to behaviourally regulate their T_{skin} . Second, physiological acclimatization does not necessarily result in a complete shift of the thermal reaction norm. Instead, it can happen selectively as in *O. quadrimaculatus*, with different extent at differing T_{skin} ranges. This is especially important to avoid overcompensation in unaffected T_{skin} ranges. Third, physiological and behavioural plasticity often work in concert and complement each other (Basson and Clusella-Trullas 2015). The differing thermal ecology of our study species results in a differing interplay between behavioural plasticity and physiological acclimatization. The ecology and habitat use of the animal thus plays a major role for the magnitude and direction of physiological adjustments.

Implications of body temperature on lizards' performance

The thermoregulatory precision and the narrow and evenly distributed frequency of activity T_{skin} in *O. quadrimaculatus* and *O. saxicola* indicate a relatively high sensitivity (narrow performance breadth) for physiological functions (Chap. 1 and 2). The low variation in activity T_{skin} indicates that both physiological and locomotory function have their optima at very similar T_{skin} . In contrast, the trade-off between physiological and ecological demands in *Z. laticaudatus* (Chap. 3) is particularly interesting to understand the behaviour-physiology nexus (Basson and Clusella-Trullas 2015). The temporal separation of physiological and ecological requirements is so far unknown for diurnal reptiles. However, nocturnal species, such as geckos, hide under the tree bark on the sunny side of the tree during daytime to gain body heat and promote physiological processes. Apart from this 'protected basking', the lizards are relatively inactive during the day. Foraging activity happens at lower night temperature, which results in suboptimal activity T_b (Huey *et al.* 1989a). This is probably not an unusual pattern for nocturnal species but in chapter three, we provide the first evidence for such a split pattern in a diurnal species with basically no restrictions in T_e .

The Middle American lizard *Ameiva festiva*, for example, also forages in the forest but basks at forest edges. However, this lizard constantly shuttles between foraging and basking in a 20-min cycle to attain higher T_b for foraging (Van Berkum *et al.* 1986). The low but broad activity T_{skin} in *Z. laticaudatus* during foraging indicates a lower thermal sensitivity and hence a flatter performance curve for locomotion whilst the thermal sensitivity for other physiological processes, such as digestion, may still be high. Angilletta *et al.* (2002) showed that lizards can exhibit different thermal optima for different physiological processes. Sinclair *et al.* (2016) came to the same result when comparing, amongst others, swimming speed and digestion rate of the western garter snake *Thamnophis elegans*. However, it remains questionable whether broader performance breadth for locomotor function is the result or the prerequisite for activity at a suboptimal T_{skin} as observed in *Z. laticaudatus*.

Potential mechanisms to compensate high refuge temperature

The general temperature increase through climate change in combination with ongoing degradation leads to a loss of thermal refugia (Lima *et al.* 2015) and chapter one shows,

that a higher crevice temperature increases the energy expenditure of *O. quadrimaculatus* during the inactive period of the day. Thus, the loss of shade and the resulting temperature increase at the lower end of the T_e range have been identified as a viable alternative explanation for the recent extinction of ectotherms (Kearney 2013). However, many species are also able to compensate for this, up to a certain degree. The choice of deeper and cooler crevices as refuge, which is a common compensatory strategy among reptiles, could counteract higher T_a . The Australian three-lined skink *Bassiana duperreyi*, for example, digs deeper burrows for its clutch at increasing T_a although this does not completely compensate for climate change (Telemeco *et al.* 2009). The garter snake *Thamnosophis elegans* selects its burrow or crevice depending on T_a . These snakes select deeper and better insulated burrows on hot days than on cooler days to avoid heat stress (Huey *et al.* 1989b). A similar behaviour is seen in the Galapagos Island iguana *Conolophus pallidus* (Christian *et al.* 1984). The children's python *Antaresia childreni* selects its nesting sites depending on temperature and humidity (Stahlschmidt *et al.* 2011). As our study species occur on rocky plateaus which are surrounded by forest, they might be able to select deeper and cooler crevices near the shady edges of the rocks to avoid increasing T_a and excessive energy expenditure.

Alternative energy saving strategies

Energy saving strategies and mechanisms that increase the chance for survival are manifold and often only become apparent through observations under natural conditions. Through brumation, the equivalent to hibernation in mammals, ectotherms can save large amounts of energy and water. Brumation is defined as a dormant state accompanied by inverse compensation in metabolism, rather than simple cold induced inactivity (Patterson and Davies 1978). Some Malagasy reptile species, such as the freshwater turtle *Pelomedusa subrufa*, brumate facultatively. If open water is available, the turtles are active year round in ponds or rivers. If the habitat is dry, they remain inactive in burrows deep in the ground and only emerge if rain occurs (Luiselli *et al.* 2011; pers. observation).

Whilst *O. quadrimaculatus* and *O. saxicola* are active year-round across their distribution range, brumation might be a possible strategy for *Z. laticaudatus* in the drier and more seasonally pronounced western portion of the island and perhaps as a response to climate warming. The western dry forest would allow for regular dormancy because higher climatic predictability would allow for better physical preparation (e.g. fat tissue) and a

higher chance of food and water availability after dormancy (Dausmann and Blanco 2016). Although there is no report for brumation in this species, unspecified general inactivity and absence of many species have been reported for the western dry forest (Andreone *et al.* 2001).

Another Malagasy reptile uses a rather extraordinary strategy: the eggs of the annual chameleon *Furcifer labordi* develop for eight to nine months during the harsh dry season and the post-hatching life-span, which includes growth, maturity and reproduction, lasts just four to five months (Karsten *et al.* 2008). On the Galapagos Islands, morphological adjustments to severe weather events have been observed in the marine iguana *Amblyrhynchus cristatus* as a response to El Niño. Individuals shrink up to 20 % of their original body length, including bone tissue, due to starvation. This phenomenon is reversible and reoccurring, and shows that even morphological plasticity is a way to cope with unforeseeable weather events (Wikelski and Thom 2000). These might be extreme examples but they show that there might be more compensatory mechanisms for reptiles under natural conditions to face environmental changes than generally assumed.

Temperature sensitivity of embryonic development

Our results from chapters one and two show that adult lizards have sophisticated mechanisms to cope with changing conditions. However, some life stages are more sensitive to environmental conditions than others. Juveniles are often rather vulnerable and embryonic development is regarded as the most sensitive life-stage because compensatory mechanisms are extremely limited and thermal conditions during incubation also affect post-hatching life-stages in ectotherms (Van Damme *et al.* 1992; Du and Ji 2006; Mitchell *et al.* 2008). Low temperature can slow embryonic development or even cause diapause, i.e. a complete development stop (Du and Shine 2015). High temperature accelerates development but laboratory experiments have shown that these hatchlings are smaller, grow slower, and have poor performance compared to cold treated eggs. An intermediate incubation temperature revealed the best balance between developmental time and post-hatch performances (Van Damme *et al.* 1992).

However, thermal sensitivity even varies during the incubation period, for example in temperature-dependent sex determination that is known for crocodiles, turtles and some lizard species (Janzen and Paukstis 1991; Mitchell and Janzen 2010). The temperature

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sensitive period lasts for 7 to 15 days, depending on the species (Lance 2009). The *Oplurus* species possess the XY genotypic sex determination system (Altmanova *et al.* 2016) but it remains unknown whether the sex of *Zonosaurus* is determined by genotype or by temperature during incubation. In the latter case, a difference in nest temperature can initiate or suppress development of one sex or the other. For example, even modest temperature increases of less than 2 °C during incubation of the painted turtle *Chrysemys picta* drastically skews the sex-ratio and an increase of 4 °C in mean nest temperature would eliminate the production of male offspring (Janzen 1994).

Surprisingly, embryos are not just passively exposed to their environmental temperature. Du *et al.* (2011) have shown that embryonic turtles are able to move within the egg and orientate their body to a heat source to gain or avoid heat. Even though the room for behavioural thermoregulation is very small, embryos are able to influence their own pre-hatching environment to a very small degree to improve their incubation period. However, this is probably not sufficient to compensate long-lasting climate change. Hence, distributional limits of our study species might not just be based on limited behavioural compensation capacity but, even more crucial, on the thermal sensitivity of embryonic development. This requires further investigation including incubation experiments and temperature measurements in natural clutches across the environmental gradient in combination with an evaluation of survival rates and sex ratios.

Importance of “thermal reality”

The flexibility of compensatory strategies depends on the exact environmental circumstances. It is of vital importance for a species' resilience whether a response is obligate or facultative. The dwarf lemur *Cheirogaleus medius*, for example, is an obligate hibernator because it inhabits a rather predictable environment (Dausmann *et al.* 2005). In contrast, the grey brown mouse lemur *Microcebus griseorufus* uses torpor and hibernation in a facultative manner depending on physical and environmental conditions (Kobbe *et al.* 2011) and the African lesser bushbaby *Galago moholi* uses daily torpor only occasionally as an emergency strategy (Nowack *et al.* 2010). Spontaneous facultative responses (in contrast to obligatory responses) to environmental changes are largely overlooked but they may yield the crux for survival. Whilst it is relatively simple to measure upper and lower thresholds for lethal T_b and performance of ectotherms in the laboratory, it is often not clear which T_b range is actually experienced in the wild because this depends on

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multiple ecological factors, such as habitat structure, severe weather events, predation pressure and competition (Huey and Slatkin 1976; Downes and Shine 1998; Herczeg *et al.* 2008; Stelatelli *et al.* 2013) and, as our study shows, foraging strategy.

Assuming that lizards always tend to achieve T_{pref} can be misleading in an ecological context, as the split patterns for *Z. laticaudatus* in chapter three show. This would lead to an over- or underestimation of the animals' thermal scope. In the case of *Z. laticaudatus*, the elevated T_{skin} in the morning might promote digestion but perhaps it is too close to the upper critical T_{skin} or energetically too expensive to be maintained throughout the day. In this case, it is difficult to assign laboratory results to the complex conditions in the wild. For example, one would assume a certain thermal preference from temperature gradient experiments with the legless lizard *Lialis burtonis* but T_b passively follows T_a under semi-natural conditions in outdoor enclosures and the lizards do not show behavioural thermoregulation at all (Wall and Shine 2008).

One of the prerequisites for standardized procedures, such as temperature gradients or MR measurements, is that the animals are post-absorptive. The duration depends on the species but lizards usually have to fast between two to five days, whilst snakes must fast up to two weeks prior to the experiment (Angilletta and Werner 1998; Clusella-Trullas *et al.* 2007; Niewiarowski and Waldschmidt 1992). However, almost all lizards show post-feeding thermophily (Gatten 1974; Gienger *et al.* 2013). Hence, the animals may have different thermal preferences and needs over time and a post-absorptive animal does not reflect ecological reality because free-ranging lizards usually have food in their guts (Niewiarowski and Waldschmidt 1992). Likewise, conclusions towards the fundamental niche from field results should be handled with caution as they are often blurred by ecological factors. In chapters one and two, the precision in thermoregulation across individuals and habitats indicates that the *Oplurus* species are not much affected by biotic factors. Conversely, if differences in mean activity T_{skin} would have occurred, the causes could have been manifold. Hence, our line of argument only works from one direction. Overall, controlled experiments reveal important information on fundamental physiological functioning, case studies from wild animals are indispensable to complement our understanding of ecological and physiological constraints.

Importance of thermal biology for conservation and extinction risk

Since almost all aspects of an ectotherms' life history are sensitive to T_b , the study of the thermal biology of ectotherms is an important conservation tool (Angilletta *et al.* 2002). It helps to understand and predict current and future distribution ranges of species and, of course, the more comprehensive the knowledge the more precise are future predictions. Temperature, whether it is T_b , T_a or T_e , is a major aspect in modelling reptiles' potential distribution and extinction risk. Usually, T_a and climate data have been used for predictions as they are widely available from weather stations and satellite remote sensing. Nowadays, the increased use of T_e has brought distribution models a huge step forward towards the thermal reality of the animals (Fig. 4.3).



Figure 4.3: Specifically designed copper models for (from left to right) *Oplurus saxicola*, *O. quadrimaculatus* and *Zonosaurus laticaudatus* to measure the operative environmental temperature. The hind legs are missing in the models for *O. saxicola* due to better correlation with live lizards in heat conductance (photo by Ole Theisinger).

However, it is an ongoing debate which variables need to be integrated into distribution models. For example, species-specific physiology has been identified to significantly enhance these predictions (Buckley *et al.* 2010). Recently, Sinclair *et al.* (2016) proposed to integrate the animals' performance curves as a variable factor into life-stage specific ecological modelling. This is a logical approach but the plasticity and variability of acclimatization capacity has not been considered in any of the models so far (Deutsch *et*

al. 2008; Kearney *et al.* 2009; Sinervo *et al.* 2010; Kearney 2013). While it is obvious that behavioural and physiological plasticity might contribute significantly to the survival of an individual, it has been largely ignored because it is highly specific, might occur spontaneously and is thus difficult to measure (Gvozdik 2012). Moreover, compensatory mechanisms may even vary within species depending on the prevalence of constraining environmental conditions (Basson and Clusella-Trullas 2015). The realized niche not just comprises ecological constraints but also flexible behavioural and physiological mechanisms to compensate for a variable combination of environmental influences. These responses might be hidden under controlled conditions, which can lead to an underestimation of an animal's compensatory capacity.

Conclusion

Behavioural thermoregulation in the two *Oplurus* species is very effective to compensate environmental differences. Nevertheless, body mass affects the thermoregulatory precision because heat conduction is faster in smaller animals. This can reduce the compensatory capacity and may lead to constraints in the geographic distribution. Furthermore, low environmental temperature can constrain the daily activity time of heliothermic lizards similarly to high temperature. In cooler habitats, the reduced time for foraging and other activities is partially balanced through lower T_b during inactivity and thus reduced energy expenditure.

Moreover, we found that foraging can affect the thermal niche of a species even more than differences in the thermal environment. The ecology and thermal preferences of lizards therefore affect the compensatory strategy. Open habitat specialists (*Oplurus* spp.) that precisely regulate their T_b throughout the day do not show physiological adjustments because behavioural compensation is sufficient to cope with different thermal environments. In contrast, lizards with shade based activity (*Z. laticaudatus*) often rely on physiological adjustments because thermoregulatory behaviour is limited. The differing ecology of these species shapes their thermal niche and thus dictates animals' responses to environmental changes.

Field studies on the ecophysiology of reptiles are still scarce but our data underline the necessity for such studies to understand the full scale of an animals' compensatory capacity and accompanied constraints in the wild. Trend-setting approaches have recently

been made to predict the extinction risk and future distribution of species using the fundamental niche (Sinervo *et al.* 2010; Kearney 2013; Sinclair *et al.* 2016). However, as the fundamental niche does not consider ecological interactions, appropriate mechanistic models require detailed information on the realized niche of species (Pacifici *et al.* 2015). Physiological measures in presence of natural abiotic and ecological factors might be challenging and time-consuming but they provide essential information to improve future predictions on the distribution and the resilience of species. This thesis demonstrates the importance of the realized niche to understand the effect of multiple ecological constraints on the thermal physiology of animals and thus highlights the integration of ecology into physiological studies.

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English language certificate



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To whom it may concern:

As a native English speaker I hereby confirm that the doctoral thesis of Ole Theisinger, titled "Thermal limits of reptiles - Ecological and environmental constraints on the thermal biology of Malagasy lizards", is written in excellent, university-level English in a clear and coherent style.

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Yours sincerely,



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Declaration on oath

I hereby declare, on oath, that I have written the present dissertation by my own and have not used other than the acknowledged resources and aids.

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