Metabolic responses to environmental variation in tropical reptiles

Dissertation with the aim of achieving a doctoral degree

at the Faculty of Mathematics, Informatics and Natural Sciences

Department of Biology

University of Hamburg

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Hamburg, 2016

Date of oral defense: 2nd March 2017

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"We will now discuss in a little more detail

the Struggle for Existence."

(Charles Darwin, On the Origin of Species)

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Summary

The compensation of environmental variation is a crucial factor for survival, especially in ectotherms, and has gained renewed attention in the face of climate change. Species have been shown to respond to changing conditions with a variety of compensatory mechanism, including behavioural and physiological adjustments, but the potential and relative importance of these mechanisms is still not fully understood. My dissertation investigates the capacity for metabolic adjustments in tropical reptiles, which has long been assumed to be limited although empirical evidence is highly variable.

A prerequisite for an understanding of compensatory mechanisms in ectotherms is information about the thermal reality of a species, especially its body temperature. The first chapter of my dissertation validates two non-invasive methods for body temperature measurements in small to medium-sized reptiles: infrared thermometers and externally attached temperature loggers. Both techniques measure skin temperature rather than core body temperature but our calibrations under semi-natural conditions and in free-ranging individuals proved that both provide a suitable alternative to conventional cloacal temperature records. These methods are easy to use under field conditions and facilitate a more rapid data collection and thus an increased sample size, the possibility of repeated measurements of undisturbed animals under natural conditions and continuous records over several days including times in refuges and during the night.

The second chapter provides an overview of observed acclimatization patterns in tropical reptiles, which range from no acclimatization to full metabolic depression. Based on the assumption that tropical environments provide rather stable conditions, this variety appears controversial but the combined consideration of thermal and energetic restrictions allows us to arrange these patterns into a scheme of decreasing metabolic costs. Our classification also describes two new patterns, selective and selective inverse acclimatization, where metabolic shifts are restricted to body temperatures below the range of activity body temperatures. The variety of constraining influences in tropical regions, including the availability of food and water as well as temperature, and their varying prevalence can adequately explain the variety of acclimatization patterns in tropical reptiles. Moreover, the consideration of the animal's thermal reality advises caution regarding the interpretation of results from laboratory experiments and shows that

an absence of metabolic responses does not necessarily allow inferences about physiological capacity.

In addition to the variety of metabolic responses in tropical reptiles in general, the third chapter of my dissertation shows that even sympatric species, which experience the same fluctuations of ambient conditions, can show contrasting metabolic responses. *Oplurus quadrimaculatus* shows no difference in metabolic rate along a steep environmental gradient from dry spiny forest to humid rainforest but clear seasonal acclimatization, while *Zonosaurus laticaudatus* exhibits adjustments to local conditions along the gradient but no seasonal compensation. We relate this inverse pattern of metabolic adjustments to differences in their foraging ecology and show that ecology can, in some cases, be an even stronger determinant of compensation mechanisms than the thermal environment. In addition, the integration of our physiological data and information about behavioural thermoregulation in these species show that the prevalence of compensatory mechanisms can change even at the intraspecific level but that behavioural responses, if available, are preferred over physiological adjustments.

Overall, my dissertation emphasises the importance of understanding an animal's thermal reality, and thus its need for compensation, before attempting to understand its compensatory capacity. Furthermore, abiotic, biotic and ecological constraints all work in concert to determine the ability of species to compensate environmental variation. This complexity of influences and responses may have led to an underestimation of compensatory potential in tropical reptiles and further research is needed especially at ecologically relevant scales, integrating natural conditions and the interplay between behavioural and physiological mechanisms.

Zusammenfassung

Die Kompensation von Umweltveränderungen ist ein entscheidender Faktor für das Überleben von Arten, vor allem von ektothermen Tieren, und dieser hat, angesichts des derzeitigen Klimawandels, wieder stark an Bedeutung gewonnen. Wechselnde Umweltbedingungen können eine Reihe von Kompensationsmechanismen hervorrufen, unter anderem Anpassungen im Verhalten und der Physiologie von Tieren. Deren Potenzial und der Stellenwert dieser Mechanismen untereinander ist allerdings noch weitgehend unbekannt. Meine Dissertation untersucht das Kompensationspotenzial von Stoffwechselanpassungen bei tropischen Reptilien, welches lange als gering angesehen wurde, obwohl empirische Studien höchst unterschiedliche Ergebnisse zeigen.

Eine Grundvoraussetzung, um Kompensationsmechanismen bei ektothermen Tieren verstehen zu können, sind Kenntnisse über ihre tatsächliche thermische Situation, insbesondere ihrer Körpertemperaturen. Das erste Kapitel meiner Dissertation beschreibt deshalb den Test zweier non-invasiver Methoden der Körpertemperaturmessung bei kleinen bis mittelgroßen Reptilien: Infrarotthermometer und extern angebrachte Datenlogger. Beide Techniken messen die Hauttemperatur der Tiere anstatt der eigentlichen Körpertemperatur. Unsere Kalibrierungen unter halb-natürlichen Bedingungen und bei freilaufenden Individuen zeigen aber, dass beide Methoden geeignete Alternativen zu herkömmlichen Messungen in der Kloake sind. Beide eignen sich gut für den Einsatz unter Feldbedingungen und ermöglichen eine schnellere Datenaufnahme und somit größere Fallzahlen, wiederholte Messungen ungestörter Tiere unter natürlichen Bedingungen und eine fortlaufende Datenaufnahme über mehrere Tage, sowie in Rückzugsorten und über Nacht.

Das zweite Kapitel bietet eine Übersicht über Akklimatisierungsmuster tropischer Reptilien, die von keinerlei metabolischer Anpassung bis hin zu einer starken Absenkung des Stoffwechsels reichen. Da die Tropen generell eher als ein klimatisch stabiler Lebensraum gelten, erscheint diese Bandbreite widersinnig. Die Integration thermischer und energetischer Einschränkungen erlaubt es uns aber, die beobachteten Muster entsprechend der damit verbundenen energetischen Kosten zu ordnen. Unsere Klassifizierung beschreibt außerdem zwei neue Muster, die selektive und die selektiv inverse Akklimatisierung, bei denen eine Verschiebung der Stoffwechselrate auf Körpertemperaturen unterhalb der

normalen Aktivitätstemperatur beschränkt ist. Die Vielfalt der limitierenden Faktoren, inklusive Futter- und Wasserverfügbarkeit sowie Temperatur, und ihre wechselnde Vorherrschaft bieten insgesamt eine passende Erklärung für die Vielfalt der Akklimatisierungsmuster bei tropischen Reptilien. Darüber hinaus zeigen unsere Ausführungen, dass manche Laborergebnisse mit Vorsicht interpretiert werden sollten und dass das Fehlen metabolischer Anpassungen nicht zwangsläufig gleichbedeutend ist mit physiologischem Unvermögen.

Neben der Vielfalt metabolischer Anpassungen bei tropischen Reptilien im Allgemeinen, zeigt das dritte Kapitel meiner Dissertation, dass selbst sympatrische Arten unterschiedliche Reaktionen zeigen können, obwohl sie den gleichen thermalen Bedingungen ausgesetzt sind. *Oplurus quadrimaculatus* zeigt keine Unterschiede in der Stoffwechselrate entlang eines steilen Umweltgradienten, von trockenem Dornenwald bis hin zum feuchten Regenwald, aber deutliche saisonale Anpassungen. *Zonosaurus laticaudatus* hingegen zeigt Anpassungen an lokale Bedingungen entlang des Gradienten, aber keinerlei saisonale Kompensation. Wir begründen diese konträren Anpassungen mit Unterschieden in der Nahrungsökologie der Tiere und zeigen somit, dass die Ökologie manchmal sogar einen stärkeren Einfluss auf das Kompensationspotenzial haben kann als die thermalen Bedingungen des Lebensraumes. Außerdem zeigen unsere physiologischen Daten in Kombination mit Informationen zum Thermoregulationsverhalten der Tiere, dass die Vorherrschaft bestimmter Kompensationsmechanismen auch innerhalb einer Art wechseln kann, dass Verhaltensanpassungen aber, wenn möglich, bevorzugt werden.

Insgesamt unterstreicht meine Dissertation die Bedeutung der tatsächlichen thermalen Situation eines Tieres und zeigt, dass es ohne Kenntnisse darüber und den daraus resultierenden Kompensationsbedarf nicht möglich ist, die Kompensationsfähigkeit der Tiere zu beurteilen. Des Weiteren beeinflussen sowohl abiotische und biotische als auch ökologische Faktoren die Fähigkeit einer Art, Umweltveränderungen zu kompensieren. Diese Komplexität verschiedener Einflüsse und Reaktionen hat möglicherweise dazu geführt, dass das Kompensationsvermögen bei tropischen Reptilien bisher unterschätzt wurde. Hierzu sind weitere Studien dringend notwendig, insbesondere ökologisch relevante, die natürliche Bedingungen und das Wechselspiel von Verhalten und Physiologie berücksichtigen.

Introduction

Environments vary across space and time and this environmental variation is directly linked to the persistence of individuals and species through its effect on animal physiology (Seebacher and Franklin 2012). Temperature and water availability, two key characteristics of an environment, directly affect physiological processes as well as the availability of resources. Changing conditions can therefore have substantial consequences for an animal's energy budget and disrupt, at least temporarily, the energy balance that animals need to maintain over time (Porter and Gates 1969). Moreover, abiotic conditions can, in extreme cases, directly affect survival by disturbing physiological functioning (Schmidt-Nielsen 1999).

Species evolved some extraordinary adaptations to cope with the impact of varying ambient conditions. For example, some terrestrial frogs survive the freezing of almost two thirds of their body fluids using cryoprotectants (Schmid 1982; Layne and Lee 1987) and the chameleon *Furcifer labordi* spends most of its life as an egg to outlive the harsh dry season in Madagascar (Karsten et al. 2008). Similarly troubled by the unpredictability of food availability during this time, several lemur species flexibly adjust their energy saving strategy from short daily torpor to long hibernation periods (Fig. 0.1; Kobbe et al. 2011; Dausmann 2014). Most notable, some species are able to reversibly shrink individual organs or even their whole body in response to extreme conditions. Arabian sand gazelles (*Gazella subgutturosa*), for example, can shrink their heart and liver to reduce evaporative water loss during long periods of drought (Ostrowski et al. 2006). And marine iguanas (*Amblyrhynchus cristatus*) can become shorter by as much as 20% within two years during extreme food scarcity (Fig. 0.1; Wikelski and Thom 2000).

Although these are illustrative examples for the potential of evolutionary responses to climatic variation, the current challenge of human-induced environmental changes seems to exceed the adaptive capacity of many species. Ecological impacts of climate change have been documented across regions and taxa (reviewed in Parmesan 2006) and future warming is predicted to cause widespread extinctions (Thomas et al. 2004; Deutsch et al. 2008; Sinervo et al. 2010; Bestion et al. 2015). Whether one agrees with dramatic terms such as 'sixth mass extinction' (Barnosky et al. 2011; Ceballos et al. 2015) and 'defaunation in the Anthropocene' (Dirzo et al. 2014) or not, populations and species are certainly disappearing at an alarming

Figure 0.1 A reddish-grey mouse lemur (*Microcebus griseorufus*) in a spiny forest in southeast Madagascar (top) and marine iguanas (*Amblyrhynchus cristatus*) on the Galápagos islands (bottom). [Photos: Wiebke Berg]

rate (Chapin et al. 2000). These species are not only a loss to biodiversity in the form of species richness but also a loss to ecological interactions and possibly ecological services (Valiente-Banuet et al. 2015). Attempts are made to reverse this trend (Seddon et al. 2014) but conservation strategies and policies will only be successful if they are based on a profound scientific understanding about the potential and limits of species to cope with varying environments.

Ectothermic species are particularly sensitive to climatic variation because they largely depend on their environment for the regulation of their body temperature (T_b) (through solar radiation, convection and conduction). Until just over 70 years ago, it was even still believed that the T_b of 'cold-blooded' reptiles passively follows the temperature regime of their environment. Fascinated by the ability of desert species to withstand extreme heat, Cowles and Bogert (1944) then demonstrated in a ground-breaking study that reptiles are able to behaviourally regulate their T_b. Thermoregulation has since been recognised as a key component of ectotherm biology and it was not long until further studies revealed that lizards are also able to adjust their physiology in response to varying environmental conditions (Lowe and Vance 1955; Dawson and Bartholomew 1956). Since those early studies, methodology advanced from T_b measurements that included shooting lizards with a pistol (Bogert 1949) to the increasing application of infrared thermography for a non-invasive assessment of T_b (Tattersall 2016). And instead of tying lizards to a stick in full sunshine until dead (Mosauer 1936), researchers now use copper models of lizards to assess the range of attainable T_b in a given habitat (Sinervo et al. 2010). These copper models are a methodological refinement from Heath's (1964) pioneering study of 'thermoregulating' beer cans, in which he placed water filled beer cans in the sun to demonstrate that a deviation of T_b from ambient temperature does not necessarily indicate active thermoregulation. These advances facilitated the identification of a variety of mechanisms and strategies that allow species to cope with changes in their environment (Huey and Slatkin 1976; Huey 1982) and the potential of these compensatory responses has become a major focus of thermal biology (Huey et al. 2009; Kearney et al. 2009; Somero 2010; Gvoždík 2012; Huey et al. 2012).

Animals generally have two options to cope with environmental variation: avoidance or tolerance. Range shifts have been observed in a wide range of species and are often attributed to global warming (Walther et al. 2002; Parmesan and Yohe 2003). However, considering the progressive fragmentation of landscapes,

dispersal will be increasingly hampered and may be additionally impaired by species interactions (Urban et al. 2013). Species are thus increasingly forced to persist *in situ* and compensate environmental changes through behavioural and physiological plasticity or genetic adaptation (Reed et al. 2011; Urban et al. 2014). These mechanisms can be distinguished based on the time-scale at which they occur (Hertz 1981). Behavioural responses are the most rapid and flexible option, followed by physiological plasticity, which facilitates reversible compensatory adjustments within the lifetime of an individual without changing the genetic makeup of a species. Finally, adaptations may evolve over several generations based on natural selection.

It is, however, questionable and an issue of ongoing debate whether genetic adaptations are able to keep pace with current environmental change (Sinervo et al. 2010; Quintero and Wiens 2013). A meta-analysis of rates of phenotypic change concludes that adaptive responses could be accelerated in anthropogenic contexts (Hendry et al. 2008). However, most of these phenotypic studies do not provide evidence for a genetic base of these changes and are thus unable to separate genetic from plastic responses (Gienapp et al. 2008). Where this distinction is made, plasticity often seems more important than genetic adaptation (Gienapp et al. 2008; Hendry et al. 2008; Hoffmann and Sgrò 2011) and indeed, many changes that have previously been attributed to genetics are now attributed to plasticity (Gienapp et al. 2008; Merilä and Hendry 2014). Genetic adaptation may therefore be less significant for species' viability than behavioural and physiological plasticity (Hoffmann and Sgrò 2011).

Behavioural thermoregulation includes, in the order of their relative importance, adjustments in activity time, shuttling between microhabitats and postural changes (Stevenson 1985). Behavioural responses are widespread in ectotherms (Angilletta 2009), and can be highly efficient in the compensation of climatic differences (Adolph 1990; Muñoz et al. 2014), provided that suitable microhabitats and sufficient spatial heterogeneity are available (Kearney et al. 2009; Sears and Angilletta 2015). Costs for behavioural thermoregulation arise mainly in the form of time that is unavailable for other activities, such as foraging or reproduction, and as energetic costs from locomotion during thermoregulatory shuttling. Thermoregulatory basking can furthermore increase an animal's predation risk. These costs are species and habitat specific but can outweigh benefits in poor

quality habitats (as defined by the cost-benefit model of thermoregulation; Huey and Slatkin 1976; but see Blouin-Demers and Nadeau 2005).

In contrast, physiological acclimatization[1] comprises mostly energetic costs for biochemical adjustments (Seebacher 2005). These biochemical adjustments can alter thermal tolerances and preferences (reviewed in Clusella-Trullas and Chown 2014; Gunderson and Stillman 2015) or influence the thermal reaction norm of physiological processes (e.g. locomotion, digestion, metabolic rate; Glanville and Seebacher 2006; Seebacher et al. 2015). The potential of physiological plasticity is generally not well understood yet. Based on Janzen's (1967) hypothesis, the capacity for acclimatization has long been assumed to be related to the extent of environmental variation that a species experiences, resulting in a limited compensatory capacity in tropical ectotherms due to more stable conditions. Many early studies on the acclimatization of metabolism seemed to confirm this expectation (Feder 1978, 1982; Tsuji 1988), whereas a recent meta-analysis revealed a contradicting pattern of increasing acclimatization capacity towards the tropics in ectotherms in general (Seebacher et al. 2015). In addition to this controversial result, the same meta-analysis reported that responses in reptiles alone are too variable to draw any conclusion about their compensatory capacity. This is particularly problematic because reptiles are highly threatened by global change (Sinervo et al. 2010; Böhm et al. 2013) and may, in reference to the global amphibian crises, even be 'the new amphibians' (Gibbons et al. 2000; Wake and Vredenburg 2008; Huey et al. 2010).

The high variability of metabolic adjustments in tropical reptiles complicates the evaluation of their compensatory capacity. Some authors are sceptical about the potential of physiological adjustments to buffer reptiles against future climate change (Huey et al. 2009). However, these predictions are still based on the assumption of a limited acclimatization capacity in tropical species (Janzen 1967). Others highlight the importance of plastic responses (Gvoždík 2012; Urban et al. 2014) and criticise the insufficient ecological relevance of experiments that impedes the detection of plasticity (Gvoždík 2012). An overarching tenor of all studies is, however, that available data is too limited (Huey et al. 2009; Urban et al. 2014), and further research is urgently needed.

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[1] The adjustment of physiological processes is referred to as 'acclimation' for shifts under laboratory conditions and 'acclimatization' for shifts under natural conditions. However, I will use acclimatization as a generic term for both unless stated otherwise.

The island of Madagascar is one of the hottest biodiversity hotspots with exceptional rates of endemism across taxa (over 98% in amphibians, reptiles and mammals) and similarly exceptional rates of deforestation (almost 40% decrease in total forest cover and almost 80% reduction in core forest from 1950s to 2000) (Myers et al. 2000; Ganzhorn et al. 2001; Harper et al. 2007). It has, moreover, recently been identified as a hotspot of species vulnerable to climate change (Pacifici et al. 2015). Especially southern Madagascar is predicted to experience further warming and drying (Hannah et al. 2008), a region which is already challenged frequently by severe drougths (Elmqvist et al. 2007) and tropical cyclones (Ganzhorn 1995).

Amidst this highly unpredictable and challenging environment, southeastern Madagascar offers a unique natural set-up, where the humid eastern and the dry southwestern portion of the island are directly connected by a small area of transitional forest. In this region, the Anosy Mountains act as a rain barrier to moist winds from the east and create an extremely steep environmental gradient on their western flanks that ranges in precipitation from 2400 mm/yr in the rainforest at higher elevations to 400 mm/yr in the spiny forest (Fig. 0.2; Goodman 1999). While rainfall can occur at any time of the year in the rainforest, precipitation is seasonal in the spiny forest with almost all rain falling during the

Figure 0.2 The Andohahela gradient in southeast Madagascar. View from the dry spiny forest across transitional forest on the mountain slopes to the humid rainforest. [Photo: Wiebke Berg]

wet season between November and April. The climatic extremes of spiny and rainforest are in some places only five kilometres apart but can differ more than 6°C in mean ambient temperature (Rakotondranary et al. 2011). They are connected by a mosaic of transitional forest patches and by gallery forest along rivers. The considerable climatic differences along the gradient coupled with seasonal effects of wet and dry season present ideal conditions to study species' responses to changing environmental conditions.
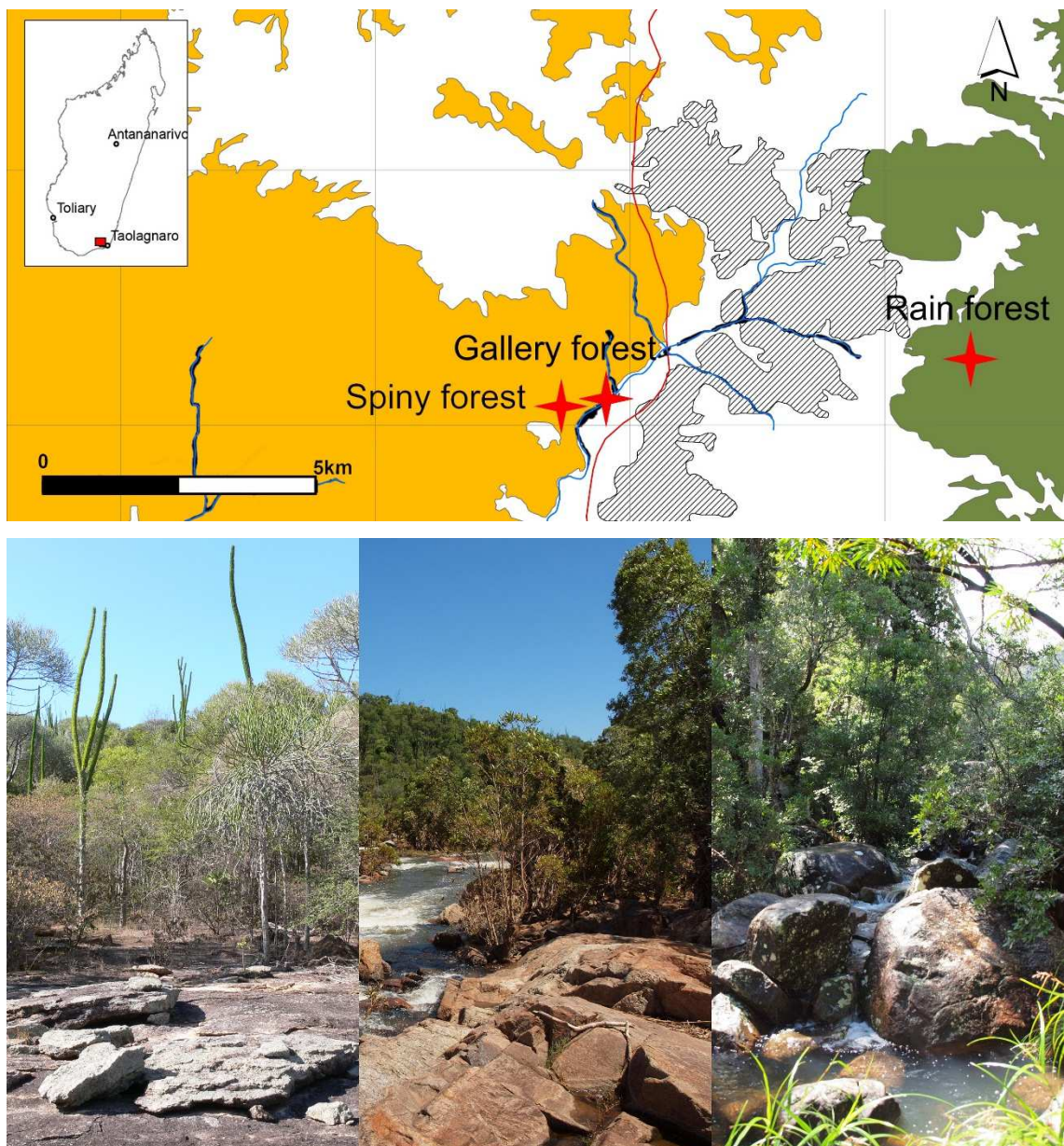
Figure 0.3 Map and photos of study sites along the environmental gradient. Colours in the map represent different vegetation forms: spiny forest (yellow, photo on the left), gallery forest (black along rivers, photo in the middle), transitional forest (shaded), savannah (white) and rainforest (green, photo on the right). Red asterisks in the map mark study sites. [Map modified from Rakotondranary et al. 2011; photos: Wiebke Berg]

My dissertation investigates the compensatory capacity of tropical reptiles in the form of metabolic responses to environmental variation at a spatial and temporal scale, i.e. along the environmental gradient and across seasons. I chose two Malagasy lizard species, *Oplurus quadrimaculatus* and *Zonosaurus laticaudatus*, which occur sympatrically on rocky sites along the entire gradient (spiny forest to rainforest; Fig. 0.3) and which are both active year-round. *Oplurus quadrimaculatus* is the largest Iguanidae of Madagascar (body mass ~80g) and an obligate saxicolous species (Fig. 0.4, top). The gerrhosaurid *Z. laticaudatus* (body mass ~120g) also inhabits large open rocks but actively searches its prey in crevices and the surrounding leaf litter (Fig. 0.4, bottom). I combine physiological measurements on these two species with ecological considerations and discuss my results in relation to data on behavioural thermoregulation in these species (Theisinger et al. in review_a,b). This integrative approach provides a comprehensive investigation of the potential and limitations of compensatory mechanisms and contributes to a better understanding about the (in)capacity of tropical reptiles to cope with changing environments. I address this topic in three chapters and will answer the following specific questions within each chapter:

1. Non-invasive monitoring of body temperature:

A prerequisite for an understanding of compensatory mechanisms in ectotherms is information about the thermal reality of a species, most of all its T_b. Body temperature has a substantial effect on metabolic rate and performance. However, changes in ambient conditions do not necessarily entail changing T_b due to behavioural thermoregulation. Moreover, as metabolic rate increases exponentially with T_b in ectotherms, oxygen consumption measurements also require parallel records of T_b. The first chapter of my dissertation evaluates two non-invasive methods for T_b measurements in small to medium-sized reptiles: infrared-thermometers and externally attached temperature loggers. Both techniques measure skin temperature rather than core T_b, so their use necessitates a validation of the accuracy of such indirect T_b measurements. Chapter one therefore investigates the following questions:

- ❖ Is skin temperature a suitable surrogate for T_b?

- ❖ Are infrared-thermometers and externally attached temperature loggers suitable for field studies?

Figure 0.4 My study species: *Oplurus quadrimaculatus* (top) and *Zonosaurus laticaudatus* (bottom). [Photos: Wiebke Berg and Ole Theisinger]

2. Differing metabolic adjustments in reptiles across the tropics:

Based on Janzen's (1967) postulation that an organism's ability to acclimatize is related to the degree of variation that this animal experiences in its environment, tropical species were long assumed to have only a limited acclimatization capacity. A recent meta-analysis, however, contradicts this latitudinal hypothesis and finds that metabolic responses in reptiles are too varied to draw a conclusion. In the second chapter of my dissertation, I therefore review available data on metabolic acclimatization from tropical terrestrial reptiles (including my own new data from *O. quadrimaculatus* and *Z. laticaudatus*) to investigate possible causes for their variable and contrasting metabolic acclimatization patterns. Thus, chapter two investigates the following questions:

- ❖ Does the observed variation of metabolic responses follow a schematic pattern?

- ❖ Are differing patterns based on differing compensatory capacities?

3. Differing metabolic adjustments in sympatric tropical reptiles:

The third chapter of my dissertation compares metabolic adjustments in the two sympatric study species on a spatial and temporal scale. Possible differences in metabolic responses to environmental variation along the gradient and between seasons provide insight into the compensatory potential of these species. Furthermore, the interspecific comparison of sympatric species within the same thermal environment provides information about possible ecological influences and how these may affect a species' compensatory potential. Chapter three investigates the following questions:

- ❖ Do these sympatric species differ in their metabolic responses to environmental variation, either on the spatial or the temporal scale?

- ❖ Are possible differences in metabolic adjustments related to differences in their ecology?

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Chapter One

Evaluation of skin temperature measurements as suitable surrogates of body temperature in lizards under field conditions

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Introduction

Temperature is a crucial factor for ectotherm biology and one of the most accurate methods to study body temperature (T_b) in reptiles is the measurement of cloacal temperature using thermocouples. Albeit being long established, this method has practical limitations as it requires capturing and handling the animal. This may falsify temperature records in smaller species if the animal moves between microhabitats during capture and impedes repeated T_b records under natural, undisturbed conditions. Furthermore, it often restricts sample sizes to small numbers per hour (e.g. Grbac and Bauwens 2001; Hertz 1992; Hertz and Huey 1981). Therefore, cloacal T_b measurements are increasingly replaced by non-invasive thermal measurements that allow repeated temperature records of undisturbed animals in their natural (thermal) environment (Tattersall and Cadena 2010). Two of these alternatives are infrared thermometers (IRT) and the attachment of external temperature loggers (ETL), e.g. Thermochron iButtons. Both methods measure skin temperature (T_{sk}) instead of the actual T_b. Although these methods clearly provide several advantages compared to measurements of

cloacal T_b, their use necessitates a validation of the accuracy of such indirect T_b measurements. IRTs allow the measurement of T_{sk} from a relatively large distance (up to 1.5 m, depending on the IRT model) without disturbing the animal. This eases the data collection considerably and facilitates larger sample sizes per hour as well as continuous surveys of single individuals. While ETLs still require the capture and handling of the animal for attachment and removal, they can repeatedly record T_{sk} for several days (and longer) of undisturbed behaviour around the clock and even in retreat sites.

Both methods have been applied in previous studies (e.g. Christian et al. 1998; Revell and Hayes 2009; Vasconcelos et al. 2012), but most of the few calibration studies of T_{sk} measurements as surrogates for T_b in reptiles were conducted under laboratory conditions (IRT: Besson and Cree 2010; Carretero 2012; Hare et al. 2007; Herczeg et al. 2006; Shine et al. 2002; Werner et al. 2005; ETL: Dubois et al. 2008; Shine et al. 2003). The laboratory results generally report T_{sk} to be an accurate estimate of T_b, except those reported by Carretero et al. (2012), who report inaccurate results from IRT records in small lacertids (four *Podarcis* spp.) and predict the bias to be even higher for field data. Available field data are scarce and results from Hare et al. (2007) show a significant relationship between T_{sk} and T_b but only with a moderate determination coefficient and sometimes large discrepancies of up to 12.9°C, possibly due to a strong dependence of IRT measurements on the orientation of the device.

The aim of our study was therefore to assess the accuracy of indirect T_b measurements in lizards of different body sizes (20 – 120g) under field conditions and to evaluate the suitability of IRT and ETL measurements as an alternative for cloacal temperature records in field studies.

Materials and Methods

We chose two iguanid lizards, *Oplurus saxicola* and *O. quadrimaculatus*, and one plated lizard, *Zonosaurus laticaudatus*, all of different body sizes and body mass, to cover a comprehensive range for our calibration study. All three species are endemic to Madagascar and occur sympatrically in rocky habitats. All are diurnal and saxicolous, although *Z. laticaudatus* also searches for its prey in leaf litter and crevices. The study took place in the Andohahela National Park in southeastern

Madagascar between November 2009 and April 2012. *Oplurus saxicola*, the smallest of the three species, reaches an average snout-vent-length (SVL ± SD) of 8.8 cm ± 0.5 cm and a body mass (BM ± SD) of 21.1 g ± 4.2 g (N = 183). *Oplurus quadrimaculatus* is the largest Iguanidae of Madagascar with a SVL of 12.8 cm ± 0.6 cm and a BM of 76.5 g ± 10.5 g (N = 310). The medium sized Gerrhosauridae *Z. laticaudatus* reaches an average SVL of 15.3 cm ± 0.78 cm and a BM of 113.5 g ± 15.9 g (N = 191; unpublished data).

Calibration measurements

We first tested the accuracy of T_{sk} measurements as surrogates for T_b by comparing ETL and IRT records with cloacal T_b measurements in captured *O. saxicola* (101 measurements from six individuals), *O. quadrimaculatus* (102 measurements from eight individuals) and *Z. laticaudatus* (84 measurements from seven individuals). After capturing the animals by noosing, we fixed a temperature logger (iButton model DS1921G; accuracy ± 0.5°C; Maxim Integrated Products Inc., San Jose, California, USA) at the rear end of the animals' backs using a small stripe of duct tape (cut and fitted around the ETL, leaving the top uncovered to avoid insulation). We used unmodified ETLs during this study, thus weighing 3.3g each. This corresponds to less than 5% of the body mass for *O. quadrimaculatus* and *Z. laticaudatus* but exceeds the generally recommended 5 – 10% of the body mass for *O. saxicola* (BM ~20g). We still attached ETLs also to the latter species for the duration of the calibration measurements in the box as the additional load did not have any negative effects on the animal's survival or well-being but provided additional information about the comparability of skin and rectal body temperature measurements. The animal was then placed into a transparent plastic box and left in the shade for at least 15 minutes before the start of the calibration. During the test, we moved the box with the animal between sun and shade to create heating and cooling curves. The ETL was programmed to record T_{sk} every minute. At intervals of 5 – 7 minutes, we took the animal from the box and immediately measured T_{sk} with the IRT (Voltcraft IR 1200 – 50 D; distance to spot ratio 50:1, range from -50°C to +1200°C, resolution 0.1°C, precision ± 1%; Conrad Electronic SE, Hirschau, Germany) from a distance of about 0.5m to approximate measurements of free ranging animals as closely as possible, which are usually taken from 1 – 1.5m distances. Following that, we recorded cloacal T_b using a

digital precision thermometer (Greisinger GTH 175/Pt; range from -199.9 to +199.9°C, resolution 0.1°C, precision 0.1°C ± 1 digit; GREISINGER electronic GmbH, Regenstauf, Germany) and returned the animal to the box. Although we avoided hand contact of the ETL and the back of the animal, where IRT records were taken, the animal (and with it the ETL) was exposed to the (differing) ambient temperature for a short time while outside the box. To ensure the comparability of T_{sk} and T_b records, we used the ETL temperature that had been stored immediately before the animal was taken from the box, the IRT record that was measured directly after that and the T_b measurement that was taken within no more than 30s after the IRT record.

IRT control measurements of free ranging animals

To further examine the relationship between T_{sk} and T_b and to ensure the usability of an IRT in the field, we also calibrated this method in free ranging animals of *O. saxicola* (N = 76) and *O. quadrimaculatus* (N = 15). After measuring T_{sk} using the IRT from distances of 1 – 1.5m, we also recorded the animals' cloacal T_b if the animals could be captured within no more than 30s. These control measurements were undertaken at different times of the day and in different microhabitats to maximise the temperature range and compensate for differing environmental conditions.

All devices have been calibrated before the measurements and a correction value was subtracted from all IRT temperatures prior to further calculations. Data were processed using IBM SPSS 20 and all means are given with standard deviation.

Results

We recorded a total of 101 paired T_b – T_{sk} calibration measurements from six individuals of *Oplurus saxicola* (7 – 22 measurements/ind.), 102 paired measurements from eight individuals of *O. quadrimaculatus* (9 – 22 measurements/ind.), and 84 paired measurements from seven individuals of *Zonosaurus laticaudatus* (4 – 19 measurements/ind.). During the calibration measurements, T_b ranged from 24.6 – 41.7°C for *O. saxicola*, 26.6 – 41.2°C for *O. quadrimaculatus* and 29.0 – 39.6°C for *Z. laticaudatus*. This is a comprehensive representation of temperatures

found in active individuals of all three species as our minimum and maximum records of free ranging individuals show similar activity temperature ranges (28.2 – 43.6°C for *O. saxicola*, 24.7 – 41.5°C for *O. quadrimaculatus* and 28.3 – 37.4°C for *Z. laticaudatus*).

External temperature loggers

T_{sk} records from ETLs are highly correlated with T_b (*O. saxicola*: Spearman correlation r_s = 0.987, p < 0.001, N = 101; *O. quadrimaculatus*: r_s = 0.933, P < 0.001, N = 102; *Z. laticaudatus*: r_s = 0.883, P < 0.001, N = 84) and show no differences between paired measurements for *O. quadrimaculatus* (t = 1.315, df = 101, p = 0.191, mean deviation 0.11 ± 0.88°C, ΔT max 2.7°C) and *Z. laticaudatus* (t = -0.637, df = 83, p = 0.526, mean deviation -0.06 ± 0.86°C, ΔT max 2.7°C). However, in *O. saxicola* paired measurements of T_b and T_{sk} differ significantly (t = 6.038, df = 100, p < 0.001, mean deviation 0.33 ± 0.55°C, ΔT max 1.4°C) (Fig. 1.1). When we assign the paired T_b – T_{sk} measurements to heating and cooling phases of the animals, we can see that the discrepancy is in most cases (98%) smaller than 1.5°C, except for phases of intense heating or cooling, when the ETL heats up or cools down faster than the animal and deviations range up to 2.7°C (Fig. 1.2).
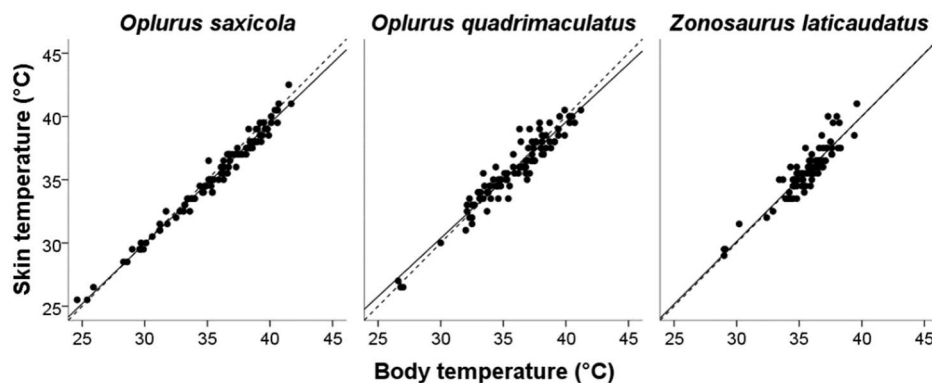
Figure 1.1 Comparison of T_{sk} measured with ETLs and T_b measured rectally with a thermocouple. The black line is the regression line (R^2 = 0.979 for *O. saxicola*; R^2 = 0.907 for *O. quadrimaculatus*; R^2 = 0.835 for *Z. laticaudatus*) and the dotted line represents a one-to-one line for reference.
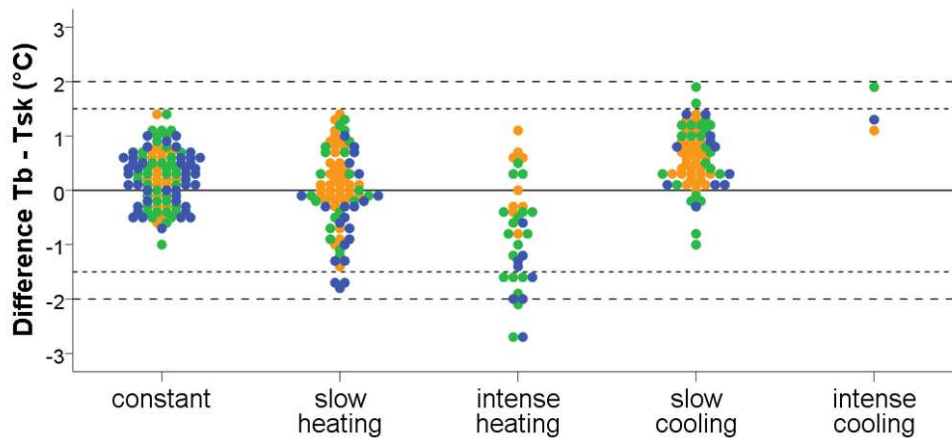
Figure 1.2 Discrepancy between T_{sk} measured with ETLs and rectal measurements of T_b according to phases of constant T_b, slow or intense heating and slow or intense cooling of the animal (exemplary also for data from IRTs). The different phases were defined based on the amount of change in T_b during the last five minutes before the measurement of T_{sk}. Constant: $\Delta T_b \leq 0.5°C$, slow heating or cooling: $\Delta T_b 1.0 - 4.5°C$, intense heating or cooling: $\Delta T_b \geq 5.0°C$. Orange dots show data from *O. saxicola*, green dots from *O. quadrimaculatus* and blue dots from *Z. laticaudatus*.
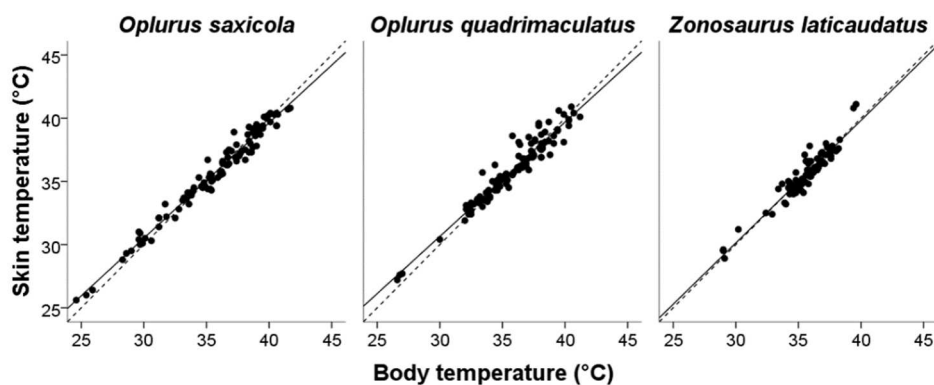
Figure 1.3 Comparison of T_{sk} measured with an IRT and T_b measured rectally with a thermocouple. The black line is the regression line ($R^2 = 0.974$ for *O. saxicola*; $R^2 = 0.926$ for *O. quadrimaculatus*; $R^2 = 0.899$ for *Z. laticaudatus*) and the dotted line represents a one-to-one line for reference.

Infrared thermometer

T_{sk} readings measured with IRTs are also highly correlated with T_b in all three species (*O. saxicola*: Spearman correlation r_s = 0.980, P < 0.001, N = 100; *O. quadrimaculatus*: r_s = 0.943, P < 0.001, N = 102; Z. laticaudatus: r_s = 0.910, P < 0.001, N = 83) and show no differences between paired measurements of T_b and T_{sk} (*O. saxicola*: t = -0.122, df = 99, p = 0.903, mean deviation -0.01 ± 0.66°C, ΔT max 1.7°C; *O. quadrimaculatus*: Wilcoxon test: p = 0.507, mean deviation -0.08 ± 0.78°C, ΔT max 2.8°C; *Z. laticaudatus* Wilcoxon test: p = 0.195, mean deviation 0.04 ± 0.64°C, ΔT max 1.9°C) (Fig. 1.3).

As in the ETL measurements, deviations of more than 2°C occur only during phases of intense T_b changes and only few measurements (2%) deviate more than 1.5°C during phases when T_b is constant or changes only slowly.
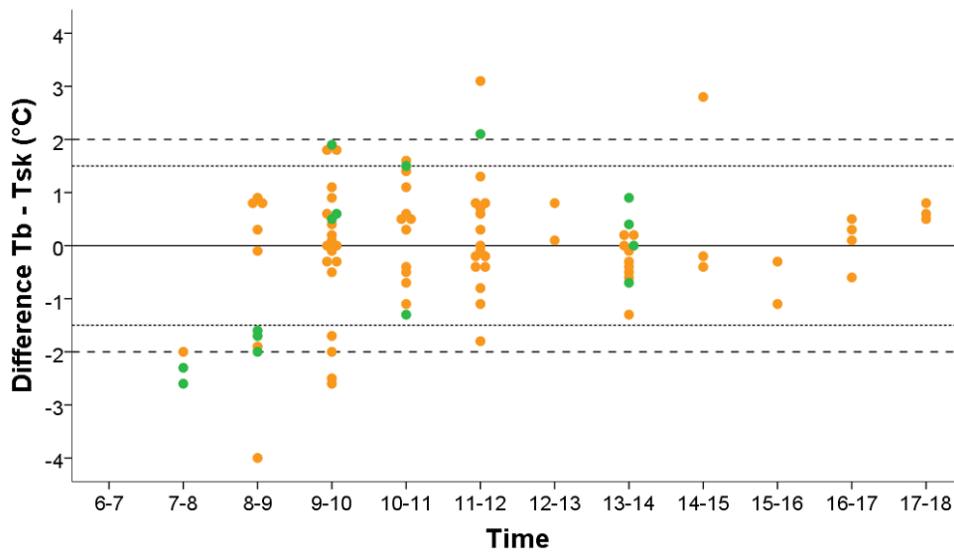
Figure 1.4 Discrepancy between T_{sk} measured with an IRT and rectal measurements of T_b in free ranging Oplurus according to the time of data collection. Orange dots show data from *O. saxicola*, green dots from *O. quadrimaculatus*.

IRT control measurements of free ranging animals

We took control measurements with the IRT of free ranging *O. saxicola* and *O. quadrimaculatus*. Both species show a high correlation of T_{sk} and T_b also under natural conditions (*O. saxicola*: Spearman correlation $r_s = 0.795$, $P < 0.001$, $N = 76$; *O. quadrimaculatus*: $r_s = 0.753$, $P < 0.001$, $N = 15$). Deviations are higher than in the calibration measurements though, with a mean deviation of $-0.02 \pm 1.14°C$ and ΔT max $4.0°C$ for *O. saxicola* and a mean deviation of $-0.29 \pm 1.57°C$ and ΔT max $2.6°C$ for *O. quadrimaculatus*. To further analyse these data, we plotted the deviations between T_b and T_{sk} according to the time of data collection (Fig. 1.4). Of all temperature records of free ranging *Oplurus* ($N = 91$), 91% deviate less than 2°C and 78% less than 1.5°C. If records from the early morning (before 0900 h), when animals often heat up 10°C or more within a short period of time, are excluded, 94% of the remaining data ($N = 78$) deviate less than 2°C and 85% less than 1.5°C.

Discussion

The suitability of ETLs and IRTs as alternatives for cloacal temperature measurements in reptiles largely depends on the applicability of T_{sk} as a substitute for T_b. Our data show a highly significant correlation between T_{sk} and T_b in all three species over a range of body sizes (20 – 120g) and neither ETL nor IRT measurements show significant differences between paired T_{sk} and T_b records in *Oplurus quadrimaculatus* and *Zonosaurus laticaudatus*. In *O. saxicola* ETL measurements of T_{sk} differ significantly from T_b records but T_{sk} measured with an IRT does not. While IRT records show an even distribution of positive and negative deviations, there is a bias in the ETL records of *O. saxicola* towards measurements with $T_{sk} < T_b$ (no bias during heating phases but $T_{sk} < T_b$ during cooling). This is a species-specific problem and due to a loose fit of some of the ETLs during cooling phases, when the animal may switch to thigmothermy and press itself flat against the ground, causing the ETL to partially loose contact with the skin. However, mean deviations are close to 0.0°C in records from both methods and across all three species. Large deviations of more than 2°C occurred only during phases of intense changes in T_b, while phases of slow heating and cooling mostly show deviations < 1.5°C in ETL and IRT measurements (Fig. 1.2). Our data from free ranging *Oplurus* confirm this observation and show large deviations with $T_{sk} > T_b$ during morning basking periods, when the animals often heat up 10°C or more

within a short period of time (Theisinger et al. unpublished data), while large deviations of $T_{sk} < T_b$ were found around midday and in the early afternoon, when the animals retreat from the heat to colder crevices (Fig. 1.4). Nevertheless, 80% of the records deviated less than 1.5°C and over 90% less than 2°C, confirming ETLs and IRTs as valuable approaches for field measurements of free ranging animals, if potential constraints by rapid changes in T_b especially in field studies are considered.

Internal gradients between the surface and the body core are considered to be negligible in models of small reptiles (< 30g; Bakken 1992). This is generally confirmed by our results from the more standardised calibration tests, during which the skin of *O. quadrimaculatus* (BM ~80g) and *Z. laticaudatus* (BM ~120g) heated up faster than the core body during phases of intense heating, while *O. saxicola* (BM ~20g) shows no clear deviation between T_{sk} and T_b (Fig. 1.2). However, data from free ranging individuals show that even animals with a BM of less than 30g may occasionally experience considerable differences between T_{sk} and T_b when undergoing intense changes in T_b (Fig. 1.4). Lags between surface and core body temperature during warming phases were also found in small lacertid lizards (BM < 10g) using an infrared camera (Luna and Font 2013). A thermal inertia between surface and core body in small species thus depends not only on the actual body size but also on the rate of heating.

The use of ETLs is mostly limited by their dimensions. Depending on the model, modifications may be possible that make the device suitable for many smaller species (e.g. in iButtons: a reduction in weight from 3g to about 1.5g; Lovegrove 2009) but might affect their functionality. Exceptions are saxicolous species like *O. saxicola*, which live in crevices barely higher than the animals themselves. An external attachment of even just a few millimetres height would restrain these animals from returning to their retreat sites. Furthermore, unmodified ETLs attached to *O. quadrimaculatus* showed such a high degree of abrasion and scratches that a modified logger, usually coated only in wax or silicone instead of a stainless steel capsule, would not last long. To further increase their accuracy, we also tried to colour the ETLs according to the animals' backs in order to mimic solar reflection and absorption. We did not find notable differences between painted and unpainted devices and thus continued with unpainted ones, which

already show a high and satisfying accuracy. It might, however, be possible to even further increase the accuracy of this method through different types of paint and colour. Compared to IRTs, ETLs offer the crucial advantage that they also record the temperature when the animal is out of sight in retreat sites or during times of inactivity and may record the completely undisturbed animal's 'thermal behaviour' over several days or even weeks, provided that the logger can be recovered.

Technical aspects to consider when using IRTs include animal size in comparison to the IRT metering point. Previous field studies on McCann's Skinks (*Oligosoma maccanni*) found a rather low relationship between T_{sk} and T_b ($r^2 = 0.586$) and great individual differences of up to 12.9°C (Hare et al. 2007) and similar problems were reported for several *Podarcis* forms (Carretero et al. 2012). Despite their effort to ensure that the target laser sights all fell on the posterior abdomen or pelvic region of the animal, Hare et al. (2007) still found a high influence of background temperature on IRT readings and reported better results if the IRT was oriented in line with the skink's body axis. Although we cannot report similar problems from our study (probably due to the larger body size of our study species), this clearly presents a limitation for IRT measurements (and should be considered when choosing a device), especially under field conditions where the difference between background and body temperature is often much greater than in laboratory studies with more consistent temperature regimes. A further consideration when using an IRT in the field is the operating temperature of the device. Depending on the device, the IRT may show abnormal temperatures if it becomes too hot (e.g. through use in direct sunlight). Although our device recovered quickly and continued to show accurate temperatures after cooling down, we recommend protecting the IRT from direct sunlight when using it in environments with extremely high temperatures. If these aspects are considered, IRTs provide a quick and easy way to measure T_{sk} in reptiles and have the advantage that measurements can be taken from larger distances without disturbing the natural behaviour of the animals (and those in close proximity). Hence a much larger number of indirect T_b measurements can be recorded per hour in contrast to conventional cloacal T_b records. For example, by implementing IRT measurements during transect walks we took up to 58 indirect T_b records per hour for *O. saxicola*. In conclusion, our results show a close relationship and high accuracy between T_{sk} and T_b in lizards of different body sizes and thus a high applicability of both

methods, ETLs and IRTs. Considerable differences between T_{sk} and T_b occurred only during phases of intense changes in T_b, and were detectable even in lizards with a BM of ~20g, suggesting that the thermal inertia depends not only on body size but also on the rate of heating. Both methods proved to be easy to use under field conditions if certain limitations are considered. We therefore generally validate the use of T_{sk} measurements as surrogates for cloacal T_b measurements in small to medium sized reptiles (20 – 120g) also for field studies, but have to emphasise the importance of restrictions related to the rate of heating, which are not necessarily apparent under standardised lab conditions. The increasing application of IRTs and other external measurements of body temperature in reptiles will expand the potential of field studies by facilitating repeated temperature records of undisturbed animals, larger sample sizes and the collection of data over several days including night times and in retreat sites.

Acknowledgements

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Author contribution

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

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Chapter Two

Acclimatization patterns in tropical reptiles: uncoupling temperature and energetics

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Abstract

The physiological compensation of changing environments through acclimatization has long been considered to be of minor importance in tropical ectotherms due to more stable climatic conditions. Contrasting this assumption are reports about a range of metabolic adjustments in tropical species, especially during the last two decades from field acclimatized animals. Metabolic rates are strongly linked to temperature in ectotherms but they also reflect energetic requirements and restrictions. We therefore postulate that the observed variety of acclimatization patterns in tropical reptiles results from an interaction of multiple influences, including food and water availability, rather than from thermal constraints alone. We present new data from two sympatric Malagasy lizards with contrasting acclimatization patterns and, complemented with an extensive literature search, discuss the variety of acclimatization patterns in tropical reptiles with regard to thermal and energetic influences. This energetic point of view allows a rearrangement of apparently controversial patterns into a scheme of decreasing metabolic costs, including two new categories for selective and selective inverse acclimatization, where metabolic shifts are restricted to body temperatures below the range of activity body temperatures.

Introduction

The study of species' responses to varying environmental conditions has a long history in biological research (Cowles and Bogert 1944) but the capacity for compensation has gained renewed attention in the face of climate change (Kearney et al. 2009; Chown et al. 2010; Somero 2010). Ectothermic species compensate environmental changes through behavioural thermoregulation or physiological adaptations (Angilletta et al. 2006) and the latter seems to be especially important if the potential for behavioural adjustments is reduced (Gunderson and Stillman 2015). Physiological responses to changes in the environment that are based on plasticity are referred to as acclimation in controlled laboratory experiments or acclimatization if they occur under natural conditions (and in the following as a generic term for acclimation and acclimatization). Based on Janzen (1967), species from the rather stable tropical environment were assumed to show only a limited capacity for physiological acclimatization in contrast to temperate species which are exposed and adapted to much greater temperature fluctuations. Although this latitudinal hypothesis has been contradicted by recent meta-analyses (Gunderson and Stillman 2015; Seebacher et al. 2015), reports about metabolic acclimatization in tropical ectotherms are scarce and controversial, ranging from no acclimatization (Feder 1982) to complete metabolic depression (Christian et al. 1999a). Acclimatization studies are generally based on changes in temperature and although metabolic rate (MR) is strongly linked to temperature in ectotherms (Angilletta 2009), it also reflects energetic requirements and restrictions. The seasonal availability of food and water, for example, is a major energetic constraint and can result in differing activity patterns and energy expenditure even between populations of the same species that experience similar thermal conditions (Christian et al. 1995). With thermal effects being less pronounced in tropical environments, energetic constraints may be more influential and can become important determinants of metabolic adjustments in tropical species (Christian and Bedford 1996).

We propose that the diversity of metabolic responses in tropical reptiles is the result of a variable interplay of thermal and energetic influences, which differ in their intensity and prevalence across the tropics. By combining new data from two sympatric Malagasy lizards and data from previous acclimatization studies on

tropical reptiles, we investigate characteristic patterns of metabolic acclimatization and relate these to possible energetic constraints.

Methods

Our study site was located in the rainforest of the Andohahela National Park (24°57'S, 46°35'E) in southeastern Madagascar. Ambient temperature ranges from 17 - 35°C during the wet season and from 14 - 33°C during the dry season (Rakotondranary et al. 2011). Our two study species occur sympatrically and are active year-round in a variety of habitats (spiny forest to rainforest). *Oplurus quadrimaculatus* is the largest species of Iguanidae in Madagascar (body mass (± SD) 76.5 ± 10.5g) and obligate saxicolous. The medium sized gerrhosaurid *Zonosaurus laticaudatus* (body mass 113.5 ± 15.9g) also inhabits large open rocks but searches its prey in crevices and the surrounding leaf litter. Mean activity body temperature (T_b) of the thermophile *Oplurus* is 37.5 ± 1.2°C, whereas the *Zonosaurus* shows a distinct bimodal T_b pattern with higher T_b (33.6 ± 1.3°C) in the morning and lower T_b (31.7 ± 2.0°C) during foraging activity (O. Theisinger, unpublished observation).

We used a portable open flow oxygen analyzer (OxBox; designed and constructed by Thomas Ruf and Thomas Paumann, Research Institute of Wildlife Ecology, Vienna) with fuel cell oxygen sensors (7OX-V CiTiceL; City Technology Ltd, Portsmouth, UK; accuracy < 0.02 vol. %) and a pull-mode respirometry set-up to conduct oxygen consumption measurements directly in the field. Measurements started in the afternoon with animals that had been captured in the morning between 0900 hours – 1200 hours and lasted for up to 24h. During this time, the natural warming from morning to afternoon, reinforced by a periodical opening of the shading tarp that covered the whole set-up, created a smooth heating curve. During the experiment, we constantly monitored the temperature inside the animal's plastic box, which served as a metabolic chamber, and finished the measurement in the afternoon when the box temperature started to decrease or ahead of schedule if an animal showed signs of overheating. We recorded the animal's skin temperature during measurements using externally attached temperature loggers (Thermochron iButtons model DS1921G; accuracy ± 1°C; weight 3.3g; Maxim Integrated Products Inc., San Jose, California, USA). Skin temperature is a suitable surrogate for T_b in these species under the given set-up (Berg et al. 2015) and we will therefore refer

to T_b rather than skin temperature in the following for an easier comparison with other studies. Using a flow rate of 40 l*h^{-1}, we sampled the animal's oxygen consumption once per minute alternated with an hourly six-minute calibration to ambient reference air. We calculated the oxygen consumption from our recordings with the following formula: MR = flow*Δ vol % O_2*10. The present analysis includes only data from the morning warming period to avoid a bias from differences between heating and cooling rates (Rismiller and Heldmaier 1985) or possible circadian influences (Bennett and Dawson 1976). We manually excluded periods of animal activity and smoothed the temperature-rate curve by calculating five minute averages.

We used non-starved rather than post-absorptive animals for our measurements because MR from fed animals provides a more ecologically relevant data base (Niewiarowsky and Waldschmidt 1992). Feeding is known to increase an animal's resting metabolic rate (RMR) and this specific dynamic action can vary at the inter- and intraspecific level (McCue 2006). However, as free ranging animals usually have food in their guts, measurements from post-absorptive animals can severely underestimate maintenance costs (Niewiarowsky and Waldschmidt 1992; Christian et al. 1997). We will therefore refer to our data as field RMR (fRMR) obtained from non-starved animals at rest during the active phase of their diurnal cycle. Our observations show no seasonal differences in foraging activity and both species defecated regularly after capture during both seasons. Although these are only indirect measures of energy intake, we are confident that the seasonal comparison of fRMR is not compromised by differences in feeding activity.

For a visual inspection of the data, we first plotted seasonal log-transformed fRMR measurements against T_b added by loess curves and their 95% confidence intervals. As our measurements include correlated data from the same individuals and an unequal number of records for each individual, we calculated a linear mixed effects model (LMM) to test for seasonal differences in log-transformed fRMR. We used the lme function in the R package nlme (Pinheiro et al. 2015) and included T_b and season as fixed factors, body mass as a covariate and individual ID as a random intercept. Body mass was averaged from before and after the experiment. We assessed the quality of the model by visually checking the model residuals with Q-Q plots and histograms. All analyses were performed in R v3.2.1 (R Development Core Team 2015). All means are given with standard deviation.

Results

We obtained 386 fRMR records from 13 *O. quadrimaculatus* and 452 fRMR records from 14 *Z. laticaudatus* distributed equally across seasons. Numbers of records per individual varied from 6 – 75 (mean = 31.0 ± 19.3) and T_b ranged from 17.5 – 41.5°C for *O. quadrimaculatus* and from 18.0 - 39.0°C for *Z. laticaudatus*.

Temperature-rate scatterplots and the corresponding loess curves indicate a seasonal divergence of fRMR at lower T_b but not at high T_b in *O. quadrimaculatus* (Fig. 2.1). To further investigate this split structure, we analysed fRMR records from this species on the one hand as a single dataset across the entire T_b range and, on the other hand, split the dataset for separated analyses across low and high T_b. We tested a range of T_b (34 - 39°C) around the meeting point of both loess curves as possible thresholds for this split. Seasons differ significantly at low T_b for all thresholds ≤ 36°C (p < 0.05, df = 10). A T_b of 36°C corresponds to the lower end of activity T_b in *O. quadrimaculatus* and also marks a threshold for behavioural changes on sunny days (O. Theisinger, unpublished observation), so a split of the dataset at 36°C also appears to be ecologically reasonable. Loess curves for *Z.*
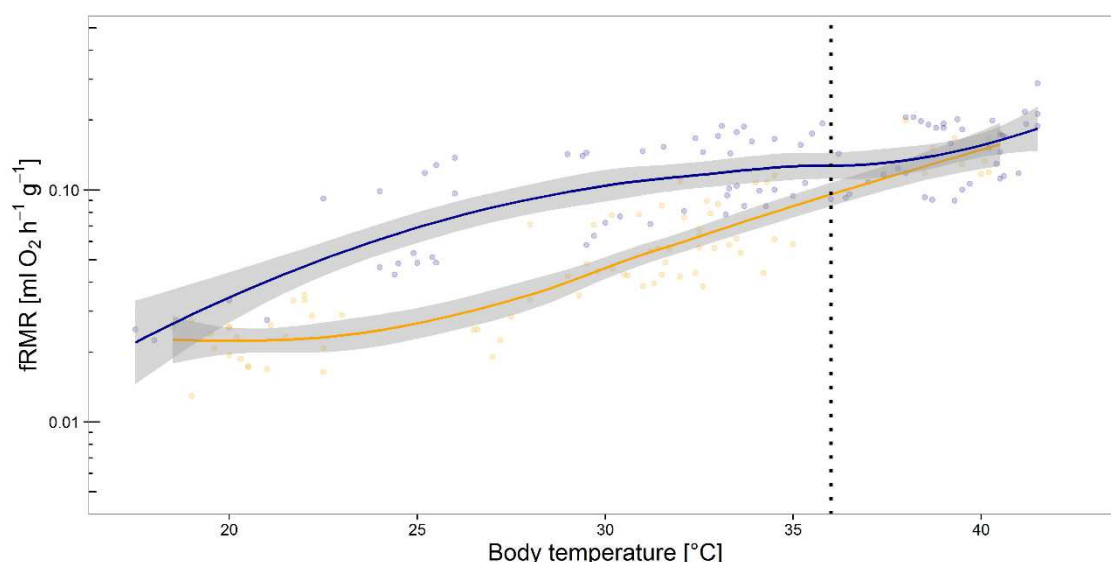
Figure 2.1 Loess curves and 95% confidence intervals of seasonal field resting metabolic rate for *Oplurus quadrimaculatus*. fRMR = field resting metabolic rate; blue = wet season; orange = dry season. The vertical dotted line marks T_b = 36°C. Multiple fRMR records per T_b from the same individual were averaged before plotting loess curves to avoid a bias in the weighting of data.

laticaudatus show no such clear pattern of diverging seasonal fRMR. We still analysed possible split data sets by gradually increasing the threshold temperature in 1°C steps across the entire range of test temperatures but found no seasonal differences above or below a certain threshold.

The effect of T_b on fRMR was always highly significant ($p < 0.001$), whereas body mass showed no significant effect in our calculations (due to the similar size of test individuals). Although figure 2.2 depicts mass-specific fRMR for ease of comparison, we used whole-animal data for calculations. The analysis of fRMR measurements across the entire T_b range in a single dataset showed no significant seasonal differences in either species ($p = 0.089$, df = 10 for *O. quadrimaculatus*; $p = 0.21$, df = 11 for *Z. laticaudatus*, Fig. 2.2). Split data sets for *O. quadrimaculatus* show significant seasonal differences at T_b below 36°C ($p < 0.05$, df = 10), but no seasonal differences above 36°C ($p = 0.80$, df = 7, Fig. 2.2).
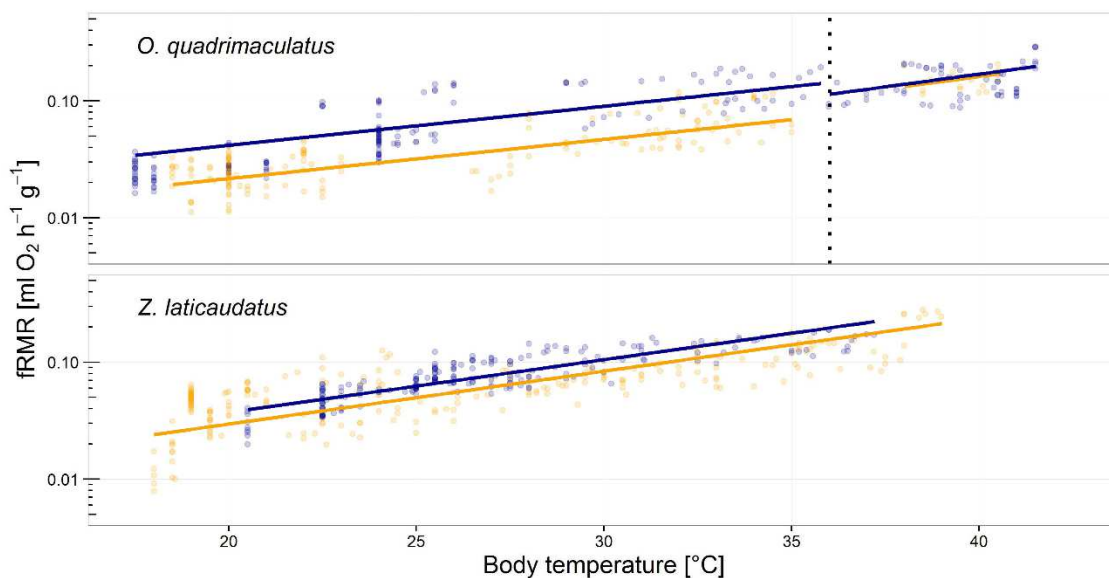
Figure 2.2 Seasonal field resting metabolic rate from *Oplurus quadrimaculatus* and *Zonosaurus laticaudatus* across a range of T_b. fRMR = field resting metabolic rate. Solid lines represent the mean population response in wet season (blue) and dry season (orange) as predicted by the linear mixed effects model (i.e. the overall mean response of individual responses), the vertical line marks the split of the dataset of *O. quadrimaculatus* at a T_b of 36°C. Seasonal fRMR differs significantly at T_b below 36.0°C ($p < 0.05$) but not above ($p = 0.80$) in *O. quadrimaculatus*, whereas *Z. laticaudatus* shows no seasonal difference in fRMR ($p = 0.21$).

Discussion

The variety of acclimatization patterns found in tropical reptiles contradicts the assumption that physiological responses are of little value in stable tropical environments (Feder 1978). While fluctuations of ambient temperature are certainly less pronounced in the tropics compared to temperate regions, other parameters, such as precipitation or food availability, can show a strong seasonality. We therefore integrate thermal aspects and energetic constraints to discuss the variety of metabolic response patterns in tropical reptiles.

Oplurus quadrimaculatus shows clear seasonal differences in fRMR at T_b below its range of activity T_b but whether a rate is elevated in one season or lowered in another (or both) can be difficult to assess in species that do not show seasonal reductions in activity (e.g. Zari 1996). *Oplurus quadrimaculatus* does not seem to experience seasonal food or water restrictions and thermoregulates precisely to a narrow range of activity T_b, at least on sunny days (O. Theisinger, unpublished observation). It therefore seems reasonable to argue against a lowered fRMR as a measure for saving energy during the dry season. However, as a heliotherm species, *O. quadrimaculatus* strongly depends on continuous basking to maintain its activity T_b. The high thermoregulatory precision is only possible on sunny days, while rainfall or cloud cover lead to reduced T_b (Fig. 2.S1). Periods of extensive cloud cover during the wet season limit basking opportunities and likely prevent *O. quadrimaculatus* from achieving high activity T_b. The higher fRMR during the wet season thus appears to be an acclimatization to compensate thermal limitations.

An elevated MR during seasons with adverse conditions has been found in several tropical ectotherms with year-round activity and is regarded as a compensatory measure to facilitate activity at lower T_b (Bennett and Dawson 1976; Tsuji 1988a). The upward shift of fRMR in *O. quadrimaculatus*, however, occurs only below the range of activity T_b. Although other studies report similar selective acclimatization at low T_b (Dutton and Fitzpatrick 1975; Wood et al. 1978; Ragland et al. 1981), few interpretations are provided for this pattern. We therefore compared literature data with activity T_b and the preferred body temperature (T_{pref}) of the respective species and found that elevated MRs generally occur only below normal activity T_b and are absent at or above this threshold (see supplementary material). Some studies report comprehensive acclimatization across all test temperatures but these studies did not include T_{pref} in their test temperatures and it thus remains

unclear whether these animals show selective acclimatization or overcompensate at higher T_b (Wheeler 1986; Zari 1996). The range of activity T_b and T_{pref} both represent temperatures at which physiological processes function as close to the thermal optimum as possible: either under natural conditions (activity T_b) or in the absence of ecological restrictions under laboratory conditions (T_{pref}; Angilletta et al. 2002). However, lower ambient temperature or limited basking opportunities may prevent species from reaching these T_b during seasons with adverse weather conditions. An elevated MR below normal activity T_b then facilitates performance near optimal levels despite lower T_b. Selective acclimatization thus expands the range of activity T_b in contrast to a shift of the whole MR curve, which would lead to overcompensation at high T_b and excessive energy expenditure during occasional warm or sunny days. Selective acclimatization still increases energetic costs at low T_b but at the same time permits year-round activity in species which are seasonally limited only by temperature but not food availability.

Many tropical ectotherms are, however, seasonally limited by reduced food resources or a shortage of water (Christian et al. 1999a). Species with reduced food availability are energetically restricted and, rather than sustain an elevated MR, need to reduce their energy demand. In addition to reduced activity levels, winter-inactive or less active species therefore often exhibit inverse acclimatization with a seasonally lowered MR during adverse conditions (Tsuji 1988a). Previous studies report two general patterns of inverse acclimatization: metabolic shifts restricted to low T_b, i.e. selective inverse acclimatization (e.g. Al-Sadoon and Spellerberg 1985b; Zari 1997) and shifts across all test temperatures, i.e. comprehensive inverse acclimatization (e.g. Ragland et al. 1981; Christian et al. 1996b, 1999b; Hailey and Loveridge 1997; Zari 1999, 2013). Similar to selective acclimatization, selective inverse acclimatization also occurs only below the threshold of activity T_b or T_{pref}, while MR at higher T_b shows no acclimatization effects. This partition offers a combination of optimal performance during activity and energy saving during inactivity. The gecko *Hemidactylus flaviviridis* for example, shows a lowered MR only at 20°C but not above. This species is inactive at temperatures below 20°C but active on warmer winter days with more than 20°C (Zari 1997). A lowering of the complete MR curve would prevent this species from being able to catch their prey during activity or reduce their ability to escape predators during foraging. Although Zari (1997) did not include data about food abundance, the selective pattern seems reasonable if food availability is reduced but still sufficient to cover

occasional days of normal activity. Comprehensive inverse acclimatization or a 'complete shutdown' on the other hand offers the greatest saving potential and is found in dormant species (e.g. Ragland et al. 1981; Hailey and Loveridge 1997) and those with a highly reduced winter activity (e.g. Christian et al. 1996b, 1999b). Although it is difficult to quantify the extent of 'reduced' concerning food consumption and activity from the available literature, data from Christian et al. (1996b, 1999b) indicate that comprehensive metabolic reductions are linked with substantial reductions in food consumption (-50% volume food in stomach; Christian et al. 1996b) and activity (-80% in the number of individuals during peak activity; Christian et al. 1999b). The limited energy acquisition requires great energy savings which can only be achieved through a complete downshift in MR and may still be accompanied by the exploitation of body fat reserves (Christian et al. 1999b). The extent of inverse acclimatization therefore depends on the extent of energetic limitation and includes selective shifts at temperatures below T_{pref} / activity T_b as well as comprehensive shifts across all T_b. Similar to a reduction in food availability, water shortages may also elicit metabolic depression (Christian et al. 1995) but available information is extremely limited in that context.

Finally, some tropical ectotherms show no metabolic acclimatization. This can, on the one hand, be an issue related to laboratory conditions, which may not necessarily provoke a species' full acclimation capacity. Direct comparisons of laboratory and field conditions are scarce but these few studies found contrasting patterns between acclimated and acclimatized individuals (Dutton and Fitzpatrick 1975; Gatten et al. 1988; Beyer and Spotila 1994). Especially species that are seasonally limited by factors other than temperature and acclimatize, for example, to compensate food shortages may not show acclimation in response to artificial temperature changes (Gatten et al. 1988). Alternatively, animals may have been captured at the 'wrong' time of the year (see *Anolis carolinensis* in Table 2.1). On the other hand, while phylogenetic factors may account for a true lack of capacity in some species, the pure absence of acclimatization does not necessarily equal an incapacity. A consideration of seasonal differences in daily T_b patterns could reveal that a species is simply unaffected by seasonal changes or that it sufficiently compensates through lowered T_b during times of inactivity. Our study species *Z. laticaudatus*, for example, is rather unaffected by limited basking opportunities due to its shade-based activity. As ambient temperature varies little between seasons and food sources seem to be sufficient, we assume that this species may not

experience energetic restrictions that require metabolic acclimatization. In other species, reductions in T_b may provide sufficient energy savings to render physiological compensation unnecessary. A 4°C reduction in night-time T_b of *Varanus mertensi*, for example, corresponds to a reduction of > 30% in standard MR, which seems sufficient to facilitate the maintenance of similar daytime T_b and activity across seasons (Christian et al. 1996c). Species with more limited energy resources may supplement a reduced activity with lower T_b (Christian et al. 2003, 2007).

In summary, we found a total of four patterns of metabolic adjustments in tropical reptiles: elevated MR at low T_b, no change in MR, lowered MR at low T_b and lowered MR across all T_b (Table 2.1). Some of these acclimatization patterns are similar to the commonly cited categories described by Precht (1958, in Prosser 1969), although his scheme is originally based on measurements of only two temperatures rather than a comparison of curves. Prosser's (1969) scheme compares MR across a range of temperatures but only describes complete shifts of entire curves. As outlined above, acclimatization curves with a sharp inflection point (i.e. acclimatization only at low T_b) are not uncommon in tropical reptiles. However, none of these studies explicitly recognized the range of activity T_b or T_{pref} as the threshold for selective and selective inverse acclimatization. Based on Precht's (1958, in Prosser 1969) scheme but adopted for temperature-rate curves across more than two test temperatures (including the range of activity T_b), we therefore summarize the observed acclimatization patterns into the following revised categorization scheme, which corresponds to a scheme of decreasing metabolic costs (Fig. 2.3, Table 2.1):

(1) *comprehensive acclimatization*

> – an elevated MR across temperatures; leads to overcompensation at high T_b [no example from tropical species found in literature]

(2) *selective acclimatization*

> – an elevated MR at low T_b but not within the range of activity T_b; found in winter-active species restricted only by lowered T_b; combines improved performance at low T_b with stable energy expenditure at high T_b

(3) *no acclimatization*

– MR similar between seasons; found in species with any level of activity which are either not influenced by constraints or compensate sufficiently through alternative mechanisms, e.g. behavioural thermoregulation

(4) *selective inverse acclimatization*

– a lowered MR at low T_b but not within the range of activity T_b; mainly found in species with reduced winter activity restricted by reduced food availability which may or may not be accompanied by lowered T_b; combines energy saving at low T_b with a stable performance level at high T_b

(5) *comprehensive inverse acclimatization*

– a lowered MR across temperatures; found in species with highly reduced winter activity and winter-inactive species restricted by severe food shortages and/or constantly low T_b; permits greatest energy savings but with decreased performance
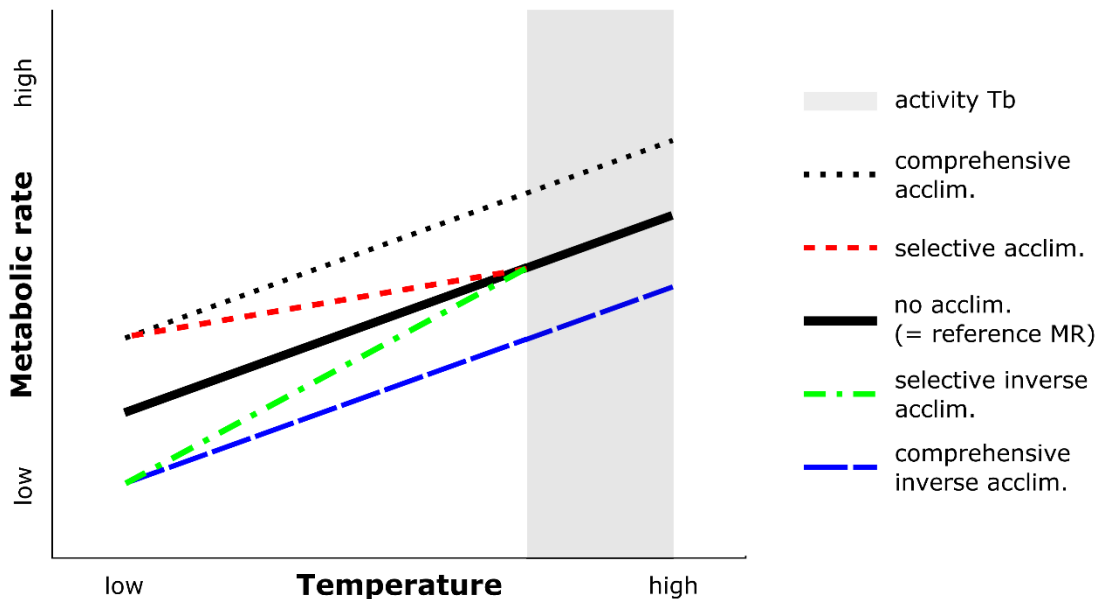
Figure 2.3 Scheme of acclimatization patterns in tropical reptiles in combination with the range of activity T_b to illustrate the threshold for selective and selective inverse acclimatization.

The common view that tropical ectotherms show little or no acclimatization responses is based on fluctuating temperatures as the most important limiting parameter (Janzen 1967) but temperature effects alone cannot explain the variety of physiological responses in tropical reptiles. The above discussion shows that additional constraints, such as the seasonal availability of food or precipitation, can be crucial determinants of metabolic adjustments. These influences vary in their intensity and prevalence across the tropics and, as our data from two Malagasy lizards show, even between sympatric species within the same habitat. This diversity of dominating influences explains the observed variety of acclimatization patterns in tropical reptiles and by integrating thermal and energetic constraints we were able to rearrange apparently controversial patterns into a scheme of decreasing metabolic costs. Acclimatization studies rarely include ecological information and data about activity levels or food availability is scarce. Yet, this energetic point of view provides a better understanding of metabolic acclimatization in tropical lizards and thus emphasises the importance of ecological perspectives for physiological studies.

Acknowledgements

Table 2.1 Patterns of metabolic acclimatization in tropical reptiles. The corresponding level of activity during seasons with adverse conditions is given for each species. (For detailed information about the range of activity T_b/T_{pref} and the experimental temperatures see supplementary material.)

Acclimatization pattern	Species	Activity level	Metabolic costs
(1) comprehensive acclimatization: - an **elevated MR across all T_b**	*no example from tropical species*		
(2) selective acclimatization: - an **elevated MR at low T_b** but not within the range of activity T_b	*Anolis carolinensis*[1,11]** *Malacochersus tornieri*[2] *Oplurus quadrimaculatus*[3] *Sceloporus olivaceus*[4]	reduced activity / active active	
- possibly selective acclimatization*:	*Acanthodactylus boskianus*[5] *Cordylus jonesi*[6]	active active	
(3) no acclimatization: - a **similar MR between seasons**	*Acanthophis praelongus*[7] *Anolis gundlachi*[8] *Mabuya striata striata*[9] *Oedura marmorata*[10] *Sceloporus jarrovi*[11] *Sceloporus variabilis*[12] *Tiliqua scincoides*[13] *Urosaurus ornatus*[14] *Varanus mertensi*[15] *Zonosaurus laticaudatus*[3]	reduced activity active active active reduced activity active reduced activity active active active	
(4) selective inverse acclimatization: - a **lowered MR at low T_b** but not within the range of activity T_b	*Chalcides ocellatus*[16] *Hemidactylus flaviviridis*[17]	reduced activity reduced activity	
(5) comprehensive inverse acclimatization: - a **lowered MR across all T_b**	*Chelonoidis carbonaria*[18] *Chlamydosaurus kingii*[19] *Cnemidophorus sexlineatus*[1] *Kinixys spekii*[20] *Lophognathus temporalis*[21] *Mabuya brevicollis*[22] *Ptyodactylus hasselquistii*[23] *Varanus scalaris*[24]	reduced activity reduced activity inactive inactive reduced activity reduced activity reduced activity reduced activity	

Sources: [1]Ragland et al. 1981; [2]Wood et al. 1978; [3]this study; [4]Dutton and Fitzpatrick 1975; [5]Zari 1996; [6]Wheeler 1986; [7]Christian et al. 2007; [8]Rogowitz 1996; [9]Patterson 1984; [10]Christian et al. 1998; [11]Gatten 1985; [12]Tsuji 1988b; [13]Christian et al. 2003; [14]Payne and Gatten 1988; [15]Christian et al. 1996c; [16]Al-Sadoon and Spellerberg 1985b; [17]Zari 1997; [18]Santos-Pinto et al. 1985; [19]Christian et al. 1996b; [20]Hailey and Loveridge 1997; [21]Christian et al. 1999b; [22]Zari 2013; [23]Zari 1999; [24]Christian et al. 1996a.

*T_{pref} not included in measurements, see Discussion.

**Anolis* collected in January show selective acclimation (Ragland et al. 1981), while *Anolis* collected in April and September show no acclimation (Gatten 1985).

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Supplementary material

Figure 2.S1 Daily profile of T_b (brown line) and ambient temperature (blue line) of *Oplurus quadrimaculatus* on a rainy day and the following sunny day, where the animal resumes its normal activity pattern. Shaded areas mark the scotophase.

opposite page and following:

Table 2.S2 List of acclimatization data for tropical reptiles. The table shows T_{pref} or activity T_b for each species, the range of test temperatures at which metabolic rate (MR) was measured and at which of those differences in MR were found among acclimatization groups as well as the corresponding acclimatization pattern.

Species	Activity during adverse season	T_{pref} / activity T_b [°C]	Test temperatures [°C]	Differences in MR at test temperature [°C]	No differences in MR at test temperature [°C]	Acclimation / acclimatization pattern	Notes	References	Additional references (if activity level / T_{pref} were not given in original publication)
Acanthodactylus boskianus	active	35-43	20,25,30,35	20,25,30,35	/	comprehensive acclim. (but test temperatues do not include T_{pref})		Zari TA (1996) Seasonal metabolic compensation in the fringe-toed lizard, *Acanthodactylus boskianus* (Reptilia: Lacertidae). J Therm Biol 21:145-150	Duvdevani I, Borut A (1974) Mean body temperature and heat absorption in four species of *Acanthodactylus* lizards (Lacertidae). Herpetologica 30:176-181
Acanthophis praelongus	reduced activity	30-33.5	18,24,30	/	18,24,30	no acclim.	seasonal difference field T_b	Christian KA, Webb JK, Schultz T, Green B (2007) Effects of seasonal variation in prey abundance on field metabolism, water flux, and activity of a tropical ambush foraging snake. Physiol Biochem Zool 80:522-533	Bedford GS, Christian KA (1998) Standard metabolic rate and preferred body temperatures in some Australian pythons. Aust J Zool 46:317-328
Anolis carolinensis	reduced activity	28-36	10,20,30	10,20	30	selective acclim.	collected in January; see difference to Gatten (1985)	Ragland IM, Wit LC, Sellers JC (1981) Temperature acclimation in the lizards *Cnemidophorus sexlineatus* and *Anolis carolinensis*. Comp Biochem Physiol A 70:33-36	Licht P. (1968) Response of the thermal preferendum and heat resistance to thermal acclimation under different photoperiods in the lizard *Anolis carolinensis*. Am Midland Nat 79:149-158
Anolis carolinensis	reduced activity	28-36	10,20,30	/	10,20,30	no acclim.	collected in April & September; see difference to Ragland et al. (1981)	Gatten RE (1985) Activity metabolism of lizards after thermal acclimation. J Therm Biol 10:209-215	Licht P. (1968) Response of the thermal preferendum and heat resistance to thermal acclimation under different photoperiods in the lizard *Anolis carolinensis*. Am Midland Nat 79:149-158
Anolis gundlachi	active	25	15,30	30 (prob. heat stress - lost weight)	15	no acclim.	altitudinal acclim.; doesn't use behavioural thermoregulation	Rogowitz GL (1996) Evaluation of thermal acclimation and altitudinal variation of metabolism in a neotropical lizard, *Anolis gundlachi*. Copeia 1996:535-542	Huey RB, Webster TP (1976) Thermal biology of *Anolis* lizards in a complex fauna: the christatellus group on Puerto Rico. Ecology 57:985-994
Chalcides ocellatus	reduced activity	28-37	10,15,20,25,30,35	10	15,20,25,30,35	selective inverse acclim.		Al-Sadoon MK, Spellerberg IF (1985a) Comparison of thermal acclimation effects on the metabolism of *Chalcides ocellatus* (desert lizard) and *Lagerta vivipara* (cool-temperate lizard). Comp Biochem Physiol A 81:939-943	
Chelonoidis carbonaria	reduced activity	27	15,20,30	15,20,30 except 15 & 20 acclim no diff at 30	15 & 20 acclim no diff at 30	comprehensive inverse acclim.		Santos-Pinto FN, Griggio MA, Russo AK, Tarasantchi J (1985) The influence of temperature acclimation on O2-consumption in the turtle *Geochelone carbonaria*. Comp Biochem Physiol A 82:859-861	Hedman H, Muñiz Pagan, D. N., and Powell, R. (2010). *Chelonoidis carbonaria* (red-footed tortoise): size and thermal biology. Herpetol Rev 41:484-485 Noss AJ, Montaño F RR, Soria F, Deem SL, Fiorello CV, Fitzgerald LA (2013) Chelonoidis carbonaria (Testudines: Testudinidae) activity patterns and burrow use in the Bolivian Chaco. S Am J Herpetol 8:19-28

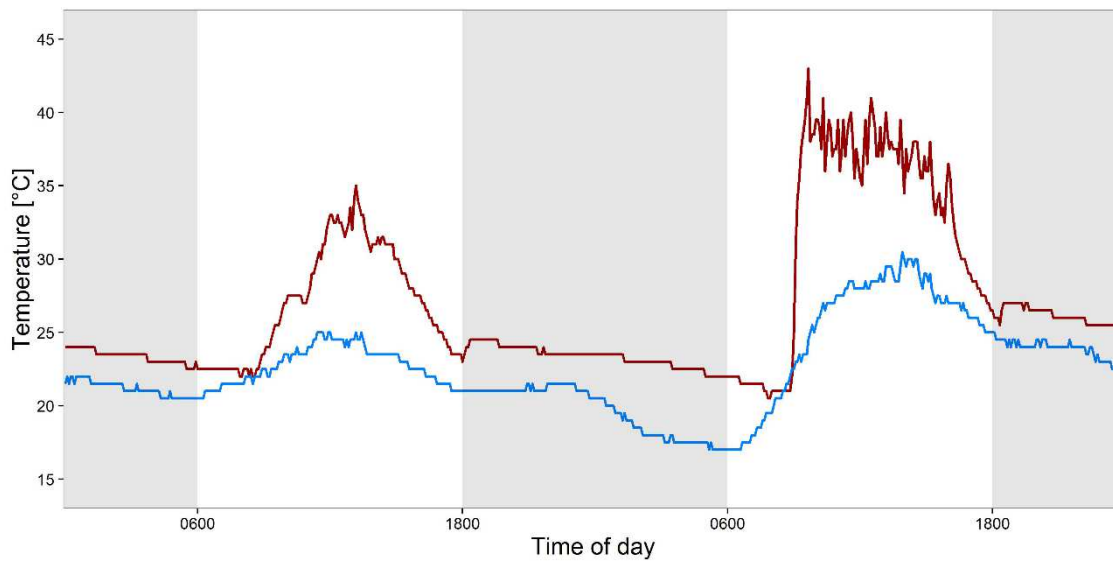| Species	Activity during adverse season	T_{pref} / activity T_b [°C]	Test temperatures [°C]	Differences in MR at test temperature [°C]	No differences in MR at test temperature [°C]	Acclimation / accclimatization pattern	Notes	References	Additional references (if activity level / T_{pref} were not given in original publication)
Chlamydosaurus kingii	reduced activity	31-34.5	18,24,30,36	all	/	comprehensive inverse acclim.	seasonal difference field T_b	Christian KA, Griffiths AD, Bedford GS (1996b) Physiological ecology of frillneck lizards in a seasonal tropical environment. Oecologia 106:49-56	Christian KA, Bedford GS (1995) Seasonal changes in thermoregulation by the frillneck lizard, *Chlamydosaurus Kingii*, in tropical Australia. Ecology 76:124-132
Cnemidophorus sexlineatus	inactive	35	10,20,30	in lab: 30; in seas. acclim.; all	in lab: 10,20; seas. acclim.: /	comprehensive inverse acclim.		Ragland IM, Wit LC, Sellers JC (1981) Temperature acclimation in the lizards *Cnemidophorus sexlineatus* and *Anolis carolinensis*. Comp Biochem Physiol A 70:33-36	in Qu Y, Li H, Gao J, Xu X, Ji X (2011) Thermal preference, thermal tolerance and the thermal dependence of digestive performance in two *Phrynocephalus lizards* (Agamidae), with a review of species studied. Curr Zool 57:684-700
Cordylus jonesi	active	33.5	20,30	20,30	/	comprehensive acclim. (but test temperatues do not include T_{pref})		Wheeler PE (1986) Thermal acclimation of metabolism and preferred body temperature in lizards. J Therm Biol 11:161-166	
Hemidactylus flaviviridis	reduced activity, inactive if Ta < 20°C	22-33; inactive if Ta < 20°C	20,25,30,35	20	25,30,35	selective inverse acclim.		Zari TA (1997) Effects of body mass, temperature, and season on resting metabolism of the nocturnal gecko *Hemidactylus flaviviridis*. Zool Middle East 14:77-86	
Kinixys spekii	inactive	27	10,15,20,25	10,15,20,25	/	comprehensive inverse acclim.		Hailey A, Loveridge JP (1997) Metabolic depression during dormancy in the African tortoise *Kinixys spekii*. Can J Zool 75:1328-1335	Hailey A, Coulson IM (1996) Temperature and the tropical tortoise *Kinixys spekii*: constraints on activity level and body temperature. J Zool 240:523-536
Lophognathus temporalis	reduced activity	35	18,24,30,36	all	/	comprehensive inverse acclim.	seasonal difference field T_b	Christian KA, Bedford G, Green B, Griffiths A, Newgrain K, Schultz T (1999a) Physiological ecology of a tropical dragon, *Lophognathus temporalis*. Austr J Ecol 24:171-181	
Mabuya brevicollis	reduced activity	31-38	20,25,30,35,40	all	/	comprehensive inverse acclim.		Zari TA (2013) Seasonal acclimation in resting metabolism of the skink, *Mabuya brevicollis* (Reptilia: Scincidae) from southwestern Saudi Arabia. J Therm Biol 38:449-453	
Mabuya striata striata	active	33.5	5,10,15,20,25,30, 35	35	5,10,15,20,25,30	no acclim.	low altitude subspecies	Patterson JW (1984) Thermal acclimation in two subspecies of the tropical lizard *Mabuya striata*. Physiol Zool 57:301-306	
Malacochersus tornieri			20,35	20	35	selective acclim.	no T_{pref} available for this species	Wood SC, Lykkeboe G, Johansen K, Weber RE, Maloiy GM (1978) Temperature acclimation in the pancake tortoise, *Malacochersus tornieri*: metabolic rate, blood pH, oxygen affinity and red cell organic phosphates. Comp Biochem Physiol A 59:155-160	

Species	Activity during adverse season	T_{pref} / activity T_b [°C]	Test temperatures [°C]	Differences in MR at test temperature [°C]	No differences in MR at test temperature [°C]	Acclimation / acclimatization pattern	Notes	References	Additional references (if activity level / T_{pref} were not given in original publication)
Oedura marmorata	active	wet: 34, dry: 31	18,24,30,36	/	all	no acclim.	seasonal difference T_{pref}	Christian KA, Bedford G, Green B, Schultz T, Newgrain K (1998) Energetics and water flux of the marbled velvet gecko (*Oedura marmorata*) in tropical and temperate habitats. Oecologia 116:336-342	
Oplurus quadrimaculatus	active	36-39	continuous 17.5 - 41.5	< 36	≥ 36	selective acclim.		this article	Theisinger O, Berg W, Dausmann KH (in review). Compensation of thermal constraints along a natural environmental gradient in a Malagasy iguanid lizard (*Oplurus quadrimaculatus*). J Therm Biol
Ptyodactylus hasselquistii	reduced activity, inactive if T_a < 20°C	21-38; inactive if T_a < 20°C	20,25,30,35	all	/	comprehensive inverse acclim.		Zari TA (1999) Seasonal acclimatization in metabolic rate of the fan-fingered gecko, *Ptyodactylus hasselquistii* (Reptilia: Gekkonidae). J Therm Biol 24:137-142	Arad Z, Raber P, Werner YL (1989) Selected body temperature in diurnal and nocturnal forms of *Ptyodactylus* (Reptilia: Gekkoninae) in a photothermal gradient. J Herpetol 23:103-108 Werner YL, Goldblatt A (1978) Body temperature in a basking gekkonid lizard, Ptyodactylus hasselquistii (Reptilia, Lacertilia, Gekkonidae). J Herpetol 12:408-411
Sceloporus jarrovi	reduced activity	34.5	10,20,30	10	20,30	selective acclim. (but only far below T_{pref})		Gatten RE (1985) Activity metabolism of lizards after thermal acclimation. J Therm Biol 10:209-215	Beal MS, Lattanzio MS, Miles DB (2014) Differences in the thermal physiology of adult Yarrow's spiny lizards (*Sceloporus jarrovii*) in relation to sex and body size. Ecol Evol 4:4220-4229
Sceloporus olivaceus	active	28-35	15,20,25,30	acclimated: 20,25,30; acclimatized: 15,20	acclimated: 15; acclimatized: 25,30	selective acclim.	in acclimatization groups: only 15 really acclimatised to 15 (inactive), others beh. thermoreg to higher T_b	Dutton RH, Fitzpatrick LC (1975) Metabolic compensation to seasonal temperatures in the rusty lizard, *Sceloporus olivaceus*. Comp Biochem Physiol A 51:309-318	Fitzpatrick LC, Hughes JL, Venables BJ (1978) Relations between preferred body temperatures and metabolic compensation in the rusty lizard *Sceloporus olivaceus*. Comp Biochem Physiol A 59:13-16
Sceloporus variabilis	active	33.5-37	10,16,35	10	16,35	no acclim.		Tsuji JS (1988b) Thermal acclimation of metabolism in *Sceloporus* lizards from different latitudes. Physiol Zool 61:241-253	in Andrews RM (1998) Geographic variation in field body temperature of *Sceloporus* lizards. J Therm Biol 23:329-334
Tiliqua scincoides	reduced activity	wet: 33, dry: 24	18,24,30,36	/	18,24,30,36	no acclim.		Christian KA, Webb JK, Schultz TJ (2003) Energetics of bluetongue lizards (*Tiliqua scincoides*) in a seasonal tropical environment. Oecologia 136:515-523	

Species	Activity during adverse season	T_{pref} / activity T_b [°C]	Test temperatures [°C]	Differences in MR at test temperature [°C]	No differences in MR at test temperature [°C]	Acclimation / accclimatization pattern	Notes	References	Additional references (if activity level / T_{pref} were not given in original publication)
Urosaurus ornatus	active	37.5	5,35	/	5,35	no acclim.	both cold and warm acclim. animals had access to 50°C T_b, tanks differed only in T_{min}	Payne JC, Gatten RE (1988) Thermal acclimation of activity metabolism in desert lizards (*Urosaurus graciosus* and *U. ornatus*). J Therm Biol 13:37-42	Licht P (1965) The relation between preferred body temperatures and testicular heat sensitivity in lizards. Copeia 1965:428-436
Varanus mertensi	active	33-35.5	18,24,30,36	/	all	no acclim.	seasonal difference T_b	Christian KA, Weavers BW, Green B, Bedford GS (1996c) Energetics and water flux in a semiaquatic lizard, *Varanus mertensi*. Copeia 1996:354-362	Christian KA, Weavers BW (1996) Thermoregulation of monitor lizards in Australia: an evaluation of methods in thermal biology. Ecol Monogr 66:139-157
Varanus scalaris	reduced activity	wet: 38, dry: 35	18,24,30,36	all	/	comprehensive inverse acclim.		Christian KA, Green B, Bedford G, Newgrain K (1996a) Seasonal metabolism of a small, arboreal monitor lizard, *Varanus scalaris*, in tropical Australia. J Zool 240:383-396	Christian KA, Bedford G (1996) Thermoregulation by the spotted tree monitor, *Varanus scalaris*, in the seasonal tropics of Australia. J Therm Biol 21:67-73
Zonosaurus laticaudatus	active	30-34	continuous 18-39	/	all	no acclim.		this article	Theisinger O, Berg W, Dausmann KH (in review). Ecological constraints in the thermal biology of heliotherm lizards. Naturwissenschaften

Author contribution

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

Hamburg,

Prof. Dr. Kathrin Dausmann

Chapter Three

Contrary metabolic responses to climatic variation across habitats and seasons in two sympatric Malagasy lizards

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Abstract

The compensation of environmental variation is a crucial factor for survival, especially in ectotherms, but our understanding about the potential and possible interactions of behavioural and physiological mechanisms is still limited. We studied metabolic responses to climatic variation between habitats and seasons in two sympatric Malagasy lizards and additionally discuss their compensatory potential in relation to behavioural thermoregulation. *Oplurus quadrimaculatus* shows no metabolic adjustments to different thermal environments but clear seasonal acclimatization, while *Zonosaurus laticaudatus* compensates differences in the thermal environment but not seasonal variation. Both species do not differ in their operative environmental temperature but differences in foraging ecology strongly influence their thermal biology (open habitat vs. shade based activity) and thus their capacity for behavioural thermoregulation. Our results show that ecology can be an even stronger determinant of compensatory capacity than the thermal environment in some species, which emphasises the importance of integrating ecological aspects into physiological studies. Furthermore, physiological adjustments seem to be rather a secondary mechanism that comes into effect if behavioural thermoregulation is restricted but the prevalence of mechanisms may change even at the intraspecific level.

Introduction

Understanding the capacity and potential of species to cope with climatic changes is one of the most pressing issues of current biology. Unless species are able to either evade or compensate for unsuitable conditions, global warming is predicted to cause widespread extinctions across taxa and especially among ectotherms (Thomas et al. 2004; Deutsch et al. 2008; Sinervo et al. 2010; Bestion et al. 2015). Considering the progressive fragmentation of landscapes that often impairs dispersal, many species will be forced to persist *in situ* and compensate environmental changes through genetic adaptation or behavioural and physiological plasticity (Travis 2003; Opdam and Wascher 2004; Urban et al. 2014). The potential and relative importance of these mechanisms are still not fully understood though. The rapid pace of global warming may exceed adaptation rates in many species (Bradshaw and Holzapfel 2006; Sinervo et al. 2010; Quintero and Wiens 2013), even though adaptive responses could be accelerated in anthropogenic contexts (Hendry et al. 2008). Plastic responses are reversible and more rapid than genetic adaptation and facilitate compensatory adjustments within the lifetime of an individual (Angilletta et al. 2002).

Behavioural plasticity is widespread in ectotherms (Angilletta 2009) and can be highly efficient in the compensation of climatic differences (Adolph 1990; Muñoz et al. 2014), provided that suitable microhabitats and sufficient spatial heterogeneity are available (Kearney et al. 2009; Scheffers et al. 2014; Sears and Angilletta 2015). The importance of physiological plasticity, on the other hand, is less clear. A recent meta-analysis of adjustments in physiological processes, for example, found an increasing compensatory capacity with decreasing latitude for ectotherms in general (Seebacher et al. 2015), which contradicts the common view that tropical ectotherms exhibit only a limited acclimatization capacity due to more stable environmental conditions (Janzen 1967; Deutsch et al. 2008). However, plastic responses are highly variable in reptiles, and especially in tropical species (Seebacher et al. 2015; Berg et al. in review), which challenges a latitudinal cline in either direction.

This high variability in reptiles may be due to a varying interplay between behavioural and physiological compensation measures. Reptile species often have a high potential for behavioural thermoregulation and these behavioural responses may reduce the need, or possibly even the capacity, for physiological adjustments

by buffering environmental variation (Huey et al. 2003; Buckley et al. 2015; Berg et al. in review). In contrast, aquatic ectotherms inhabit an environment with high thermal conductance, where the behavioural compensation of temperature fluctuations is severely limited. Many of these species therefore show pronounced physiological responses (Johnson and Bennett 1995, Wilson et al. 2007, Condon et al. 2010) and an overall greater capacity for physiological acclimatization than terrestrial ectotherms (Seebacher et al. 2015). Behavioural and physiological mechanisms are not mutually exclusive though. Combined responses can optimize an organism's performance (Glanville and Seebacher 2006; Hadamová and Gvoždík 2011; Seebacher 2005) and a recent investigation of the 'behaviour-physiology nexus' showed that the prevalence of behavioural and physiological responses may even vary seasonally within a single species (Basson and Clusella-Trullas 2015). The observed variety of physiological adjustments in reptiles could therefore also be based on differences in realized plasticity rather than varying capacity.

A better understanding of physiological compensation is in part hindered by a scarcity of empirical data. Available data is often heavily geographically biased and especially tropical regions are highly undersampled (Pacifici et al. 2015; Seebacher et al. 2015). In addition, compensatory mechanisms have mostly been studied individually which ignores possible interactions of behaviour and physiology. However, studies that integrate behavioural and physiological aspects are even more scarce and further research is needed with a special focus on tropical reptiles.

The island of Madagascar is not only a biodiversity hotspot (Myers et al. 2000) but has also recently been identified as a hotspot of species vulnerable to climate change (Pacifici et al. 2015). Southeastern Madagascar offers a unique natural set-up where an extremely steep environmental gradient connects cool rainforest and hot dry spiny forest within only a few kilometres. The considerable climatic differences coupled with seasonal effects of wet and dry season present ideal conditions to study species' responses to changing environmental conditions. We measured metabolic rate, as a key component of animal physiology, in two sympatric lizards (*Oplurus quadrimaculatus* and *Zonosaurus laticaudatus*) to assess their potential for metabolic compensation and possible drivers of physiological (in)capacity. We investigated whether these species differ in their metabolic response to climatic variation across habitats and seasons and whether

possible differences relate to ecological differences between these species and/or an interaction with behavioural thermoregulation.

Methods

Study site & study species

Our study took place in the Andohahela National Park (24°57'S, 46°35'E) in south-eastern Madagascar. The area comprises a steep environmental gradient at the southern slopes of the Anosy Mountains from rainforest at higher elevations in the east across a mosaic of transitional forest patches and gallery forest along rivers to dry spiny forest in the west. The climatic extremes of spiny and rainforest are in some places only five kilometres apart but can differ more than 6°C in mean ambient temperature (T_a) and range in precipitation from 2400 mm/yr in the rainforest to 400 mm/yr in the spiny forest (Goodman 1999; Rakotondranary et al. 2011).

We chose two lizard species for our studies which occur sympatrically on rocky plateaus along the entire gradient (rainforest to spiny forest) and which are both active year-round. *Oplurus quadrimaculatus* is the largest Iguanidae of Madagascar (body mass ± SD: 76.5 ± 10.5g) and an obligate saxicolous species. The sit-and-wait forager feeds mainly on small flying insects and thermoregulates precisely to a high activity body temperature (T_b) that ranges between 36 - 39°C (Theisinger et al. in review_1). The gerrhosaurid *Zonosaurus laticaudatus* (body mass ± SD: 113.5 ± 15.9g) also inhabits large open rocks but actively searches its prey in crevices and the surrounding leaf litter. Its diet is more varied including insects, small vertebrates and invertebrates as well as flowers and fruit. This species shows a lower and broader range of activity T_b (30 - 34°C) and, moreover, a distinct daily pattern that is divided into a morning basking period with slightly higher T_b and a period of relative thermoconformity during subsequent foraging activity (Theisinger et al. in review_1).

Ambient temperature

We installed data loggers (Thermochron iButtons model DS1921G; accuracy ± 1°C; Maxim Integrated Products Inc., San Jose, California, USA) in each habitat type in full shade and protected from wind and rain at approximately 50cm height. We recorded T_a every 30 minutes from January – April 2011 (wet season 2011),

September – November 2011 (dry season 2011) and January – April 2012 (wet season 2012). For comparisons of T_a over the course of the day, we averaged each half hour record across seasons for a habitat comparison (Fig. 3.1), per season for each habitat for a comparison of seasons (Fig. 3.2, left) and per month for an exemplary illustration of T_a variation between months (Fig. 3.2, right). Habitat differences were first calculated for each half hour record of each day and subsequently averaged across seasons.

Metabolic rate

We conducted oxygen consumption measurements in the field using a portable open flow oxygen analyser (OxBox; designed and constructed by Thomas Ruf and Thomas Paumann, Research Institute of Wildlife Ecology, Vienna). Measurements started in the afternoon with animals that had been captured in the morning between 0900 hours – 1200 hours and lasted for up to 24h. We used the natural warming from morning to afternoon, added by a periodical opening of the shading tarp that covered the whole set-up, to obtain a smooth heating curve. We constantly monitored the temperature inside the animal's plastic box, which served as a metabolic chamber, and finished the experiment in the afternoon when the box temperature decreased or ahead of schedule if an animal showed signs of overheating. As skin temperature proved to be a suitable surrogate for T_b in these species under the given set-up (Berg et al. 2015), we used this non-invasive method to record the animal's skin temperature during measurements with externally attached Thermochron iButtons and will refer to records as T_b for ease of comparison with other studies. We used a flow rate of 40 $l*h^{-1}$ and sampled the animal's oxygen consumption once per minute alternated with an hourly six-minute calibration to ambient reference air. We calculated the oxygen consumption from our recordings with the following formula (MR = metabolic rate): MR = flow* Δ vol % O_2*10. The present analysis includes only data from the morning warming period to avoid a bias from differences between heating and cooling rates (Rismiller and Heldmaier 1985) or possible circadian influences (Bennett and Dawson 1976). We manually excluded periods of animal activity and smoothed the temperature-rate curve by calculating five minute averages.

We used non-starved, rather than post-absorptive, animals for our measurements because metabolic rate from fed animals provides a more ecologically relevant data

base (Niewiarowski and Waldschmidt 1992). Feeding is known to increase an animal's resting metabolic rate and this specific dynamic action can vary at the inter- and intraspecific level (McCue 2006). However, as free ranging animals usually have food in their guts, measurements from post-absorptive animals can severely underestimate maintenance costs (Niewiarowski and Waldschmidt 1992; Christian et al. 1997). We will therefore refer to our data as field resting metabolic rate (fRMR) obtained from non-starved animals at rest during the active phase of their diurnal cycle. Our field observations show no differences in foraging activity between habitats or seasons and both species defecated regularly after capture. Although these are only indirect measures of energy intake, we are confident that our comparisons of fRMR are not compromised by differences in feeding activity.

We collected data in the spiny and gallery forest from March – April 2011 (wet season 2011) and in the spiny, gallery and rainforest from September – November 2011 (dry season 2011) and January – April 2012 (wet season 2012). Data from the rainforest were also analysed for a classification of acclimatization patterns in tropical reptiles in Berg et al. (in review).

Statistical analysis

We tested for differences in metabolic rate between habitats and seasons using a linear mixed model (LMM) to account for correlated data from the same individuals and an unequal number of records for each individual. We used the lme function in the R package nlme (Pinheiro et al. 2015) and included T_b, body mass and habitat (spiny forest / gallery forest / rainforest) respectively season (wet 2011 / dry 2011 / wet 2012) as fixed factors and individual ID as a random intercept. Body mass was averaged from before and after the experiment. We assessed the quality of the model by visually checking the model residuals with Q-Q plots and histograms. All analyses were performed in R v3.2.1 (R Development Core Team 2015). All means are given with standard deviation.

Results

We analysed 757 fRMR records from 34 individuals of *O. quadrimaculatus* and 1350 fRMR records from 43 individuals of *Z. laticaudatus* with T_b ranging from 14.5 – 43.5°C for *O. quadrimaculatus* and from 13.5 – 39.8°C for *Z. laticaudatus*. *Oplurus quadrimaculatus* has been shown to exhibit acclimatization restricted to

T_b below the range of activity T_b, with a threshold at 36°C (Berg et al. in review). This pattern is termed selective acclimatization if metabolic rate is elevated at T_b below the range of activity T_b, and selective inverse acclimatization if metabolic rate is lowered at T_b below the range of activity T_b. We therefore split our data from this species and conducted separate analyses for records below and above the threshold. All LMM calculations confirm that T_b has a highly significant effect on fRMR in both species with increasing T_b resulting in increased fRMR ($p < 0.001$; Table 3.S1, Table 3.S2).

Ambient conditions

Records of T_a along the gradient in Andohahela show considerable differences between habitat types, with daily T_a fluctuations from 21.9 – 34.3°C in the spiny forest, from 22.4 – 31.0°C in the gallery forest and from 21.2 – 27.0°C in the rainforest (Fig. 3.1). Habitat differences are most distinct during daytime hours but much less pronounced at night with deviations between hot spiny forest and cooler rainforest ranging from 0.6 ± 1.3°C in the early morning to 7.6 ± 3.2°C around midday (daily mean difference 3.6 ± 2.5°C). Seasonal differences in T_a are generally more pronounced in minimum T_a rather than mean or maximum T_a (Fig. 3.2, left). However, southern Madagascar is subject to strong interannual fluctuations including long lasting droughts as well as cyclones. Monthly variations of T_a often exceed seasonal fluctuations during the wet season (Fig. 3.2, right) but are relatively stable during the dry season.
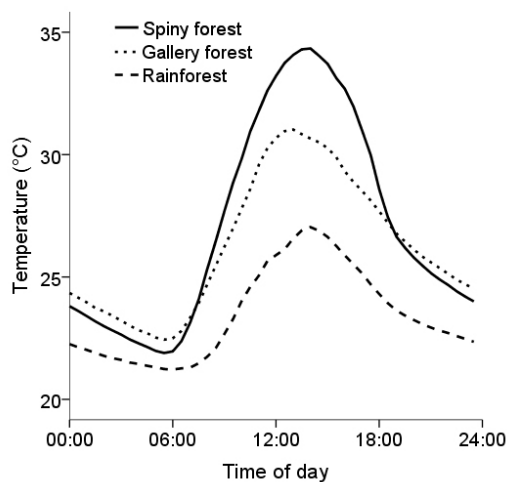
Figure 3.1 Ambient temperature over the course of the day in each habitat type. Spiny forest = solid line, gallery forest = dotted line, rainforest = dashed line. Lines are drawn from half-hourly records of ambient temperature that were averaged across seasons for each habitat type.
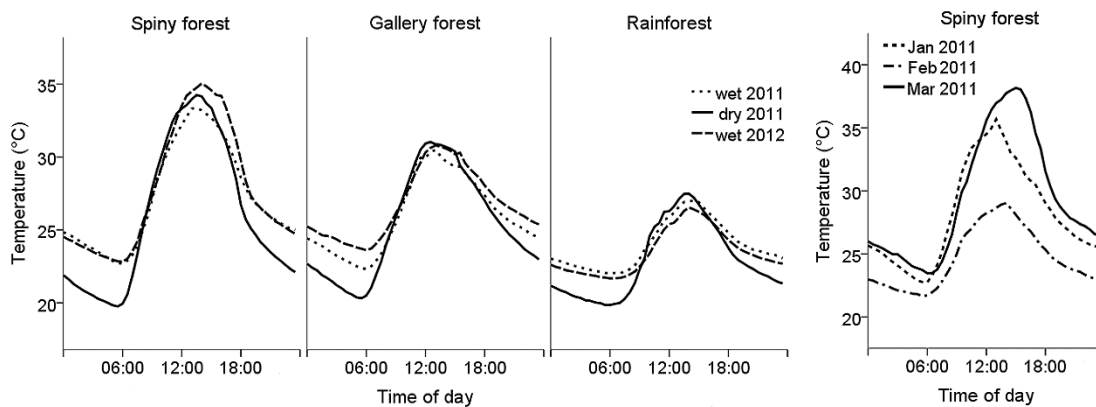
Figure 3.2 Mean ambient temperature (T_a) over the course of the day per season in each habitat type (left) and exemplary monthly variation during the wet season in the spiny forest (right). Seasonal differences in T_a are most pronounced in minimum T_a with little variation in maximum T_a. Monthly T_a variations during the wet season often exceed seasonal variations, especially if cyclones affect ambient conditions (e.g. cyclone Bingiza hit southeast Madagascar in February 2011).
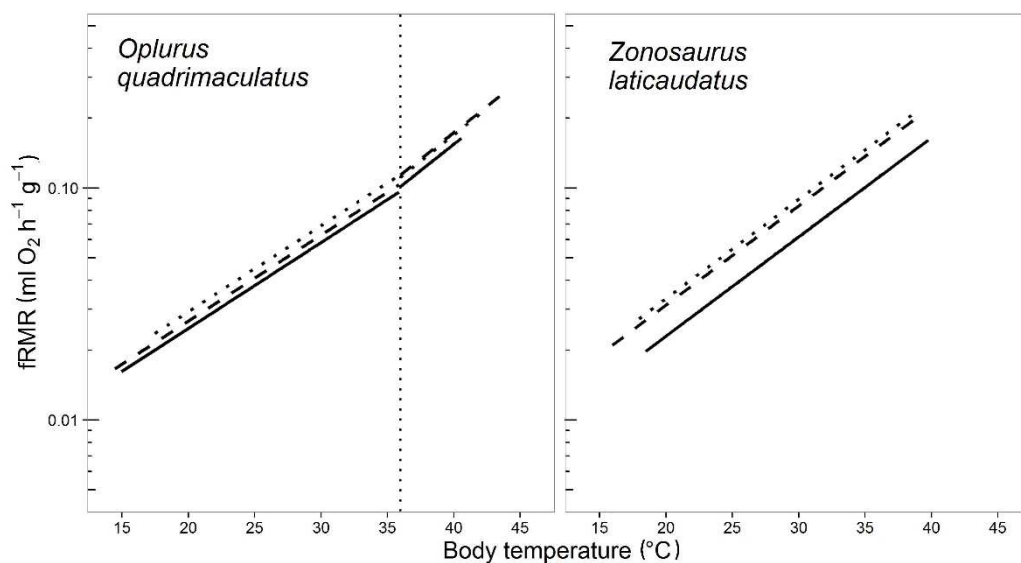
Figure 3.3 Habitat comparison of field resting metabolic rate (fRMR) for *Oplurus quadrimaculatus* (left) and *Zonosaurus laticaudatus* (right). Spiny forest = solid line, gallery forest = dotted line, rainforest = dashed line. The vertical line marks the threshold for selective (inverse) acclimatization in *O. quadrimaculatus*. Field resting metabolic rate does not differ between habitats in *O. quadrimaculatus* but is significantly lower in the spiny forest than in gallery and rainforest (p < 0.05 resp. p < 0.01) in *Z. laticaudatus*.

Habitat comparison of fRMR

Despite pronounced climatic differences along the gradient, *Oplurus quadrimaculatus* does not show differences in fRMR between habitat types, neither below nor above a T_b of 36°C (Fig. 3.3, Table 3.S1). *Zonosaurus laticaudatus*, on the other hand, exhibits a significantly lower fRMR in the spiny forest compared to gallery forest ($p < 0.05$) and rainforest ($p < 0.01$) but gallery forest and rainforest individuals do not differ in their fRMR (Fig. 3.3, Table 3.S1).

Seasonal comparison of fRMR

Adverse weather conditions restricted data collection in the spiny and gallery forest during the wet season 2012 and these data sets were excluded from the seasonal analysis. We thus conducted the following seasonal comparisons of fRMR for both species: 1) spiny forest: wet season 2011 – dry season 2011; 2) gallery
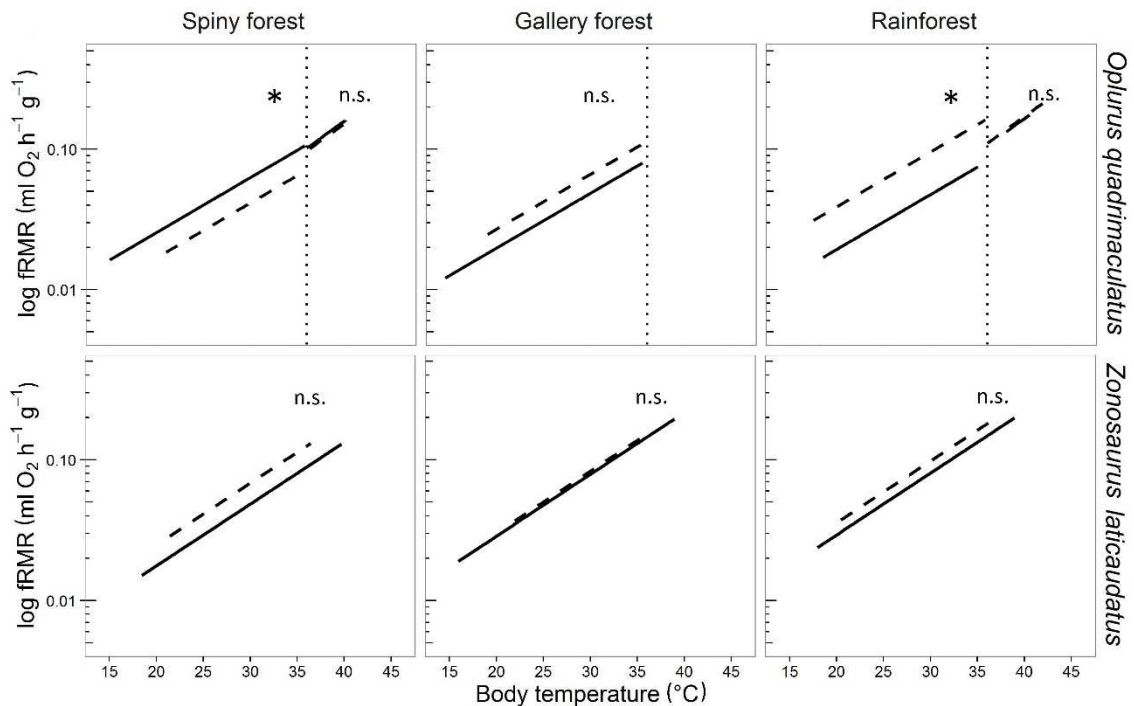
Figure 3.4 Seasonal comparison of field resting metabolic rate (fRMR) for *Oplurus quadrimaculatus* (top) and *Zonosaurus laticaudatus* (bottom) in the different habitat types. Dry season = solid line, wet season = dashed line. The vertical line in the top graphs marks the threshold for selective (inverse) acclimatization in *O. quadrimaculatus*. *Oplurus quadrimaculatus* shows metabolic acclimatization in the dry forest and the rainforest at body temperatures below 36°C ($p < 0.05$; marked by an asterisk) but not in the gallery forest or at body temperatures above 36°C (n.s. = not significant). *Zonosaurus laticaudatus* shows no seasonal acclimatization in any of the habitats.

forest: wet season 2011 – dry season 2011; 3) rainforest: dry season 2011 – wet season 2012.

Oplurus quadrimaculatus shows selective inverse acclimatization, with a significantly lower fRMR at T_b below 36°C, during the wet season in the spiny forest (p < 0.01) and selective acclimatization, with a significantly higher fRMR at T_b below 36°C, during the wet season in the rainforest (p < 0.05). We observed no seasonal differences in fRMR in the gallery forest at T_b below 36°C and data was insufficient for higher T_b (Fig. 3.4, Table 3.S2). *Zonosaurus laticaudatus* shows no seasonal acclimatization in any of the habitats (Fig. 3.4, Table 3.S2).

Discussion

Ectotherm species can exhibit behavioural or physiological adjustments to compensate varying environmental conditions but the potential and relative contribution of these modifications is largely unknown (Basson and Clusella-Trullas 2015). Our study on metabolic adjustments along an environmental gradient and across seasons reveals inverse patterns between two sympatric Malagasy lizards. *Oplurus quadrimaculatus* shows no differences in fRMR between habitats but clear seasonal acclimatization, while *Z. laticaudatus* exhibits adjustments to local conditions along the gradient but no seasonal compensation.

Metabolic responses across habitats
Both species occur sympatrically on rocky plateaus that are scattered with crevices and thus offer ample opportunity for behavioural thermoregulation. There is no interspecific difference in the operative environmental temperature, i.e. the range of obtainable T_b in a given habitat (Bakken 1976), but a marked decrease in operative temperature from spiny forest to rainforest (Theisinger et al. in review_2). However, these thermal differences between habitats elicit physiological adjustments only in *Z. laticaudatus* but not in *O. quadrimaculatus*.

Previous intraspecific comparisons of populations that inhabit differing environments provide similarly varied results regarding physiological compensation. Some species show distinct metabolic differences (Beaupre 1996; Angilletta 2001) and others resort to changes in activity and preferred T_b (Van Damme et al. 1989), while some show only behavioural rather than physiological

adjustments (Gvoždík 2002). Physiological responses can be limited if thermal differences between habitats are too low to induce physiological adjustments, if thermal differences are buffered by behavioural thermoregulation, or by phylogenetic constraints (Van Damme et al. 1990; Rogowitz 1996; Gvoždík and Castilla 2001).

Despite their sympatric occurrence and an equal range of operative temperature, both species differ markedly in their ecology with pronounced effects on their thermal biology. *Oplurus quadrimaculatus* is strictly heliothermic and spends most of the day on open rocks, where it is able to combine foraging with precise thermoregulation. This open habitat ecology allows *O. quadrimaculatus* to maintain similar T_b during activity along the entire Andohahela gradient (Theisinger et al. in review_2), despite an almost 4°C decrease in daily mean T_a and a more than 7°C decrease in daily maximum T_a from spiny forest to rainforest. A similar extraordinary effectiveness of behavioural thermoregulation has been described in *Anolis* lizards from Hispaniola (Muñoz et al. 2014) and seems sufficient in *O. quadrimaculatus* to render metabolic adjustments unnecessary along the gradient (Fig. 3.3).

Zonosaurus laticaudatus, on the other hand, is highly constrained by its foraging ecology, which restricts most of its activity to crevices and leaf litter. This species mostly thermoconforms except for a daily morning basking period (Theisinger et al. in review_1). Its shade based activity limits the potential for behavioural thermoregulation and exposes *Z. laticaudatus* to differing shade and crevice temperatures along the gradient. We therefore assume that the observed lower fRMR in the spiny forest reflects a compensation of higher T_b due to higher crevice temperatures in the spiny forest, which would otherwise increase metabolic costs (Fig. 3.3). The spiny forest in Andohahela nevertheless constitutes a thermal limit for *Z. laticaudatus* and marks the boundary of its geographic distribution. Foraging ecology thus constrains the compensatory capacity of this species and it can only resort to physiological adjustments, even though the environment theoretically provides ample opportunity for behavioural thermoregulation.

The contrasting metabolic responses from *O. quadrimaculatus* and *Z. laticaudatus* to thermal differences along the gradient can therefore be explained by a differing capacity for behavioural thermoregulation. Although we cannot entirely exclude

phylogenetic influences for our study species, a similar contrast between thermoregulating and non-thermoregulating species regarding compensatory strategies has also been observed in closely related *Anolis* lizards (Hertz 1981; Hertz and Huey 1981; Hertz 1992). The open habitat species *A. roquet*, *A. cybotes* and *A. cristatellus* all show behavioural responses to environmental changes, while the forest inhabitant *A. gundlachi* is a thermoconformer that compensates physiologically along an altitudinal gradient. Our findings corroborate the hypothesis that a greater physiological capacity is found in species with limited potential for behavioural thermoregulation. Such thermoregulatory limitations have previously mainly been related to restrictions from the thermal environment, i.e. forest or aquatic habitats (Hertz 1992; Seebacher 2005; Gunderson and Stillman 2015). Our results show, however, that ecological constraints can be an equally strong determinant of compensatory capacity even if the thermal environment is not limiting, which highlights and expands the importance of ecological influences on thermoregulation (Huey and Slatkin 1976; Blouin-Demers and Weatherhead 2002).

Metabolic responses across seasons

Seasonal data from *O. quadrimaculatus* reveal metabolic acclimatization at both ends of the gradient, despite this species' great capacity for behavioural thermoregulation. The direction of acclimatization is reversed between spiny forest and rainforest (Fig. 3.4), so the underlying limitations seem to differ between both climatic extremes.

The rainforest population of *O. quadrimaculatus* faces limited basking opportunities during the wet season due to extensive rain and cloud cover. This can significantly affect an animal's performance through lowered operative temperatures (Kingsbury 1994; Clusella-Trullas et al. 2011), even if T_a is relatively constant across seasons (Fig. 3.2, left). The thermal challenge of low T_b during the wet season is therefore compensated through an elevated fRMR, which improves performance at low T_b and permits year-round activity in this lizard even during times when the capacity for behavioural thermoregulation is limited (Bennett and Dawson 1976; Tsuji 1988).

In contrast, the spiny forest provides ample opportunity for thermoregulatory basking with little rainfall even during the wet season (400 mm/yr; Goodman

1999). With low seasonal variation in ambient conditions and no seasonal reductions in foraging or overall activity (pers. obs.), it can be difficult to assess which season requires compensation. However, our wet season data in the spiny forest were collected in March 2011, an exceptionally hot period during this season (Fig. 3.2, right). The lower fRMR may therefore represent a compensation of high T_b during inactivity, which would otherwise increase energetic costs (Christian et al. 1999, Kearney et al. 2009). Moreover, as the metabolic shift in *O. quadrimaculatus* is restricted to T_b below the range of activity T_b (selective inverse acclimatization, Berg et al. in review), it combines energy saving when the animal is inactive with the maintenance of optimal performance during activity.

Zonosaurus laticaudatus, on the other hand, shows no seasonal acclimatization in any of the habitats. We assume that this is based on an absence of thermal limitations. As daytime T_a varies little between seasons (Fig. 3.2, left), and is likely to be even more stable in crevices, the shade based activity of this species may result in relatively stable daytime T_b across seasons. This would render acclimatization unnecessary, similar to the absence of acclimatization of resting metabolic rate in the semiaquatic lizard *Varanus mertensi* (Christian et al. 1996). This assumption is further supported by the fact that *Z. laticaudatus* does show metabolic adjustments along the gradient. These are most likely based on plasticity rather than genetic adaptation because our study sites in spiny and gallery forest were only about one kilometre apart, a distance that can easily be covered within an individual's lifetime and thus speaks against a genetic differentiation between populations. Although we cannot entirely rule out a phylogenetic inertia, it seems more plausible that the absence of metabolic acclimatization in *Z. laticaudatus* is due to a lack of necessity rather than a lack of capacity.

Ecological differences therefore also explain the contrary patterns of seasonal acclimatization in these two lizards. The open habitat ecology of the thermoregulating *O. quadrimaculatus* makes this species more susceptible to changes in solar radiation, whereas the shade and crevice based existence of *Z. laticaudatus* buffers this species against seasonal changes to a certain extent.

Interactions of behavioural and physiological mechanisms
An absence of physiological responses, as found in *O. quadrimaculatus* along the gradient, has led to the proposition that the behavioural buffering of climatic

variation could reduce the selection pressure for physiological adjustments and may ultimately lead to reduced physiological compensation capacities (Huey et al. 2003; Huey et al. 2012; Buckley et al. 2015). *Oplurus quadrimaculatus* strongly depends on behavioural compensation along the gradient, which could indeed indicate a lack of physiological capacity. However, our data from seasonal fRMR measurements demonstrate that *O. quadrimaculatus* has the capacity for physiological compensation but only resorts to it if behavioural measures are severely restricted. The same pattern has been found regarding preferred T_b in the lizard *Psammodromus algirus*, which varies between seasons but not with altitude (Díaz et al. 2006). These results show that the absence of physiological responses does not necessarily imply an inability but may also result from a lack of necessity, e.g. if environmental variation is sufficiently compensated by behavioural thermoregulation (Adolph 1990; Muñoz et al. 2014).

On the other hand, physiological responses are often found in species with limited potential for behavioural thermoregulation, such as aquatic or forest species (Lowe et al. 2010; Huey et al. 2012; Gunderson and Stillman 2015). In our study, *Z. laticaudatus* shows metabolic adjustments along the gradient because it does not thermoregulate behaviourally and *O. quadrimaculatus* resorts to seasonal acclimatization if behavioural responses fail. Does this mean that the physiological compensation of environmental changes is generally a secondary mechanism and only found if behavioural options are limited? Species with sufficient potential for behavioural thermoregulation usually exhibit little physiological variation (Hertz and Huey 1981; Rogowitz 1996; Gvoždík 2002). Furthermore, nocturnal geckos, with limited potential for behavioural thermoregulation at night, thermoregulate their T_b during the day (Huey et al. 1989; Kearney and Predavec 2000). This indicates that animals resort to behavioural mechanisms whenever they are available.

The most valuable insight, however, comes from studies that incorporate behavioural and physiological aspects at the intraspecific level. *Sceloporus undulates* shows little variation in thermal tolerances between seven populations with effective thermoregulation, while an eighth population with less potential for behavioural compensation shows physiological adjustments (Buckley et al. 2015). The lizard *Cordylus oelofseni* varies between mainly behavioural compensation in summer and metabolic acclimatization in winter, depending on the potential for

behavioural thermoregulation (Basson and Clusella-Trullas 2015). These results support the assumption that behavioural thermoregulation is the preferred compensatory mechanism, while physiological compensation rather functions as a secondary mechanism if behavioural options are limited.

In conclusion, our data show that ecological constraints can have a strong influence on the compensatory potential of species. Differences in foraging ecology and the associated differences in microhabitat use force our sympatric study species to rely on different compensation mechanisms despite living in a similar thermal environment. It is thus important to integrate information about the thermal reality and the realized thermal niche of a species to improve predictions about the capacity of species to compensate environmental changes.

Furthermore, our data show that the prevalence of compensatory mechanisms may change within species depending on the scale of environmental variation. Thermoregulating species in particular may also resort to physiological mechanisms if thermoregulation becomes too costly or limited. We would therefore like to advise caution as an observed absence of physiological acclimatization does not necessarily imply a physiological incapability. This is especially important to consider in predictions about the vulnerability of species in the face of climate change because the compensatory potential of physiological mechanisms, especially in tropical species, might have been underestimated.

Acknowledgements

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Supplementary material

Table 3.S1 Model summaries for habitat comparison. Each data set was run twice: (a) to test gallery and rainforest data against spiny forest data and (b) to test rainforest data against gallery forest data (spiny forest data was also included in the second run but results are a replicate of (a)). Results for body temperature (T_b) and body mass (BM) are replicates and only shown once for (a) and (b). N = number of records of field resting metabolic rate for each habitat; the number of individuals is given in brackets.

data set		parameter	N	*p*-value
Oplurus quadrimaculatus	(a)	reference (spiny forest)	137 (10)	
		gallery forest	115 (7)	0.68
$T_b < 36°C$		rainforest	311 (13)	0.44
	(b)	reference (gallery forest)		
		rainforest		0.81
	(a & b)	T_b		< 0.001
		BM		0.33
$T_b \geq 36°C$	(a)	reference (spiny forest)	65 (10)	
		gallery forest	58 (8)	0.57
		rainforest	81 (10)	0.44
	(b)	reference (gallery forest)		
		rainforest		0.93
	(a & b)	T_b		< 0.001
		BM		0.03
Zonosaurus laticaudatus	(a)	reference (spiny forest)	401 (15)	
		gallery forest	494 (13)	0.02
		rainforest	455 (15)	0.006
	(b)	reference (gallery forest)		
		rainforest		0.73
	(a & b)	T_b		< 0.001
		BM		< 0.001

Table 3.S2 Model summaries for seasonal comparison. Tb = body temperature; BM = body mass; N = number of records of field resting metabolic rate for each season with number of individuals given in brackets.

data set	parameter	N	*p*-value
Oplurus quadrimaculatus			
Spiny forest $T_b < 36°C$	reference (wet 2011)	84 (4)	
	dry 2011	30 (4)	0.02
	T_b		< 0.001
	BM		0.07
Spiny forest $T_b \geq 36°C$	reference (wet 2011)	30 (4)	
	dry 2011	22 (4)	0.99
	T_b		< 0.001
	BM		0.47
Gallery forest $T_b < 36°C$	reference (wet 2011)	70 (3)	
	dry 2011	35 (3)	0.34
	T_b		< 0.001
	BM		0.21
Gallery forest $T_b \geq 36°C$	*data insufficient*		
Rainforest $T_b < 36°C$	reference (dry 2011)	169 (5)	
	wet 2012	142 (8)	0.04
	T_b		< 0.001
	BM		0.42
Rainforest $T_b \geq 36°C$	reference (dry 2011)	17 (3)	
	wet 2012	64 (7)	0.86
	T_b		< 0.001
	BM		0.16
Zonosaurus laticaudatus			
Spiny forest	reference (wet 2011)	241 (6)	
	dry 2011	96 (7)	0.15
	T_b		< 0.001
	BM		0.002
Gallery forest	reference (dry 2011)	269 (6)	
	wet 2012	211 (6)	0.13
	T_b		< 0.001
	BM		0.95
Rainforest	reference (dry 2011)	241 (8)	
	wet 2012	214 (7)	0.12
	T_b		< 0.001
	BM		0.80

Author contribution

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

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Prof. Dr. Kathrin Dausmann

Discussion

Understanding the capacity of species to cope with environmental changes is one of the most pressing issues of current biology. Species have been shown to respond to changing conditions with a variety of compensatory mechanism (Walther et al. 2002; Bradshaw and Holzapfel 2006; Hofmann and Todgham 2010; Huey et al. 2012) but how and why species exhibit one or the other of these mechanisms is still not well understood (Luiselli and Akani 2002; Bovo et al. 2012). In my dissertation, I investigate the capacity for metabolic compensation in tropical reptiles, which has long been assumed to be limited (Janzen 1967; Ghalambor et al. 2006) although empirical evidence is highly variable (Seebacher et al. 2015).

Non-invasive monitoring of body temperature

A prerequisite for an understanding of compensatory mechanisms in tropical reptiles is information about their body temperature (T_b) under natural conditions. Field T_b provide insight into the thermal reality of an animal and without such knowledge, we can neither understand nor predict the effect of changing environments. In addition, as metabolic rate increases with increasing T_b (Bennett and Dawson 1976), oxygen consumption measurements at variable temperatures require a constant monitoring of T_b and this can be challenging under field conditions.

The conventional method to record T_b in reptiles are cloacal temperature measurements using thermocouples. This method has several practical limitations though and is increasingly replaced by non-invasive methods, such as infrared thermometers, thermographic cameras or externally attached temperature loggers (Tattersall and Cadena 2010; Tattersall 2016). All these devices measure skin temperature rather than core T_b, so their suitability largely depends on the relationship between these two parameters. In small reptiles (< 30g), the internal gradient between core and surface temperature is considered to be negligible (Bakken 1992) but the thermal inertia in large ectotherms can result in considerable differences (Stevenson 1985; Paladino et al. 1990).

Chapter one validates the use of infrared thermometers and externally attached temperature loggers for the three lizard species *Oplurus saxicola*, *O. quadrimaculatus* and *Zonosaurus laticaudatus* (Fig. 4.1). Skin temperature records proved to

be generally suitable surrogates for T_b measurements in these small to medium sized lizards (body mass *O. saxicola* ~20g, *O. quadrimaculatus* ~80g and *Z. laticaudatus* ~110g) and both methods proved to be easy to use under field conditions.

However, our data from free ranging *O. saxicola* show that even lizards with less than 30g body mass may exhibit considerable differences between surface and core T_b during periods of intensive heating, for example during the morning basking period. Similar lags between surface and core T_b during warming phases were also found in some small lacertid lizards (body mass < 10g) using an infrared camera (Luna and Font 2013). A thermal inertia between surface and core body in small species thus depends not only on the actual body size but also on the rate of heating. Infrared thermometers have previously been used in several smaller species (e.g. *Goniurosaurus kuroiwae kuroiwae* (body mass ~8g), Werner et al. 2005; *Zootoca vivipara* (body mass up to 6g), Herczeg et al. 2006), and sometimes even without prior calibration against core T_b (Christian et al. 1998). Our proof of a thermal inertia during intense basking even in small species is highly important for future field studies because rapid heating may be part of the usual daily T_b pattern in thermoregulating species (Theisinger et al. in review_a).

The non-invasive monitoring of T_b nevertheless presents an important technological progress because it facilitates a more rapid data collection and thus increased sample sizes, the possibility of repeated measurements of undisturbed animals under natural conditions and continuous records over several days including times in refuges and during the night.

Such information about daily T_b patterns provides crucial insight into the thermal ecology of species (e.g. thermoconforming vs. thermoregulating; Theisinger et al. in review_b) and plausibly explains the contrasting acclimatization patterns that are described in chapters two and three. Field T_b can be used to assess the accuracy and efficiency of behavioural thermoregulation (Hertz et al. 1993) and, in combination with metabolic rate measurements, allow detailed calculations of maintenance costs (Kearney und Porter 2004). Furthermore, temperature loggers also provided valuable insight into the overwintering ecology of the Gopher Tortoise (*Gopherus polyphemus)* by compiling continuous temperature and activity profiles over several months when tortoises were mostly hidden in burrows (Harris et al. 2015). And infrared cameras allow to distinguish temperature

Figure 4.1 Skin temperature measurements using an infrared thermometer on *Zonosaurus laticaudatus* (top) and with an externally attached Thermochron temperature logger on *Oplurus quadrimaculatus* (bottom). [Photos: Wiebke Berg]

differences among body regions even in small lizards, which showed that these species are able to physiologically regulate the heat distribution across their body (Sannolo et al. 2014; Lunghi et al. 2016).

Differing metabolic adjustments in reptiles across the tropics

Varying T_b have a direct impact on the energy expenditure and performance of ectotherms based on the temperature dependence of physiological processes (Clarke 2004; Dell et al. 2011; Schulte 2015). Acclimatization shifts the temperature-rate curve of physiological processes and can thus adjust an animal's performance and energy expenditure to varying environmental conditions (Huey 1982; Schulte et al. 2011; Huey et al. 2012). Based on Janzen's (1967) postulation that an organism's ability to acclimatize is related to the degree of variation that this animal experiences in its environment, tropical species were long assumed to have only limited acclimatization capacity (Ghalambor et al. 2006; Deutsch et al. 2008). This hypothesis was supported by early studies, which found that temperate species show metabolic adjustments, whereas tropical ones do not (Feder 1978, 1982; Tsuji 1988a). However, with a (slow) increase of empirical studies from tropical regions, evidence for the acclimatization capacity of tropical reptiles has become highly variable (Seebacher et al. 2015). The number of studies is still limited enough though that controversial patterns could be dismissed as 'exceptions to the rule'. This lack of clarity is particularly concerning because tropical ectotherms are predicted to be most vulnerable to climatic changes. Many of these species live already close to their thermal limits with a small thermal safety margin, i.e. the difference between the animal's maximum heat tolerance and current ambient temperature (Deutsch et al. 2008; Huey et al. 2009; Sunday et al. 2014).

Chapter two of my dissertation shows that available data on tropical terrestrial reptiles (including new data from *O. quadrimaculatus* and *Z. laticaudatus*) can be arranged into distinct categories, which correspond to a scheme of decreasing metabolic costs: (1) comprehensive acclimatization, (2) selective acclimatization, (3) no acclimatization, (4) selective inverse acclimatization and (5) comprehensive inverse acclimatization. For the first pattern however, no example is available from tropical reptiles.

'No acclimatization' (the pattern predicted for tropical ectotherms by Janzen (1967)) is only one of the four patterns that are described for tropical reptiles and these patterns are consistent enough across species that a general incapacity is questionable. We find that the variability of energetic restrictions in the tropics presents a more reasonable explanation for the diversity of patterns, which, at the same time, advises caution regarding the interpretation of results from laboratory experiments. Our energetic point of view furthermore permits a conclusive explanation of 'selective acclimatization' and 'selective inverse acclimatization', two patterns that previously lacked interpretation.

Selective and selective inverse acclimatization

The direction and magnitude of metabolic compensation has previously been classified using a scheme from Precht (1958). However, his scheme is based on a comparison of metabolic rate at only two temperatures. Prosser (1969) described shifts of temperature-rate curves across a range of temperatures but only complete shifts of entire curves. Our data from *Oplurus quadrimaculatus*, however, shows that this species acclimatizes only at T_b below its range of activity T_b but not within this range. We introduce the term 'selective acclimatization' for this pattern because only part of the temperature-rate curve is adjusted during acclimatization. This pattern is energetically reasonable, as an elevation of the entire curve would result in excessive energy expenditure at normal activity T_b. A selectively elevated metabolic rate improves performance at lower T_b, while energy expenditure at higher T_b remains constant. Accordingly, a selectively lowered metabolic rate (termed 'selective inverse acclimatization') facilitates energy saving during inactivity at low T_b but the maintenance of optimal performance at higher T_b.

Such selective patterns of acclimatization have been noted in other species (Dutton and Fitzpatrick 1975; Wood et al. 1978; Ragland et al. 1981) but did not receive further attention. However, these patterns are energetically favourable in species with moderate thermal or energetic restrictions. Selective acclimatization is advantageous if T_b is temporarily decreased but not low enough to enforce inactivity. Selective inverse acclimatization, on the other hand, is beneficial to combine energy saving with the opportunity to occasionally resume normal activity.

Variability of energetic restrictions in the tropics

Metabolic rate is a measure of energy transformation and metabolic acclimatization facilitates the compensation of energetic limitations. Such limitations arise if the balance between energy supply and energy demand is disturbed, for example as a consequence of seasonal food shortage. Moreover, based on the exponential increase of metabolic rate with temperature, high T_b can lead to excessive energy expenditure, while low T_b reduce the animal's energy turnover and thus its performance (Little and Seebacher 2016).

Seasonal reductions of resources and pronounced temperature fluctuations are strongly linked at high latitudes. Ectotherms in temperate regions thus experience food restrictions and low T_b simultaneously and it is not surprising that compensatory metabolic responses mostly reflect energy savings during inactivity (Patterson and Davies 1984). In contrast, food availability is often (but not always) linked to precipitation rather than ambient temperature in tropical regions (Congdon 1989; Previtali et al. 2009). Seasonal fluctuations of all three parameters - temperature, precipitation and food supply - vary considerably in their intensity across the tropics (Visher 1922; Legates and Willmott 1990; Feng et al. 2013) and all three parameters have been shown to provoke acclimatization in tropical reptiles (Sievert et al. 1988; Christian et al. 1995, 1999; McCue 2007). Without the dominating effect of temperature, these differing influences vary in their prevalence and result in differing degrees of energetic constraints between species. We illustrate in chapter two how the seemingly controversial metabolic responses in tropical reptiles match these differing constraints and provide alternative explanations for a lack of acclimatization in tropical reptiles. This includes the compensation of varying ambient conditions through behavioural thermoregulation or the simple absence of marked environmental variation.

This energetic point of view, in contrast to a pure focus on temperature changes that commonly dominates acclimatization studies, can thus adequately explain the unexpected variety of acclimatization patterns in tropical reptiles. Moreover, the integration of the animal's thermal reality, i.e. the experienced variation in T_b, shows that an absence of metabolic responses does not necessarily allow inferences about physiological capacity.

Ecologically unrealistic experiments

A further possible source of variance in metabolic responses lies in the experimental set-up of acclimation[2] studies. Metabolic compensation has been studied extensively for decades (e.g. Wells 1935) but the biological relevance of experimental set-ups has only rarely been questioned (Tsuji 1988b; de Andrade 2016).

The common protocol for acclimation studies provides that animals are kept at constant temperatures for a certain acclimation period before metabolic rate is measured at a set of test temperatures. However, ectotherms rarely experience constant temperatures under natural conditions. Comparative studies with acclimation to constant and fluctuating temperature cycles reveal differing responses in a variety of physiological traits, including metabolic rate (Carey 1979; de Andrade 2016), preferred T_b (Hadamová and Gvoždík 2011; Gvoždík 2012), thermal tolerances (Feldmeth et al. 1974), running speed (Šamajová and Gvoždík 2010), development rates and survival (Paaijmans et al. 2013) and cell growth and proliferation (Podrabsky and Somero 2004). Insufficient acclimation times may also lead to an underestimation of physiological capacities and especially tropical species may show slower responses due to slower seasonal changes in their natural environment (Pintor et al. 2016). Finally, season has been shown to affect acclimation responses (Mayhew 1965; Harri and Hedenstam 1972; Dunlap 1973, 1980; Gregory 1982), which provides a plausible explanation why individuals of the lizard *Anolis carolinensis* show acclimation effects under laboratory conditions if collected in January (Ragland et al. 1981) but not if collected in April and September (Gatten 1985).

Experimental temperature changes alone therefore do not seem to be sufficient to provoke the full physiological capacity in tropical reptiles and factors other than temperature have been suggested as a driver of acclimatization, for example food or water availability (Clarke 1993; Christian et al. 1999; McCue 2007). The incorporation of biological reality into laboratory experiments is often challenging but laboratory studies of metabolic responses in tropical reptiles may be particularly impaired by the complexity of influencing parameters. One possibility to overcome these difficulties is the study of naturally acclimatized animals (e.g.

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[2] I specifically distinguish between 'acclimation' to laboratory conditions and 'acclimatization' to natural conditions in this paragraph, rather than using a generic term for both.

Zari 1996, 1999). This might provide a better insight into the compensatory capacity of these species but is more labour intensive and more difficult regarding the assessment of decisive parameters.

Differing metabolic adjustments in sympatric tropical reptiles

Chapter two illustrates how differing energetic restrictions based on a variable interplay of temperature, precipitation and resource availability result in highly variable patterns of metabolic acclimatization in tropical reptiles. However, even sympatric species that experience the same fluctuations of ambient conditions can show contrasting metabolic responses, as shown in chapter three of my dissertation. *Oplurus quadrimaculatus* shows no differences in metabolic rate along the environmental gradient from dry spiny forest to humid rainforest but clear seasonal acclimatization, while *Zonosaurus laticaudatus* exhibits adjustments to local conditions along the gradient but no seasonal compensation. Furthermore, the direction of seasonal acclimatization in *O. quadrimaculatus* differs depending on the thermal environment. Animals in the cloudy rainforest need to compensate seasonal thermal restrictions from limited basking opportunities and thus show elevated metabolic rates. In the hot spiny forest, on the other hand, seasonal increases in ambient temperature lead to higher T_b during inactivity and *O. quadrimaculatus* compensates the resulting increased energy expenditure with lowered metabolic rates.

The inverse patterns of metabolic adjustments in these two sympatric lizards can be related to differences in their foraging ecology and show that ecology can, in some cases, be an even stronger determinant of compensation mechanisms than the thermal environment. In addition, the integration of our physiological data and information about behavioural thermoregulation in these species (Theisinger et al. in review_a,b) shows that the prevalence of compensatory mechanisms can change even at the intraspecific level but that behavioural responses, if possible, are preferred over physiological adjustments.

Ecological influences on compensatory capacity

The compensatory capacity of a species depends on a variety of factors, including biochemical and energetic limitations (Campbell et al. 2010; Hofmann and Todgham 2010), developmental conditions and phylogenetic restrictions (Hertz et

al. 1983; Somero 2005; Seebacher et al. 2012; Hoffmann et al. 2013) and the thermal environment for the provision of basking and cooling sites (Angilletta et al. 2002b), which can be adversely affected through human habitat alterations (Stellatelli et al. 2013). An additional factor that received less attention so far in physiological studies are ecological constraints. Interspecific competition for space and/or food as well as predation can curtail an animal's microhabitat use (Fuentes and Cancino 1979; Losos et al. 1993; Downes and Shine 1998; Herczeg et al. 2008), which can in turn affect its thermoregulatory potential if access to basking sites is limited (Huey 1974; Gillis 1991).

We observed such a restricting effect in *Zonosaurus laticaudatus* based on foraging ecology. This species actively forages in crevices and surrounding leaf litter, which confines most of its activity to shady microhabitats and consequently limits its potential for behavioural thermoregulation. As a result this species has to compensate hot ambient conditions in the spiny forest physiologically through adjustments in metabolic rate. In contrast, its sympatric conspecific *O. quadrimaculatus* is a sit-and-wait forager on open rocky plateaus with sufficient opportunity for basking to compensate environmental differences along the gradient behaviourally. However, an inverse pattern is seen across seasons: the thermoregulating *O. quadrimaculatus* is sensitive to weather changes and cloud cover and resorts to metabolic acclimatization between seasons, whereas *Z. laticaudatus* in its crevices is rather unaffected by changes in solar radiation and therefore shows no seasonal compensation. Although both species often bask side by side in the morning (Fig. 4.2), differences in foraging activity and diet result in a clear separation of microhabitats used for the remainder of the day, which in turn has a substantial effect on their compensatory capacity.

Previous studies mostly documented how thermoregulation requirements influence the selection for specific microhabitats (Adolph 1990; Grover 1996; Blouin-Demers and Weatherhead 2001) and restrict foraging activity (Gvoždík 2002; Kearney et al. 2009). In contrast, our data show that foraging and associated microhabitat restrictions can also limit the thermoregulatory potential of a species. In this case, ecology is an even stronger determinant of compensatory potential than the thermal environment, which would theoretically provide ample opportunity for behavioural thermoregulation for *Z. laticaudatus*. This result highlights and expands the importance of ecological influences on compensatory mechanisms (Huey and Slatkin 1976; Blouin-Demers and Weatherhead 2002).

Figure 4.2 A couple of *Oplurus quadrimaculatus* and a couple of *Zonosaurus laticaudatus* basking side by side in the morning. [Photo: Wiebke Berg]

Relative importance of behavioural and physiological mechanisms

Behavioural thermoregulation and physiological acclimatization are two fundamentally different strategies (maintenance of stable T_b vs. maintenance of a stable thermal reaction norm) and have therefore long been studied separately. Huey (1990) furthermore suggested a separation of both mechanisms according to the time scale of environmental fluctuations. Behavioural changes are rapid and effective in the avoidance of short term stress, whereas acclimatization is beneficial as a response to more long term, e.g. seasonal, fluctuations. Nevertheless, several recent studies show that behavioural and physiological mechanisms can work in concert (Angilletta et al. 2002a; Seebacher 2005). Such interactions can increase the compensatory capacity of species, for example if the physiological lowering of the thermal optimum increases the time window for behavioural thermoregulation (Hadamová and Gvoždík 2011). The relative importance of both mechanisms and how they influence each other is not entirely clear though (Basson and Clusella-Trullas 2015) and a contrasting position proposes that behavioural thermoregulation may reduce the selection pressure for physiological adjustments by buffering the impact of environmental variation (known as the 'Bogert effect'; Bogert 1949; Huey et al. 2003; Buckley et al. 2015).

Our spatial and temporal comparisons of metabolic adjustments in *O. quadrimaculatus* and *Z. laticaudatus* show that the potential for behavioural thermoregulation substantially affects metabolic adjustments. The absence of physiological adjustments in *O. quadrimaculatus* along the gradient is related to a strong dependence on behavioural thermoregulation. While this appears to be in agreement with the Bogert effect, our seasonal data show that these lizards are very well capable of metabolic adjustments. Similarly, Basson (2015) found that the lizard *Cordylus oelofseni* shows no physiological plasticity in summer when behavioural responses are prevalent. This is not based on physiological incapacity though because these lizards do exhibit metabolic acclimatization in winter when behavioural options are limited. These results show that the prevalence of mechanisms may change even within species and corroborate our conclusion from the second chapter that an absence of physiological compensation does not necessarily reflect physiological incapacity.

Furthermore, Buckley (2015) reports that, while physiological responses are absent in seven thermoregulating populations of *Sceloporus undulates*, an eighth populations with less potential for behavioural thermoregulation does show greater thermal tolerances. Considering these results, it seems that the buffering effect of thermoregulation certainly weakens the need for physiological compensation. We would, however, argue that this may merely postpone physiological adjustments rather than eliminate the capacity because intra- and interspecific comparisons show that physiological mechanisms often come to the fore if behavioural options are limited (Hertz 1981; Kam et al. 2001; Wu et al. 2007; Basson and Clusella-Trullas 2015; Buckley et al. 2015).

Physiological adjustments thus seem to function rather as a secondary mechanism, while behavioural thermoregulation appears to be of primary importance. This is further supported by the fact that even nocturnal geckos thermoregulate behaviourally during the day using 'protected basking' under tree bark to minimise predation (Huey et al. 1989).

Conclusion

Species are currently facing unprecedented rates of environmental change and our understanding about the impact of these changes and possible compensatory mechanisms is still alarmingly limited. Two aspects emerge from this dissertation as being of major importance for future research: the thermal reality of an organism and the variability of compensatory responses at the inter- and intraspecific level.

Predictions about the impact of changing climates are often based on large-scale meteorological data (Bakken and Angilletta 2014; de Andrade 2016) and mean ambient temperature (e.g. Deutsch et al. 2008). However, these parameters often differ substantially from the thermal reality of animals, which is based on microclimatic differences and the heterogeneity of a habitat (Sears and Angilletta 2015) as well as the animal's ability to make use of these differing microsites. Influences such as cloud cover or habitat fragmentation can markedly change thermal conditions for an ectotherm even if ambient temperature is unaffected (Clusella-Trullas and Chown 2014; Tuff et al. 2016), and even organisms that are exposed to identical conditions can have a differing body temperature (T_b) due to differences in body size and shape or colouration (Stevenson 1985; Stewart and Dixon 1989; Helmuth 2002). Studies therefore increasingly integrate operative temperature rather than ambient temperature (Kearney and Predavec 2000; Sinervo et al. 2010; Sunday et al. 2014). However, even operative temperature is sometimes insufficient to understand the thermal reality of an animal because it describes the theoretically attainable range of T_b in a given habitat, whereas the realized T_b is additionally influenced by a multitude of biotic and ecological constraints (Theisinger et al. in review_b).

The assessment of an animal's T_b under natural conditions can be extremely challenging, as has been illustrated by Helmuth (2002) for organisms in the complex intertidal environment. Methods described in chapter one of my dissertation provide a great advance in this respect, not only for the assessment of ectotherm T_b but also for studies on mammals (McCafferty 2007; Nie et al. 2015) and plants (Chaerle et al. 1999; Jones et al. 2009).

The importance of integrating the thermal reality of an animal is furthermore demonstrated in chapters two and three of my dissertation. Without information on

T_b and their variation under natural conditions, an absence of physiological adjustments has often been interpreted as a lack of capacity, even though stable T_b may simply render physiological compensation unnecessary. We therefore first need to understand an animal's thermal reality, and thus its need for compensation, before attempting to understand its compensatory capacity.

Moreover, chapters two and three show that a multitude of abiotic, biotic and ecological constraints act together and therefore provoke equally varied compensatory responses. The complex and variable interplay of influences and mechanisms is now increasingly realized, not only for ectothermic reptiles and insects (Helmuth et al. 2005; Angilletta et al. 2006) but also for mammals and birds (McKechnie 2008; Boyles et al. 2011). This has important implications for the field of animal physiology because it emphasises the need for comprehensive field studies (Costa and Sinervo 2004). Studies that integrate both behavioural and physiological mechanisms and investigate adjustments at different scales in time and space are still extremely scarce but they provide essential mechanistic insight that would not be available from laboratory experiments (Basson and Clusella-Trullas 2015).

Overall, metabolic adjustments provide a great energy saving potential, especially in tropical regions (Christian et al. 1999). However, the complexity and variability of metabolic responses may have led to an underestimation of compensatory capacities in tropical reptiles and further research is needed especially at ecologically relevant scales, integrating natural conditions and the interplay between behavioural and physiological mechanisms. It may ultimately be exactly this variability that marks the crux for species' survival.

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<u>Acknowledgements</u>

This whole project is not just my own and would never have been possible without the support and endurance of so many others…

First, I would like to thank Kathrin Dausmann for her great supervision, for all her helpful advice and her support during field work and data analysis. This dissertation would not have been possible without you!

I am deeply grateful to Jörg Ganzhorn for taking me to Hamburg, which had a far greater impact on my life than just doing a PhD. Thank you for all your advice, your cheerful wisdom and for being a constant source of urgently needed motivation.

Special thanks also go to Jacques Rakotondranary and Tolona Andrianasolo for so much fun in Tana, for fantastic support with Malagasy authorities and for their annual supply with superbly conserved Mango Zanzibar.

Another big thank you to our field crew: to Maharavo for his assistance with the data collection, for walking back and forth from spiny forest to rainforest but also for tsangatsangana amin'ny tendrombohitra; to Manzo for safeguarding everyone and everything; to Fety and Norihy for rice and beans, scrambled egg with ginger and finally tortillas and for a lot of laughter in the camp; to Refa for his assistance and all them for patiently going with us through good times and wet times (of which we had plenty).

Thank you to Roberto Rakoto and his crew for logistics and transport and for saving us last minute from cyclone Bingiza.

I am grateful for financial support from Evangelisches Studienwerk e.V., the German Research Association (DFG) and for material support from UHU and Mitsubishi uni.

Thanks to the Malagasy authorities (Madagascar National Parks and the Ministère des Eaux et Forêts Madagascar) for the permission to conduct field work in the Andohahela National Park.

Finally and most importantly, I would like to thank my family for their endless support:

My parents for always being there for me, no matter what I do or where I am. Driving back and forth from Lippstadt to Paris six times in the hope for an uncancelled flight during the eruptions of Eyjafjallajökull is just one of the countless examples of your self-sacrificing support. Without you I would not be who I am!

My fantastic son for being so understanding and enduring until his parents' PhDs are finally submitted.

And most of all, I am grateful to Ole Theisinger for the best time in Madagascar ever, for saving my life after that one in a million most stupid mosquito bite ever, for being who you are and for being with me. I am looking forward to sitting on a bench with you (or will it be a hammock?) in fifty years' time and remembering all those adventures that we went through together!

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 Wiebke Berg, titled "Metabolic responses to environmental variation in tropical reptiles", is precisely written in excellent English at a high level appropriate for the dissertation.

Yours sincerely,

James Turner

Declaration of oath

I hereby declare on oath that the work in this dissertation is my own and that I have not used other than the acknowledged resources and aids.

Hamburg, 14.12.2016

Wiebke Berg