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Patterns in Physiological Trait Variation Delineate Potential Impacts of Climate Change on Ectotherms

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DECLARATION ON ETHICS

This research presented and reported in this thesis was conducted in compliance with the National Health and Medical Research Council (NHMRC) Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 7th Edition, 2004 and the Qld Animal Care and Protection Act, 2001. The proposed research study received animal ethics approval from the JCU Animal Ethics Committee (Approval Numbers # A1675, A1755, and A2076) and additionally by the animal ethics committee of the University of Sydney for research on animals collected in New South Wales (Approval Number # L04/10-2011/3/5617).

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Thesis Abstract

Understanding the physiological and behavioural mechanisms that limit species' distributions is essential to our understanding of species' evolutionary physiology, as well as our ability to predict differential impacts of climate change. Despite the re-emerging interest in physiological determinants of large-scale biogeographic patterns (macrophysiology), substantial knowledge gaps remain in our understanding of the drivers of differential evolution of physiological traits and the potential for these traits to limit species' ability to cope with climatic extremes. Ectotherms are of particular concern, because they make up most of the world's biodiversity (invertebrates, fish, amphibians, reptiles, and plants) and are highly susceptible to spatial and temporal variation in thermal regimes. Problems in the relevant literature include;

(i) Focus on the presence or absence of biogeographic patterns rather than their underlying mechanisms,
(ii) Inadequate or inconsistent, assessment of phenotypic plasticity (acclimation potential) across studies used for meta-analyses,
(iii) Limited knowledge on how behaviour modifies exposure to extremes,
(iv) Under-appreciation of desiccation risk as a limiting factor in addition to thermal constraints,
(v) Lack of studies on comprehensive sets of thermal traits within a phylogenetically and methodologically controlled frame work, and
(vi) Lack of knowledge on which, of the many, traits affected by temperature are physiologically and geographically the most limiting to ectothermic organisms.

In Chapter I, I use published data to review the validity of one of the most heavily debated biogeographic pattern, Rapoport's Rule, and its underlying mechanism, the Climatic Variability Hypothesis. I provide a novel approach to testing the Climatic Variability Hypothesis and show that it applies even to taxa that do not follow the pattern of Rapoport's Rule.

In Chapter II, I describe the complete acclimation process of critical thermal minimum temperatures in tropical ectotherms to establish the length and extent of this process and assess the degree to which previous studies may underestimate thermal tolerances because of inconsistent, short acclimation times. I show that acclimation of lower thermal limits, which contribute substantially to estimates of thermal tolerance breadth, can take more than 16 weeks to complete, even in a tropical ectotherm with little natural exposure to cold conditions. Current estimates of thermal tolerance based on inconsistent, partial acclimation, consequently greatly bias our estimates of thermal tolerances.

Chapter III examines how behavioural hydroregulation reduces exposure of "dry-skinned" ectotherms to conditions that promote high desiccation rates. Active hydroregulation is present in dry-skinned ectotherms from tropical rainforests and desiccation avoidance clashes with thermoregulation, placing individuals in thermally suboptimal conditions and likely reducing activity times in dry conditions.

In Chapter IV, I provide a comprehensive study of inter- and intraspecific variation in fully acclimated thermal traits in a clade of small, dry-skinned ectotherms from Eastern Australia across a natural geographic gradient. Water loss rates, metabolic rates, critical thermal minima, thermoregulatory behaviour and performance parameters vary along the latitudinal gradient in temperature. However, upper thermal limits, although often used to predict vulnerability of tropical and temperate organisms to climate change, do not vary with latitude.

Chapter V assesses interactions between thermoregulatory behaviour and thermal dependence of performance to establish how the former may buffer exposure to extremes. The combined effects of behavioural hydroregulation and thermoregulation may buffer species against environmental variability and enable them to occur in conditions far outside their physiological tolerance limits. While decreasing risk of overheating and desiccation, these behaviours may, however, greatly limit potential activity time and, therefore, fitness. Vulnerability of ectotherms to increasing temperatures may be determined by increases desiccation, as well as by impacts of reduced activity times and increased metabolic expenditure on species' energy budgets, rather than simply by risk of overheating. Substantial intraspecific variation in metabolic rate and lower thermal limit suggests strong selection pressures on these traits.

Chapter VI considers which physiological or behavioural traits best predict species' potential to extend into climatic extremes. Metabolic compensation to cold and cold tolerance are the best predictors of species' potential to extend into colder regions, while physiological heat tolerance is not a significant predictor of mean or maximum air temperatures species extend into, but rather appears to predict tolerance to high radiation levels. Overheating is, therefore, likely to affect ectotherms through species' capacity to deal with high levels or frequency of exposure to radiation, rather than high ambient air temperatures. Water loss rates are a significant predictor of species' potential to extend into dry habitats, even in dry-skinned ectotherms, and are underappreciated in their relevance for species' vulnerability to climate change, which is predicted to not only increase temperatures, but also moisture deficit and frequency of drought.

Future studies should assess variation in desiccation resistance across geographically and taxonomically broader scales to enhance our ability to predict impacts of climate change. This study provides fine-scale, novel insight into determinants of limits to species' current and future distribution and is the most comprehensive analysis of thermal trait variation in a taxon to date.

"When you realize the value of all life, you dwell less on what is past and concentrate more on the preservation of the future."

– Dian Fossey

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General Introduction

Macrophysiology, the study of the underlying physiological and climatic mechanisms behind large scale geographic and temporal patterns in species' distributions (Gaston et al. 2009), has recently re-emerged as a field of immense interest because of its relevance for the impact of climate change on the world's biodiversity (Kearney and Porter 2004, Deutsch et al. 2008, Sunday et al. 2014). A multitude of biogeographic patterns in species' abundance and distribution have been considered over the last two centuries (Bergmann 1848, Wallace 1860, Bartholomew 1958, Brown 1984, Stevens 1989, Huey and Bennett 1990, Holt 2003 , Clusella-Trullas and Chown 2014, Sunday et al. 2014), and are thought to result from variation in physiological, anatomical, phenological and behavioural traits (Gaston 2003). Despite this our understanding of what drives the differential evolution of physiological traits and how species' range extents are limited by their differential physiological capacities is surprisingly limited. If we want to predict how climate change will impact species' range dimensions and survival, we urgently need to rethink how we use currently observed patterns to predict future responses and address substantial knowledge gaps in our understanding of how species deal with extremes through physiology and behaviour.

One of the most heavily debated biogeographical patterns is Rapoport's Rule, which predicts that species occurring at higher latitudes will have larger latitudinal range extents (Stevens 1989). The underlying mechanism thought to give rise to this pattern is described by the Climatic Variability Hypothesis (CVH), which states that species at higher latitudes, where environmental variability is greater (Müller 1982), should evolve broader environmental tolerances and, therefore, be able to achieve larger range extents along the latitudinal gradient in mean climate and climatic variability (Stevens 1989, Gaston and Chown 1999). The CVH is of particular importance with respect to species' differential vulnerability to climate change, because it directly implies that species confined to the tropics should have narrower tolerances than temperate species and are, thus, more vulnerable to changes in climatic conditions (Deutsch et al. 2008, Huey et al. 2009). This is presumably exacerbated by the fact that they are already closer to their upper thermal limits and that rising temperatures (IPCC 2013) will therefore expose them to detrimental temperature extremes (Deutsch et al. 2008). The broad biogeographic pattern predicted by Rapoport's Rule has been tested for in numerous studies, often without assessing the validity of the underlying mechanism or even the presence of the appropriate climate patterns within the study area that could elicit such a pattern (Fu et al. 2004, Almeida-Neto et al. 2006, McCain and Bracy Knight 2013). However, the validity of the proposed underlying mechanism (CVH) has rarely been tested explicitly (Addo-Bediako et al. 2000, Calosi et al. 2008, Calosi et al. 2010). It should not be surprising then, that the evidence for Rapoport's Rule is equivocal, with strong patterns in some studies but none, or even the reverse of predictions in others (Rohde et al. 1993, Gaston et al. 1998), leading to continued debate and contention. The study of biogeographic patterns and their generality is the essential basis for hypotheses on the mechanisms driving such patterns. However, continued debate over the existence of such patterns has limited value when both evidence for and against them has been collated in detail, because our priority should be to identify and understand the underlying physiological mechanisms that lead to such divergent lines of evidence on the generality of the patterns.

Because critical thermal limits and physiological thermal tolerance breadth are often used as a proxy for species' differential ability to deal with climatic variability, the accurate and standardized measurement of these values is crucial. However, values in the literature are obtained using widely varying methodologies and, particularly, acclimation times. Our knowledge of the length and extent of the acclimation process of thermally linked physiological traits is mostly limited to a relatively old literature that suggests that upper thermal limits of some ectotherms acclimate within a few days (Hutchison and Maness 1979, Lutterschmidt and Hutchison 1997), while metabolic rates take almost two weeks to complete acclimation in snakes (Blem and Blem 1990). Presumably because minimum temperatures vary more geographically than do maximum temperatures (Müller 1982), tolerance of low temperature also varies more geographically than does high temperature tolerance (Huey et al. 2009, Clusella-Trullas and Chown 2014) and contributes more to overall thermal tolerance breadth. However, not much is known about the time-course of cold acclimation of critical thermal minimum temperatures in ectotherms: detailed descriptions of the complete process of acclimation of critical thermal limits is limited to a few studies in invertebrates (Mellanby 1939, Edney 1964, Weldon et al. 2011, Allen et al. 2012) and vertebrate ectotherms (Brett 1944, Brett 1946, Hutchison and Maness 1979, Blem and Blem 1990) and acclimation responses vary greatly among taxa (Weldon et al. 2011, Allen et al. 2012). Consequently, the accuracy, comparative relevance and hence predictive value of thermal tolerance measurements across studies using hugely variable acclimation regimes (different temperatures and acclimation times anywhere between zero and up to seven weeks of acclimation (Murrish and Vance 1968, Corn 1971, Wheeler 1986, Kattan and Lillywhite 1989, Kaufmann and Bennett 1989, Terblanche et al. 2007, Angilletta 2009, Calosi et al. 2010, Clusella-Trullas and Chown 2014) is questionable and the effect of such variation in methodology on data comparability has been acknowledged repeatedly since the 1930s (Mellanby 1939, Hutchison 1976).

Temperature is generally thought to be the main physical factor limiting species' distributions, and especially ectotherm species' distributions, which make up the bulk of the world's biodiversity (Deutsch et al. 2008, Kearney et al. 2009). While it is well established that a multitude of traits can affect range limits and distributions, many current studies, especially those on interspecific patterns in differential vulnerability to climate change, focus largely on physiological tolerances as a potentially limiting trait (e.g. Deutsch et al. 2008), Although some recent studies have added considerations of behavioural thermoregulation into their work (Sunday et al. 2014, Buckley et al. 2015). The common focus on physiological heat tolerances inherently suggests an under-appreciation of the fact that environmental thermal tolerances can be limited by traits other than physiological thermal tolerances. Among others, these are (i) standard metabolic rates, which determine how much energy is expended at a certain temperature (i.e. species with higher metabolic rates may be able to exploit lower temperatures but those with low metabolic rates may expend comparatively less energy in hot environments; Tsuji 1988, Dillon et al. 2010), (ii) preferred body temperatures, which determine activity times and therefore potential for energy assimilation (Kearney and Porter 2004, Kearney et al. 2013), (iii) precision of thermoregulation, which determine risk of overeating in a heterogeneous environment (Vickers et al. 2011), (iv) performance during chosen activity times, i.e. within the range of selected body temperatures, and last, but not least, (v) desiccation resistance (Kearney et al. 2013), which is linked to temperature as well as to hydric conditions. In dry-skinned vertebrate ectotherms, water loss rates and, even more importantly, especially behavioural avoidance of desiccation (active hydroregulation) have received little attention to date, despite their potential to limit activity times as well as spatial distributions (Kearney et al. 2013). Despite the growing interest in impacts of changes in temperature there is a lack of assessments of inter- and intraspecific variation in a comprehensive set of fully acclimated thermal traits, such as the ones listed above, in model taxa of closely related, ecologically similar species occurring along a natural climatic gradient.

Lastly, there are two similar, but distinct questions that should be considered when studying physiological determinants of species' distributions: (i) what drives the differential evolution of physiological traits across natural climate gradients, and (ii) which traits limit species' abilities to deal with extremes at distribution boundaries the most. While the first question has been the focus of many studies on comparative physiology (Clusella-Trullas et al. 2011, Sunday et al. 2011, Clusella-Trullas and Chown 2014, Sunday et al. 2014), the second is of greater interest when determining species' range limits and vulnerability to climate change. Many recent studies on mechanistic models of species distributions address the second question to some extent by correlating current limits of range extents to physiological traits (Kearney and Porter 2004, Kearney et al. 2010, Kearney et al. 2013). However, comprehensive studies determining which thermal traits best predict species' potential to occur into climatic extremes, as well as which climate dimensions have the greatest potential to be geographically (rather than just physiologically) limiting, are urgently needed. If, for example, a climate variable does not vary much geographically, it is unlikely to limit species' ranges differentially, even if it is physiologically highly relevant.

This thesis aims to address all of the knowledge gaps outlined above. First, I review the controversial debate on the existence of the pattern predicted by Rapoport's Rule and provide a novel approach to how the validity of its proposed underlying mechanism, the Climatic Variability Hypothesis, rather than the resulting biogeographic pattern itself can be adequately assessed (Chapter I; published as Pintor, AFV, Schwarzkopf, L, and Krockenberger, AK 2015. Rapoport's Rule: do climatic variability gradients shape range extent? Ecological Monographs **85**(4):643-659). I then describe the complete time-course and extent of acclimation of lower thermal limits in a tropical ectotherm (Chapter II; published as Pintor, AFV, Schwarzkopf, L, and Krockenberger, AK 2016. Extensive Acclimation in Ectotherms Conceals Interspecific Variation in Thermal Tolerance Limits. PLOS ONE 11(3): e0150408. doi:10.1371/journal.pone.0150408) and discuss the consequences for interpreting studies currently in the literature. Next, I test for hydroregulation in a tropical rainforest ectotherm (Chapter III; in press as Pintor, AFV, Schwarzkopf, L, and Krockenberger, AK. Hydroregulation in a Tropical Dry-Skinned Ectotherm. Oecologia) and discuss how avoidance of detrimental desiccation rates can affect potential activity times and reduce fitness by restricting individuals to thermally suboptimal microhabitats. In the Chapter IV and V I provide a comprehensive analysis of inter- and intraspecific variation in fully acclimated thermal traits (critical thermal limits, thermoregulatory behaviour, metabolic rates, water loss rates and endurance) in thirteen species of closely-related, ecologically-similar ectotherms from Eastern Australia (rainbow skinks; genera *Carlia* and *Lygisaurus*). This represents the most comprehensive study of geographic variation in thermal traits within a taxon to date. I conclude my thesis by determining the best predictor traits of species' ability to extend into climatic extremes along geographically-limiting thermal as well as hydric gradients (Chapter VI) and provide directions for better assessments of ectotherm species' differential vulnerability to climate change. My results provide novel, detailed insight into thermal trait variation across climate gradients and indicate future directions in the field of macrophysiology.

-Chapter I-

Rapoport's Rule: Do Climatic Variability Gradients Shape Range Extent?

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Abstract

The trend of increasing latitudinal range sizes of species towards higher latitudes, known as Rapoport's Rule, has been highly controversial in the literature since it was first proposed by Stevens in 1989. We contend that the question of interest is not whether general global patterns occur, nor whether they support or refute Rapoport's Rule, but whether the mechanism thought to underlie such patterns, the Climatic Variability Hypothesis, is supported. The Climatic Variability Hypothesis suggests that taxa originating from environmentally variable habitats, such as those at high latitudes and altitudes, should evolve wider environmental tolerances, and consequently establish wider distributions along climate gradients than taxa originating from relatively stable habitats. We applied a novel approach, incorporating measures of temperature variability across habitats within species' ranges into models of range size distributions, to determine whether the Climatic Variability Hypothesis applied to three clades of medium-sized ectotherms (lizards) distributed over Australia. Our results show that the Climatic Variability Hypothesis is supported, even in taxa that do not exhibit a traditional Rapoport Effect, due to complex, non-unidirectional climatic gradients in our study area. The results highlight the strong impact of climatic variability on species' physiological tolerances and their associated geographic distributions.

Introduction

Rapoport's Rule, the trend for species' latitudinal range sizes to increase with increasing latitude, has been the subject of controversy over the last two decades. Since the pattern was first noted by Stevens (1989), and then contested by Rohde (1993), the number of articles citing Stevens' (1989) paper has increased from over 300 in 2006 (Stauffer and Rohde 2006) to 655 at present (http://www.scopus.com/, 11/03/2015). The controversy around this ecological "rule", which has been increasingly recast as an "effect" in recent literature (Gaston et al. 1998, Ribas and Schoereder 2006, Beketov 2009), is mostly fuelled by (i) equivocal evidence, in combination with criticism of the statistical methods used to demonstrate the pattern initially, (ii) arguments about the underlying mechanisms, and (iii) limited incorporation of source-sink dynamics into interpretations. Using distributions of several speciose genera of Australian skinks, we summarize and evaluate the most relevant literature and arguments surrounding these three components of the debate, propose new methodological approaches, and contend that the true value of patterns predicted by rules or effects in biogeography lies in clarifying the underlying mechanisms affecting the distributions of taxa rather than supporting or refuting a specific rule (Gaston et al. 2009).

Rapoport's Rule – Evidence & Methodology

Stevens (1989) originally described Rapoport's rule by plotting the mean latitudinal range extent of all species of various taxa in North America occurring in 5° latitudinal bands across the continent, and found supporting evidence for the trend in trees, marine molluscs, freshwater and coastal fishes, reptiles and amphibians, mammals, and non-migratory birds. Subsequently, the rule was expanded to also describe variations in range extent along elevational and bathymetric gradients (Stevens 1992, 1996). Stevens suggested that high environmental variability at higher latitudes (as well as at higher elevations and shallower ocean depths) would select for organisms with broad environmental tolerances, which could, therefore, occur over wider latitudinal ranges along gradients in environmental conditions (the Environmental or Climatic Variability Hypothesis; see Gaston et al. 1998, Gaston and Chown 1999, Addo-Bediako et al. 2000, Calosi et al. 2010). He supported this suggestion with data on variability in temperature, which generally increases with latitude in both hemispheres (Stevens 1989). He also noticed that the pattern did not hold for migratory birds, which may avoid selection for broad environmental tolerances by moving (Stevens 1989). Clearly, Stevens (1989) never suggested that Rapoport's Rule should be true for all organisms, even though the lack of consistency across taxa is a common basis for criticism of the rule (see Gaston et al. 1998). Rather, he proposed that the Rule should influence taxa that (i) occur in locations where climatic variability changes along a gradient (such as latitude, elevation or other spatial gradients), and that (ii) cannot avoid experiencing most of this variability (Stevens 1989). In reality, patterns may be complicated or obscured by overlays of gradients along different spatial axes, or there may be modification of the variability experienced by different taxa because of their behavior and ecology. In addition, species-specific variation in susceptibility to climatic variation may complicate patterns produced at higher taxonomic levels. The pattern's presence or absence can, therefore, be used to determine the mechanisms underlying species' range size. Closer examination of species that vary from the expected pattern for their group could, therefore, lead to a better understanding of the influence of current and past climate patterns and also provide some predictive capacity for the future. For example, they might be used to predict evolutionary responses to climate change. We first need, however, to appropriately describe and analyse the pattern.

Stevens' method (described above) suffered from several statistical problems (Rohde et al. 1993, Gaston et al. 1998). One problem was non-independence of the latitudinal categories: most species included in the analysis occurred in more than one 5° latitudinal band. Wide-ranging species occurred in more latitudinal bands than restricted species, giving wide-ranging species more influence in the analysis. The effect of non-independence is exacerbated by latitudinal gradients in species richness, which can cause great differences in the number of data points included for each group in each latitudinal category. In addition, species' range-size distributions within each latitudinal band are right-skewed, making the grouping mean an inappropriate representation of central tendency (Roy et al. 1994, Gaston et al. 1998).

To address problems associated with Stevens' methodology, Rohde et al. (1993) applied a different method, plotting the latitudinal range extent of species against their latitudinal midpoint (the "midpoint method"), either as independent data points or in latitudinal midpoint categories. Using this method, he found that latitudinal range extent of marine and freshwater fishes is greatest at low (rather than high) latitudes, contrasting with Stevens (1989) who found no consistent Rapoport Effect in marine environments (in accordance with his prediction that bathymetric variability gradients overlying latitudinal gradients would conceal the effect). The midpoint method was, however, flawed, much like Stevens' method, because the latitudinal midpoint of a species' range constrains maximum potential latitudinal range size. In any given sampling area, endemic species with midpoints close to the boundaries of the predefined area are necessarily restricted to a small maximum potential latitudinal range extent, since they can by definition not extend further in one direction from the midpoint than in the other and are therefore restricted in their range size by the geometric boundaries of the study area. As a result, if a species' midpoint lies two latitudinal degrees from a continent's northern boundary, it cannot have a range extent greater than four latitudinal degrees. Species with midpoints in the middle of an area, however, will have the largest possible range extents (Colwell and Hurtt

1994, Blackburn and Gaston 1996, Lyons and Willig 1997, Ribas and Schoereder 2006, Šizling et al. 2009). This constraint means that species' realized ranges could occur anywhere within a triangle (Colwell and Hurtt 1994) determined by latitudinal, elevational or other spatial boundaries in the area being examined, as potential range extents increase from the edges of an area towards the middle. This triangle effect can sometimes be concealed if species' midpoints are clustered in one side of the triangle (i.e., if a taxon does not occur throughout the whole area), potentially leading to a strong apparent Rapoport Effect or reverse Rapoport Effect (depending on the part of the study area to which the taxon is restricted). Thus, trends may appear stronger than they are, because of triangular geometrical constraints on the data, especially if the data is restricted to one half of the triangle. Thus, Rapoport Effects should not be analysed using linear regression, because potential data spread increases towards intermediate x-values and then decreases again towards high x-values. Methodologies that substitute distal point for midpoint analysis (e.g. Pagel et al. 1991) have similar problems.

Surprisingly, given these problems of interpretation, the midpoint and distal point methods have been regularly used to determine whether Rapoport Effects occur in spite of, and without mention of, these well-known constraints (e.g., Letcher and Harvey 1994, Smith et al. 1994, Hughes et al. 1996, Meliadou and Troumbis 1997, Price et al. 1997, Fleishman et al. 1998, Sanders 2002, Husak et al. 2003, Macpherson 2003, Fu et al. 2004, Cruz et al. 2005, Mora and Robertson 2005, Almeida-Neto et al. 2006, Bhattarai and Vetaas 2006, Hausdorf 2006, Morin and Chuine 2006, WanJun et al. 2010, Abellán and Ribera 2011, Guerrero et al. 2011, Hu et al. 2011, Pincheira-Donoso 2011, Thieltges et al. 2011, Novillo and Ojeda 2012, Lee et al. 2013). One attempt to deal with these constraints used the proportion of observed range extents as compared to the potential range extents at specific midpoints (Blackburn and Gaston 1996). However, using this method, information on actual range sizes is lost (e.g., a relatively restricted species at low latitudes may be attributed the same value for proportional range extent in this analysis as a species with a midpoint at intermediate latitudes that ranges across half the study area). Because of this, small variations in actual range size of species at both ends of the study area will carry greater weight in the proportional analysis than substantial variations in range size of species distributed more centrally within the study area. So, the species with the largest potential range extents will have the least weight in the analysis, even though they may be the ones of greatest interest. Furthermore, the choice of midpoints as a descriptive variable for range position appears arbitrary, since range midpoints are not representative of factors determining range size, such as the species' center of abundance (which should be in the middle of the species' niche rather than its geographic extent; Brown 1984, Brown et al. 1996), degree of current climatic variability experienced by the species (Stevens 1989), or the biogeographical origin of the species' ancestors (which we might expect to be representative of the climatic variability experienced by the species' ancestors).

Some recent studies have addressed these statistical challenges by using randomization to compare results to appropriate null models (Lyons and Willig 1997, Diniz-Filho and Tôrres 2002, Ribas and Schoereder 2006, Beketov 2009, Davies et al. 2011, Morin and Lechowicz 2011, 2013), by using more advanced grid-cell analyses over a two-dimensional landscape (Smith et al. 1994, Meliadou and Troumbis 1997, Ruggiero et al. 1998, Hawkins et al. 2006, Ruggiero and Hawkins 2006, Luo et al. 2011, Morin and Lechowicz 2011, Whitton et al. 2012, Morales-Castilla et al. 2013, Morin and Lechowicz 2013) accounting for more complex climate patterns than simple latitudinal gradients, or by using quartile analyses to assess the impact of different groups of range sizes within the data set (e.g., by using only restricted-range species, (Amend et al. 2013, McCain and Bracy Knight 2013, Morin and Lechowicz 2013). However, evidence for Rapoport's Rule remains equivocal. Finally, determining the role of the essential underlying mechanism for the pattern, the influence (or lack thereof) of the Climatic Variability Hypothesis, often seems forgotten in the process.

The Climatic Variability Hypothesis

The most commonly accepted mechanism thought to cause the evolution of larger range sizes at higher latitudes is described by the Climatic Variability Hypothesis (CVH), as postulated by Stevens (1989). The CVH proposes that, because higher latitudes tend to have more variable climates, in closely related species with similar ecologies, species at higher latitudes have been selected for broader environmental tolerances (see **Table 1.1**). Those species should then have broader fundamental niches, and consequently be capable of persisting across a broader range of climatic conditions along a latitudinal climate gradient. This train of inferences is consistent with Janzen's (1967) predictions that mountain passes pose a greater physiological barrier to organisms in the tropics, because tropical organisms should have narrower fundamental niches than temperate species, making elevational climate gradients relatively steeper. It is important to clarify that "climatic variability" under this definition is the variability in the specific area from which the species originated, not necessarily the absolute variability across the entire current range of the species, which necessarily increases with increasing range size. If climatic conditions vary with latitude, then any species occurring over a large latitudinal range will, by definition, experience a greater overall range of climatic conditions, but not necessarily in any given habitat. Gradients in mean climatic conditions as well as climatic variability occur across latitude, elevation and ocean depth in several parts of the world, at least with respect to temperature (Janzen 1967, Snyder and Weathers 1975, Müller 1982, Stevens 1989, 1992, 1996). However, many studies on Rapoport's Rule or the CVH do not quantify the gradients in their

Table 1.1 Definitions of some terms used in this chapter.

Term:	**Synonyms:**
Range Extent	Range, Range Size

Definition:

The dimensions of a species' range, measured from one extreme to the other along a scale such as latitudinal range (difference between maximum and minimum latitude of occurrence), elevational range (difference between maximum and minimum elevation of occurrence), etc.

Term:	**Synonyms:**
Midpoint	Range Centre

Definition:

The midpoint between the two extremes of a species range, such as mid-latitude (the half-way point between maximum and minimum latitude of occurrence) or mid-elevation (the half-way point between maximum and minimum elevation of occurrence), etc.

Term:	**Synonyms:**
Environmental Niche	Environmental Tolerances, Environmental Niche Breadth

Definition:

The range of environmental conditions tolerated by a species, such as the highest and lowest temperatures within a species range and the difference between the two. In this chapter two different types of environmental tolerances are referred to: (i) a species' overall tolerance across its geographic range, measured as the difference between the maximum and minimum temperature experienced within that range, and (ii) the maximum annual variability (MAV) experienced by the species at any location within its range. These are influenced by and often indicative of but not synonymous to the fundamental niche to physiological tolerances and can be modified extensively from the latter two through thermoregulatory behaviour and dispersal rates. It can be further modified by biotic interactions into a realized niche.

Term:	**Synonyms:**
Fundamental Niche	Fundamental Niche Breadth

Definition:

The subset of abiotic conditions acting on a species that it can physiologically tolerate, in ectotherms especially with respect to variables affecting body temperature. This niche space is determined by physiological thermal limits (critical thermal maximum and minimum body temperature; absolute physiological tolerances) but also by water loss rates, metabolic rates, performance, life history traits, etc.

Term:	**Antonym:**
Source	Sink
Definition:	**Definition:**
Geographic areas, environmental niche space or fundamental niche space (depending on context), where a species can experience positive population growth.	Geographic areas, environmental niche space or fundamental niche space (depending on context), where a species cannot experience positive population growth but where low population numbers are maintained because of dispersal.

study areas (e.g. Fu et al. 2004, Almeida-Neto et al. 2006, McCain and Bracy Knight 2013), which is critically relevant to interpretation of results. Gradients in other climatic variables, such as humidity or precipitation, may also be strongly relevant, depending on the taxon, but are rarely measured in this context (Gaston and Chown 1999). In addition, differential capabilities of taxa to avoid unfavorable climatic conditions through migration, dormancy, or thermoregulatory behavior are usually not addressed. More importantly, however, evidence for the crucial component of the CVH, namely the correlation of niche breadth with range size or range position, or both, along latitudinal, elevational or bathymetric gradients, is surprisingly sparse (Calosi et al. 2008, Calosi et al. 2010). Only one study has assessed the relationship of thermal tolerance to both range size and range position across a reasonable number of closely related and ecologically similar species (Calosi et al. 2010). There is still a lack of studies across taxa and environments accounting for phylogeny, ecological similarity, acclimation potential, actual underlying climate patterns including environmental variables other than temperature, as well as presence or absence of a Rapoport Effect and comparing tolerances with range size as well as range position.

The first step towards a comprehensive understanding of species' range size distributions, and the underlying mechanisms shaping them, is to correlate the current range extents of ecologically similar organisms within a taxon with appropriately assessed differences in the variability of the relevant climate factors they experience, as a direct test of the CVH. To our knowledge, the only study that has done so appropriately to this date is a recent study on range sizes in North American trees (Morin and Lechowicz 2013). Adding measures of actual measured niche breadth (i.e., taking a mechanistic approach; Calosi et al. 2010) to models based on current occurrence (Morin and Lechowicz 2013) should ultimately be the goal.

In summary, if we want to assess the validity of the CVH as a basis for Rapoport's Rule, we need to ensure its premises are met, i.e. (i) there must be a spatial gradient in mean climatic conditions and climatic variability (e.g., latitudinal variation) in the study area, (ii) the taxon used to test the CVH should include ecologically similar species affected similarly by environmental factors (Calosi et al. 2010), and (iii) the physiological or at least environmental tolerances of species should either be measured directly, or estimated appropriately by using indicators such as maximum variability experienced in any one location. Only if these premises are met can we accurately assess the validity of the CVH. Whether a Rapoport Effect is present in a taxon is, in this respect, of little relevance and should solely depend on whether the direction of gradients in climatic variability in an area represents the average global trend outlined by Müller (1982) and Stevens (1998).

Source-Sink Dynamics

The logic of the CVH assumes that there are source-sink dynamics in animal populations. In short, simplified source-sink dynamics (without the effect of biotic interactions) suggest that a species' range consists of (i) a source habitat, which is within the limits of the species' fundamental niche, where the species can experience positive population growth, and (ii) sink habitats, outside the species' fundamental niche, into which individuals may disperse. Sink populations persist only because of repeated immigration from sources, because birth rates in such habitats are too low to maintain positive population growth (Pulliam 1988, 2000, Holt 2009). The corollary of this is that in a stable source habitat, species experience selection for a narrow niche. It follows that they will (i) have difficulty persisting in sink habitats for extended periods, (ii) experience selection against high dispersal rates, as individuals that disperse into sinks will have lower fitness, and (iii) have difficulty adapting to sink conditions, because of genetic swamping from the source (Holt 1985, Pulliam 1988, Holt 2003). In the absence of long-term changes in climatic conditions, these species' tolerance ranges and, consequently, geographical ranges, should contract to an increasingly restricted source habitat (Holt 1985, Pulliam 1988, Holt 2003). We expect, however, that species from variable source habitats, will be adapted to a broad range of conditions, making dispersal into sinks less detrimental to their fitness, or even beneficial, since they may persist in sinks if conditions in their source habitat become unfavorable, after which they could reinvade source habitat (e.g. Puschendorf et al. 2011). These species may, therefore, be selected for high dispersal rates and not suffer genetic swamping in sinks, leading to range expansion, and large geographical range sizes (Holt 1985, Pulliam 1988, Holt 2003).

Complex source-sink dynamics cause several theoretical and methodological problems when analyzing species' range sizes and their distribution. First, we need to clearly define species' ranges. The realized niche of a species, and, therefore, its range, may be either (i) smaller than the fundamental niche or potential range because of biotic interactions and dispersal barriers, but it may also be (ii) larger than the fundamental niche or potential range because of the presence of sinks (Pulliam 1988). With regard to the CVH, range size is assumed to be a representation and direct consequence of niche breadth, or, in other words we assume that the extent of a species' current source habitat represents the variability in the historical source habitat of its ancestors. Because a species' current range, estimated using occurrence data, may over- or underestimate the geographic range over which it could potentially experience positive population growth based on climate conditions, support for the CVH could either be obscured or enhanced. More importantly, however, to accurately reflect range position we should use a reference point that reflects conditions in the historical source habitat of the species' direct ancestors, which, if we assume the CVH is correct, should be the most variable habitat in a species' range in its biogeographic history. Since this is an impossible measure to obtain, due to changing climates and range sizes (historically as well as currently), the best measure we can use to assess whether the degree of variability experienced by a species in any one location translates into its potential to cover a greater breadth of climate conditions geographically, is the most variable habitat in a species' current range. There remains the risk that this habitat may, in fact, represent sink conditions, because the most extreme current occurrence point is most likely not the habitat of optimal population growth. We believe, however, that it is the best available measure to use to estimate tolerances of source populations according to the theoretical premises of the CVH.

In summary, a thorough approach to unravelling the story behind Rapoport's Rule and the climatic variability hypothesis should involve answering several questions in a logical sequence. These are: (1) What are the patterns in relevant climate factors and climatic variability across a given area to which a taxon is likely to be exposed? (2) Does the taxon show a trend in range size distributions consistent with expectations based on climate (i.e. does the climatic variability hypothesis apply)? (3) Are differences in climatic tolerances related to patterns in range size, or is climate variability buffered behaviorally, ecologically or through genetic sub-structuring of physiological traits across ranges (local adaptation)?

In this study, we first examine the climatic gradients experienced by a group of closely related, ecologically similar, species of reptiles, and test for a Rapoport Effect using both Stevens' (1989) method and the mid-point method, to allow for comparison with previous studies and to highlight how presence or absence of a Rapoport Effect measured through previous methodologies contrasts with results from direct tests of the CVH. We do this by using a novel approach correlating maximum temperature variability within each species' range with its current range extent. This approach allows a direct test of the CVH, examining whether species that exhibit greater latitudinal and elevational range extents also have greater tolerance of annual temperature variation, as estimated based on the greatest variability experienced in any one habitat within their range. This allows for clarification of whether the presence or absence of Rapoport Effects reported in previous literature has any relevance to the validity of the underlying mechanism of interest, the CVH. We predict that the absence of a Rapoport Effect, in itself, has little relevance for the validity of the CVH and can be explained by deviations of regional climate patterns from global latitudinal climate gradients. Assessing the validity of underlying mechanisms of biogeographical "rules" may consequentially be of much greater relevance than whether these rules exist unequivocally across taxa.

Methods

As our study system we used the three largest monophyletic lineages of lygosomine skinks endemic to the Australian mainland, namely the *Carlia* clade (Eugongylus group; genera *Carlia*, *Lygisaurus* and *Liburnascincus*), the *Egernia* clade (*Egernia* group; genera *Egernia*, *Bellatorias*, *Liopholis*, *Lissolepis*, *Tiliqua* and *Cyclodomorphus*) and the genus *Ctenotus* (Sphenomorphus group; genus *Ctenotus*). Species in the *Carlia* clade are mostly restricted to the Australian East Coast (AEC), while the other two clades used in this study occur over most of Australia, including the arid interior. *Carlia* and *Ctenotus* are small- to medium-sized, oviparous, largely diurnal species. The *Egernia* clade is quite different from the other two clades in both anatomy and physiology. They are viviparous, large, and some species are nocturnal (Wilson and Swan 2008).

Species' distributions (from museum records) and climate information were downloaded from the Atlas of Living Australia (http://www.ala.org.au/explore/species-maps/, 06/10/2011). Information from climate layers was accessed through the Atlas of Living Australia, which uses climate layers from external sources, by downloading the value at the specific occurrence records of each species. The climate variables used in the analysis were "Temperature – annual mean (Bio01; Hijmans et al. 2005 ; see http://www.worldclim.org/bioclim)", "Temperature – max absolute mean max" (Williams et al. 2010), "Temperature – min absolute mean min" (Williams et al. 2010) and "Elevation". Latitude and Longitude for each occurrence point were downloaded as well. All other values used were calculated from these. The annual temperature range for each occurrence was calculated as the difference between "Temperature – min absolute mean min" and "Temperature – max absolute mean max". Museum Records were compared with distribution maps in Wilson and Swan (2008) and definite outliers excluded (i.e., single occurrence records far outside the known distribution, likely to be identification errors). Latitudinal and Elevational Midpoints were calculated as the mid-distance between maximum and minimum values for latitude and elevation for each species separately. Latitudinal and elevational range extents were calculated as the difference between maximum and minimum values for latitude and elevation, respectively.

Some species of the clades we examine extend into Papua New Guinea (PNG) or Tasmania. Extralimital species were excluded from the analysis. This was done mostly due to lack of climate data for PNG and to avoid the influence of island size on species' range limits, i.e., truncation of potential ranges. Data for some species were missing from online museum records and these species were also excluded.

Two traditional approaches were applied for comparison of tests of the CVH with Rapoport Effects as measured by earlier studies: the midpoint method and Stevens' (1989) method. To illustrate geometric limitations on the midpoint method using independent data points, latitudinal and elevational midpoints were plotted against latitudinal and elevational range extents. For Stevens' Method (latitude and elevation), the mean latitudinal and elevational range extent was plotted for all species present in each of the six 5° latitudinal and nine 250m elevational bands. Patterns were analysed using Spearman's rank correlation coefficient, in accordance with Stevens' analysis of the same pattern (Stevens 1992). Since these preliminary tests revealed a latitudinal Rapoport Effect in two out of the three clades but – in contradiction with this - an elevational Rapoport Effect in all three clades, the selected clades were an ideal selection to compare with results from more direct tests of the CVH.

To assess the validity of the CVH as the main proposed mechanism causing Rapoport Effects in some taxa, the annual temperature variability of the most variable occurrence point (Maximum annual variability - MAV) for each species was plotted against latitudinal range extent for the three clades separately. It is important to note, however, that the ranges of wide ranging species are more likely, by random chance alone, to include locations with high environmental variability. Therefore, we compared the plot of the maximum annual variability versus latitudinal range to a null model. The null model was generated by combining all occurrence points for all species in each clade, and randomly selecting the same number of sampling locations for climatic variability as were recorded for each species (data for different clades were kept separate for this analysis). The maximum annual variability for each set of randomized data points for each species was recorded. 10,000 repetitions of this randomization were performed for each species to obtain a dataset of 10,000 expected MAVs for each species based on chance alone. If our randomizations showed greater expected maximum variability than that measured for a species in ≥ 95% of 10,000 simulations, we concluded the species was significantly less tolerant (i.e., occupied significantly less variable habitats) than expected by chance alone for a species of that particular range size. Since most measured maximum annual variabilities were significantly lower than expected maximum annual variability, we inferred that the observed trend of restricted species occurring in less variable habitats was not due to sampling artefacts caused by the size of the range. To assess whether different species differed in their experienced MAV more or less from the mean expected MAV, we used a linear model to analyse the relationship between the logged difference between the two measures and species' range sizes. If the CVH applies, restricted species were expected to not only occur in less variable habitats, but also to show stronger deviation of the experienced variability from the randomly expected variability. The results of our direct tests of the CVH were compared to the results from traditional methodologies assessing the presence of Rapoport Effects to see whether the presence or absence of the effect, as reported by previous studies, could indeed be used as a test of the CVH with any reliability, or not.

Statistical analysis was performed in R (R Core Team 2011). We used linear mixed effects models ([R] package nlme, lme function, Pinheiro et al. 2013) to allow us to include clade as a random effect. Models of best fit were determined by comparing Akaike Information Criterion (AIC) values and Log Likelihood Ratio Tests. Where inclusion of the clade identity as a random effect improved the model significantly, results are reported for each clade separately.

Results

Climate

Absolute mean maximum and minimum temperature, as well as maximum annual temperature range, as a measure of climatic variability, varied significantly with latitude along the Australian East Coast at sea level (**Fig.1.1**; linear regression for absolute mean maximum with latitude: $P = 0.034$ and $R^2 = 0.30$, absolute mean minimum $P < 0.001$ and $R^2 = 0.74$, and absolute annual range $P < 0.001$ and $R^2 = 0.66$, respectively). At higher latitudes there was a larger range of temperatures than at lower latitudes, mostly caused by a steeper gradient in minimum temperatures (**Fig.1.1**). However, examination of maximum annual variability (MAV; the difference between absolute mean minimum and maximum temperatures) across species' ranges spanning large parts of the continent revealed that gradients were not unidirectional. Whereas a species with an East Coast distribution (*Carlia vivax*; **Fig.1.2B**) experiences a latitudinal gradient in variability that is complicated only slightly by longitudinal changes in variability, more widely distributed taxa such as *Ctenotus robustus* (**Fig.1.2A**) experience much lower variability along the southern coastline than in the highly variable arid center.

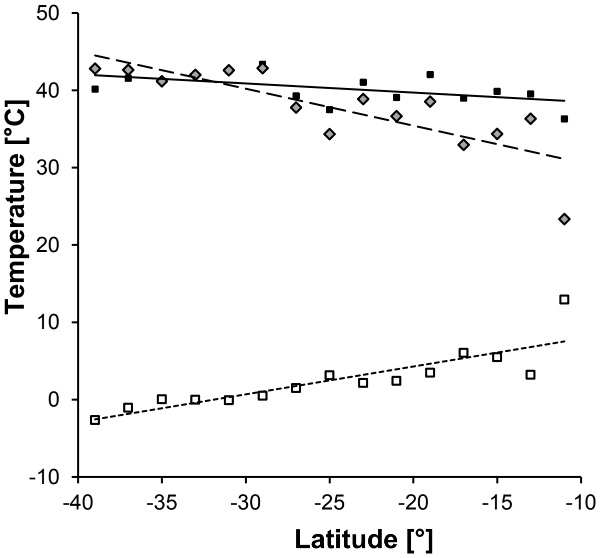

Fig.1.1 Correlation between latitude and absolute mean maximum (filled squares, solid line) and absolute mean minimum temperatures (open squares, dotted line) and absolute annual temperature range (grey diamonds, dashed line) along the Australian East Coast at 0 m elevation AMSL (linear regression for absolute mean max with $P = 0.034$ and $R^2 = 0.300$, absolute mean min with $P < 0.001$ and $R^2 = 0.741$, and absolute annual range with $P < 0.001$ and $R^2 = 0.661$, respectively)

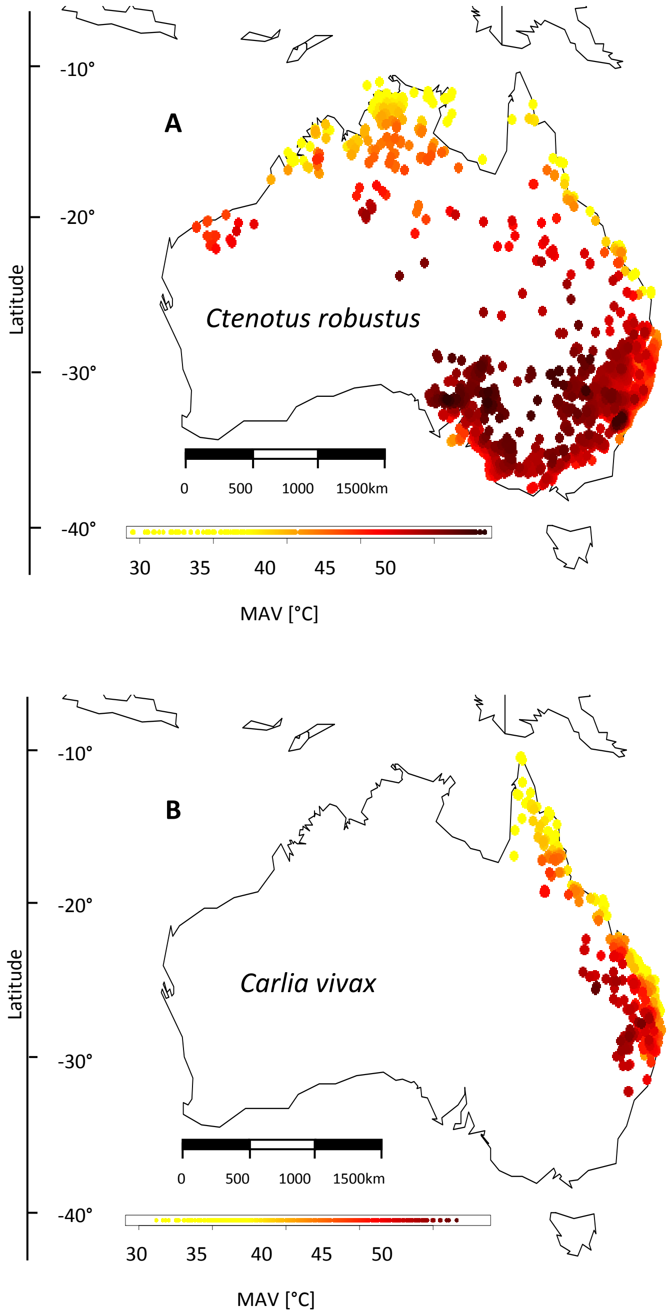

Fig.1.2 Maximum Annual Variability (MAV) measured as difference between absolute mean minimum and absolute mean maximum temperatures [°C] in two species with different distributions. *Ctenotus robustus* extends into more variable habitats inland (**A**), while *Carlia vivax* displays a coastal distribution more representative of the expected latitudinal gradient in variability (**B**).

Rapoport's Rule in the Carlia, Ctenotus & Egernia clades

When the three most speciose monophyletic clades in the *Eugongylus*, *Sphenomorphus* and *Egernia* groups were analysed separately using traditional methodologies, several patterns emerged (**Fig.1.3A**). The *Carlia* clade is restricted to mid- to low latitudes with an apparent clustering of restricted species at low latitudes, the *Ctenotus* clade is distributed throughout the whole study area within a triangular space of potential range sizes at different latitudinal midpoints, defined by geometric restrictions exerted by midpoints on potential range sizes, and members of the *Egernia* clade occur more frequently at higher latitudes. With respect to elevation (**Fig.1.3B**), all three clades' species were clustered, with most ranges starting at sea level and extending to variable extents into higher elevations, leading to a spread of midpoints along the lowest possible range extent in the lower half of the triangular space of potential range extents, with the exceptions of the restricted high elevation species *Cyclodomorphus praealtus* and *Liopholis guthega* from the *Egernia* clade.

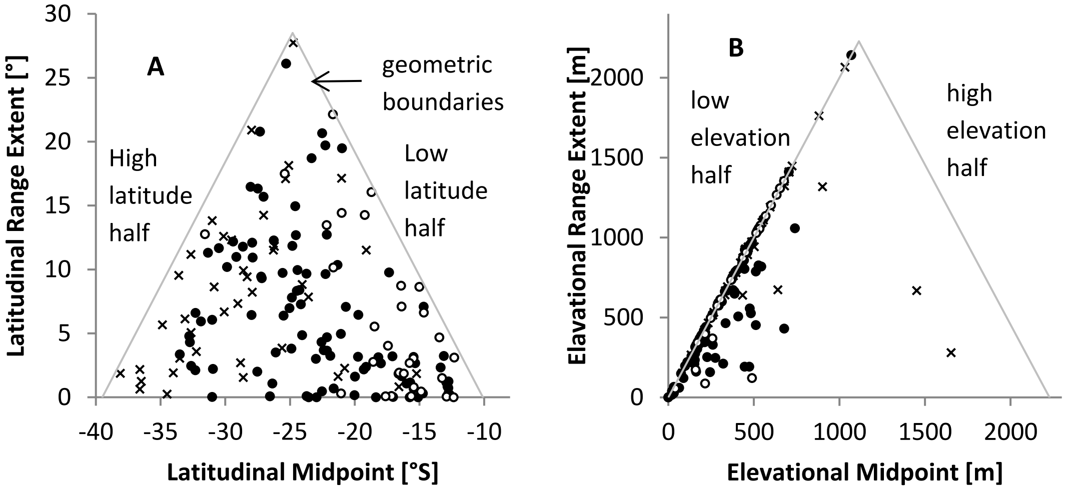

Fig.1.3 Relationship between Latitudinal Range Extent vs. Latitudinal Range Midpoint (**A**) and Elevational Range Extents vs. Elevational Range Midpoint (**B**) for species within the *Carlia* (open circles), *Ctenotus* (closed circles) and *Egernia* (crosses) clades endemic to the Australian mainland.

Using Stevens' method, we found that range sizes decreased significantly with latitude in the *Carlia* (**Fig.1.4 A**; Spearman's rank correlation coefficient; $\rho = -0.438$; $P < 0.001$) and *Ctenotus* clades (**Fig.1.4 B**; $\rho = -0.264$; $P < 0.001$). These two clades exhibited a positive Rapoport Effect, while the trend in the *Egernia* clade was reversed (a negative or reversed latitudinal Rapoport Effect) with species exhibiting larger mean ranges at lower latitudes (**Fig.1.4 C**; $\rho = 0.208$; $P < 0.05$). There was a positive elevational Rapoport Effect, as range size increased towards higher elevations in all three clades (**Fig.1.5 A-C**; $\rho = 0.414$, $\rho = 0.478$ and $\rho = 0.299$ for the *Carlia*, *Ctenotus* and *Egernia* clade, respectively, with $P < 0.001$ for all clades). The

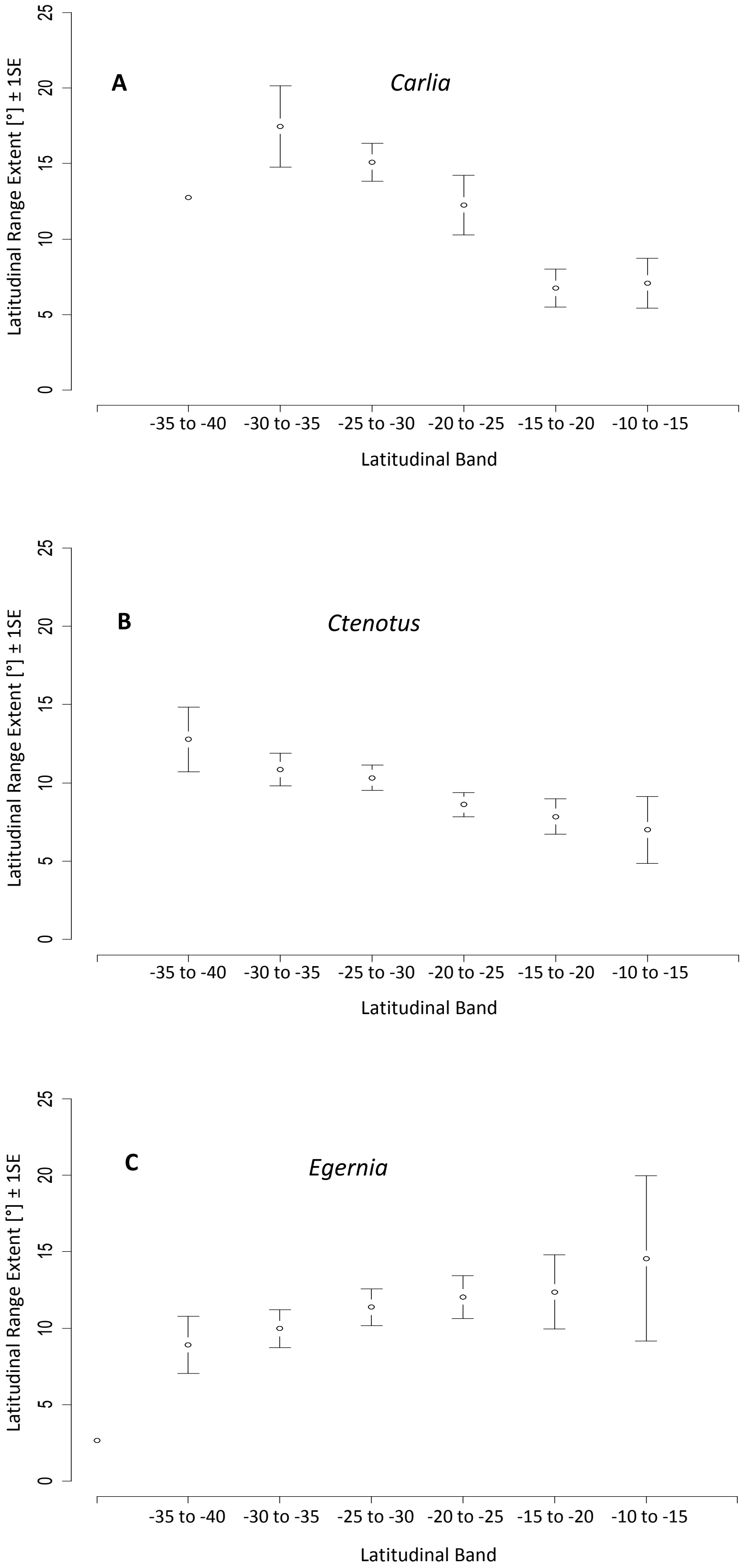

Fig.1.4 Mean latitudinal range extent of all species occurring in each 5° latitudinal band (Stevens' method) for the (A) *Carlia*, (B) *Ctenotus* and (C) *Egernia* clades, respectively. Spearman's rank correlation coefficient revealed significant correlations in A (ρ = -0.438; $P <$ 0.001), B (ρ = -0.264; $P < 0.001$), C (ρ = 0.208; $P < 0.05$).

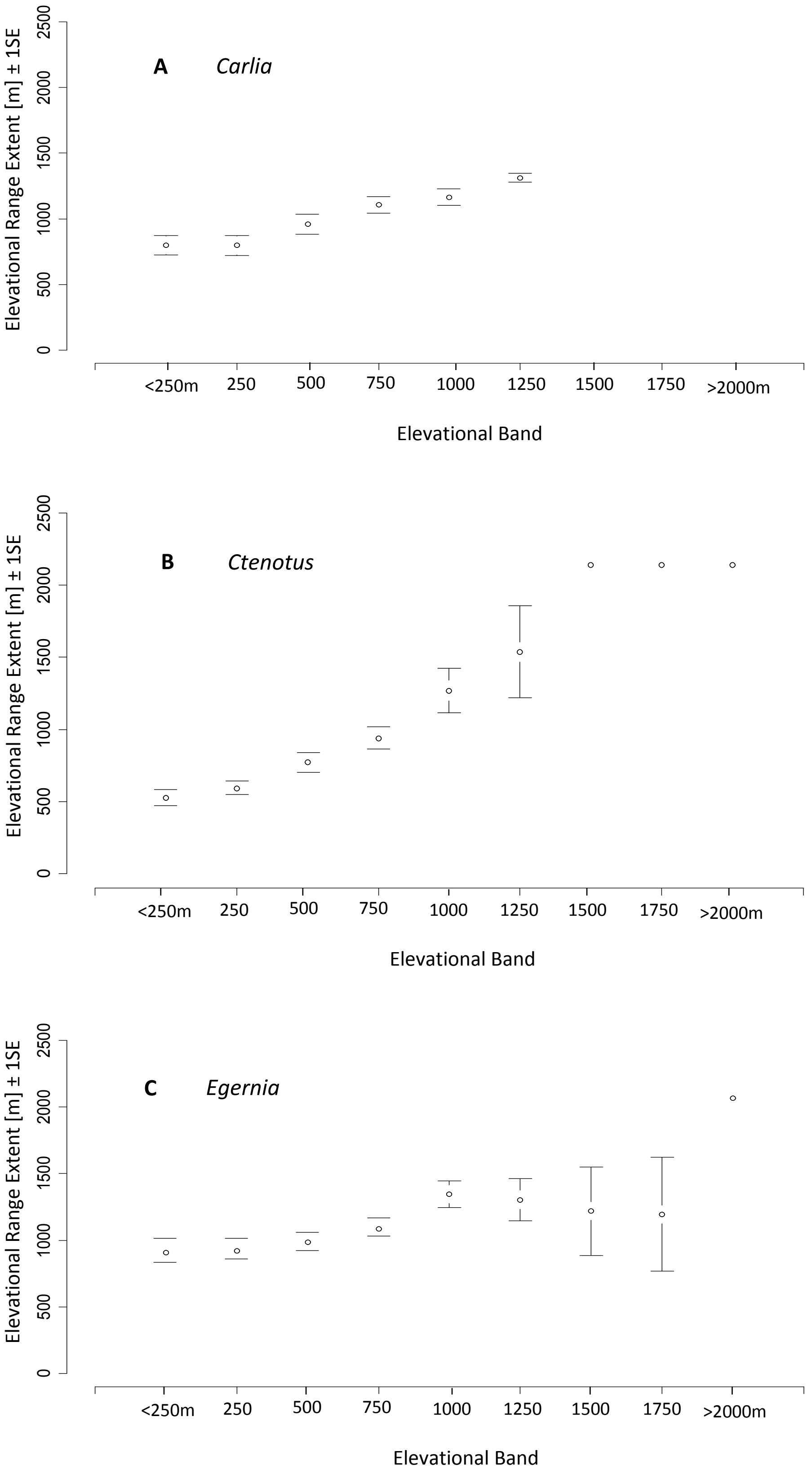

Fig.1.5 Mean altitudinal range extent of all species occurring in each 250 m altitudinal band (Stevens' method). Spearman's rank correlation coefficient revealed significant correlations for the *Carlia* (A; $\rho = 0.414$; $P < 0.001$), *Ctenotus* (B; $\rho = 0.478$; $P < 0.001$) and *Egernia* (C; $\rho = 0.299$; $P < 0.001$) clade, respectively.

Positive elevational Rapoport Effect in the *Egernia* clade occurred in contrast to the negative latitudinal Rapoport Effect in the same clade, while trends in the other two clades were consistent.

When absolute annual temperature range (Maximum Annual Variability; MAV) at the most variable occurrence point of each species (as a measure of estimated environmental tolerance) was plotted against latitudinal and elevational range extent (**Fig.1.6 A**, **B** and **C** and **Fig.1.6 D**, **E** and **F**, respectively), they were significantly positively correlated in all three clades for both latitudinal and elevational ranges (Spearman's rank correlation; *Carlia*: $\rho_s = 0.668$ and $P < 0.0001$ for latitude and $\rho_s = 0.638$ and $P < 0.0005$ for elevation; *Ctenotus*: $\rho_s = 0.802$ and $P < 0.001$ for latitude and $\rho_s = 0.706$ and $P < 0.0001$ for elevation; *Egernia*: $\rho_s = 0.729$ and $P < 0.0001$ for latitude and $\rho_s = 0.399$ and $P < 0.001$ for elevation). This agrees with Rapoport Effects observed for the *Carlia* and *Ctenotus* clades for latitude, but contrasts with the decrease of range size at higher latitudes (reverse latitudinal Rapoport Effect) in the *Egernia* clade. Most species' estimated tolerances are significantly narrower than expected by chance alone (i.e., for most species >95% of randomizations resulted in broader estimated tolerances than the observed MAV suggests), with the exception of 3 out of 29 (10%) *Carlia*, 10 out of 89 (11%) *Ctenotus*, and 9 out of 45 (20%) *Egernia* species (**Fig.1.7 A** and **B**).

The difference between measured and mean randomized MAV for each species decreased with species' range size latitudinally, as well as elevationally (**Fig.1.8 A** and **B**), indicating that the more restricted a species was in both dimensions, the narrower its estimated tolerance compared to results from the randomizations, while more wide-ranging species approached tolerance breadths expected by chance alone. The model of best fit describing the negative correlation of the log transformed difference between measured and randomized MAV with increasing range size, was a linear mixed model with a different intercept (*a*) for each clade for latitudinal patterns, indicating that the pattern varied among clades (**Fig.1.8 A**; AIC = 287.56, $t_{135} = -10.81$, $P < 0.0001$). It was a significantly better fit than the simpler model for all clades combined (AIC = 308.01, Log-Likelihood ratio = 25.61, $P < 0.0001$). Accounting for a different intercept and slope (*b*) for each clade in a more complex model did not significantly improve the fit (AIC = 288.41, Log-Likelihood ratio = 3.15, $P = 0.21$). Tolerances were narrowest in *Carlia*, followed by *Ctenotus*, and widest in *Egernia*. *Carlia* exhibited the greatest discrepancy between measured and expected MAV, and therefore appeared to be most affected by the latitudinal gradient in temperature variability (linear regression; *Carlia*: $a = 2.38$, $b = -6.54*10^{-2}$, $F_{1,24} = 19.2$, $P < 0.001$, $R^2 = 0.42$; *Ctenotus:* $a = 2.04$; $b = -1.20*10^{-1}$, $F_{1,76} = 79.23$, $P < 0.001$, $R^2 = 0.50$; *Egernia*: $a = 1.53$, $b = -7.74*10^{-2}$, $F_{1,33} = 26.52$, $P < 0.001$, $R^2 = 0.43$). For elevation (**Fig.1.8 B**), the best linear mixed effects model (lowest AIC) also had different intercepts and slopes for the three clades (AIC = 339.31, $t_{138} = -3.24$, $P < 0.005$). Varying the slopes and

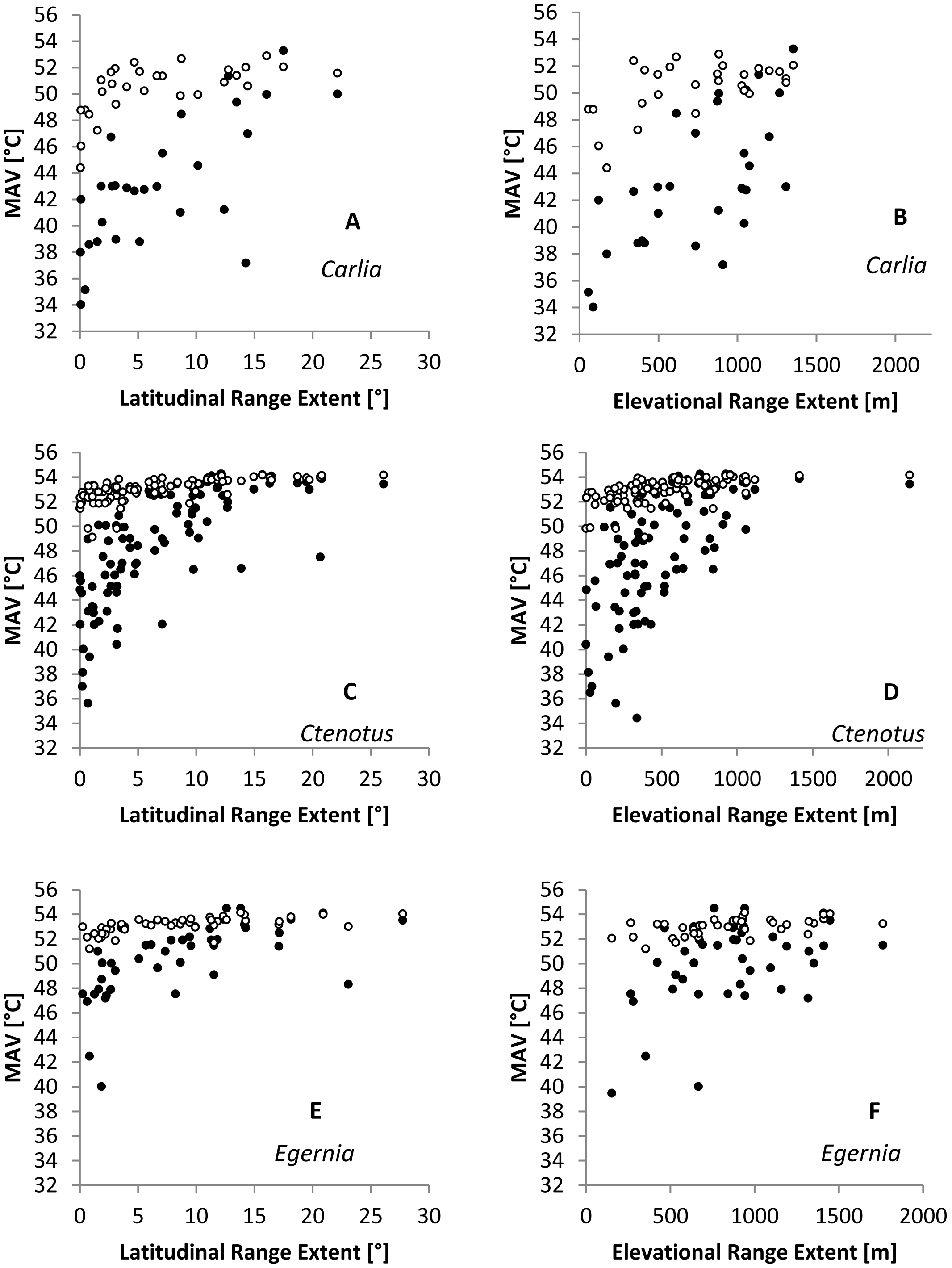

Fig.1.6 Absolute annual temperature range at each species' most variable occurrence point (Maximum Annual Variability; MAV) as a measure of climatic variability as proposed by the climatic variability hypothesis for different latitudinal (**A**, **C** and **E**) and altitudinal (**B**, **D** and **F**) range extents of species in the *Carlia* (**A** and **B**), *Ctenotus* (**C** and **D**) and *Egernia* (**E** and **F**) clades (closed circles). Open circles represent the expected MAV for each species if MAVs were the result of differences in sampling effort or sample size for species with different range sizes (mean MAV for 10,000 randomization results for each species). Spearman's rank correlation coefficient and significance levels were for measured MAVs were as follows: (**A**: $\rho = 0.668$; $P < 0.0001$; **B**: $\rho = 0.638$; $P < 0.0005$; **C**: $\rho = 0.802$; $P < 0.001$; **D**: $\rho = 0.706$; $P < 0.0001$; **E**: $\rho = 0.729$; $P < 0.0001$; **F**: $\rho = 0.399$; $P < 0.001$).

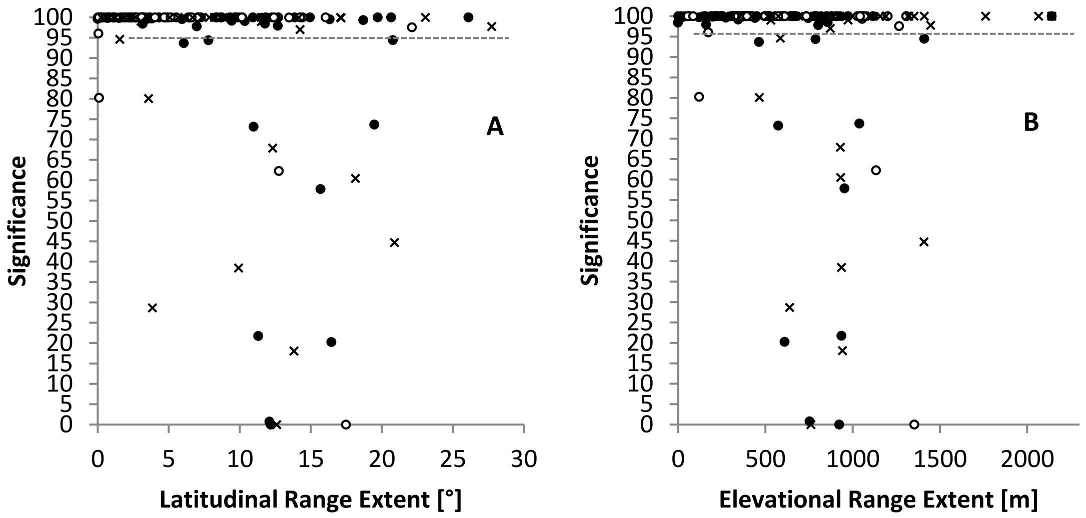

Fig.1.7 Percent of randomization MAV results at different (**A**) latitudinal and (**B**) altitudinal range sizes for each species in the *Carlia* (open circles), *Ctenotus* (closed circles) and *Egernia* (crosses) clades that lie above the real MAV value for that species. Values above 95% are considered significant. A small proportion (3 of 29 species representing the *Carlia* clade, 10 of 89 species representing the *Ctenotus* clade and 9 of 45 species representing the *Egernia* clade) in this study do not have significantly lower MAVs than expected based on randomizations.

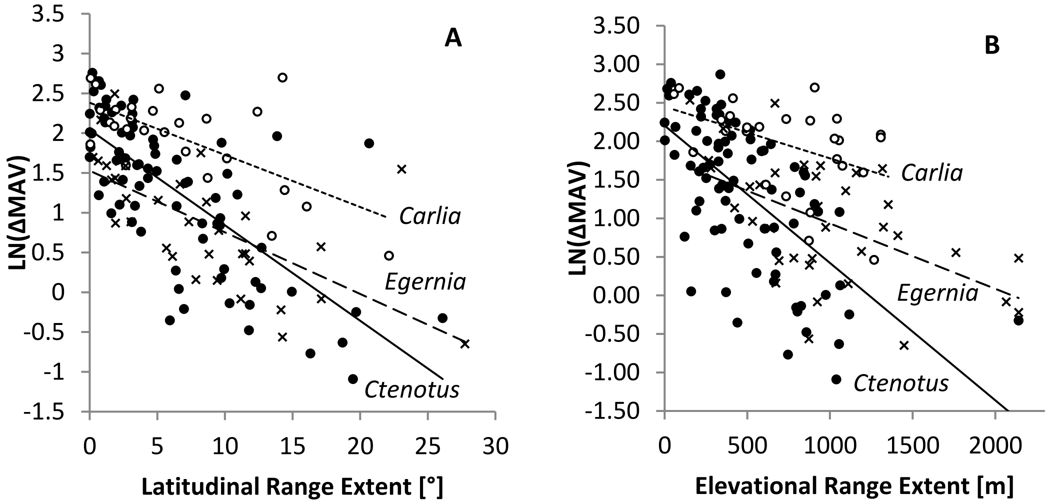

Fig.1.8 Negative linear correlations between (**A**) latitudinal as well as (**B**) altitudinal range sizes, and the natural logarithm of the absolute difference between the mean randomized MAVs and the real MAVs for each species (only species that showed statistically significant differences were included). All regressions were significant for latitude for the *Carlia* (open circles; dotted line; a = 2.38, b = -6.54*10^{-2}, $P < 0.001$; $R^2 = 0.42$), *Ctenotus* (closed circles; solid line; a = 2.04; b = -1.20*10^{-1}; $P < 0.001$; $R^2 = 0.50$) and *Egernia* clades (crosses; dashed line; a = 1.53; b = -7.74*10^{-2}; $P < 0.001$; $R^2 = 0.43$) as well as for elevation for the *Carlia* (a = 2.45; b = -6.72*10^{-4}; $P < 0.05$; $R^2 = 0.16$), *Ctenotus* (a = 2.20; b = -1.78*10^{-3}; $P < 0.001$; $R^2 = 0.40$) and *Egernia* clades (a = 1.78; b = -8.47 * 10^{-4}; $P < 0.001$; $R^2 = 0.27$).

intercepts by clade improved the fit significantly, compared to the simpler model combining data for all clades (AIC = 361.06, Log-Likelihood ratio = 27.75, $P < 0.0001$) and compared to a simpler model varying intercept only by clade (AIC = 342.21, Log-Likelihood ratio = 6.90, $P < 0.05$). Differences in intercepts showed the same pattern as for latitude for the three clades, however slope also varied and was steepest for the *Ctenotus* clade (linear regression; *Carlia: a* = 2.45, $b = -6.72*10^{-4}$, $F_{1,24} = 5.94$, $P < 0.05$, $R^2 = 0.16$; *Ctenotus: a* = 2.20, $b = -1.78*10^{-3}$, $F_{1,78} = 52.65$, $P < 0.001$, $R^2 = 0.40$; *Egernia: a* = 1.78, $b = -8.47 * 10^{-4}$, $F_{1,34} = 13.81$, $P < 0.001$, $R^2 = 0.27$). With respect to elevation, *Ctenotus* approached the expected tolerance breadth predicted by randomizations more quickly with increasing range size than the other two clades. This result is similar to trends in the latitudinal analysis for which slope was greater, but not significantly greater for *Ctenotus* (see above).

Discussion

Our novel approach revealed that, despite the absence of a latitudinal Rapoport Effect in one of the clades, the CVH was supported in all three clades. The larger the species' latitudinal and elevational range sizes, the greater their tolerance breadth as measured by the maximum annual variability (MAV) they experienced within their range (**Fig.1.6**). This was true for latitudinal as well as elevational range extents. Additionally, the more restricted a species was, the more its MAV differed from that expected by chance alone, whereas wide-ranging species occurred in areas closer to the maximum variability they would be expected to experience by chance alone (**Fig.1.8**). This pattern occurred, independently of whether restricted species in a clade occurred, on average, at lower latitudes (*Carlia*; traditional Rapoport Effect) or not (*Egernia*; reverse Rapoport Effect). Thus, range size in all three clades increased with temperature variability: more wide ranging species in each clade did indeed occur in habitats that required greater climatic tolerances due to greater overall variability in temperatures. Furthermore, even though wide ranging species had smaller MAVs than expected by chance, the difference between the maximum variability they experienced and the variability they would experience if their occurrence records were randomly distributed was relatively small, while restricted species occurred in habitats with substantially less variability than expected by chance. This was true in all clades regardless of whether a Rapoport Effect was observed or not, and suggests that the capability of a species to tolerate greater variability does allow it to spread further along climate gradients, whether gradients are linear or more complex across a landscape. This provides strong support for the CVH. These results show that even though traditional methodologies used to test for a Rapoport Effect may suggest that the CVH does not apply in some taxa, these methodologies do not, in fact, provide a relevant test of the CVH, because regional climate patterns across the area within which a taxon occurs do not always follow the average global trend of decreasing variability towards lower latitudes (**Fig.1.2**).

The strong trend for restricted species to occupy significantly less climatically variable environments, means that geographic range restriction in the examined taxa was likely due to narrow tolerances rather than to biotic interactions, habitat specificity or dispersal barriers. While the association between species' ranges and tolerance ranges seems obvious, it is not necessarily so. For example, when spatial climate gradients are complex rather than linear, increased tolerance of variability may not necessarily translate into a potential to spread proportionately further in any particular direction. This may be one of the reasons why the *Carlia* clade in this study showed the strongest trends (**Fig.1.6** and **1.8**), since it was the only clade that occurred over an area (the Australian East Coast) that exhibits a relatively clear, one-dimensional latitudinal climate gradient. Similarly, as thermal variability is generally determined by variation in minimum rather than maximum temperatures (see **Fig.1.1**), a species adapted to a highly variable habitat may have difficulty surviving in a more stable habitat defined by longer periods of high temperatures or in areas with higher peak temperatures (common in Australia). On the other hand, species adapted to stable (often warm, tropical) habitats could spread into more variable (often temperate or arid) habitats, by extending dormancy during colder periods, by egg retention or by viviparity (allowing them to avoid restrictions on minimum temperatures for egg incubation) by shifting activity periods away from the hottest time of day (Gordon et al. 2010), or by developing nocturnality. Similarly, selection of stable microhabitats, such as moist leaf litter, may enable a species with narrow tolerances to spread over large areas, provided that suitable refuge microhabitats are available, even without any change in the physiological tolerance of individuals. Last, but not least, adaptation to a widespread habitat type or vegetation type, and effects of biotic interactions may define range size more than climate variability in some taxa. In this respect, it is interesting that even in the *Egernia* clade, in which both viviparity (which could buffer them from temperature constraints on egg incubation) and nocturnality (which could buffer them from diurnal temperature extremes in the habitat) are common, climatic variability and range size were significantly correlated. More importantly, however, this trend occurred in the *Egernia* clades even though a latitudinal Rapoport Effect was absent. This, and the fact that latitudinal and elevational Rapoport Effects contradicted each other in this clade, emphasizes the point that even though some biogeographical patterns and their variations can serve us as an indicator of underlying mechanisms, ultimately it is these mechanisms that are of interest.

Our findings emphasize the importance of quantification of underlying climate patterns for the correct interpretation of species' range size distributions. For example, there is a suggestion in the literature that Rapoport's Rule is weaker or non-existent in the southern hemisphere, and absent in Australia (Smith et al. 1994, Hughes et al. 1996). However, we show that any apparent absence of the effect in Australia is irrelevant, because even though a gradient in absolute mean maximum and absolute mean minimum temperatures occurs with latitude along the Australian East Cost (AEC) at sea-level (**Fig.1.1**), climate patterns across the whole continent are much more complex with minimum and maximum temperatures varying considerably at any given latitude (**Fig.1.2**). Because of this, annual temperature range is wider in many central areas, compared to coastal areas. Once we are aware that latitudinal patterns in climatic variability are not as uniform in Australia as suggested by the global average latitudinal trends and generally weaker than in the northern hemisphere, failure to find a Rapoport Effect in the southern hemisphere in some taxa is, in itself, of little relevance and does not refute the CVH. It is, therefore, not surprising that the only clade exhibiting a strong latitudinal Rapoport Effect in this study was *Carlia*, which is restricted to parts of the continent in which a clear latitudinal gradient in climatic variability actually occurred (**Fig.1.2 B**). The pattern was less pronounced, or even reversed in other clades distributed over areas with less consistent climate gradients, while it was surprisingly consistent across elevational gradients.

Since elevational climate gradients are generally more consistent than latitudinal climate gradients, the uniformity of patterns in species' range size distributions across this dimension, supports the interpretation above that a lack of consistency in observed trends across latitude are caused by less distinct climate gradients across this dimension, rather than because the CVH does not apply. In this respect, if a Rapoport Effect was to be used as an indication of the validity of the CVH at all, patterns along elevational gradients may be the only ones associated with some reliability in this respect. However, since range sizes are truncated at the minimum elevation present within a study area, most species' elevational ranges tend to cluster or "pile up" along the lower elevation geometric boundary of the study area (see midpoint plot in **Fig.1.3 B**). This is likely due to a secondary effect of species present at low elevations sorting themselves along the elevational gradient based on tolerances acquired in different latitudinal source habitats (see the "alternative rescue effect" as described by Almeida-Neto et al. 2006). Such sorting can lead to an apparently strong Rapoport Effect that is not driven by the evolutionary mechanisms proposed in the elevational component of the CVH, which predicts that species with origins at different elevations with different climatic variability will extend in both directions up and down the elevational gradient based on their tolerances.

The results of tests of a Rapoport Effect in comparison to direct tests of the CVH showcase how little relevance the presence or absence of a Rapoport Effect analysed according to traditional methodologies can have to the validity of the CVH. If the actual climate patterns in the study area are used in analysis, the negative Rapoport Effect observed in *Egernia* (greater range sizes in more northerly latitudes) accurately reflects the climate patterns in its southerly distribution, because climatic variability increases from cold but relatively stable areas along the southern coastlines towards the highly variable arid interior, into which species in this clade extend to different degrees. The distribution of *Egernia* range sizes is therefore consistent with expectations of the Climate Variability Hypothesis, as confirmed by its adherence to the predicted relationship of increasing range size with habitat variability (**Fig.1.6**). The significant effect of climate on range sizes in this clade, which should be buffered against some of the influence of climatic variability by nocturnality and viviparity, demonstrates the substantial influence of climatic variability on range sizes, and emphasizes how misleading assessment of range size distributions solely based on the presence or absence of a Rapoport Effect can be. In this respect, the detection of a Rapoport Effect in the *Ctenotus* clade was surprising, since its distribution spans several complex climate gradients including the arid interior of Australia rather than a single unidirectional one (see **Fig.1.3 A**). However, the fact that this effect was nevertheless relatively weak in this clade confirms that such complex climate gradients can conceal the presence of relationships between range size and climatic variability to some degree, unless a direct test of the CVH is used, as in our study.

In addition to the strong support provided here for the CVH, we detected several other important trends. First, species in the three clades responded differentially to changes in climatic variability. For both latitude and elevation, *Carlia* showed a stronger response to stability of habitats (i.e., larger ΔMAV values) than did *Ctenotu*s and *Egernia* (respectively), indicating that species occurred in consistently less variable habitats than predicted by randomizations. Thus, *Carlia* species may either be more evolutionarily susceptible to climatic variability, or may have evolved a clearer trend in range size distributions due to a stronger and more defined natural climate gradient across the clade's distribution. An inability to adapt to high climate variability, and to be more influenced by such variability, is also consistent with their absence from highly variable habitats in the arid interior, and may stem from the ancestral rainforest origin of the clade (Couper et al. 2005, Dolman and Hugall 2008). Recently, various authors have suggested that ectotherms that evolve in low-variability habitats, such as rainforests, may be less adaptable in the face of past or future climate changes (Deutsch et al. 2008, Huey et al. 2012). On the other hand, *Egernia* species appear relatively resilient in this respect, as may be expected based on their larger body size and viviparity, which may buffer effects of temperature fluctuation to some degree. Secondly, it emerged from our analysis that some species with small ranges (in each clade) occurred in surprisingly variable habitats (**Fig.1.7 A** and **B**). A small number of exceptions is not necessarily a contradiction to the broader validity of the climatic variability hypothesis but, instead, may give us insight into other mechanisms that may also be relevant in restricting realized geographic ranges. Potential reasons for such restrictions could include biotic interactions, pressures from predation or competition, physical barriers to dispersal, and substrate or habitat specialization. The exceptions are, therefore, of great interest for further research on mechanisms restricting ranges in addition to physiological limitations.

There is one caveat that should be noted. The original interpretation of the climatic variability hypothesis was that the maximum variability tolerated by a species reflects that of the source habitat (Stevens 1989). Mechanisms shaping physiological tolerances and range sizes are dynamic, however, meaning that species could originate from a relatively stable source and gradually adapt to more variable conditions as range extension becomes favorable (for example due to high competition in the source habitat). There is support for the notion that species' ranges are limited more by biotic than abiotic interactions at the more climatically stable end of their ranges, and mainly by abiotic variables at the more variable end (Dobzhansky 1950, Stevens 1992, Brown et al. 1996, Martin 2001). Similarly, historic climate change may have selected some species to expand their tolerances beyond the variability of their evolutionary source habitat. These mechanisms are difficult to distinguish and, in most cases, it is only possible to say whether range size reflects the maximum variability experienced by species today, not whether they originated from the most variable spot or gradually evolved to tolerate more variable conditions. A likely example of this is the *Carlia* clade, which has a tropical evolutionary origin (Couper et al. 2005, Dolman and Hugall 2008) but includes some very wide ranging species reaching high latitudes in temperate regions in NSW and Victoria (*Carlia vivax, Carlia tetradactyla, Lygisaurus foliorum*). We, therefore, need to appreciate that there may not be a single mechanism describing the way all species evolved with respect to changes in physiological tolerances and shifts in range size and location. Such considerations warrant further research into the complexity of these mechanisms, especially if we want to use past evolutionary changes in tolerances and ranges to predict consequences of anthropogenic climate change with respect to species' potential to adapt or adjust their distributions.

This study presents strong support for the validity of the CVH in three clades of small, closely related clades of ectotherms comprising species with similar ecologies. We aimed to employ a direct test of the CVH by incorporating actual climate patterns across our study organisms' area of distribution into analyses rather than merely assessing the presence of generalized biogeographic patterns. Such analyses are essential if we want to understand the mechanisms underlying species' range limits and distributions, and are vital for our understanding of influences of future climate change on species. However, all correlative studies on species' distributions and climate patterns can benefit from expansion using actual measured physiological qualities, i.e. by taking a more mechanistic approach (Kearney and Porter 2004). There is consequently an urgent need to expand the body of literature comparing experimentally determined physiological traits such as thermal tolerances, metabolic rates or desiccation resistance to biogeographic patterns (see Calosi et al. 2008; 2010), rather than inferring them from current species' distributions, if we want to understand the full complexity of the mechanisms shaping species' ranges. Further research is therefore needed in this direction to expand on results from the present study.

In conclusion, we have provided a direct test of the CVH determining the underlying mechanisms of Rapoport's Rule and demonstrating the need to expand our understanding of factors driving range size limits in the biogeographical past, now, and with respect to future adaptation to climate change. Investigation of factors associated with patterns in range size distributions require clear exposition of the underlying questions, rather than repeated testing of the superficial pattern. Confirming or refuting a global pattern, without reference to the likely underlying mechanisms, does not advance our understanding. We hope to put an end to the debate over the existence of Rapoport's Rule, and encourage others to look more deeply into macro-ecological and physiological patterns rather than focus on reporting trends without examining mechanisms.

-Chapter II-

Extensive Acclimation in Ectotherms Conceals Interspecific Variation in Thermal Tolerance Limits

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Abstract

Species' tolerance limits determine their capacity to tolerate climatic extremes and limit their potential distributions. Interspecific variation in thermal tolerances is often proposed to indicate climatic vulnerability and is, therefore, the subject of many recent meta-studies on differential capacities of species from climatically different habitats to deal with climate change. Most studies on thermal tolerances do not acclimate animals or use inconsistent, and insufficient, acclimation times, limiting our knowledge of the shape, duration and extent of acclimation responses. Consequently patterns in thermal tolerances observed in meta-analyses, based on data from the literature are based on inconsistent, partial acclimation and true trends may be obscured. In this study we describe time-course of complete acclimation of critical thermal minima in the tropical ectotherm Carlia longipes and compare it to the average acclimation response of other reptiles, estimated from published data, to assess how much acclimation time may contribute to observed differences in thermal limits. Carlia longipes decreased their lower critical thermal limits by 2.4°C and completed 95% of acclimation in 17 weeks. Wild populations did not mirror this acclimation process over the winter. Other reptiles appear to decrease cold tolerance more quickly (95% in 7 weeks) and to a greater extent, with an estimated average acclimation response of 6.1°C. However, without data on tolerances after longer acclimation times available, our capacity to estimate final acclimation state is very limited. Based on the subset of data available for meta-analysis, much of the variation in cold tolerance observed in the literature can be attributed to acclimation time. Our results indicate that (i) acclimation responses can be slow and substantial, even in tropical species, and (ii) interspecific differences in acclimation speed and extent may obscure trends assessed in some meta-studies. Cold tolerances of wild animals are representative of cumulative responses to recent environments, while lengthy acclimation is necessary for controlled comparisons of physiological tolerances. Measures of inconsistent, intermediate acclimation states, as reported by many studies, represent neither the realised nor the potential tolerance in that population, are very likely underestimates of species' physiological capacities and may consequently be of limited value.

Introduction

Climate patterns within species' geographic distributions shape their physiological traits and tolerances (Stevens 1989, Holt 2003 , Andrews and Schwarzkopf 2012). Most notably, expectations for physiological tolerances are expressed in the climatic variability hypothesis, which proposes that species originating from more environmentally variable habitats along a climatic gradient (such as latitude or elevation) evolve broader environmental tolerances and consequently spread to occupy a larger range size along such gradients (Stevens 1989, 1992, Pintor et al. 2015). Tropical organisms, on the contrary, are expected to have narrower tolerances and limited acclimatisation potential (Stevens 1989, Terblanche et al. 2007, Pintor et al. 2015).

With respect to latitudinal gradients in climate patterns, the upper limits of temperature variability (i.e. maximum temperatures, and species' tolerances thereof), have recently received particular attention because of the impending threat of anthropogenic climate change and the prediction of limited capacity of species to deal with rising temperatures (Deutsch et al. 2008, Vickers et al. 2011, Sunday et al. 2014). However, latitudinal changes in temperature variability arise mainly from geographic variation in minimum temperatures, while maximum temperatures vary comparatively little with latitude (Müller 1982, Stevens 1989). Consequently, differences in tolerance breadth, often assumed to be indicative of species' overall sensitivity to thermal variability (Stevens 1989), are largely driven by differences in cold tolerances (Huey et al. 2009, Clusella-Trullas and Chown 2014). Adaptations related to tolerance of low temperatures are therefore suggested by some authors to limit current distributions more than heat tolerance and published data is consistent with this (Sunday et al. 2011), especially in ectotherms, because of their limited capacity for active metabolic heat production. Consequently, ectotherms are limited by colder temperatures at high latitudes (Shine 1999, Kearney and Porter 2004, Kearney et al. 2008) and many species that extend into temperate regions have only achieved these range expansions through mechanisms such as viviparity (Shine and Bull 1979, Shine 1999), brumation periods over winter months (Aleksiuk 1976), metabolic compensation or inverse acclimation of metabolic rates at low temperatures (Tsuji 1988), lowered critical minimum temperatures or other specialised cold adaptations (Storey and Storey 1992, Ramløy 2000). Out of these adaptations, tolerances of low temperatures, particularly, appear to show a clear trend across latitude (Addo-Bediako et al. 2000, Kimura 2004, Sunday et al. 2011, Sunday et al. 2014), reflecting geographic trends in minimum temperatures (Müller 1982, Addo-Bediako et al. 2000).

Since minimum temperatures vary greatly across seasons, especially at higher latitudes, critical thermal minimum temperatures (CT_{min}) are likely to acclimate substantially in many organisms.

It is known that CT_{min} acclimate in a range of organisms (Angilletta 2009, Sunday et al. 2011, Weldon et al. 2011), that they do so to a greater extent than critical maximum temperatures (Brett 1946, Allen et al. 2012, Clusella-Trullas and Chown 2014) and that they change more slowly than upper thermal limits (Layne and Claussen 1982). However, the shape and exact duration of this and other acclimation responses is not well studied. The acclimation responses that have been studied in some detail are limited to metabolic rates, which acclimate within approximately 14 days in some snakes (Blem and Blem 1990), critical thermal maxima, which acclimate rapidly (within 1-4 days) in some ectotherms (Hutchison and Maness 1979, Lutterschmidt and Hutchison 1997, Allen et al. 2012), critical thermal minima in insects, which can show substantial acclimation ressponses within hours or days (Allen et al. 2012), and other measures of heat and cold tolerance such as survival, heat knock down times and cold coma recovery times in fishes (Brett 1946) and insects (Weldon et al. 2011), which show substantial interspecific and inter-axa differences in the time required to complete acclimation (several hours to several weeks). The importance in recognizing such variation in acclimation responses when comparing tolerances among species and taxa has been acknowledged repeatedly since the 1930s (Mellanby 1939, Hutchison 1976). However, acclimation responses of thermal limits appear greater and more flexible with respect to cold tolerance as compared to heat tolerance (Weldon et al. 2011).

Because temperature increases beyond thermal optima rapidly approach lethal limits (Deutsch et al. 2008, Angilletta 2009), upper thermal limits may need to acclimate rapidly in many circumstances to allow for immediate survival of sudden short term rises in temperature. Lower temperatures may restrict activity times in colder seasons (Kearney et al. 2008) but are less detrimental in the short term, and critical thermal limits lie far below thermal optima around which individuals thermoregulate (Angilletta 2009, Vickers et al. 2011). Consequently acclimation of lower thermal limits may be much slower, to match gradual seasonal changes rather than short-term diurnal fluctuations (Huey and Bennett 1990). This matches observations from the insect literature that suggests that daily short term temperature fluctuations are physiologically compensated for by rapid “hardening” (Lee Jr 1989) while seasonal temperature changes require slower but more substantial acclimation responses (Kimura 1988). This may be added to by reduced reaction rates at low temperatures, which likely make slow cold acclimation not only favourable ecologically but also restrict organism’s potential to evolve fast cold acclimation responses (Belehradek 1957). Similarly it has been suggested that plastic responses are only favoured when temperature changes are predictable (e.g. temperate seasonal changes) but not in unpredictable or stable (e.g. tropical) habitats (Bradley 1978, Gabriel et al. 2005) but whether tropical ectotherms can accommodate the comparatively small, yet nevertheless notable, seasonal variations in minimum temperatures through acclimation is not well studied. These gaps in our knowledge of acclimation potential and acclimation processes are potentially of great importance, because researchers routinely use critical thermal limits measured under laboratory conditions, and thermal tolerances based on these, as indicators of species' vulnerability to climate change (Deutsch et al. 2008, Sunday et al. 2012), and as a factor potentially limiting species' range extents (Addo-Bediako et al. 2000, Sunday et al. 2012) and for other inferences about their physiology (Lutterschmidt and Hutchison 1997).

If acclimation of some physiological traits provides a mechanism to adjust gradually to long-term seasonal changes, they are probably completed only after extensive exposure to a specific temperature regime. Experimental acclimation of animals in the laboratory after capture is often not performed at all, or only applied for a widely varying number of weeks (usually anything up to 7 weeks; Murrish and Vance 1968, Corn 1971, Wheeler 1986, Kattan and Lillywhite 1989, Kaufmann and Bennett 1989, Terblanche et al. 2007, Angilletta 2009, Calosi et al. 2010, Clusella-Trullas and Chown 2014, Gunderson and Stillman 2015), which may be sufficient for some taxa with fast acclimation rates, such as insects (Weldon et al. 2011), but insufficient for taxa with slower acclimation responses, such as some vertebrate ectotherms (Brett 1946). It has, for example, been shown that some vertebrate ectotherms from climatically different habitats display identical thermal tolerances when acclimated for substantial time periods (Yang et al. 2008) and that any differences among wild populations would, therefore, result from their extensive acclimation potential rather than from adaptive differences. Many of our estimates of species tolerances may, consequently, be underestimates, or at least highly inconsistent across studies. Because previously published values of thermal tolerances are used extensively in meta-studies (Clusella-Trullas and Chown 2014, Sunday et al. 2014, Gunderson and Stillman 2015), understanding the limitations imposed by partial and inconsistent acclimation is crucial. Establishing estimates of the length of time required for different physiological traits to acclimate completely is essential to indicate adequate methodologies, to standardise within and among studies and to assess comparability of values from different studies.

In this study we aimed to describe the time-course and extent of acclimation to cold temperatures in a restricted-range tropical lizard (*Carlia longipes*) and to compare these processes with estimates of average acclimation potential and time required to complete acclimation across reptiles based on published data. We expected that: (i) acclimation responses to cold temperatures can be lengthy and substantial, and are completed in a similar amount of time that gradual temperature trends across seasons would take to manifest; (ii) differences in cold tolerances among reptiles – and potentially in other taxa - observed in the literature are more representative of differences in acclimation time than of true interspecific differences; and (iii) acclimation only occurs if animals are forced into prolonged periods of low temperatures, because acclimation to temperatures that are not representative of true seasonal trends would be disadvantageous. These expectations were confirmed, indicating that lengthy and substantial acclimation may confound results of current meta-studies on thermal limits and vulnerability to climate change.

Materials & Methods

Animal collection and husbandry. *Carlia longipes* is a tropical scincid species restricted to the area between approximately Gordonvale (-17.1°S) and Cooktown (-15.5°S) in Tropical North Queensland, Australia. Overall, 45 individuals were used for the experiments in this study. A first group of individuals of *Carlia longipes* were captured at the end of the warm, wet season (May) 2012, before seasonal decreases in temperature are likely to elicit a cold acclimatisation response. Individuals were housed separately in plastic containers (300 x 200 x 100 mm) with mesh lids on a substrate of commercially available potting soil and leaf litter dried at 60°C. Water was provided ad libitum and food (crickets: *Acheta domestica*) was provided three times per week. Immediately after animal collection, containers were placed in a constant temperature room, with air temperature of 26°C, on aluminium shelving that was cooled at one end by cold water pumped through aluminium tubes. The setup produced a cold acclimation regime with a thermal gradient between the back and the front of each container ranging from 18 to 22°C inside the containers. A gradient was used instead of a constant acclimation regime because constant acclimation regimes have negative effects on *Carlia* spp. (Schwarzkopf, pers. com.). Animals were observed to remain at the warmer side of the containers for the first few days but then used the whole range of thermal conditions within the containers for the remaining acclimation time. Body size ranged from 0.68 g to 12.76 g with a mean of 4.88 g.

Acclimation of critical thermal minimum. At various intervals (0, 1, 2, 3, 4, 6, 8, 12, 16, and 43 weeks) from the start of acclimation, i.e. from the time animals were first subjected to a low temperature regime of 18-20 °C, the CT_{min} of a subset of the animals was determined. At intervals between 0 and 8 weeks, CT_{min} was quantified for a previously untested subset of individuals (3 to 4 naïve individuals) as well as for groups that had been tested in any of the previous weeks. This allowed us to assess any potential effects of repeated testing on acclimation. As a result, the number of individuals increased in groups tested at longer acclimation times, with 21 individuals in the final group at 8 weeks acclimation. In week 12 and 16, only these 21 individuals from previously tested groups were tested again, because we originally did not expect acclimation to continue beyond 8 weeks, and therefore did not prepare additional naïve groups for these longer time intervals. A random subset of 12 individuals was re-tested at 43 weeks.

The number of times an individual had been tested did not have a significant effect on the final model (see results) and therefore the lack of naïve groups from the last time intervals (12, 16 and 43 weeks) had no potentially confounding effect on our results. For the "43 week" interval we used a pooled group of animals with acclimation periods beyond 8 months (36 to 49 weeks) with an average acclimation time of 43 weeks. This interval was included to provide an estimate of final CT_{min} (at an interval beyond any seasonal periodicity possibly experienced in the wild).

From May 2012, a new group of 3 to 8 individuals was captured at bimonthly intervals to examine potential acclimatisation of CT_{min} in the wild. Overall, 3 naïve wild individuals were tested in May, 8 in July, 6 in September, 4 in November, 5 in January and 5 in March. Variation in numbers resulted from variation in capture success. CT_{min} of these animals was measured within 24 hours of capture and they were subsequently released. The high abundance of *Carlia longipes* at our capture site at James Cook University, Cairns, made it highly unlikely we would accidentally recapture the same individual in different months.

For CT_{min} experiments, individuals were separately placed in a cylindrical plastic container inside a temperature-controlled cabinet and left for 30 minutes at 18°C, to ensure that all individuals had the same body temperature before the start of the experiments. Following this, the air temperature in the cabinet was cooled, resulting in gradual temperature decrease within the container of approximately 0.2°C per minute, which was continuously recorded with a thermocouple (Type T). Animals were flipped on their back every 60 seconds by slowly rolling the container. CT_{min} was defined as the temperature at which loss of righting response occurred, at which point air temperature within the container was recorded. To estimate lizard body temperature from air temperature in the container, a calibration correction was determined by measuring the body temperature of five museum specimens of rainbow skinks of different sizes (range: 0.47 to 10.96g; obtained from a collection at James Cook University) while air temperature in the container was decreased, to establish the relationship between body mass and the time-lag of body temperature behind air temperature at the cooling rate we used. For this, museum specimens were rehydrated in saline at 4 °C for 2 days and their skin dried prior to experiments to avoid excessive evaporative cooling. The maximum average lag of body temperature behind air temperature for the largest animal (a *Carlia longipes* specimen of 10.96g body mass) was 0.6 °C. Calibration experiments were performed exactly as CT_{min} experiments, including turning the animals every 60 seconds. The resulting correction was then applied to CT_{min} values of live animals. All temperature readings were recorded on a Sable Systems TC1000 thermocouple reader using electronic thermocouples (type T), which had all previously been calibrated on the same mercury in glass immersion thermometer with a precision of ±

0.05C (0.1C marks; NATA calibrated). Body mass in grams was recorded to two decimal places on a laboratory scale. Museum specimens stored in ethanol were rehydrated at 4°C in saline for 3 days before the calibration experiment.

Data on warm acclimated and partially cold acclimated CT_{min} of other reptiles was obtained from a recently published meta-study on acclimation potential of critical thermal limits (Gunderson and Stillman 2015). We obtained measures of cold acclimated CT_{min}, acclimation temperatures used by the cited studies, acclimation time, and acclimation response ratios (ARR) for CT_{min} values. We used the ARR to calculate the warm acclimated CT_{min} of each species (these were not provided in the supplementary material and had to be obtained through calculation). We cross referenced all calculations with the source studies to ensure that values were accurate representations of the original values reported by the cited studies. This gave us two values of CT_{min} for each of 22 reptile species, warm acclimated CT_{min} and CT_{min} at a certain acclimation time.

Environmental temperatures.

Nocturnal (i.e. minimum) microhabitat temperatures for *Carlia longipes* were recorded in each month when naïve wild individuals were collected over a 14 to 20 day period at 5 am (i.e. one measurement per day before sunrise) using Thermochron® iButtons® placed in 8 different locations representative of microhabitats utilised by the species (pers. obs.). These included two locations under leaf litter (one under vegetation and one in the open), two locations 10cm above the leaf litter (one under vegetation and one in the open), two locations in rock crevices (one under a rock that was in the sun during the day and one under a shaded rock), one on top of a rock that was in full sun during the day and one 10cm above the same rock. These locations are representative of microhabitats that individuals are actively foraging in as well as locations for potential refuge with different degrees of temperature buffering compared to ambient air temperatures. Because only nocturnal microhabitat temperatures were being used, there was no need to account for effects of solar radiation on the sensors. All iButtons® were calibrated using the same mercury in glass immersion thermometer as used for calibration of the electronic thermocouples used in CT_{min} experiments. The recorded microhabitat temperatures were compared to temperature records from weather stations (CAIRNS AERO at 16.87°S and 145.75°E; http://www.bom.gov.au/climate/data/ accessed 10.6.2014).

Statistical Analysis.

Data was analysed in R 2.13.2 (R Core Team 2014). The acclimation process of *Carlia longipes* was analysed using non-linear mixed effects models (package lme4, function nlmer; Bates et al. 2011) to allow for inclusion of animal as a random effect *a priori* to account for the repeated measured design. The model fitted was an exponential decay function of the form

Eq.2.1 $CT_{min}\ [°C] = A + b * e^{(c*t_a)}$

where A was the asymptote approached as CT_{min} decreased and t_a was acclimation time in weeks. This model enabled us to estimate final CT_{min} as the asymptote approached by the response function. The initial model included acclimation time, body mass and number of tests previously performed on each individual (because we used a repeated measures design) as potential predictor variables. The final model was determined by step-wise backwards selection using AIC. The function "nlmer" obtains parameter estimates for the final model of best fit using restricted maximum likelihood. Because naïve wild individuals did not vary in CTmin across seasons, all naïve wild individuals were pooled in the unacclimated starting group at 0 weeks acclimation.

The same function as used for *Carlia longipes* was fitted to the acclimation response of other reptiles (Gunderson and Stillman 2015), but without body mass or number of previous tests as covariates (because they were not reported in the published data set). Final models were highly dependent on starting values used for the estimation of the asymptote of the model (i.e. the estimated final CT_{min}), because data was only available for a limited, initial part of the response (i.e. the first three weeks) and only for two time-points per species (one warm acclimated and one partially acclimated value at 14 to 28 days acclimation time). We therefore fitted multiple models with starting values in 1°C intervals between 7°C (the mean CT_{min} of all data points) and -3°C (the mean estimated CT_{min} of all species if the percentage of acclimation completed during their acclimation time was identical to that completed by *Carlia longipes* in the same time). All models converged to one of three different end models. The end model with the lowest AIC was considered the best model and used to describe the estimated, minimum average acclimation response. Species ID was included *a priori* as a random effect in all models. Partial R^2 of the fixed effects was compared to the overall R^2 of the final model to assess the how much of the model fit was explained by acclimation time rather than by the random species effect (Nakagawa and Schielzeth 2013).

Results

When acclimated to a constant low temperature regime of 18-22°C, CT_{min} in *C. longipes* rapidly decreased over the first few weeks and subsequently approached a predicted asymptote of 6.8-7.6°C (depending on body mass), representative of a drop of 2.4°C compared to that of animals collected from the wild (9.67 ± 0.89 °C; mean ± SE). The final model describing the acclimation process of CT_{min} in *Carlia longipes* included body mass ($\Delta AIC = -3.71$; $\chi^2 = 5.71$; $p < 0.02$; **Fig 2.1**; **Table 2.1**) in addition to acclimation time. The CT_{min} of larger animals was, on average, higher than the CT_{min} of smaller animals. The number of times the experiment had been performed on an individual did not significantly affect CT_{min} ($\Delta AIC = 1.23$; $\chi^2 = 0.77$; $p = 0.38$; **Table 2.1**).

Table 2.1 AIC of different models fitted to the acclimation response of captive *Carlia longipes*. The model with lowest AIC was the one including an exponential plus constant term for acclimation time plus an additional body size term.

	Model formula for fixed terms	AIC
1	$a + b^*e(c^*t_a)$	87.30
2	**$a + b^*e(c^* t_a) + d^* m_b$**	**83.59**
3	$a + b^*e(c^* t_a) + d^* m_b + e^*$number of tests	84.82

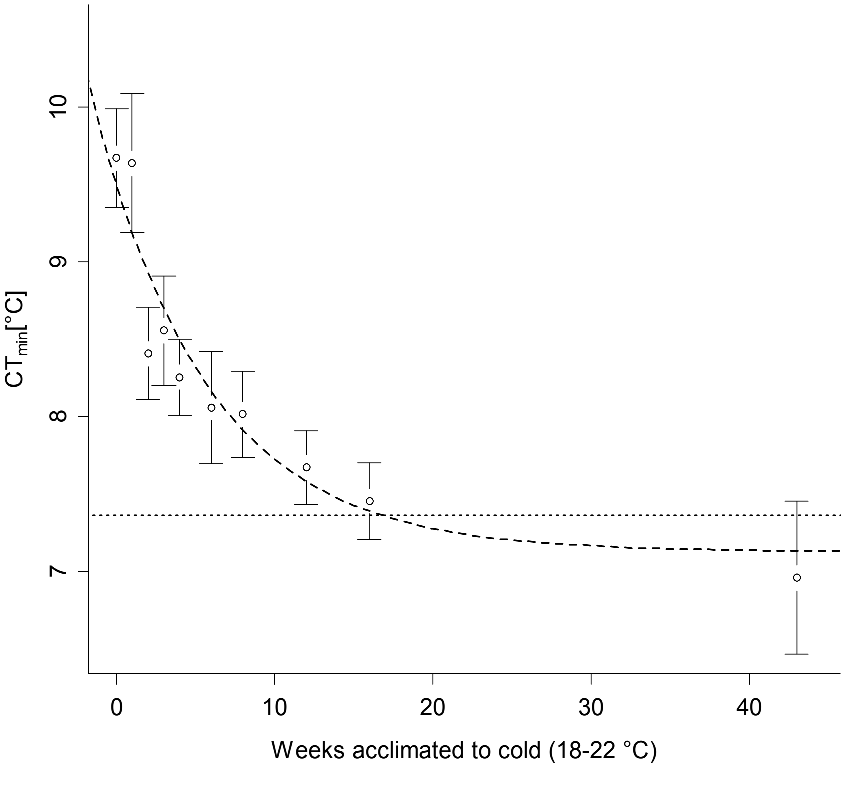


Fig 2.1. Acclimation of CT_{min} in captive *Carlia longipes*. Critical thermal minimum temperatures [°C± 2SE] of captive individuals of *Carlia longipes* acclimated to a temperature regime of 18-22°C for different time periods measured in weeks. Every week, 3 to 4 previously untested individuals were added to the experimental group and other individuals retested. Past 8 weeks acclimation, only previously tested individuals were used. Predictor line for an average sized animal (4.88g) is shown (dashed). Values approach an asymptote of 7.13°C for an average sized animal, with 95% (7.36 °C) of this acclimation completed after 17 weeks (dotted line).

The acclimation process was best described by the exponential equation

Eq.2.2 $CT_{min}\,[°C] = (6.8 + 0.068 * m_b\,[g]) + 2.37 * e^{(-0.14 * t_a\,[weeks])}$

with acclimation time (t_a) in weeks and body mass (m_b) in gram as predictor variables, where the expression “6.8 + 0.068 * m_b” represents the body-mass-specific asymptote. The intra-class correlation coefficient (Goldstein 1986, Burton et al. 1998), which can lie between values of zero and one and is a measure of how much of the residual variation of the model is accounted for by the random effect, was less than 0.01. This indicates that the random effect explained little of the residual variation. Consequently, the fixed effects explained most of the variation in CT_{min}.

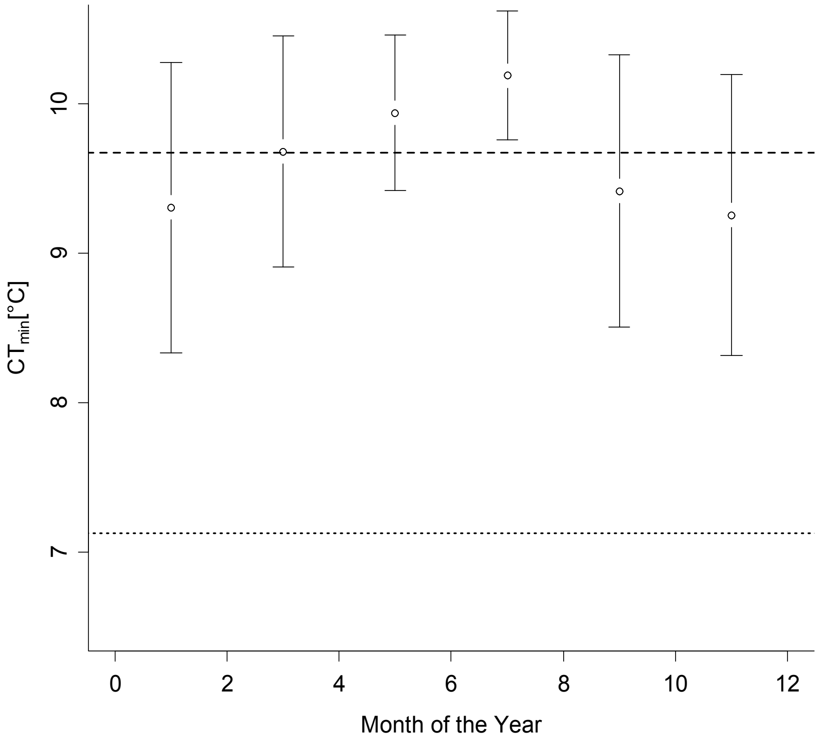


Fig 2.2. Acclimation of CT_{min} in wild *Carlia longipes*. Critical thermal minimum temperatures [°C ± 2SE] of wild individuals (3 to 8 individuals per group) of *Carlia longipes* collected in different months of a year. No statistically significant differences were observed (ANOVA: $F_{(5, 25)}$=1.05, p=0.4). Lines show mean CT_{min} of wild animals (9.67 °C; dashed line) and predicted cold acclimated CT_{min} of an average-sized animal of 4.88 g body mass (7.13 °C; dotted line).

The CT_{min} of individuals of *C. longipes* collected in the wild every second month over a one year period did not differ significantly among months (**Fig 2.2**; ANOVA: $F_{(5, 25)}$=1.05, p=0.4), although a trend toward a gradual increase in mean CT_{min} was observed from January to July, followed by a similar but opposite trend in CT_{min}, with differences of less than 1°C between the highest and lowest mean CT_{min} recorded. Body mass was not significantly correlated with CT_{min} in un-acclimated animals overall (linear regression; $F_{1,29} = 0.027$, $p = 0.87$), or if collection month was included as a random effect ($F_{1,24}=0.0021$; $p=0.96$; linear mixed effects model using R package nlme; Pinheiro et al. 2013).

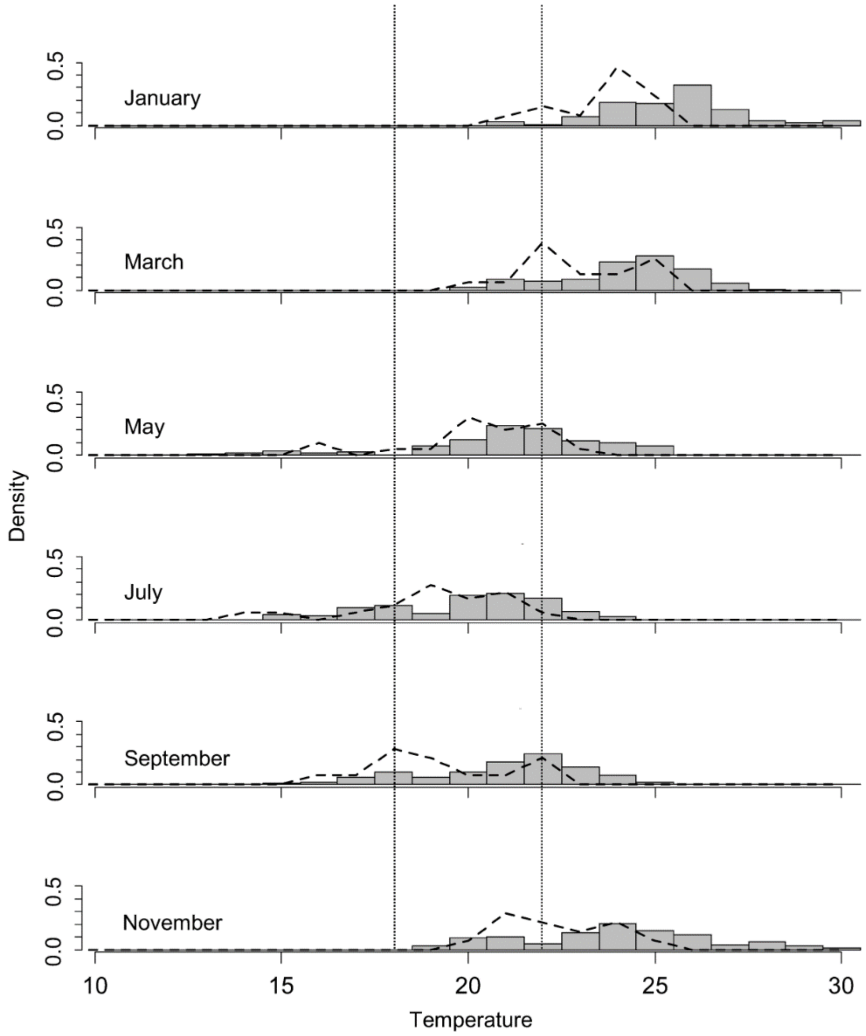


Fig 2.3. Minimum habitat temperatures of *Carlia longipes*. Density distribution of habitat temperatures (histograms) and minimum temperatures recorded by the Bureau of Meteorology (dashed lines) at different times of year. Habitat temperatures represent records collected at 5 am every day (coldest time of day) over a two-week period in eight different microhabitats representative of the locations that *Carlia longipes* is commonly observed in. Vertical dotted lines show acclimation regime used in this study.

Habitat temperatures available to *C. longipes* (**Fig 2.3**) at the coldest time of day consistently included higher temperatures than the minimum temperatures recorded by BOM for Cairns over the same time period. In the coldest month recorded for this year, habitat temperatures and minimum temperatures dropped to approximately 14.5°C (14.6 and 14.4°C, respectively) but while BOM minimum temperatures stayed at 22.2°C or lower, experimentally measured habitat temperatures were spread over a broader temperature range and reached 24.4°C (usually under rocks that had been in the sun during the day; pers. obs.). The interquartile range of habitat temperatures in the coldest month at the coldest time of day was 18.2-21.6°C with a median 20.4°C, which is similar to the temperature range of 18-22°C used for experimental acclimation in this study.

The average acclimation process of 22 other reptile species, based on the model with the lowest AIC fitted to previously published values of hot acclimated and partially cold acclimated CT_{min} [38] was estimated to be more substantial at 6.1°C but faster, with 95% of the projected acclimation response completed in 7.3 weeks (**Fig 2.4**).

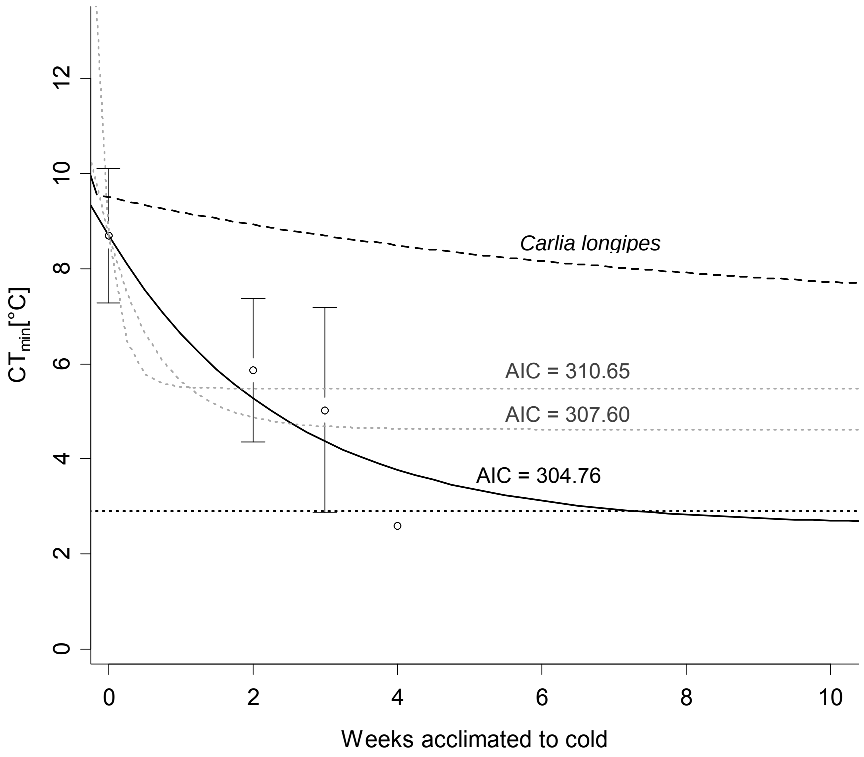


Fig 2.4. Acclimation of CT_{min} in 22 reptile species. Critical thermal minimum temperatures [°C ± 2SE] of 22 warm acclimated (0 days acclimation) and partially acclimated (2 to 4 weeks acclimation) reptile species. Solid line shows predictions by linear mixed effects model with lowest AIC. Values approach an asymptote of 2.61°C. 95% of acclimation is completed at 7.3 weeks (dotted black reference line). Predictions by other models of inferior fit (dotted grey lines) and acclimation response of *Carlia longipes* (dashed black line) are shown for comparison.

The model of best fit was described by the following equation:

Eq.2.3 $CT_{min}\ [°C] = 2.61 + 6.09 * e^{(-0.41 * t_a\ [weeks])}$

It had a significantly lower AIC (304.76) than the other two models based on different asymptote starting values (AIC= 310.65 and 307.60). The final model was highly dependent on starting values for the model asymptote and additional data at longer acclimation times would likely change estimates of final acclimation state. The intra-class correlation was low at 0.02. The model explained 40.2% of the variability of the data, 39.1% of which was explained by acclimation time. The random effect of species ID had a negligible effect on the model fit.

Discussion

The acclimation response of *Carlia longipes* (**Fig 2.1**), as well as that of other reptiles (**Fig 2.4**), was substantial, and prolonged and the latter explained the bulk of the interspecific differences observed in the literature, as indicated by the 39.1% of variation explained by acclimation time alone (partial R^2), as compared to 40.2% explained by the whole model including the random species-specific effect. True interspecific differences may, therefore, be concealed if data from the literature is used without accounting for differences in acclimation time because the amount of variation in CT_{min} across acclimation states is substantial compared to the amount of final interspecific differences. This is exacerbated by other methodological differences among studies that can potentially influence acclimation responses, such as the varying acclimation temperatures used to elicit the response (Gunderson and Stillman 2015) or the effects of different cooling and heating rates used when testing thermal limits (Terblanche et al. 2007, Allen et al. 2012). Prolonged acclimation responses and, especially, differences among species in the time period required to complete acclimation can present a significant problem in meta-analyses, which use data from studies with inconsistent methodologies, and this is supported by our comparison of the response of *C. longipes* to the mean response of other reptiles. Ideal acclimation extent and time for CT_{min} is likely driven by how quickly seasons change in a species' habitat and by the maximum temperature difference between seasons (Bradley 1978, Gabriel et al. 2005). Even inclusion of acclimation time as a covariate in meta-analyses (Gunderson and Stillman 2015) may, therefore, be insufficient to account for differences in acclimation time because some species may be fully acclimated at three weeks, while others (such as *C. longipes*) take much longer to complete acclimation, i.e. the response cannot be assumed to be comparable among species and cannot be accounted for without detailed knowledge of interspecific differences in acclimation speed and extent.

The acclimation process observed in this study confirmed our predictions that tropical organisms are capable of accommodating environmental changes through substantial physiological changes, despite the relatively stable nature of their habitat. Additionally, their acclimation response may be lengthier than for other organisms, because seasonal changes are slow and cold acclimation is likely avoided unless they are exposed to prolonged low temperature regimes. This is in stark contrast to the rapid acclimation responses in critical thermal maximum temperatures observed in other ectotherms (e.g., some amphibians acclimate in approximately 48 hours; Hutchison and Maness 1979). However, since even short exposures to high temperatures can be lethal, a more immediate response to hot than to cold conditions is not surprising (Huey and Bennett 1990).

Critical thermal minimum temperatures in the geographically restricted tropical ectotherm *C. longipes* decreased significantly over an extended time period and approached an asymptote approximately 2.4°C below initial values after 17 weeks of continuous cold acclimation (**Fig 2.1**), with 95% of acclimation completed at 17 weeks after initiation of the acclimation response. Gradual, residual acclimation continued for much longer. This supports our hypotheses that physiological plasticity in at least this tropical organism is stronger than typically appreciated and that acclimatisation as a mechanism should track long-term seasonal changes, rather than short-term temperature fluctuations (Huey and Bennett 1990), especially in a habitat where potential for cold acclimatisation may only be advantageous occasionally, in particularly cold years. Minimum annual temperatures in Cairns have occasionally dropped to 6.2°C (coldest record since 1942; CAIRNS AERO at 16.87°S and 145.75°E; http://www.bom.gov.au/climate/ data/ accessed 10.6.2014), which is close to the asymptote of 6.8°C approached by very small individuals of *C. longipes* in this study. It is, therefore, likely that the observed acclimation potential directly reflects selection pressures on *C. longipes* populations over longer periods, especially because tolerance of winter temperatures may be highly relevant in a tropical organism that, contrary to many temperate organisms, doesn't avoid such temperatures through complex brumation behaviours (Aleksiuk 1976).

Smaller individuals exhibited lower CT_{min}. It has previously been shown that smaller reptiles in some species acclimate more rapidly and to a greater extent (Blem and Blem 1990). However, in other reptiles smaller individuals have been found to be less cold resistant if not acclimated (Xu and Ji 2006). Perhaps smaller reptiles require greater tolerance to temperature extremes because they are thermally less buffered, but inconsistent results in the scientific literature warrant further exploration of this possibility, especially since acclimation times applied in that body of literature are inconsistent and short. Naivety of young individuals in selecting thermally optimal microhabitats could also render them more susceptible to environmental temperature fluctuations, making greater absolute tolerance necessary. CT_{min} did not vary with body mass in wild populations, most likely because acclimatisation states in wild animals exposed to different temperatures regimes vary too much to detect statistically significant trends. Further research on the thermal makeup of microhabitats available to or selected by individuals of different sizes within a species may prove valuable to shed light on in thermal physiology and thermoregulation.

Regarding thermoregulation, habitat temperatures (**Fig 2.3**) show that, in the study year, temperatures only fell to about 14°C in winter, which is a relatively warm winter in Cairns (median minimum annual temperature of 10.2°C, CAIRNS AERO at 16.87°S and 145.75°E; http://www.bom.gov.au/climate/data/ accessed 10.6.2014). Microhabitat temperatures in retreats were centred around a median of 20.4°C with an interquartile range of 18.2-21.6°C, similar to the temperatures used for experimental acclimation in this study. However, these temperatures represent the coldest time of day in the coldest month of the year rather than a continuous exposure. Furthermore, temperatures up to 24.4°C were available to animals in suitable microhabitats, even at this time. Considering that most *Carlia* species thermoregulate behaviourally around preferred body temperatures of 25.5 – 32.3°C (Vickers et al. 2011), individuals are likely to select higher temperatures throughout the day and may find refuge in the warmest microhabitats available to them at night (Andersson et al. 2010). Under the conditions observed here, it is therefore highly likely that individuals could, and would, have avoided exposure to low temperatures – and therefore to acclimatisation - whenever possible. This is especially true if this species does not brumate and individuals are attempting to stay active near their optimum temperatures throughout the year. The lack of acclimatisation in wild populations (**Fig 2.2**) was very likely a direct result of this, although a slight, non-significant seasonal trend in CT_{min} was observed and larger sample sizes may reveal slight acclimatisation responses in the wild. Stronger decreases in winter temperatures during colder years might nevertheless induce significant acclimatisation responses in wild populations.

Critically, our results suggest that records of temperature tolerance in the literature are most likely underestimates in most cases. Organisms are usually experimentally acclimated for 0-2 weeks and rarely up to 7 weeks (Murrish and Vance 1968, Corn 1971, Wheeler 1986, Kattan and Lillywhite 1989, Kaufmann and Bennett 1989, Terblanche et al. 2007, Angilletta 2009, Calosi et al. 2010, Clusella-Trullas and Chown 2014, Gunderson and Stillman 2015). If acclimation processes generally follow the time-course we found in *C. longipes* (and the best model of acclimation response in reptiles suggests that the period is extended; **Fig 2.4**), these acclimation periods are insufficient to capture true tolerances to minimum temperatures. In *C. longipes*, 7 weeks would only capture around 62% of the full acclimation potential. Across the broader range of species considered from the literature, at least 7 weeks would be required to complete 95% of acclimation whereas the longest acclimation period of the 22 species for which data was available was 4 weeks. Note also that the form of final models derived was strongly dependent on the starting parameters (see Material and Methods) and we restricted those to similar levels as found in the actual data. More extensive data at longer times would likely extend the period and degree of acclimation represented by the best model here. Generally, measurements of both complete and entirely unacclimated states have relevance if it is acknowledged which of the two is being measured. Unacclimated measures, i.e. CT_{min} of animals immediately after capture will have ecological relevance as measures of susceptibility to extremes of habitat temperatures integrated across their actual (even if not fully defined) recent acclimatisation history, including environmental limits and behavioural responses. Alternatively, lengthy (complete) acclimation in the laboratory provides good estimates of overall physiological tolerances for comparison among species. However, partial acclimation and, especially, comparison of physiological tolerances of species using different degrees of partial acclimation, has little immediate descriptive or predictive relevance, and may disguise patterns of interest. Metastudies using values from studies with greatly varying methodologies regarding acclimation should be aware of this, especially since interspecific variation in CT_{min} has a greater influence on variation in overall thermal tolerance breadth than variation in CT_{max} (Addo-Bediako et al. 2000, Huey et al. 2009, Clusella-Trullas and Chown 2014).

If one aims to use CT_{min} values to make inferences on species' thermal tolerance breadth or potential distribution limits, either prolonged acclimation of study animals or some form of estimate of the expected acclimation response that can be used to predict final acclimation states are necessary for reliable results. Inaccurate inferences, at least in ectotherms, are less likely to be a problem if CT_{max} maxima only were used, as the time-course of potential acclimation is rapid (Hutchison and Maness 1979) and the degree of acclimation is less (Sunday et al. 2011). However, restricting inferences to CT_{max} has limited value, as they vary less geographically (Addo-Bediako et al. 2000, Sunday et al. 2011) and geographic distributions in ectotherms are largely limited by cold tolerances (Shine 1999, Kearney and Porter 2004, Kearney et al. 2008).

We have shown that acclimation responses can be more substantial and more prolonged than implied by the short and inconsistent time periods used in the literature , while animals in the wild appear to avoid acclimation by appropriate microhabitat selection when possible (Bogert 1949). In contrast to suggestions in the literature (Buckley et al. 2015), the substantial acclimation potential of *C. longipes* suggests that thermoregulation does not preclude their adaptation to low temperatures, despite buffering acclimation responses. Our study is, to our knowledge, one of very few studies describing the shape of the acclimation response for a physiological trait, and the only one examining cold acclimation of lower (rather than upper) thermal limits, over a prolonged time period in a vertebrate ectotherm (Hutchison 1961, Brattstrom and Lawrence 1962, Hutchison and Ferrance 1970, Hutchison and Maness 1979). The extent to which the cold acclimation response depends on acclimation temperatures, diurnal fluctuations in acclimation temperature or diurnal rhythms in photoperiod is not well known (Huey and Bennett 1990) and further research in this direction would improve our ability to predict the impacts of long-term changes in environmental conditions. The slow, extensive acclimation abilities observed here need be taken into account when inferences are made based on published values for physiological traits.

-Chapter III-

Hydroregulation in a Tropical Dry-Skinned Ectotherm

In press as "Pintor, Schwarzkopf & Krockenberger (in press). Hydroregulation in a Tropical Dry-Skinned Ectotherm. Oecologia."

Abstract

While temperature effects on species' vulnerability to climate change are well studied, desiccation effects receive comparatively little attention. In addition, we poorly understand the capacity of ectotherms, and especially reptiles, to control water loss rates behaviourally by selecting suitable microhabitats. This study examined water loss rates and behavioural hydroregulation in the tropical rainforest skink Carlia rubrigularis to assess whether this dry-skinned ectotherm actively avoids desiccation and whether trade-offs occur between desiccation avoidance and selection of optimal temperatures, as previously shown in amphibians. Higher temperatures elicited humid refuge choice despite placing individuals in suboptimal thermal conditions, as indicated by preferred substrate temperatures. This finding emphasizes the importance of water loss even for taxa traditionally assumed to be highly desiccation resistant, and highlights this factor's potential influence on vulnerability to climate change by limiting activity times or by restricting individuals to thermally suboptimal microhabitats.

Introduction

Most ectotherms are highly susceptible to the climatic conditions to which they are exposed, because they have limited capacity to thermoregulate physiologically using metabolic heating or evaporative cooling, and thus use behavioural thermoregulation. Some recent studies predicting vulnerabilities of ectotherms to climate change suggest that tropical ectotherms are highly susceptible to environmental temperature increases, because they are currently experiencing temperatures that are already close to optimum temperatures for performance and to critical thermal maxima (Deutsch et al. 2008). Forest dwelling ectotherms might be particularly vulnerable in this respect because they are often thermoconformers (Huey et al. 2009).

Water loss rates (WLRs) are directly influenced by environmental temperatures (Warburg 1965, Dmi'el 2001) and are consequently likely to increase as temperatures rise with climate change (IPCC 2013), depending on species' capacity to mitigate environmental warming through behavioural thermoregulation (Sunday et al. 2014, Buckley et al. 2015). Changes in hydric conditions will likely contribute to such climatic effects on water balance but are less well understood than temperature changes: predictions include complex spatial and temporal variation globally (Suppiah et al. 2007), decreases in dry season precipitation and increases in moisture balance deficit across most of Australia (Hughes 2003, IPCC 2013). Even though environmental variables connected to water balance, such as humidity and precipitation, have long been known to affect the activity, abundance and distribution limits of some dry-skinned ectotherms (Bursell 1957, Messenger 1959, Andrewartha and Birch 1960), and even though WLRs vary among closely related species from habitats of different aridity even in comparatively desiccation resistant ectotherms such as reptiles (Hillman et al. 1979, Neilson 2002), measures of WLRs are not usually included in studies assessing the vulnerability of dry-skinned ectotherms to climate change with the exception of some recent work on insects (Chown et al. 2011). Especially in reptiles, interspecific differences in WLRs as well as the potential capacity to resist or avoid desiccation through behavioural mechanisms, are greatly understudied, despite their potential to be of great importance to the vulnerability of ectotherms to changes in their environment (Chown et al. 2011, Kearney et al. 2013).

While behavioural thermoregulation has been studied extensively in ectotherms (see (Angilletta 2009), active behavioural hydroregulation of individuals has received less attention (Davis and DeNardo 2010, Tracy et al. 2014) and has, so far, been studied largely in amphibians (Bundy and Tracy 1977, Tracy et al. 1993, Bartelt et al. 2010) and invertebrates (Tracy et al. 1979, Prange 1996). Whether reptiles employ behavioural hydroregulation to avoid desiccation, however, is largely unknown, despite some indications that increased desiccation levels modify temperature choice and other behaviours that influence water loss, such as gaping (Tattersall and Gerlach 2005, Scarpellini et al. 2015). Additionally, some reptiles select periods of high precipitation for activity (Davis and DeNardo 2010) or retreat to humid burrows during dry periods (Wilms et al. 2010), which further highlights the possible importance of hydroregulation in this taxon and warrants more detailed research.

If increasing temperatures pose a particular risk to tropical forest reptiles because they are thermoconformers (Huey et al. 2009), increased desiccation rates induced by higher body temperatures may exacerbate such vulnerability. It is therefore of great interest whether reptile species from tropical forest habitats, where water is currently not limiting but may become limiting in the future, are not only thermoconformers but also hydroconformers, or whether they can mitigate combined effects of high temperature and low humidity on desiccation through behavioural mechanisms.

The aim of this study was, therefore, to (i) quantify active behavioural thermoregulation as well as hydroregulation in a tropical rainforest lizard (*Carlia rubrigularis*), and to (ii) estimate the species' desiccation resistance across temperatures. We predicted that *C. rubrigularis* would avoid desiccation at high temperatures by actively choosing humid refuge sites.

Methods

Adult rainbow skinks (*C. rubrigularis)* were collected by hand at different locations between Bramston Beach (-17.35°S, 146.02°E) and Mossman (-16.44°S, 145.36°E) and acclimated to a 28-32°C temperature regime at 60%-90% relative humidity (conditions were identical for all individuals but varied across days because cages were sprayed with water every 3 days to maintain high humidity levels) for more than 17 weeks before experiments. Such extensive acclimation was necessary because previous work on rainbow skinks has shown that some physiological traits require 17 weeks to complete acclimation (Chapter II). Animals were housed in 30 (D) x 20 (W) x 10cm (H) plastic containers with mesh lids. Potting mix and leaf litter were dried at 65 °C and used as substrate and retreat sites. Lizards were fed crickets several times per week and provided with water *ad libitum*.

Preferred substrate temperatures of 9 warm acclimated individuals (mean body mass = 2.71 g; range: 1.63 - 3.53 g) fasted for two days prior to experiments was measured within a 2-m thermal gradient with an aluminium base and opaque Perspex walls, heated at one end and cooled at the other by Peltier plates (temperature range from 5°C to 65°C). White sand paint was applied to the base to provide a more natural substrate, while optimizing contrast. Lizards were introduced into the hot end, to facilitate quick temperature selection away from unsuitable temperatures, and were left in the gradient for one hour to adjust to experimental conditions. Subsequently, photographs were recorded every 60 seconds for 24 hours from above, under a 12h:12h day:night regime. Dim red light was provided at night. Start times were chosen at random between 7:00 and 22:30. For the analysis, only the first two hours of recording were used, unless start time was after 17:00, in which case the first two hours in the morning (7:00-9:00) were analysed instead. Measurements from the dark photophase were not used. Using ImageJ (http://imagej.nih.gov/ij/) lizard positions (distance from cold end of gradient to the point between lizard front legs in pixels) were measured for each photo. The position of ten iButtons® (model 1922L), which measured the temperature at 20 cm intervals along the gradient every 60 seconds, were recorded in the same manner. Using a polynomial calibration curve on the temperatures recorded by the iButtons® in the gradient during the experiment (see APPENDIX I), lizard positions were converted into selected substrate temperature at each 60-second interval. For each lizard, the median of the 120 measurements collected over two hours was calculated and used for further analysis. Upper and lower limits of set point range of each individual was calculated as the 25% and 75% quartile of the 120 temperatures. The median of all values of the median preferred substrate temperature, 25% quartile and 75% quartile across individuals was recorded as the species' preferred substrate temperature, lower, and upper limits of set point range, respectively. Some animals became inactive during the recording and positioned themselves at very cold temperatures over extended time periods. If inactivity was suspected, the measurement was repeated several weeks later and the first measurement was discarded. Similarly, if shedding was noted within seven days of the experiment, that instance was discarded and the measurement was repeated.

Behavioural hydroregulation was measured by testing lizards' choice of refuge in 30D x 20W x 10H plastic containers with mesh lids and sand substrate. Each container had two refuges placed at opposite corners. Refuges consisted of microfiber cloths placed on sand-filled 9-cm diameter plastic dishes. One refuge had a wet cloth cover and moist sand, while the other was kept dry. Animals were introduced to containers near the dry refuge. Containers were then placed in a temperature- and humidity-controlled cabinet at ten different temperatures between 18°C and 36°C in randomized order. The position of the container in the cabinet (wet or dry hide towards the back) was also randomized. Humidity in the cabinet was set to 20% (the lowest possible setting with a stable resulting cabinet humidity). The same 10 animals (mean body mass = 2.78 g, range: 2.12-3.87 g) were tested at each temperature. Experiments were started at 17:00 ± 1h. The fraction of individuals that had chosen the wet refuge was recorded after 16 h. If animals were sitting on top of a refuge, they were assumed to have recently emerged from this refuge that morning and refuge choice was recorded accordingly. No food or water was offered during experiments but animals were well hydrated up to the start of experiments. Hygrobuttons® randomly placed in four of the wet and four of the dry refuges, respectively, in each experiment recorded differences in humidity and temperature between the two refuge options. Humidity in the open was recorded as well.

Refuge choice was analysed using logistic mixed effects generalized linear models in R (Bates et al. 2015, R Core Team 2014). The fixed effects component of the starting model included temperature and body mass as potential predictors of wet refuge selection, as well as a random term for individual ID, because the same 10 individuals were used at each of the ten temperatures. Backwards step-wise selection on fixed terms according to AIC and log-likelihood ratio tests was used to eliminate insignificant terms.

WLR was measured in 9 warm acclimated individuals (mean body mass = 2.83 g, range: 2.12-3.64 g) at 20 and 30°C in constant darkness in transparent plastic chambers for 16 h overnight. Atmospheric air was dried to under 5% relative humidity (as measured by Hygrobuttons®) using a scrubber containing silica gel pearls and was pumped through each chamber to maintain a constant low relative humidity. These two experimental conditions (20 and 30 °C at 5% relative humidity) were equivalent to 2.22 and 4.03 kPa vapour pressure deficit (VPD) of the air. After animals had adjusted to experimental conditions in the chamber for one hour, each lizard was weighed with its chamber, and again the following morning. The difference in mass over 16 h was converted to WLR as percentage of body mass. To avoid overestimation of WLRs, measurements were discarded if animals defecated during the experiment, shed within seven days after the experiment, or were very active at the time of morning measurements. Such animals were re-tested after at least 2 days in case of defecation or activity and after at least 7 days post shedding. The relationship of water loss in mg over 16h with vapour pressure deficit was analysed using mixed linear models in R (package nlme; Pinheiro et al. 2013, R Core Team 2014) to allow for the inclusion of individual as a random effect. The model included a term for body mass to account for size differences in water loss. Log transformed values of water loss and body mass and untransformed values for temperature were used based on the well-known shape of the relationship between the variables (Withers et al. 2000).

Results

Median preferred substrate temperature of *C. rubrigularis* was 32.9 °C (range: 29.7-37.3; **Fig. 3.1**). The set point range was between 31.1 (range: 27.9-36.8) and 35.2 (range: 29.9-38.6). There was no significant effect of body mass on preferred substrate temperature (linear

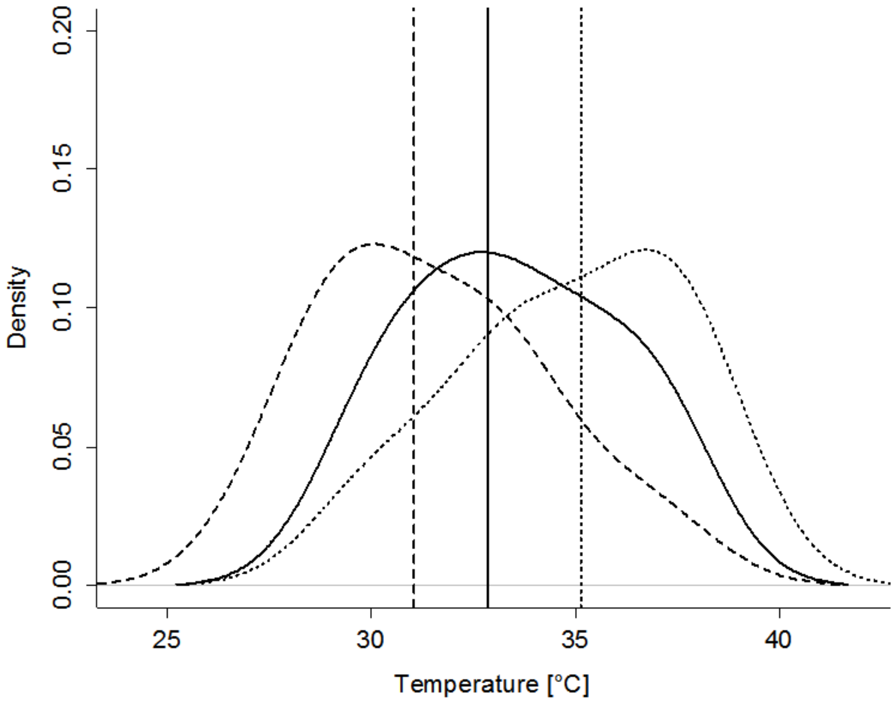


Fig.3.1 Density plot of median preferred substrate temperatures (solid line, median indicated by solid vertical line), lower (dashed line, median indicated by vertical dashed line), and upper (dotted line, median indicated by vertical dotted line) limits of set point range (median 50% of selected substrate temperatures).

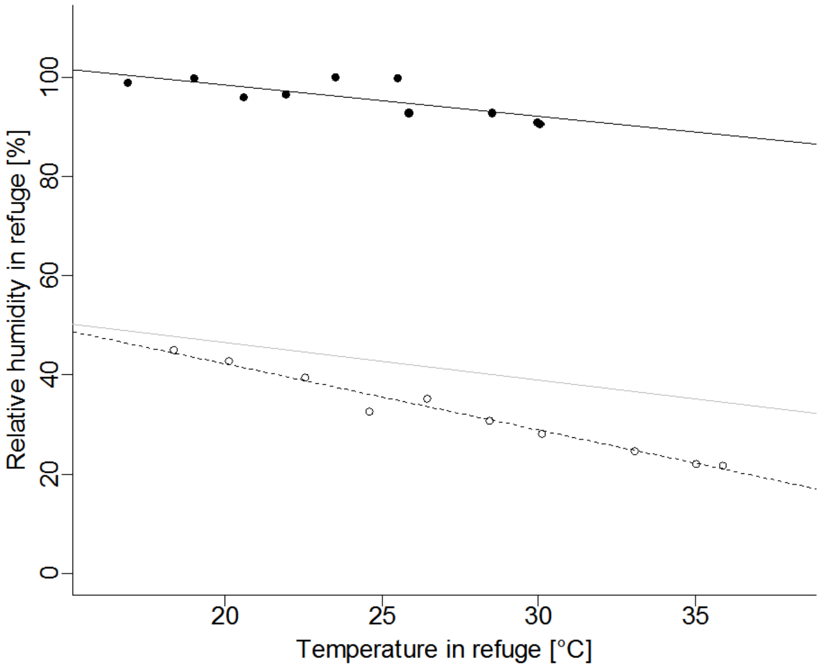


Fig. 3.2 relative humidity in the wet (●; ——) and dry (o; - - -) refuges across different temperatures in the same refuges, as well as humidity in the open at different controlled cabinet temperatures (——). Humidity increased with temperatures in both refuge categories but always remained high (>90%) in the wet and substantially lower (<45%) in the dry refuges.

regression; $F_{(1,7)} = 0.51$, $p = 0.50$), lower quartile of selected temperatures (linear regression; $F_{(1,7)} = 0.15$, $p = 0.71$) or upper quartiles of selected temperatures (linear regression; $F_{(1,7)} = 1.25$, $p = 0.30$).

Relative Humidity in dry and wet refuges was 32.0 ± 3.4% and 95.7 ± 3.0% (M ± SD, n=4 for both wet, and dry categories). Mean relative humidity decreased linearly with temperature in both refuge types (linear regression; wet refuge: $F_{(1,8)} = 11.58$, $p < 0.01$; dry refuge: $F_{(1,8)} = 285.7$, $p < 0.001$) but always remained above 90% in wet and below 45% in dry refuges, i.e. humidity in dry refuges was consistently lower than in wet refuges (**Fig. 3.2**). Temperatures were consistently lower in wet refuges.

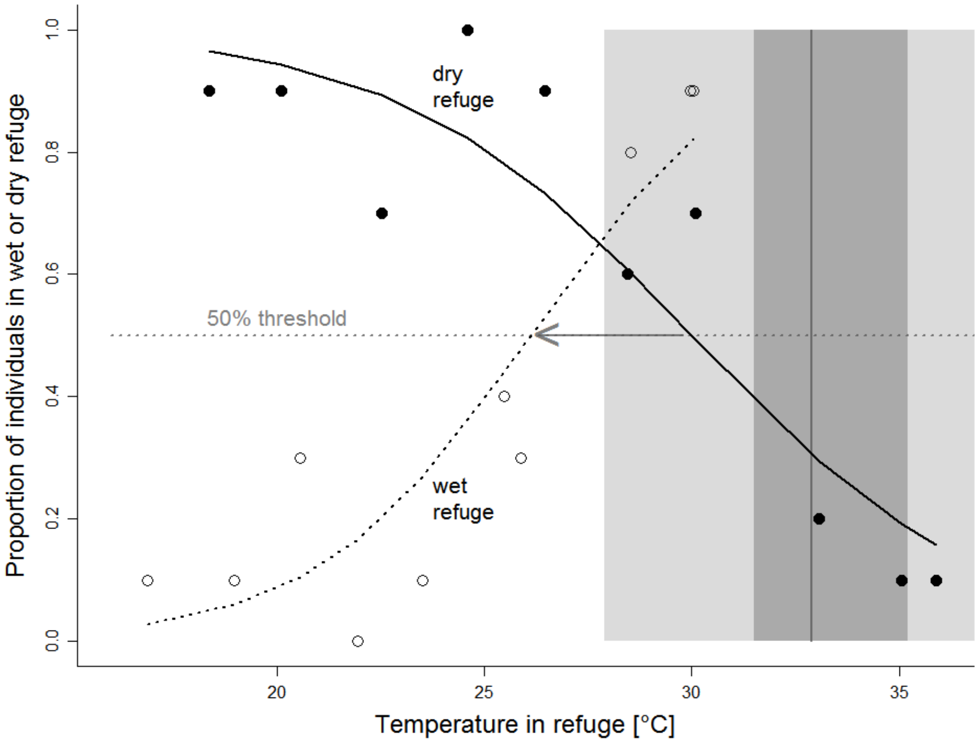


Fig.3.3 Proportion of individuals selecting wet (o; - - -) or dry refuges (●; ──) at different temperatures, including fitted values of logistic regressions using temperature the respective refuge as predictor of refuge choice. The median preferred substrate temperature (32.9 °C) dark grey vertical line), and median set point range of *C. rubrigularis* (31.5 to 35.2 °C; dark grey shaded area), as well as the temperature range delimited by the lowest and highest of the set point range boundaries recorded for any individuals (27.9 to 38.6; light grey shaded area) are shown for comparison with temperatures in chosen refuges. When the temperature in dry refuges reached 30 °C, the majority of individuals (>50%; horizontal grey dotted line) began to select wet refuges instead, and thereby placed themselves in temperatures further below the species' range of preferred temperatures.

Temperature was a significant predictor of the proportion of individuals in the wet refuge, whether temperatures in dry (logistic generalized linear mixed effects model, $Z = 5.06$, $p < 0.0001$) or wet refuges (logistic generalized linear mixed effects model, $Z = 5.08$, $p < 0.0001$)

were used as predictor variables (**Fig. 3.3**). Including body mass as an additional predictor did not improve these models significantly (ΔAIC < 0.5, χ^2 = 2.24, p = 0.13) . Above 30°C in the dry refuge, equivalent to a VPD of 3.05 kPa, over 50% of individuals selected the wet refuge, where the temperature was only 26°C at this point (VPD = 0.24 kPa). This temperature (30°C) at which dry refuge avoidance began was below the median preferred substrate temperature of the *C. rubrigularis* (32.9 °C) and below the lower limit of set point ranges of more than half of all examined individuals (see **Fig. 3.1** for comparison). When temperatures in dry refuges were increased to within the set point range of *C. rubrigularis* (31.1 °C to 35.2 °C), an increasing majority of individuals selected the wet refuge, where temperatures (approximately 26 °C to 30°C) were well below the species' set point range.

There was a significant correlation of log transformed WLR over 16 h with vapour pressure deficit (df = 6; t = 9.87; p < 0.001) as well as with log transformed body mass (df = 6; t = 3.70, p = 0.01; mixed linear model with individual as random effect). The relationship was described by the following equation:

Eq. 3.1 $\ln\,(WLR\ [mg]) = 2.97 + 0.31 * VPD\ [kPa] + \ln\,(BM^{0.62}\ [g])$

Where WLR is the water loss rate over 16h, VPD is vapour pressure deficit at the test temperatures at 5% relative humidity, and BM is body mass.

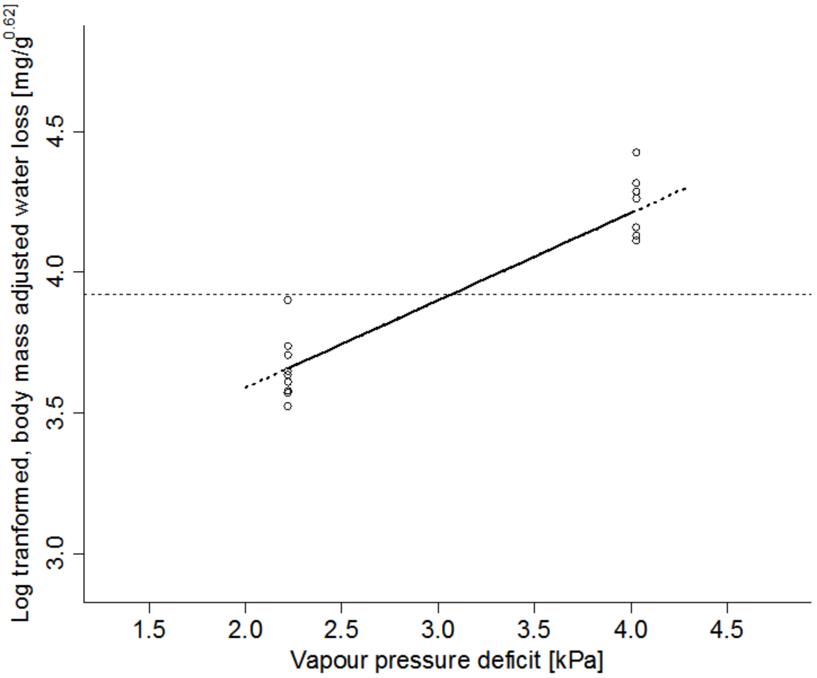


Fig. 3.4 Body mass adjusted WLR at different VPD (calculated from measurements at 20°C and 30°C with RH ~ 5%), including predictor line of linear mixed effects model (solid), and extrapolation from the model (dotted). Predicted WLR at the voluntary threshold of 3.05 kPa would be 3.4% over 16 h or 0.2% per hour for an average sized animal (horizontal dashed line).

Body mass adjusted water loss rate (**Fig. 3.4**) is consequently described by the following rearranged equation:

Eq. 3.2 $$\ln\ (WLR\ [mg]/BM^{0.62}\ [g]) = 2.97 + 0.31\ *\ VPD\ [kPa]$$

According to this equation, the WLRs of an average sized animal of 2.83 g at a VPD of 3.05 kPa (equivalent to 30 °C and 28% relative humidity in the dry refuge; see **Fig. 3.2**), at which more than 50% of animals took refuge in a wetter refuge, would be 95.63 mg. This equates to 3.4% of body mass over 16 h, or 0.2% per hour. By choosing a wet refuge of approximately 26 °C and 93% relative humidity instead (see **Fig. 3.2**), the resulting vapour pressure deficit would have been 0.24 kPa, resulting in an estimated water loss of 40.02 mg, or 1.4% of body mass over 16h (0.09% per hour). While selection of a wet refuge under these conditions would have resulted in placement in a thermally suboptimal condition outside the set point range of the species (see **Fig. 3.1**), such a choice would have also more than halved the water loss rate.

Discussion

With increasing temperature, more rainbow skinks (*C. rubrigularis)* selected wet over dry refuges (**Fig. 3.3**). This could be interpreted as thermoregulatory behaviour, because temperatures were consistently lower in the wet refuge. However, the set point range of *C. rubrigularis* range lies between 31.1 and 35.2 °C (**Fig. 3.1**), whereas individuals began choosing a wet refuge around 30°C. Therefore, the majority of individuals selected wet refuges with temperatures well below their preferred temperature, even though temperatures within their preferred temperature range were available in the dry refuges. Avoidance of high temperatures is, consequently, unlikely to be the cause of the observed preference. Thus, individuals in our experiment behaviourally thermoregulated when desiccation stress was low and selected dry (warmer) refuges at lower temperatures (**Fig. 3.3**). However, when the temperature in dry refuges approached their preferred temperature, they increasingly selected wet refuges at suboptimal temperatures and, thus, clearly avoided desiccation. This strongly suggests that these tropical rainforest skinks exhibit behavioural hydroregulation, either by selecting conditions that reduce desiccation in the first place, or by lowering their thermal preference when desiccated, similar to responses observed in other reptiles (Ladyman and Bradshaw 2003). Both represent a form of active hydroregulation, either through preventative or through responsive behaviour. Contrary to previous suggestions (Huey et al. 2009), the tropical forest species in this study was, therefore, neither a thermoconformer, nor a hydroconformer, and the two behavioural mechanisms for optimization of thermal and hydric state clashed.. Such behavioural trade-offs between optimization of hydration and thermal state are similar to those observed in amphibians (Bundy and Tracy 1977, Tracy et al. 1993, Köhler et al. 2011) and invertebrates (Tracy et al. 1979, Prange 1996) but have to date received much less attention in reptiles (Ladyman and Bradshaw 2003, Scarpellini et al. 2015).

At 30°C and 28% RH, when most individuals began to avoid the drier, thermally more suitable refuge, VPD reached 3.05 kPa. This suggests that there may be a voluntary desiccation threshold, either expressed as a water loss rate that is avoided (preventative) or as a maximum desiccation level that is tolerated before avoidance behaviours are initiated (responsive). If hydroregulation is preventative, our results suggest a voluntarily accepted hourly water loss rate of 0.2% of body mass as the threshold for *C. rubrigularis*. However, there may alternatively be an absolute desiccation threshold, that when reached elicits avoidance behaviour. The desiccation level at which half of the individuals in our study selected a low VPD refuge was about 3.4% of body mass over the experimental period. However, in absence of definite observations of the time point at which individuals changed their refuge preference and corresponding measurements of desiccation state at that point, a reliable desiccation threshold cannot be determined. Comparison of our results with data from other species would be of great interest to establish whether closely related species with different water loss rates have similar desiccation avoidance behaviours, whether trade-offs between hydroregulation and thermoregulation influence species' distributions and activity times and what the exact decision mechanisms used by reptiles for hydroregulation (e.g. preventative vs. responsive) are.

Desiccation is likely to exacerbate the risks associated with high temperatures (Vickers et al. 2011) and may increase the vulnerability of species to climate change. Climate change will lead to increases in temperature as well as changes in precipitation, cloud cover, and relative humidity (IPCC 2013). High temperatures are strongly relevant to species' vulnerability, due to abrupt decreases in fitness to the right of the thermal optimum in most ectotherm species (Deutsch et al. 2008, Angilletta 2009). However, increased temperatures may also be detrimental because of associated increases in water loss, especially if humidity or water availability decreases as temperatures rise (Bartelt et al. 2010). Synergies between temperature and desiccation could directly expose reptiles to lethal conditions but will also have indirect impacts if individuals are forced to seek hydric refuges (Kearney et al. 2013), thus potentially decreasing activity time (Bartelt et al. 2010, Kearney et al. 2013) and suitable habitats. Additionally, and perhaps more importantly, optimal temperatures may be avoided if conditions are too dry, necessarily leading to activity in suboptimal thermal conditions and, therefore. As opposed to the direct lethal effect of high temperatures or low humidity, these more subtle effects may severely reduce fitness, and could represent a substantial threat to species from habitats where aridity or seasonality increases with climate change. This is in accordance with recent predictions suggesting that environmental conditions are likely to change along several axes, leading to novel combinations of conditions and making environmental risks hard to predict (Williams and Jackson 2007, Williams et al. 2007).

In conclusion, we found strong behavioural hydroregulation in a reptile, trading off with thermoregulation. Comparative studies on reptiles from different habitats in combination with models predicting activity times and potential distributions based on these two interacting mechanisms are needed, to further clarify the likely impact such behaviours may have on different species' responses to climate change. Voluntary desiccation thresholds of species are of particular interest in this context. Especially in reptiles, the relevance of water loss is often underestimated (Kearney et al. 2013). The effects of changing temperature, however, should not be assessed in isolation from other climate variables.

-Chapter IV-

Latitudinal Trends in Ectotherm Physiology Suggest Complex Adaptive Pressures on Thermal Traits other than Heat Tolerance

Abstract

Thermal trait variability across spatial, temporal and evolutionary dimensions contributes to geographic distributions, to patterns in activity times and to adaptation potential. Physiological differences of species with different range locations and extents may predict their future vulnerability to climate change. Despite the importance of understanding these patterns, and despite the growing body of literature on the topic, major knowledge gaps remain. Specifically, despite the extensive literature on geographic trait variation within some taxa, there is still a need for more comprehensive studies examining inter- and intraspecific variability of a whole suit of relevant physiological and behavioural traits within well-defined model taxa. Such model taxa should include large numbers of closely related, ecologically similar species to allow for detection of the drivers of fine scale physiological differentiation. Here I provide such a study on a group of small Australian ectotherms and establish a frame work for future studies on evolutionary physiology within this relatively new model taxon. I assessed differences in metabolic rates, water loss, preferred temperatures, set point ranges, thermal limits, and acclimation potential across a latitudinal climate gradient among thirteen closely related species using linear mixed effects and phylogenetically generalized least squares models. All traits except upper thermal thresholds showed latitudinal trends. Notably, the latter is the trait usually focused on when assessing differences in vulnerability to climate change. Acclimation responses were observed in most and intraspecific variability in some of the measured traits. Water loss and heat tolerance exhibited substantial residual variation not explained by latitude. My results suggest that (i) absolute thermal tolerances alone do not accurately reflect latitudinal differences in vulnerability to climate change, (ii) substantial intraspecific variation in some and low phylogenetic signal in most traits indicate ongoing adaptation in this taxon, and (iii) evolution of some important physiological traits may be driven by climate variables other than those with clear latitudinal gradients. These observations emphasize the need for further studies examining the influence of thermal trait variability on species' limits across gradients more complex than latitude and provide, to my knowledge, one of the most comprehensive analyses of thermal trait variation within a taxon to date. The growing body of literature on physiology and phylogeny within rainbow skinks as well as their tropical ancestral origin, defines this taxon as a promising new model system for the assessment of processes driving the adaptation of tropical taxa to increasing climatic variability.

Introduction

Spatial and temporal variation in physiological, behavioural and anatomical traits has been studied for decades as one of the main drivers of the location and extent of species' potential distributions (Bergmann 1848, Wallace 1860, Bartholomew 1958, Brown 1984, Stevens 1989, Huey and Bennett 1990, Gaston 2003, Holt 2003 , Clusella-Trullas and Chown 2014, Sunday et al. 2014). Species' physiological potential to deal with spatial and temporal temperature variability affects their geographic range extent (Stevens 1989, Gaston 2003), their abundance within their range (Brown 1984, Brown et al. 1996), their occurrence patterns within heterogeneous environments (Brown 1984, Brown et al. 1996), their activity times (Kearney and Porter 2004), and their adaptability to climatic change throughout their evolutionary and biogeographic history (Huey et al. 2012). This is particularly relevant in ectotherms, which make up the bulk of the world's biodiversity (Deutsch et al. 2008, Kearney et al. 2009).

The main dimension along which gradients in climate are thought to limit species' distributions most clearly is latitude, presumably leading to physiological differences among species with different range positions and potential range extents along this gradient (Stevens 1989, Clusella-Trullas and Chown 2014). The gradual change in mean, maximum and minimum temperatures and, consequently, in temperature variability across latitudes in most parts of the world (Müller 1982) makes this dimension an ideal natural model system in which to determine how temperature influences the evolution of physiology and how, in turn, physiology restricts taxa to different extents along this gradient. Some of the most commonly discussed biogeographic and macrophysiological patterns resulting from latitudinal gradients in temperatures are (i) the increase in range size of organisms towards higher latitudes (Rapoport's Rule; Stevens 1989), driven by broader environmental tolerances of high latitude species exposed to greater local climatic variability (Climatic Variability Hypothesis; Chapter I; Stevens 1989, Gaston and Chown 1999, Pintor et al. 2015), (ii) the increase in body size towards the poles, presumably driven by selection for reduced heat loss via a lower surface to volume ratio (Bergman's Rule; Blackburn et al. 1999, Pincheira-Donoso et al. 2008), and the increase in extremity size towards warmer climates, allowing for greater heat loss in warm and reduced heat loss in cold climates (Allen's rule; Allen 1877, Symonds and Tattersall 2010). Some of these patterns have been observed along gradients other than latitudinal ones, for example elevational and bathymetric gradients (Stevens 1992, 1996). However, predicted trends along those dimensions are complex, consequently harder to assess and so they are less powerful as a natural model.

In addition to spatial variation, the importance of temporal variation in macrophysiology is increasingly being recognized (Clusella-Trullas and Chown 2014). Unfortunately, many studies on thermal trait variation are meta-analyses and therefore inherently include data from studies using different acclimation times, besides other important differences in methodologies (Chapter II). It can consequently be difficult to assess inter- and intraspecific thermal trait variation without detailed knowledge on the contribution of such methodological differences, especially because the time required to complete acclimation is only assessed in a small subset of studies (Chapter II; Brett 1944, Brett 1946, Lutterschmidt and Hutchison 1997, Weldon et al. 2011). Consequently, without a complete or systematic understanding of such plasticity the physiological potential of organisms may be underestimated and misrepresented. Inclusion of consistent acclimation regimes, at least within a certain model taxon, may therefore provide substantial additional insight not only into variation of thermal traits but also into geographic variation in acclimation potential of those traits. Additionally, another form of temporal variation of interest are long term, evolutionary changes in thermal physiology. Because population differentiation can indicate the potential for ongoing adaptation, examining population differences in addition to interspecific trait variation is highly relevant in this context (Hoffmann et al. 2002).

While there is a huge literature on each of the above mentioned topics, there are only few model taxa in which both inter- and intraspecific variation as well as acclimation potential across a broad range of thermal traits have been assessed within the same methodological and taxonomical framework. In fact, there are only a limited number of model taxa with well-known phylogenies including a large number of ecologically similar species that have been thoroughly studied with respect to thermal trait variation. Most such taxa are insects, especially dipterans (Hoffmann et al. 2002, Sgro et al. 2010, Overgaard et al. 2011, Kellermann et al. 2012, Overgaard et al. 2014), while vertebrate ectotherms are underrepresented. Probably the only extensively studied vertebrate ectotherm taxon with respect to inter- and intraspecific variability of thermal traits are *Anolis* lizards (Hillman and Gorman 1977, Hertz et al. 1979, Hillman et al. 1979, van Berkum 1988, Dmi'el et al. 1997, Leal and Gunderson 2012, Velasco et al. 2016) and data on this taxon comes from a variety of smaller studies with differing methodologies (which is of concerns because methodology greatly influences measures of thermal traits in some ectotherms; Chapter II; Terblanche et al. 2007, Santos et al. 2011), each covering only a limited number of species or populations within the same experimental framework. Additionally, most studies within these taxa focus on thermal tolerances and studies on a whole suit of thermal traits within the same study taxon are rare. There is, consequently, a need for the establishment of additional model taxa and extensive data sets on inter- and intraspecific patterns in thermal trait variation within these, if we want to establish a better and more general understanding of the mechanisms driving thermal trait adaptation.

Because of the severe impacts that climate change is predicted to have on the world's biodiversity (Pearson and Dawson 2003), understanding the connection between thermal physiology, thermoregulation, and species' distributions has become crucial to predicting and mitigating negative effects abundance and diversity (Kearney et al. 2009, Sunday et al. 2014, Buckley et al. 2015). Ectotherms may be particularly vulnerable to future exposure to temperature extremes, as well as reductions in activity times consequent on avoidance of such extremes (Deutsch et al. 2008, Kearney et al. 2009) and ectotherm vulnerability to increasing temperatures may be especially high in the tropics (Huey et al. 2009), where current tolerances are predicted to be narrow and thermal safety margins are small (Deutsch et al. 2008). Although we know that ectotherms are limited by environmental temperatures in many ways, the mechanisms shaping their thermal physiology and distributions are still controversial (Chapter I; Gaston 2003, Pintor et al. 2015). Additionally, there are few high quality, comparative studies on geographic variation in comprehensive sets of thermal traits within a methodologically and phylogenetically controlled framework (Calosi et al. 2008, Calosi et al. 2010, Clusella-Trullas and Chown 2014) with the exception of the extensive work on some insect taxa and *Anolis* lizards mentioned above. Especially the establishment of new model taxa with tropical origins that have previously adapted to increases in thermal variability (either through adaptation to historical climate change *in situ* or through range expansions into more variable habitats) is of particular interest in this context, because how these tropical taxa from thermally stable habitats, which appear to be of particular concern in the context of climate change (Deutsch et al. 2008, Huey et al. 2009), have adapted to increasing variability in the past may indicate how they will respond to similar changes in the future.

When predicting species' distributions, the set of potentially relevant thermal traits is large and naturally includes, but is not limited to, thermal tolerances. The multitude of traits influencing range sizes and distributions has received considerable attention in the past (see Gaston 2003). However, the focus of many recent studies on thermal trait variation has focused predominantly on thermal tolerances (Sunday et al. 2012), especially upper thermal thresholds (Deutsch et al. 2008, Calosi et al. 2010, Hoffmann et al. 2013, Clusella-Trullas and Chown 2014), because mean temperatures around the globe are predicted to increase under anthropogenic climate change (IPCC 2013) and because, intuitively, heat tolerance is of great concern in a warming world. This focus on upper thermal limits, especially when related back to changes in mean temperatures, might have some limitations; (i) mean temperatures are not a good predictor of extremes, are mostly driven by variation in minimum temperatures, and have little relevance to thermal tolerances (Dillon et al. 2010, Clusella-Trullas et al. 2011), (ii) minimum temperatures and lower tolerance thresholds vary more, geographically, than maximum temperatures and upper thermal limits (Müller 1982, Huey et al. 2009, Clusella-Trullas et al. 2011, Clusella-Trullas and Chown 2014), making it questionable whether the slight variation in upper limits will lead to substantially different impacts on species from different latitudes , (iii) many ectotherms are likely to be more affected by exposure to solar radiation than by high ambient air temperatures (Sunday et al. 2014), (iv) most ectotherms exhibit strong and effective behavioural avoidance of exposure to extreme temperatures (Vickers et al. 2011, Sunday et al. 2014, Buckley et al. 2015) and, lastly, (v) there are a multitude of other thermal traits that impact fitness under different temperature regimes (see below; Hofmann and Todgham 2010) but receive little attention in the context of climate change. Most notably, the importance of water loss in this context has recently been reviewed in insects (Chown et al. 2011) but remains poorly studied in other dry-skinned ectotherms, such as reptiles. Thus, the assumption that variations in CT_{max} in isolation from other physiological and behavioural traits determine resilience to climate change and changes in future range extents lacks a clear supporting mechanism and this is increasingly being recognized (Chown et al. 2011, Clusella-Trullas and Chown 2014, Sunday et al. 2014, Buckley et al. 2015).

Because exposure to high temperatures is highly and rapidly detrimental (Vickers et al. 2011, Vasseur et al. 2014), and because fitness is optimal within a narrow range of temperatures around the physiological thermal optimum temperature (T_{opt}), preferred body temperatures (PBT) and ranges of selected body temperatures around PBT (set point range; SPR; Angilletta 2009) buffer the effect of climatic variability (Bogert 1949, Sunday et al. 2014, Buckley et al. 2015) if suitable microhabitats are available for behavioural thermoregulation (Huey and Stevenson 1979, Kearney et al. 2013). Species from highly variable habitats are likely to evolve narrow SPRs around their T_{opt}, i.e. thermoregulate more precisely, because the risk and associated cost of accidental exposure to thermal extremes is greater (Vickers et al. 2011, Vasseur et al. 2014). Species from thermally stable (e.g. tropical) or well-buffered habitats with little exposure to radiation (e.g. forests) tend to thermo-conform and have a broader SPR (Hertz 1974, Huey and Slatkin 1976, Huey et al. 2009). Similarly, species that have a high exposure to high temperatures, tend to evolve a higher T_{opt} and higher, coevolved PBTs (Angilletta 2009) (which are often used as a proxy for T_{opt}). Species most at risk of overheating if frequency of high temperatures or exposure increases will, consequently, be those with relatively low T_{opt}, PBTs not far below, or even above, T_{opt} and broader SPR. Perhaps counterintuitively, species with precise thermoregulation and narrow SPR will be less likely to be exposed to dangerous temperatures but still at risk of severe decreases in activity times if sufficiently buffered microhabitats are no longer available for such precise thermoregulation (Kearney et al. 2013). Consequently, PBT and SPR are highly relevant to overheating risk as well as energy assimilation, and therefore, to geographic distribution and vulnerability to climate change (Kearney and Porter 2004).

Reproduction by individuals and consequently population growth can only occur where sufficient energy can be assimilated during activity to cover the energetic costs of maintenance and reproduction (Kearney et al. 2008). Because metabolic rate is temperature dependent, cold climates are sometimes said to favour higher metabolic rates at a given temperature (i.e. high temperature specific metabolic rate or Standard Metabolic Rate; (Bullock 1955, Feder 1976), especially in colder seasons, to allow for longer potential activity periods (metabolic compensation; Bullock 1955, Tsuji 1988). Warmer climates, on the other hand, have been suggested to favour lower metabolic rates at a given temperature (i.e. low Standard Metabolic Rates) to reduce energy expenditure (Dillon et al. 2010). However, there has been debate about the universal applicability of metabolic cold adaptation in some taxa (Clarke 1980) and a more detailed examination of latitudinal patterns in metabolic rate in reptiles is therefore warranted. Additionally, in habitats where prolonged periods of inactivity are required periodically (e.g. during winter), decreased metabolic rates during brumation (inverse acclimation; Tsuji 1988, Christian et al. 1999) might allow for minimal energy expenditure when activity is not possible, analogous with hibernation in endotherms. Consequently, the differential potential to up-regulate metabolic rates in colder climates be a highly limiting factor to ectotherm distributions (Kearney et al. 2008). Similarly, metabolic rates may influence range sizes with respect to habitat productivity, because high metabolic rates facilitate quicker growth but low metabolic rates enable species to exist in low resource environments (Bauer et al. 1991). Birds have also been suggested to be geographically limited by their metabolic rates, with high latitude range boundaries representing a point at which further increases in metabolism cannot be compensated by increased energy intake (Root 1988). While such increases in metabolism in endotherms are directly related to a need for increased heat production and are, therefore, not equivalent to the kind of changes observed in ectotherm metabolic rates, increased metabolic rates in cold climates in the context of metabolic cold adaptation or acclimation may nevertheless have similar implications for ectotherms. Latitudinal trends in metabolic rates may, therefore, reflect differences in habitat productivity instead. Lastly, with respect to climate change, species with higher Standard Metabolic Rates are likely to be particularly vulnerable (Dillon et al. 2010), if predicted temperature increases across their geographic range are large and especially if coupled with decreased activity times. Standard metabolic rates and their acclimation potential are therefore a thermally dependent trait potentially limiting species' distributions in many possible ways. This is increasingly recognized in studies on mechanistic models of species' distributions (Kearney and Porter 2004) but there are few studies assessing the implications of inter- and intraspecific trends in metabolic rates for vulnerability to climate change in reptiles (Dillon et al. 2010).

Lastly, the relevance of desiccation resistance to species' distributions is under-appreciated in the recent literature on climate change impacts (Chown et al. 2011), given the temperature dependence of water loss rates. It has been shown that taxa from drier habitats exhibit lower water loss rates, even in "dry-skinned" ectotherms (Hillman et al. 1979, Neilson 2002), and that lizards actively avoid desiccation at high temperatures and low humidities (Chapter III; Scarpellini et al. 2015). Consequently, lethal (and sub-lethal) limits in water loss as well as hydroregulation may affect fitness and activity times similarly to thermal limits and thermoregulation. Despite this, water loss and its potential to limit the distributions of dry-skinned ectotherms is chronically understudied and, with the exception of work on insects, has only recently been receiving increased attention in the context of climate change (Chapter III; Chown et al. 2011, Kearney et al. 2013). Because climate change is forecast to not only result in increased temperatures, but also changes in precipitation, moisture deficit and cloud cover, impacts may be substantial and especially threaten species with high water-loss rates, or those already close to their desiccation threshold.

Here I provide a comprehensive analysis of thermal trait variation along a latitudinal climate gradient, among ecologically similar species in a monophyletic clade of small ectotherms with tropical ancestral origin and establish a detailed baseline data set for this taxon using a consistent methodology, to establish it as a potential model taxon for future research on inter- and intraspecific thermal trait variation. I quantified thermal tolerances (critical thermal minimum and critical thermal maximum), behavioural thermoregulation (preferred body temperature and set point range), energy expenditure (standard metabolic rate), and desiccation resistance (water loss rate at different temperatures). I examined the correlation of each of these thermal traits with latitudinal position of species' ranges and latitudinal range extents. Furthermore, I tested whether traits of populations within species differed in relation to their latitudinal position within the species' range (evolutionary plasticity) and whether hot or cold acclimation affected each trait (phenotypic plasticity). Based on this, I assessed the likelihood of different traits to currently limit species' ranges based on the amount of plasticity (necessity for within-lifetime adjustments of a trait), interspecific (adaptation) and intraspecific (ongoing adaptation) latitudinal variation. Lastly, I used these observations to discuss the likely influence of climate change on tropical and temperate, as well as restricted and wide ranging ectotherms. Specifically, I hypothesized that all traits would be affected by latitudinal variation and predicted that (i) the potential of species to extend into colder, high latitude environments would correlate strongly with cold tolerances, while the effect of range size or position on upper thermal limits would be weak, (ii) low latitude species would thermoregulate around higher preferred body temperatures (indicative of higher thermal optima in environment with high frequency of warm temperatures) and have broader set point ranges (indicative of tendency to thermoconform in low variability environments), (iii) towards higher latitudes standard metabolic rates would increase and acclimate more (compensation); (iv) water loss rate would be a significant determinant of range sizes and positions and, lastly, (v) latitudinal patterns within species would be analogous to the patterns between species, because thermal selection pressure on small, poorly buffered ectotherms are likely to be strong enough to lead to ongoing, local adaptation in limiting thermal traits. In accordance with this, I expected phylogenetic signal to be low in all measured traits. This is, to my knowledge, the most extensive and comprehensive experimental study on latitudinal variation in physiological traits within a reptile taxon to date.

Methods

The thirteen species tested in this study were from the genera *Carlia* (nine species; *Carlia dogare*, *C. jarnoldae*, *C. longipes*, *C. munda*, *C. rubrigularis*, *C. schmeltzii*, *C. storri*, *C. tetradactyla*, *C. vivax*,) and *Lygisaurus* (four species; *Lygisaurus aeratus*, *L. foliorum*, *L. laevis* and *L. rococo*), which constitute a monophyletic clade of small, mostly leaf litter dwelling skinks with extreme variation in latitudinal range size and range position (Dolman and Hugall 2008, Wilson and Swan 2008). The species selected for this study were distributed along the Australian East Coast, which is characterised by clear gradients in mean, minimum, and maximum temperatures, and temperature variability (Chapter I; Pintor et al. 2015). Species were chosen based on their East Coast distributions (i.e. not extending into the arid interior of Australia; except for *Carlia munda*) to reduce potential effects of multidimensional climate gradients on measured variables. The Climatic Variability Hypothesis applies to this clade, as species occurring in more variable habitats have wider latitudinal ranges (Chapter I; Pintor et al. 2015). This clade, therefore, provides an ideal model to examine the effect of thermal trait variation on latitudinal range size and position. The number of populations collected per species varied. Some highly restricted species were collected from only one location, more wide ranging species were collected from up to three locations across their latitudinal distribution. Generally, an attempt was made to collect more widely distributed species from the centre of their range as well as from both latitudinal extremes within their range (see Appendix IV). Population effects were assessed based on the deviation of sampling locations from the average location of the species' range, i.e. as an additional continuous predictor of traits, modifying average species trends as described by species' range position (latitudinal midpoint), rather than a categorical description of population ID.

Animals were housed in 30 x 20 x 10cm (DWH) plastic containers with a mesh lid on a substrate of potting mix and leaf litter, and were fed crickets and a calcium and vitamin D_3 supplement. Water was provided *ad libitum*. Animals received UV light one day per week (Reptile One UVB 10.0 Fluorescent Light Tube). Half of all animals from each population were acclimated to a hot (28-32°C) and half to a cold (18-22°C) temperature regime for a minimum of sixteen weeks (Chapter II; Pintor et al. 2016) at constant light dark cycles (12h:12h). Animals were not used for experiments when shedding, because shedding substantially raises standard metabolic rate in reptiles (Pintor et al. 2010) and may affect other physiological measurements. Thus, most individual animals experienced each of the experiments outlined below, except when shedding prevented it. Metabolic rates, water loss rates and preferred body temperatures were determined first, as these were the least stressful experiments and were unlikely to influence acclimation. Following this, critical thermal minimum (CT_{min}) was determined. Critical thermal maxima (CT_{max}) were determined last, to prevent any effects of this more stressful test on results of other experiments, because exposure to hot temperatures can lead to rapid acclimation of physiological traits (Hutchison and Maness 1979).

Standard Metabolic Rate (SMR) of each individual was measured at 20 and 30°C using positive-pressure flow-through respirometry, at a flow rate between 3 and 10 ml/min of dried atmospheric air, depending on lizard body mass (body mass range: 0.2-10.9g). Seven animals were tested sequentially, with one empty chamber as a reference. Excurrent air from each experimental chamber was analysed separately by switching between chambers via a multiplexer (Sable Systems Respirometry Multiplexer V2.0) and measured for 60 min per chamber with a 30 min reference interval between chambers. The experiments were performed in the dark over night between 17:00 and 9:00 to ensure animals were resting. If chambers were measured more than once during this period, the lowest measurement was used in the analysis. If activity was suspected, due to fluctuations in oxygen traces, or if animals shed within seven days of the test, the test was repeated and the lowest measurement was used in the analysis. Individuals were not fed for at least two days prior to testing, to ensure that measurements were not influenced by the heat increment of feeding, which can be substantial in reptiles (Pintor et al 2010). Air flow, carbon dioxide, and oxygen concentration of excurrent air was measured (in that order) using a Sable Systems Foxbox (Sable Systems, http://www.sablesys.com/products/field-line/foxbox-respirometry-system). Example traces are shown in Appendix V. Oxygen concentration in the air always remained above 20.5% and mostly remained over 20.8% for typical measurements of inactive individuals, which were typically between 0.05% and 0.1% under reference concentrations. Equipment was calibrated to adjust for drift every day before start of experiments. Water was removed from the air using silica gel before measurement of flow and carbon dioxide and oxygen, and carbon dioxide was removed using Ascarite before oxygen measurements. The experiment was performed in a temperature and humidity controlled cabinet (Thermoline Scientific, model TRH-460-GD-D/L). Data acquisition and subsampling were controlled with WartHog LabHelper$^{©}$ software (http://warthog.ucr.edu/) via a Sable System UI-2 digital to analog converter. Oxygen consumption was calculated using the following formula as implemented by WartHog LabAnalyst X Universal$^{©}$ software (http://warthog.ucr.edu/):

Eq. 4.1 $VO_2 = STP * FR * ((FiO_2\text{-}FeO_2) - FiO_2 * (FeCO_2\text{-}FiCO_2) + FeO_2 * FeCO_2) / (1\text{-}FiO_2)$

where STP = a factor that converts measured to standard conditions of 0°C and 101.325 kPa, FiO_2 and FeO_2 = the fraction of the incurrent (measured in the reference chamber) and excurrent air made up of oxygen, $FiCO_2$ and $FeCO_2$ = the fraction of the incurrent and excurrent air made up of carbon dioxide, FR = the flow rate. The mean of the lowest 20 min of each measurement of oxygen consumption [µl/min] was used as the final value of SMR for each individual.

Water loss rate (WLR) was measured at both 20 and 30°C, in the dark (to prevent activity) within transparent plastic chambers over a 16 hour period during the inactive period (night), while pumping dried atmospheric air (<5% relative humidity) through the chamber. Animals were weighed, placed in the chamber and left to acclimate to experimental conditions for one hour. Subsequently, the chamber containing each lizard was weighed, left overnight (approximately 18:00 to 9:00), and reweighed in the morning. The difference in mass was converted to WLR in mg/h. To avoid overestimation of WLRs, measurements were repeated and the lower of the measured value used if animals defecated during the experiment, if they shed within seven days of the experiment, or if they were very active during the morning measurement. Repeat tests occurred after at least 2 days in case of defecation or activity and after at least 7 days post shedding. To verify that water loss measurements based on weight loss was not confounded by metabolic weight loss, I estimated metabolic weight loss for a subsample of lizards (warm acclimated *Carlia rubrigu*laris individuals) and compared it to water loss rates in the same group of lizards. Depending on whether animals were assumed to metabolise lipids, protein or carbohydrates, the percent of overall mass loss caused by metabolic mass loss accumulated to between 1.7% and 4.1% for an average sized individual of 2.83g body mass. The contribution of metabolic mass loss to my estimates of water los was therefore negligible.

Preferred body temperature was measured within a 2-m thermal gradient with an aluminium base and opaque Perspex walls, heated at one end and cooled at the other by Peltier plates (temperature range from 5°C to 65°C). White sand paint was applied to the base to provide a more natural substrate, while optimizing contrast. Lizards were introduced into the hot end, to facilitate quick temperature selection away from unsuitable temperatures, and were left in the gradient for one hour to adjust to experimental conditions. Subsequently, photographs were recorded every 60 seconds for 24 hours from above, under a 12h:12h day:night regime. Dim red light was provided at night to enable photography. Start times were chosen at random between

7:00 and 22:30. For the analysis, only the first two hours of recording after the initial one hour of familiarization with the gradient were used, unless start time was after 17:00, in which case the first two hours in the morning (7:00-9:00) were analysed instead. Measurements from the dark photophase were not used. Using ImageJ (http://imagej.nih.gov/ij/) lizard positions (distance from cold end of gradient to the point between lizard front legs in pixels) were measured for each photo. The position of ten iButtons® (model 1922L), which measured the temperature at 20 cm intervals along the gradient every 60 seconds, were recorded in the same manner. Using a polynomial calibration curve on the temperatures recorded by the iButtons in the gradient during the experiment (see Appendix I for calibration curve and image of the thermal gradient), lizard positions were converted into selected substrate temperature at each 60-second interval. For each lizard, the median of the 120 measurements collected over two hours was calculated and used for further analysis. Set Point Range of each individual was calculated as the difference between the 25% and 75% quartile of the 120 temperatures recorded. Animals were fasted for two days prior to the experiment. Some animals became inactive during the recording and positioned themselves at very cold temperatures over extended time periods. If inactivity was suspected, the measurement was repeated several weeks later and the first measurement was discarded. Similarly, if shedding was noted within seven days of the experiment, that instance was discarded and the measurement was repeated. It has been shown that distributions of selected temperatures in thermal gradients can be influenced by thermal dependence of motion, at least in very small ectotherms (Dillon et al. 2012). However, such effects lead to an accumulation of animals at the cold end of the gradient and presume constant movement. In this study, most animals did not move constantly but rather selected a temperature and only explored the warmer and colder ends of the gradient periodically. Similarly, I did not observe an accumulation of animals at the gradient ends. Observed selected temperatures are, therefore, unlikely to reflect thermal dependence of motion.

CT_{min} was measured as in described in Chapter II. Individuals were separately placed in a round plastic container inside a temperature controlled cabinet and left for 30 minutes at 18°C to standardise individual starting body temperature. A wet sponge in the container raised humidity to maximize rate of heat transfer as well as to prevent desiccation. Air temperature in the cabinet was cooled, creating a gradual temperature decrease within the container of approximately 0.2°C per minute, which was recorded continuously with a thermocouple. To estimate lizard body temperature from air temperature in the container, a calibration correction was determined by measuring the body temperature of five museum specimens of rainbow skinks of different sizes (range: 0.47 to 10.96g; obtained from a collection at James Cook University) while air temperature in the container was decreased, to establish the relationship between body mass and the time-lag of body temperature behind air temperature at the cooling rate we used. For this, museum specimens were rehydrated in saline at 4 °C for 2 days and their skin dried prior to experiments to avoid excessive evaporative cooling. The maximum average lag of body temperature behind air temperature for the largest animal (a *Carlia longipes* specimen of 10.96g body mass) was 0.6 °C. Calibration experiments were performed exactly as CT_{min} experiments, including turning the animals every 60 seconds. The resulting correction was then applied to CT_{min} values of live animals. All temperature readings were recorded on a Sable Systems TC1000 thermocouple reader using electronic thermocouples (type T), which had all previously been calibrated on the same mercury in glass immersion thermometer with a precision of ± 0.05C (0.1C marks; NATA calibrated). Animals were flipped on their back every 120 seconds by rolling the container until the air temperature in the container reached 14°C, and every 60 seconds thereafter. CT_{min} was defined as the temperature at which loss of righting response occurred. To estimate lizard body temperature from air temperature in the container, the relationship between body mass and cloacal body temperature lag was obtained using lizard museum specimens of different sizes preserved in ethanol (obtained from a collection at James Cook University), which had been rehydrated at 4°C in saline for 3 days. CT_{min} of live animals were corrected for that lag using the relationship derived. For CT_{max} experiments, methodologies were the same as for CT_{min} except that the starting temperature was 34°C, and animals were flipped on their back every 120 seconds until temperature had increased to 38°C and every 60 seconds thereafter. For CT_{max} fewer animals were tested, as this was the most stressful test. A separate calibration to the one used for CT_{min} experiments was performed on the lag of body temperature behind air temperature during CT_{max} experiments. Body size of the five specimen used for the calibration ranged from 0.22 to 11.96g and the maximum lag observed in the largest animal was 1.2 °C.

Latitudinal Midpoint of species' ranges and latitudinal range extents were calculated from occurrence points for each species downloaded from the Atlas of Living Australia (http://spatial.ala.org.au/, 10/07/2014). Museum Records were compared with distribution maps in the "Complete Guide to Reptiles of Australia" (Wilson and Swan 2008) and clearly erroneous outliers excluded. I excluded single occurrence records far outside the known distribution (likely identification errors) and records outside species' distributions due to recent taxonomic divisions of species with geographically distinct ranges. For example, populations recorded as *Carlia longipes* in northern Cape York and Arnhem land, which have recently been reclassified as *Carlia sexdentata* (Donnellan et al. 2009), were not included in the analyses. Latitudinal range extents were calculated as the difference between maximum and minimum values for latitudinal occurrence points and latitudinal midpoints as the mid-distance between these latitudinal extremes (expressed as negative values because they were located in the southern hemisphere: less negative or "larger" values reflect more tropical locations).

Deviations of individuals' capture locations from the species' midpoints (population deviations) were calculated as the difference, in latitudinal degrees, between the collection latitude of individuals and the midpoint of species' ranges. Deviations from midpoints that were directed north towards the tropics were expressed as positive values, deviations towards high latitudes as negative values.

All data exploration, analyses, and model validations were performed in R (R Core Team (2014) using two separate approaches, linear mixed effects (LME) models (including species as a random effect) and phylogenetic generalized least squares (PGLS) models in the package nlme (Pinheiro et al. 2013). Models were fitted to individual data, i.e. all data points shown represent values of individuals included in the analysis. Phylogenetic correlation structures were created using the most recent published phylogeny of the clade (Pyron et al. 2013) and implemented in the R package ape (Paradis et al. 2004). *L. rococo* was excluded from phylogenetic analyses as it was not included in any published phylogeny and therefore could not be used to create the correlation structure. Initial models for both approaches included the three geographical covariates; latitudinal midpoint of the species that individuals belonged to, latitudinal range extents of the species, and population deviation from midpoints (the latitudinal deviation of each individual's collection location from the species' midpoint), as well as log transformed body mass of individuals. A factor covariate for acclimation treatment (hot or cold) and interaction terms for acclimation treatment and each of the above main terms was included to test whether acclimation state influenced estimates of latitudinal trait variation. Similarly, I included an interaction term between latitudinal midpoint and population deviation to allow for differences in population structure between species from different latitudes. These variables and interactions were included in all initial models. Furthermore, treatment temperature and its interaction with latitudinal midpoint and acclimation treatment was included as a potential predictor of standard metabolic and water loss rates, and time of day and its interaction with latitudinal midpoint and acclimation treatment was included as a potential predictor of preferred body temperature and set point range. Some of the dependent variables were log transformed due to observed skew in the data (see **Tables 4.1-4.4**). Model selection on the full models was performed by step-wise backwards selection using AICs and log-likelihood ratio tests. After the model with the lowest AIC was found, non-significant terms (on the 5% level) remaining in the model were excluded using log-likelihood ratio tests. If a term improved the AIC of the model but did not improve the model fit significantly based on log-likelihood ratio tests, it was excluded from the final model.

For LME models, it was decided *a priori* to include species ID as a random intercept effect in all models. In models of preferred body temperature and set point range, I additionally allowed the slope of the model with time of day to vary among species (random intercept and slope).

This was necessary because preferred body temperature at any given time of day may depend on preferred body temperature at a previous time of day, and this potential temporal autocorrelation needs to be accounted for in a mixed effects model. Intraclass correlation coefficients (ICC; Goldstein 1986, Burton et al. 1998), were calculated to assess how much of the residual variation of each model was explained by the random effect. ICCs above 0.5 indicated high residual variation between species, not explained sufficiently by the fixed effects and warranting future exploration of other potential explanatory variables. In addition, partial R^2 of the fixed effects was compared to the overall R^2 of the final model to assess the improvement attributed to the random effect (Nakagawa and Schielzeth 2013). Once final models of best fit were found, they were refitted using standardized dependent and independent variables to obtain standardized correlation coefficients to aid with comparison of effect size. Both, unstandardized and standardized coefficients are reported in the results.

For PGLS models, a "corPagel" correlation structure (Pagel 1999, Freckleton et al. 2002) was used to estimate the degree of phylogenetic signal (λ), which can assume a value between 0 (no phylogenetic signal) and 1 (strong phylogenetic signal; Halsey et al. 2006). Resulting models were compared to models assuming complete accordance with a Brownian motion model of evolution using likelihood ratio tests to assess whether observed trends were significantly stronger than they would be if based purely on phylogeny. PGLS models were fitted to assess the degree of phylogenetic signal and to assess potential shortcomings of LME models. However, since results from both approaches were congruent and since only LME models allowed for the inclusion of *L. rococo*, LME models were used for final predictions.

Results

Phylogenetic signal in almost all traits was low ($\lambda<0.55$), with the single exception of water loss rates ($\lambda = 0.79$). In all cases, phylogenetic signal was significantly weaker than assumed under a Brownian motion model of evolution ($\lambda=1$; likelihood ratio tests; $p<0.005$ for all traits). Differences between PGLS models and LME models are explained where appropriate. The intraclass correlation in LME models was low (<0.5) for most traits except WLR, indicating that most of the variation was explained by the fixed effects. LME and PGLS models resulted in nearly identical end models (see **Table 4.1-4.4**), except that latitudinal range extent remained a significant predictor of standard metabolic rate (SMR) and water loss rate (WLR) when phylogenetic relationships were accounted for. Latitudinal range extent was not a significant predictor of any other trait. Latitudinal midpoint, however, was a significant predictor of all traits except CT_{max}. Additionally, there was significant latitudinal variation between populations for SMR as well as CT_{min}. Acclimation treatment had a significant effect on all traits except median PBT.

Thermal Tolerances

The best model describing variation in critical thermal minimum (CT_{min}) included; log transformed body mass, latitudinal midpoint, and population deviation from midpoints, as well as acclimation regime and an interaction between acclimation regime and each of the other main terms (**Table 4.1**).

Table 4.1 Predictor coefficients for best-fit linear mixed effects models (LME) and phylogenetic generalized least squares models (PGLS) fitted to critical thermal minima and maxima.

Model	LME			PGLS	
Trait	Estimate	SE	St. Estimate	Estimate	SE
Critical thermal minimum [°K] (n=275)					
Intercept	282.81	0.44	-0.62	282.74	0.45
- Log body mass [g]	-0.051	0.13	-0.024	-0.0063	0.15
- Acclimation treatment [hot or cold] (hot)	0.82	0.45	1.43	0.84	0.46
- Latitudinal midpoint [lat. deg. south]	0.12	0.022	0.39	0.12	0.022
- Population deviation from midpoint [lat. deg.]	0.05	0.019	0.11	0.048	0.019
- Log body mass [g] * Acclimation treatment [hot or cold] (hot)	0.43	0.14	0.20	0.43	0.15
- Latitudinal midpoint [lat. deg. south] * Acclimation treatment [hot or cold] (hot)	-0.063	0.022	-0.20	-0.062	0.022
- Population deviation from midpoint [lat. deg.] * Acclimation treatment [hot or cold] (hot)	-0.056	0.027	-0.14	-0.057	0.027
Critical thermal maximum [°K] (n=217)					
(Intercept)	315.54	0.12	-0.41	315.61	0.17
- Log body mass [g]	-0.39	0.11	-0.42	-0.49	0.10
- Acclimation treatment [hot or cold] (hot)	0.59	0.079	0.67	0.62	0.079
- Log body mass [g] * Acclimation treatment [hot or cold] (hot)	-0.25	0.085	-0.27	-0.24	0.084

Note: latitudinal midpoint was measured in degrees south. More negative values represent higher, more southerly latitudes. A positive correlation with latitude therefore represents an increase in the dependent variable towards the north.

Smaller animals were more cold tolerant when hot acclimated. Large animals, however, acclimated their cold tolerances more to the cold temperature regime (i.e. exhibited greater phenotypic plasticity), resulting in very similar absolute cold tolerances for species of different body size (**Fig.4.1**). High latitude species had lower CT_{min} in both acclimation regimes and greater acclimation potential (difference between cold and hot acclimated values) (**Fig.4.2**). Similar to differences observed among species, higher latitude populations within species also had greater acclimation potential and were, as a result, overall more cold tolerant. The intraclass correlation coefficient (ICC) was small (0.11) and only 3% out of an overall R^2 of 71% was explained by the random effect. Phylogenetic signal was low at $\lambda=0.14$ and significantly different from 1 (likelihood ratio test; $\chi^2(1)= 8059.686$; $p<0.0001$).

The best model for critical thermal maximum temperatures included only log transformed body mass, acclimation regime and an interaction term between the two (**Table 4.1**). Large bodied animals were less heat tolerant and did not acclimate much, while smaller animals were more tolerant of hot temperatures and additionally increased their CT_{max} by up to 1 °C in the hot acclimation treatment (**Fig.4.3**). None of the predictor variables relating to latitudinal position or range size were significant. The ICC was intermediate at 0.40 and 26% of the overall R^2 of 61% were explained by the random effects, indicating substantial residual variation between species not explained by the included predictor variables (intraclass correlation coefficient) and a reasonably high amount of residual variation not explained by the model (overall R^2). Phylogenetic signal was relatively low ($\lambda=0.44$) and significantly different from 1 (likelihood ratio test; $\chi^2(1)= 6219.122$; $p<0.0001$).

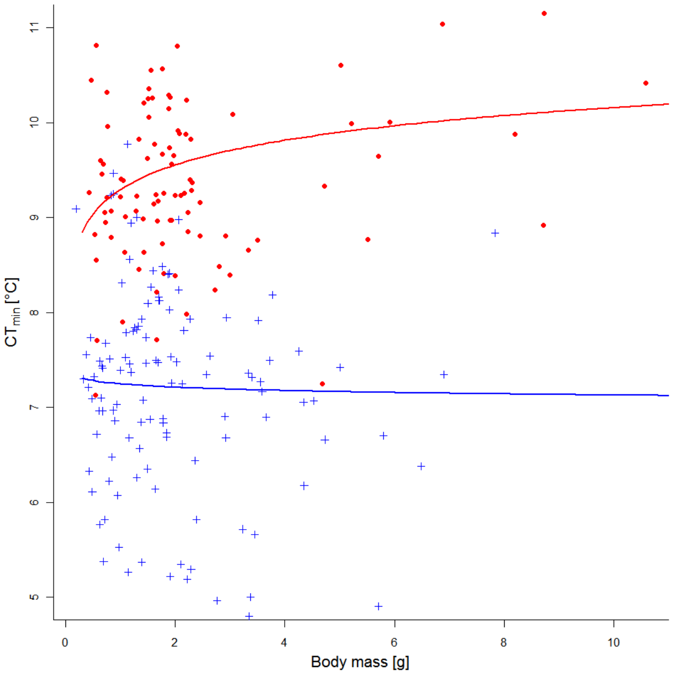


Fig.4.1 Predicted CT_{min} and data for hot (●; ——) or cold (+; ——) acclimated individuals of different body size. Smaller animals were more cold tolerant when hot acclimated but cold acclimated animals did not show much body size variation in CT_{min}.

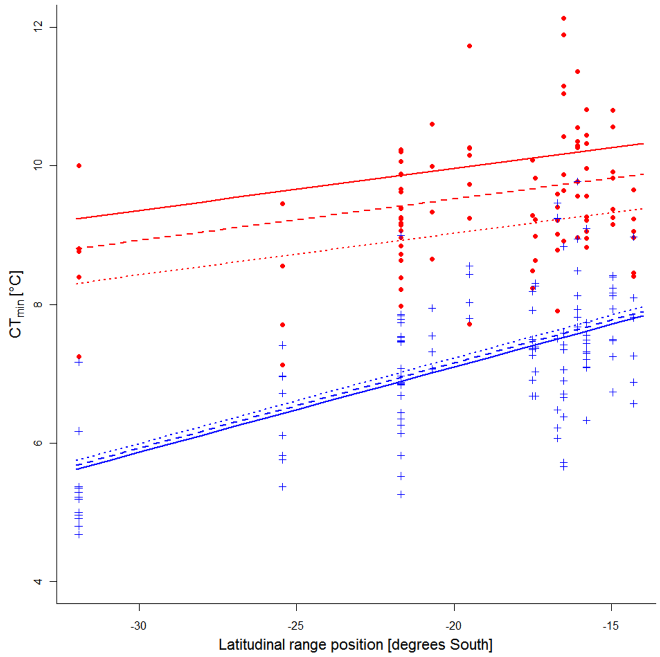


Fig.4.2 Predicted CT$_{min}$ and data for small (.....), medium (- - -) and large (——) species from different latitudes when hot (●; ——) or cold (+; ——) acclimated. CT$_{min}$ decreased toward higher latitudes and did so more for cold than for hot acclimated animals. Smaller animals were more cold tolerant when hot acclimated but cold acclimated animals did not show much body size variation in CT$_{min}$.

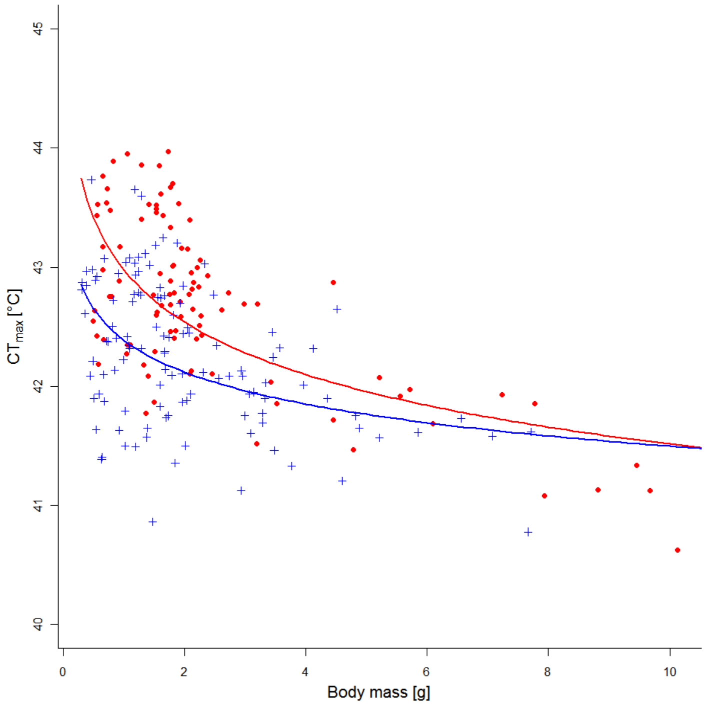


Fig.4.3 Predicted CT$_{max}$ and data for hot (●; ——) and cold (+; ——) acclimated individuals of different body mass. Smaller animals were more heat tolerant and acclimated their CT$_{max}$ more. Heat tolerance is likely more important in small animals with less thermal inertia.

Preferred Body Temperatures

The final model for median preferred body temperature (PBT) included significant terms for time of day, latitudinal range position and an interaction between the two (**Table 4.2**).

Table 4.2 Predictor coefficients for best-fit linear mixed effects (LME) and phylogenetic generalized least squares models (PGLS) fitted to median preferred body temperatures and set point ranges.

Model	LME			PGLS	
Trait	Estimate	SE	St. Estimate	Estimate	SE
Preferred body temperature [°K] (n= 321)					
(Intercept)	297.97	1.73	-0.0088	298.04	1.80
- Time of day [decimal h]	0.56	0.12	0.47	0.57	0.13
- Latitudinal midpoint [lat. deg. south]	-0.21	0.078	-0.08	-0.20	0.080
- Latitudinal midpoint [lat. deg. south] * Time of day [decimal h]	0.013	0.0055	0.11	0.014	0.0056
Log set point range [°K] (n=321)					
(Intercept)	-3.65	1.25	-0.29	-3.80	0.92
- Acclimation treatment [hot or cold] (hot)	1.67	0.44	0.54	1.67	0.45
- Time of day [decimal h]	0.29	0.083	0.28	0.031	0.066
- Latitudinal midpoint [lat. deg. south]	-0.14	0.058	-0.012	-0.14	0.039
- Time of day [decimal h] * Acclimation treatment [hot or cold] (hot)	-0.085	0.032	-0.29	-0.086	0.033
- Latitudinal midpoint [lat. deg. south] * Time of day [decimal h]	0.011	0.0038	0.19	0.011	0.0028

Note: latitudinal midpoint was measured in degrees south. More negative values represent higher, more southerly latitudes. A positive correlation with latitude therefore represents an increase in the dependent variable towards the north.

PBT increased with time of day. High latitude species had higher morning PBTs than tropical species. However tropical species increased their PBT more throughout the day leading to similar PBTs for all species in the afternoon (**Fig.4.4**). Predicted PBTs in the morning and afternoon ranged from 32.6 °C to 33.8 °C, respectively, in temperate species, and from 30.7 °C to 34.4 °C in tropical species. Inclusion of body mass improved the model marginally (ΔAIC= 0.71), but not significantly (log-likelihood ratio = 2.71, p = 0.10) so mass was excluded from the final model. Range size, population or acclimation regime had no significant effect. The ICC was very small (0.04) and only 3% out of an overall low R^2 of 24% were explained by the random effect. Although the fixed effects explained most of the model fit, the low R^2 indicates substantial individual (not species-specific) variation in PBT around the predictor lines despite the clear trends identified by the model. Phylogenetic signal was very weak (λ=0.043) and significantly different from 1 (likelihood ratio test; $\chi^2(1)$= 9267.554; p<0.0001).

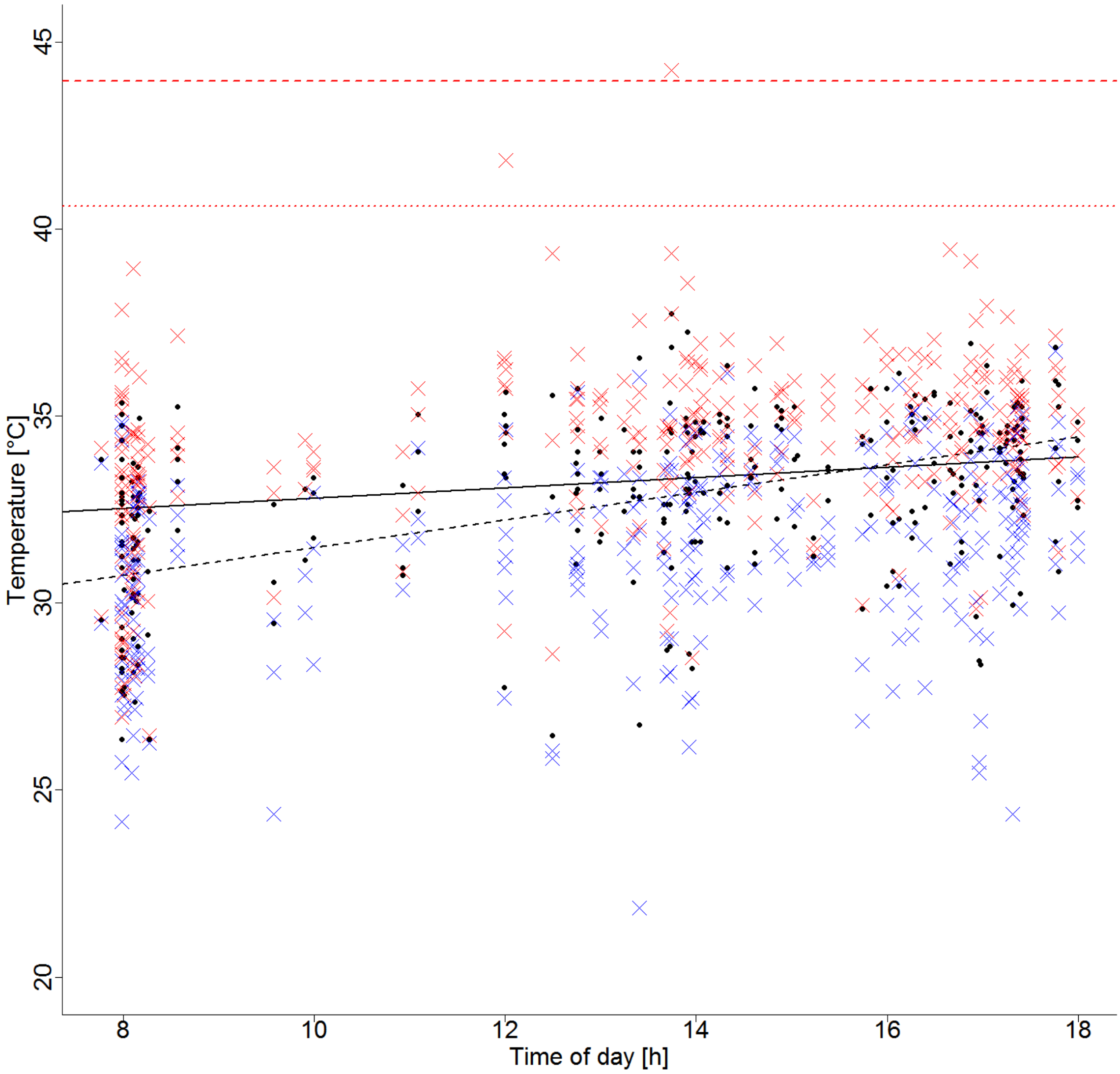


Fig.4.4 Median (●), upper (**x**) and lower (**x**) quartiles of selected body temperatures across times of day (decimal h) showing all animals used in this study, including predictor lines for median selected body temperature of a species with a high (——, midpoint of *Carlia tetradactyla*) and low (— — —; midpoint of *Carlia dogare*) latitudinal midpoint. Low latitude species thermoregulated at lower temperatures in the morning than high latitude species but increased their preferred body temperature more with time of day, leading to similar preferred body temperatures in the afternoon. All species thermoregulated below the minimum (**……**) and maximum (**———**) hot acclimated CT_{max} recorded in this study.

The log-transformed set point range (SPR) around the median PBT, defined as the interquartile range of the temperatures selected by individuals (Angilletta 2009), was best described by a model including; time of day, latitudinal midpoint of species ranges and acclimation regime, as well as interactions between midpoint and time of day, and acclimation regime and time of day (**Table 4.2**).

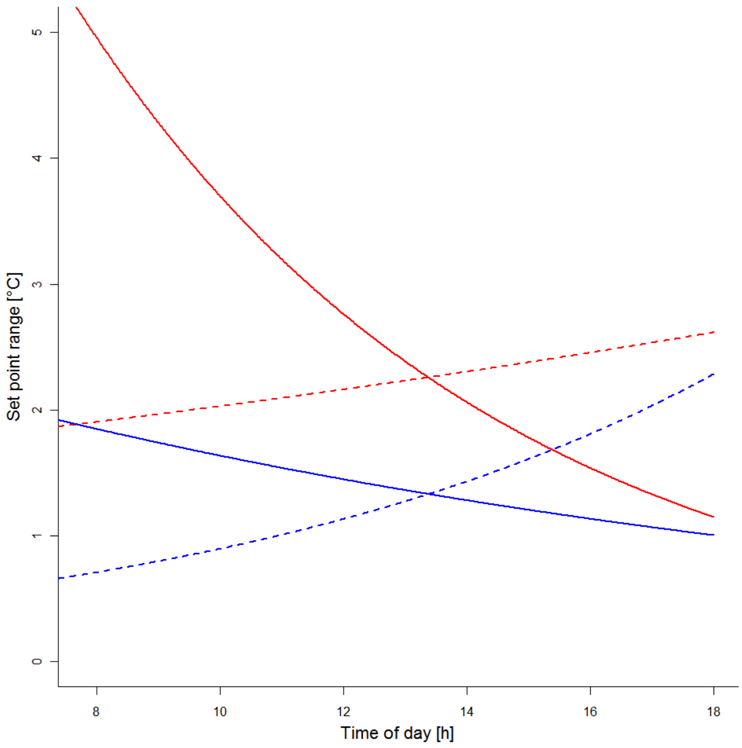


Fig.4.5 Predicted breadth of set point range at different times of day for hot (—) and cold (—) acclimated animals. Solid lines represent a temperate species, dashed lines represent a tropical species. Hot acclimated animals had a broader set point range, i.e. thermoregulated less carefully around their median preferred body temperature. On average, tropical species increased their set point range with time of day, while temperate species decreased it. However, there was substantial residual variation not explained by the fixed effects of this model and predictive power of these estimates is therefore low.

In spite of the absence of acclimation effects on median PBTs, hot acclimated animals consistently displayed broader SPRs than cold acclimated animals. This trend was slightly less pronounced in the afternoon than in the morning (**Fig.4.5**). Tropical species thermoregulated more precisely than temperate species in the morning. However, this difference became less pronounced at later times of day and eventually reversed. For temperate species, the estimated breadth of selected temperatures decreased with time of day from 5.7 °C to 1.1 °C for hot acclimated animals and from 2.0 °C to 1.0 °C for cold acclimated. For tropical species, estimated SPR increased from 1.8 °C to 2.6 °C (hot acclimated) and 0.6 °C to 2.3 °C (cold acclimated). The ICC was relatively low (0.37) but 31% of the overall R^2 of 42% was explained by the random effects. Despite significant average trends, residual intraspecific differences explained a substantial portion of the model and the residual variability explained neither by species-specific nor by the chosen fixed effects was still high. Similar to PBT, phylogenetic signal for SPR was very low (λ=0.011) and significantly different from 1 (likelihood ratio test; $\chi^2(1)$= 9253.818; p<0.0001).

Standard Metabolic Rate

The model of best fit for log-transformed standard metabolic rate included body mass (log-transformed), temperature, latitudinal range position, relative latitudinal position of populations (deviation of capture location from the species' latitudinal midpoints) and acclimation regime, as well as interaction terms between (i) acclimation regime and body mass, (ii) acclimation regime and latitudinal species' position, (iii) acclimation regime and relative latitudinal population position, and (iv) latitudinal species' position and position of populations relative to species' position (**Table 4.3**). Latitudinal range extent was only a significant predictor of metabolism in PGLS models.

Table 4.3 Predictor coefficients for best-fit linear mixed effects models (LME) and phylogenetic generalized least squares models (PGLS) fitted to standard metabolic rates.

Model	LME			PGLS	
Trait	Estimate	SE	St. Estimate	Estimate	SE
Log standard metabolic rate [µl O_2/min] (n=644)					
(Intercept)	-21.41	0.51	0.055	-21.33	0.56
- Log body mass [g]	0.83	0.031	0.80	0.81	0.036
- Temperature [°K]	0.072	0.0016	0.48	0.072	0.0017
- Acclimation treatment [hot or cold] (hot)	0.14	0.070	-0.097	0.14	0.071
- Latitudinal midpoint [lat. deg. south]	-0.0092	0.0080	-0.049	-0.028	0.013
- Latitudinal range extent [lat. deg.]	**NA**	**NA**	**NA**	**-0.020**	**0.0082**
- Population deviation from midpoint [lat. deg.]	0.14	0.024	0.028	0.15	0.024
- Log body mass [g] *					
Acclimation treatment [hot or cold] (hot)	-0.073	0.023	-0.070	-0.074	0.024
- Latitudinal midpoint [lat. deg. south] *					
Acclimation treatment [hot or cold] (hot)	0.0093	0.0033	0.061	0.0090	0.0034
- Population deviation from midpoint [lat. deg.] *					
Acclimation treatment [hot or cold] (hot)	0.011	0.0040	0.060	0.011	0.0041
- Latitudinal range midpoint [lat. deg. south] *					
Population deviation from midpoint [lat. deg.]	0.0069	0.0010	0.19	0.0072	0.0011

Note: latitudinal midpoint was measured in degrees south. More negative values represent higher, more southerly latitudes. A positive correlation with latitude therefore represents an increase in the dependent variable towards the north.

Metabolic compensatory acclimation to cold was greater in larger animals. Cold acclimation increased metabolic rate consistently at both experimental temperatures. More temperate species had higher metabolic rates than low latitude species when cold acclimated, but not when hot acclimated (**Fig.4.6**). Extreme low latitude species did not increase their metabolic rate when cold acclimated (no compensation).

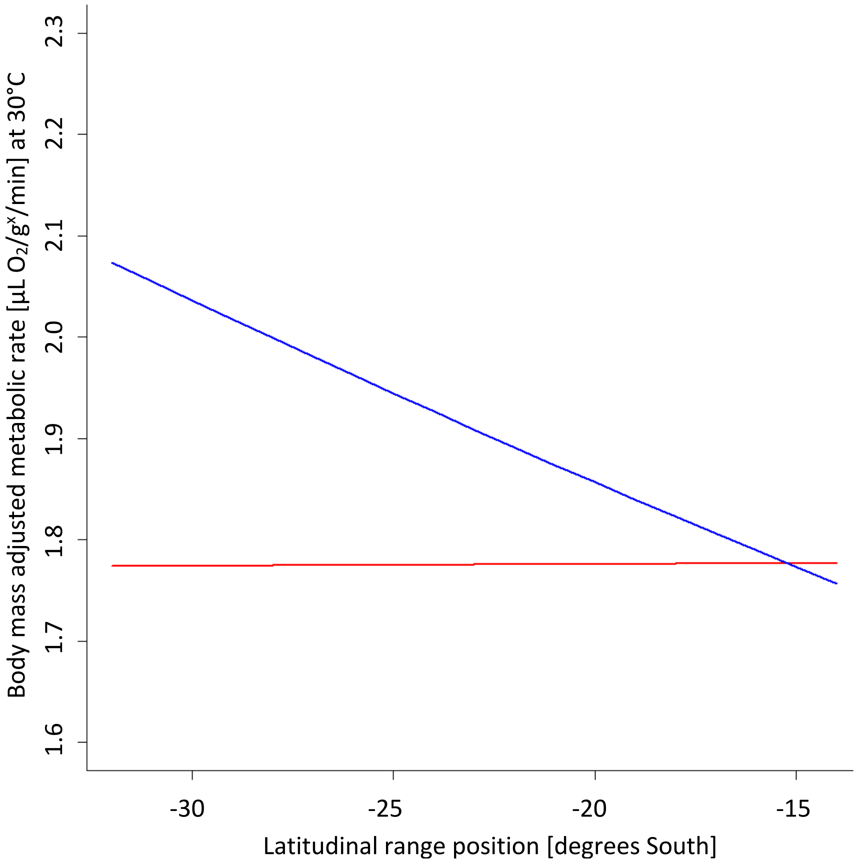


Fig.4.6 Predicted body mass adjusted standard metabolic rate at 30°C for hot (—) and cold (—) acclimated animals from different latitudes. Due to the interaction between body mass and acclimation regime, the exponent for body mass adjustments varied between the two (0.77 for hot and 0.84 for cold acclimated animals). More negative numbers along the x-axis represent higher, more temperate latitudes. Average metabolic rate of hot acclimated animals was similar across latitudes but cold acclimated metabolic rate was higher at high latitudes.

Populations from higher latitudes within temperate species had higher metabolic rates, whether hot or cold acclimated, but the trend was more pronounced after cold acclimation (**Fig.4.7**). More tropical species showed smaller differences between populations. For species at very low latitudes (i.e. with midpoints at latitudes below 17°S) the trend appeared reversed, leading to lower metabolic rates in high latitude populations within tropical species' ranges. However, the intraspecific trends were driven mostly by large variation in metabolism among populations of high latitude species. Low latitude *Carlia* and *Lygisaurus* species in this clade are mostly restricted (Chapter I; Pintor et al. 2015) and extrapolation of results beyond population deviations possible in reality would be inappropriate. Consequently, predicted among population differences in metabolic rates of tropical species likely overestimate real differences (see **Fig.4.2).** The ICC of the random effect (species) was relatively low (0.28). Similarly, the fixed effects alone explained 88% of the variance, while fixed and random effects combined increased the R^2 by only 3% (to 91%). The fixed effects explained most of the interspecific differences. Phylogenetic signal was intermediate at $\lambda=0.55$ but significantly different from $\lambda=1$ (likelihood ratio test; $\chi^2(1)= 18861.6$; $p<0.0001$).

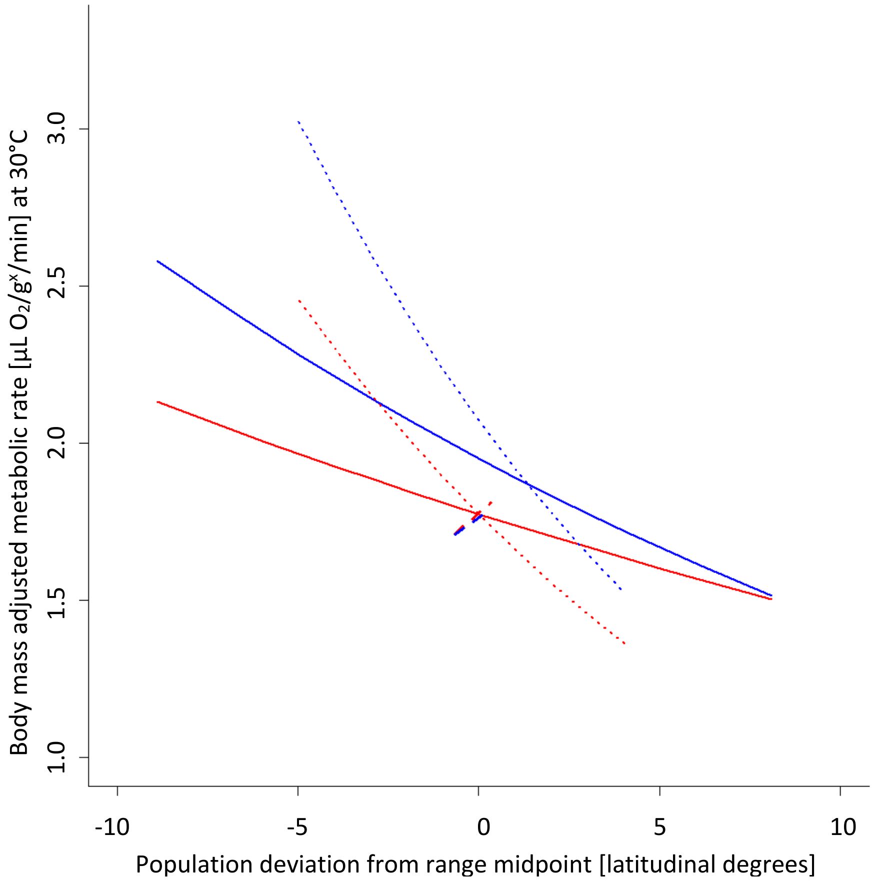


Fig.4.7 Predicted body mass adjusted standard metabolic rate at 30°C for hot (—) and cold (—) acclimated animals from different populations within species' ranges. The exponent for body mass adjustments varied between the hot (0.77) and cold (0.84) acclimation regime. Positive x-axis values represent lower, negative values higher latitude populations, compared to the species' latitudinal range midpoint (0). Predictions are shown for a high latitude species according to range position and size of *Carlia tetradactyla* (dotted lines), a wide ranging mid-latitude species (range position and size of *Lygisaurus foliorum*, solid lines) and a restricted low latitude species (*Carlia dogare*, dashed line). Population trends are mostly driven by intraspecific latitudinal variation in high latitude species, while low latitude species show little intraspecific variation across their usually small range. Predictions for restricted tropical species, therefore, have to be made with caution.

Water Loss Rate

The final model of best fit for log-transformed water loss rate included body mass (log-transformed), temperature, species' latitudinal range position, acclimation regime and interaction terms for acclimation regime and body mass as well as acclimation regime and latitudinal midpoint (**Table 4.4**). Latitudinal range extent was only a significant predictor of water loss in PGLS models.

Table 4.4 Predictor coefficient estimates and standardized estimates for best-fit linear mixed effects models (LME) and phylogenetic generalized least squares models (PGLS) fitted to water loss rates.

Model	LME			PGLS	
Trait	Estimate	SE	St. Estimate	Estimate	SE
Log water loss rate [mg H2O/h] (n=639)					
(Intercept)	-24.84	0.86	-0.022	-25.27	0.94
- Log body mass [g]	0.84	0.048	0.69	0.87	0.050
- Temperature [°K]	0.083	0.0024	0.46	0.083	0.0024
- Acclimation treatment [hot or cold] (hot)	0.70	0.098	0.11	0.70	0.10
- Latitudinal midpoint [lat. deg. south]	-0.0057	0.025	-0.032	-0.047	0.032
- Latitudinal range extent [lat. deg.]	**NA**	**NA**	**NA**	**-0.044**	**0.020**
- Log body mass [g] *					
Acclimation treatment [hot or cold] (hot)	-0.13	0.032	-0.11	-0.14	0.032
- Latitudinal midpoint [lat. deg. south] *					
Acclimation treatment [hot or cold] (hot)	0.026	0.0047	0.15	0.026	0.0048

Note: latitudinal midpoint was measured in degrees south. More negative values represent higher, more southerly latitudes. A positive correlation with latitude therefore represents an increase in the dependent variable towards the north.

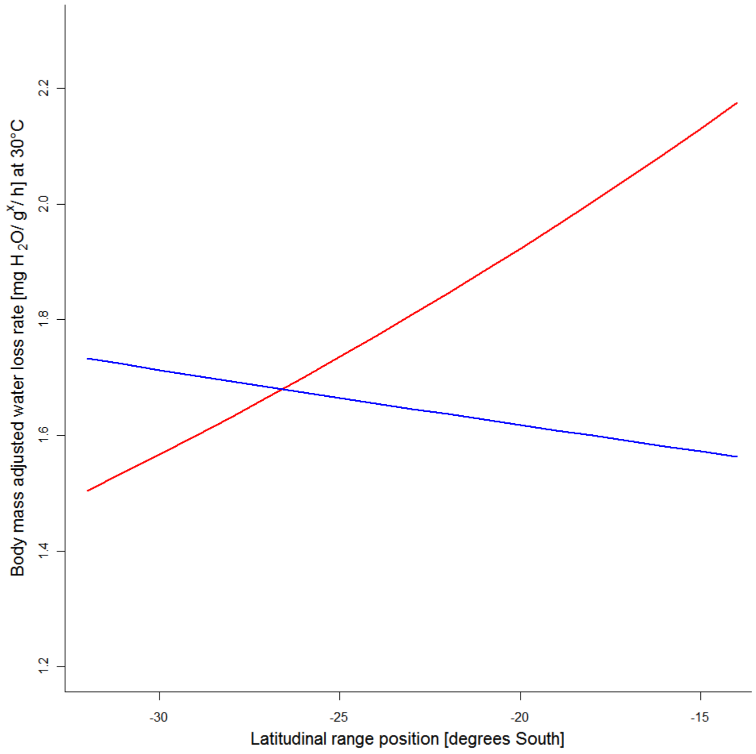


Fig.4.8 Predicted body mass adjusted water loss rate at 30°C for hot (—) and cold (—) acclimated animals from different latitudes. Due to the interaction between body mass and acclimation regime, the exponent for body mass adjustments varied between the two (0.71 for hot and 0.84 for cold acclimated animals). More negative numbers along the x-axis represent more temperate latitudes. Water loss rate decreased in cold acclimated animals at low latitudes but decreased with hot acclimation at high latitudes.

Water loss rate decreased slightly towards tropical latitudes for cold acclimated animals, while increasing in the same direction for hot acclimated animals (**Fig.4.8**). There was no significant intraspecific latitudinal trend in water loss rates. The intraclass correlation was relatively high (0.67) and a relatively large percentage (24%) of the overall R^2 of 89% was explained by the random effect, indicating substantial residual variation between species that was not explained by the chosen fixed effects. Similarly, phylogenetic signal was higher than for other traits (λ=0.79) but still significantly different from λ=1 (likelihood ratio test; $\chi^2(1)$= 18604.17; p<0.0001).

Overall, most traits exhibited plasticity and interspecific latitudinal variation (except CT_{max}). However, only standard metabolic rate and CT_{min} exhibited ongoing intraspecific adaptation (**Table 4.5**). Phylogenetic signal was generally low, as was ICC (except for WLR). Model fits explained most of the variation in the data for most traits except for with regard to thermoregulatory behaviour (PBT and SPR).

Discussion

This current study represents, to my knowledge, the most comprehensive analysis to date of variability in physiological traits within a vertebrate ectotherm taxon across large spatial scales (latitude) as well as across short-term temporal scales (phenotypic plasticity or acclimation), evolutionary scales (intraspecific variation or adaptation potential), and allometric scales (body size). By establishing an extensive baseline data set on thermal trait variation in rainbow skinks using a consistent methodological framework, this study provides the basis for ongoing future research on this relatively new model system and the physiological counterpart to the extensive phylogenetic and phylogeographic work currently done on the same taxon (Stuart-Fox et al. 2002, Phillips et al. 2004, Couper et al. 2005, Dolman and Moritz 2006, Dolman and Hugall 2008, Donnellan et al. 2009, Potter et al. 2016). The tropical ancestral origin of this model taxon (Couper et al. 2005, Dolman and Hugall 2008) has the potential to facilitate a better understanding of the processes involved in adaptation to increases in climatic variability. Nearly all physiological traits tested in this study showed plasticity (except for median preferred body temperatures) and clear latitudinal trends (except for critical thermal maxima; **Table 4.5)**, suggesting adaptive benefits of being able to adjust most thermal traits throughout individuals' lifetime and additional selection pressures causing trait differentiation among species extending into climatically different latitudinal regions.

Table 4.5. Summarized predictors of thermal traits, sorted into 4 categories: 1. plasticity (acclimation or body size effects), 2. evolution along latitudinal climate gradients (among species), 3. ongoing evolution along latitudinal climate gradients (within species), and 4. other terms included only in some models *a priori*. 5. Also shown are the phylogenetic signal of each trait (λ; i.e. the tendency to be evolutionarily conserved), the intraclass correlation coefficient (ICC; i.e. the amount of residual variation explained by the random species effect), and the pseudo-R^2 (i.e. the amount of the variance in the data that was explained by the model rather than by residual variation between individuals).

Predictor	Plasticity	Body Mass	Body Mass*Plasticity	Latitude	Latitude*Plasticity	Population	Population*Plasticity	Latitude*Population	Time	Time*Latitude	Time*Plasticity	Temperature	Phylogenetic signal (λ)	Residual Interspecific Variation? (ICC)	Residual individual Variation (R^2)
Trait	1			2		3			4				5		
Metabolic Rate	X	X	X	X	X	X	X	X				X	Low (0.55)	Low (0.28)	Low (91%)
CT_{min}	X	X	X	X	X	X	X						Low (0.14)	Low (0.11)	Low (71%)
Water Loss	X	X	X	X	X							X	High (0.79)	High (0.67)	Low (89%)
Set Point Range	X			X					X	X	X		Low (0.01)	Low (0.37)	Med (42%)
Preferred Body Temp.				X					X	X			Low (0.04)	Low (0.04)	High (24%)
CT_{max}	X	X	X										Low (0.44)	Low (0.40)	Med (61%)

Additional ongoing adaptation to latitudinal climate gradients (as suggested by intraspecific latitudinal trait variation) was only indicated in analyses of standard metabolic rates and critical thermal minima. In rainbow skinks, these traits therefore appear to be of high importance for species' distributions across latitudinal gradients and, consequently, have the potential to play a larger role in differentially limiting species' distributions than other traits. Especially for a taxon with tropical origin, it is not surprising that changes facilitating cold adaptation (i.e. physiological cold tolerance and metabolic compensation for cold; Tsuji 1988) may have experienced particularly strong selection for in species that have expanded into more temperate latitudes. While critical thermal minima appear to limit species' ranges towards colder, higher latitudes, critical thermal maxima did not appear to be driven by latitudinal temperature gradients and are therefore unlikely to be a good measure of differential vulnerability of species from different latitudes to climate change in this taxon. If physiological heat tolerance plays an integral role in resistance to rising air temperatures, geographic differences in the severity of climate change impacts may, therefore, stem from differences in the magnitude of change rather than from differences in current tolerance. Similarly, differential interaction of rising environmental temperatures at sub-lethal levels with traits that do show latitudinal variation, such as preferred body temperature, metabolic rates, and water loss rates may have greater relevance in this respect. Effects of high temperatures on species' fitness via their impact on traits other than physiological heat tolerance may also explain the apparent underfilling of species potential ranges towards the warmer end of their range extents (Sunday et al. 2012). However, residual variability in critical thermal maxima and, especially, water loss rates, not explained by the fixed effects included in my models, suggests that variation in these two traits may additionally be driven by other variables that do not exhibit latitudinal gradients and were not explicitly explored in this study. Likely candidates are climate variables such as solar radiation, vapour pressure deficit, or aridity, which have the potential to be physiologically limiting by affecting water loss (Chown et al. 2011) or body temperature but may exhibit more complex geographic patterns than the latitudinal gradient in thermal conditions. Differential vulnerability of species across gradients other than latitude and identification of climate variables that are most geographically and physiologically limiting under current and future climate regimes urgently require further assessment.

Most traits, furthermore, exhibited high levels of phenotypic plasticity (metabolism, water loss, set point range, CT_{min}, and CT_{max}), highlighting the importance of standardizing or accounting for acclimation state in comparative physiological studies (Chapter II; Pintor et al. 2016). Trends among populations within species were similar to those among species for some traits (metabolic rate and CT_{min}), indicating strong, ongoing selection on these traits, as well as substantial residual adaptation potential within the *Carlia* and *Lygisaurus* genera, at least with respect to cold adaptation. The relatively low phylogenetic signal in most traits suggests that the observed trends are not as evolutionarily conserved as suggested by previous findings that indicated evolutionary conservatism in many thermal traits of ectotherms (Huey et al. 2009, Clusella-Trullas et al. 2011, Clusella-Trullas and Chown 2014). However, this may be due to the comparatively rapid recent radiation and diversification of the taxon (Dolman and Hugall 2008).

Thermal Tolerances

We found that temperate species, and more temperate populations within species, were more cold tolerant and acclimated their cold tolerance more than did tropical species and populations

(**Fig.4.2**). The largest acclimation response of CT_{min} measured in this study was approximately 4.5 °C and was exhibited by the highest latitude populations of the highest latitude species from this study (*Carlia tetradactyla*). This is an agreement with findings of previous studies (Clusella-Trullas and Chown 2014), which suggest that CT_{min} varies greatly with latitude because it is correlated with gradients in mean annual temperature (Clusella-Trullas et al. 2011), which show strong latitudinal trends, mostly driven by changes in minimum temperatures (Chapter I; Müller 1982, Pintor et al. 2015). CT_{max} did not, however, exhibit any latitudinal patterns, despite acclimating by up to approximately 1 °C in some species.

CT_{max} has recently received much attention because global warming may push species beyond their upper thermal tolerances, particularly in the tropics, where environmental conditions are already closer to maximum tolerances (Deutsch et al. 2008, Huey et al. 2009). However, maximum temperatures vary less with latitude than minimum temperatures (Chapter I; Müller 1982, Pintor et al. 2015), CT_{max} varies less with latitude than CT_{min} (Huey et al. 2009, Clusella-Trullas and Chown 2014), and predicted average temperature increases are likely to be defined more by increased night-time temperatures than increased day time temperatures, leading to a decrease in diurnal temperature ranges (Easterling et al. 1997). Thus, variation in CT_{min} may be a more important determinant of tolerance breadth and range extent than CT_{max} and CT_{max} is unlikely to be a good indicator of differential sensitivity to increases in mean air temperatures. This is particularly so if tropical species thermoregulate to maintain a large behavioural thermal safety margin throughout much of the day (as suggested by lower morning PBTs). Whether differences in PBT translate into larger thermal safety margins, however, depends on latitudinal trends in thermal optima and therefore needs to be confirmed using performance data. Nevertheless, variation in CT_{max} and exposure to lethally high temperatures may be less relevant to species' differential vulnerability to high air temperatures than is often assumed.

Nevertheless, the reasonably high residual interspecific variation in CT_{max} not explained by the fixed terms in my model (ICC=0.40; partial R^2=26 out of R^2=61) suggests that other factors driving the evolution of CT_{max} need to be further explored. Operative temperatures of ectotherms are very different from ambient air temperatures (Sunday et al. 2014). Possible candidate variables in this context are, therefore, geographic (not latitudinal) variation in radiation levels or different levels of exposure to radiation as well as reduced efficiency of thermoregulation across different habitats.

Despite the lack of latitudinal trends in CT_{max}, the trait exhibited substantial acclimation potential in this study (**Fig.4.3**) and has been shown to acclimate relatively quickly over the time course of one or two days in other vertebrate ectotherms (Hutchison and Maness 1979), while CT_{min} undergoes a slow acclimation process over several weeks or months at least in some *Carlia* spp. (Chapter II; Pintor et al. 2016). It is therefore likely that variability in CT_{max} represents tolerance to short term extremes (e.g., high radiation or occasional heat waves) to facilitate survival (Huey and Stevenson 1979), while acclimation of CT_{min} increases the potential for activity, or at least decreases the risk of exposure to lethally low temperatures during inactivity, in prolonged cold seasons. This is supported by the observation that both, CT_{min} and CT_{max}, were correlated with body mass. Smaller individuals were more heat tolerant and additionally increased their heat tolerance more when hot acclimated (**Fig.4.3**), which suggests that reduced thermal inertia in small individuals necessitates greater tolerance to short term fluctuations in body temperature (Pincheira-Donoso et al. 2008).

Preferred Body Temperatures

Preferred body temperatures and set point ranges determine when and where species will be active and buffer them against negative effects of climatic variability on performance (Sunday et al. 2014, Buckley et al. 2015). I suggest that we need to distinguish between two dimensions of thermoregulatory precision. Accuracy (ability to maximize performance) of thermoregulatory behaviour influences fitness by determining how close to their thermal optimum species chose to be (Huey and Slatkin 1976). Because thermal optima do not vary with latitude in the clade examined in this study (Chapter V; mean hot acclimated thermal optimum = 31.6 °C), observed differences in median PBT represent differences in thermoregulatory accuracy. Precision (breadth of selected temperatures) of thermoregulatory behaviour, influence the risk of exposure to unfavourable, and potentially lethal conditions (high precision=lower risk), but simultaneously limits species' activity times (low precision=broader potential activity windows), and consequently their potential for energy assimilation and potentially their distributions (Huey and Slatkin 1976, Kearney et al. 2009). There is, consequently, very likely a trade-off between maximizing time used for energy assimilation (Huey and Slatkin 1976) and minimizing the risk of exposure (Vickers et al. 2011, Vasseur et al. 2014). Our ability to predict vulnerability to climate change is therefore intricately linked to our knowledge of species' intrinsic thermoregulatory capacity as well as the extrinsic conditions impacting on this capacity (e.g. availability of a range of thermally distinct microhabitats).

Thermoregulatory accuracy (i.e. how well species maximize their fitness) requires detailed knowledge of the thermal dependence of their performance and cannot be assessed in this study. However, I determined two kinds of thermoregulatory precision: the range of median PBTs selected by species across their diurnal cycle in thermoregulatory behaviour, and their set point range at any specific time of day. Diel rhythms in preferred body temperatures occur in a range of reptile species (Hutchison and Maness 1979, Innocenti et al. 1993, Angilletta et al. 1999, Ellis et al. 2006, Clusella-Trullas and Chown 2014). However, how diel rhythms differ among species and what may drive these differences has, to my knowledge, not previously been examined.

My results suggest that tropical species select lower body temperatures in the morning than temperate species, but increase their PBT with time of day, leading to a broader range of overall PBTs (**Fig.4.4**). However, thermoregulation of tropical species is precise at any given time of day (SPR is less than 2.6°C) and more so in the morning (SPR <2.0°C), while temperate species show a broad SPR in the morning (up to ~5°C) but thermoregulate very precisely in the afternoon (SPR <1.5; **Fig.4.5**). These results, in combination, suggest that tropical species exploit a broader range of thermal conditions throughout the day (potentially maximizing activity times by exploiting lower temperatures in the morning and higher ones in the afternoon) but, simultaneously, thermoregulate very precisely at any given time of day (potentially reducing the risk of accidental overheating). Temperate species, however, operate at consistently high temperatures throughout the day and are less heat cautious throughout much of the day (large SPR), considering that their CT_{max} is similar to that of tropical species. Range expansion into thermally more variable, high latitude habitats may have, therefore, been facilitated partially by an increase in thermal boldness. However, the fixed effects in my models only explained a very small percentage of the variation in the SPR data despite describing clear, significant trends. The substantial residual variability in SPR as well as PBT, therefore, warrants further exploration of other variables potentially affecting thermoregulatory behaviour more strongly (e.g. habitat type, boldness, developmental plasticity, etc.) than latitudinal gradients.

My results contradicted my hypothesis of higher PBTs and less precise thermoregulation in the tropics and suggest that tropical species are rather "heat cautious", thermoregulating at relatively low body temperatures for much of the day. However, not accounting for diel rhythms in PBT could easily disguise such patterns because the widely varying PBTs recorded across the day could be interpreted as a sign of thermoconforming (Huey et al. 2009). Since critical thermal maxima do not vary across latitudes (**Fig.4.3**), their experienced body temperatures likely lie further away from their critical thermal maxima than is often assumed when basing thermal safety margins on ambient (and especially mean ambient) air temperatures (Deutsch et al. 2008). High frequency of hot temperatures and high radiation levels may increase the risk of overheating in the tropics and favour a cautious approach to thermoregulation. The notion that more precise thermoregulation is driven by higher current risk of overheating is supported by my observation that cold acclimated animals consistently selected a narrower range of temperatures (i.e. were more cautious thermoregulators) than hot acclimated animals, possibly because of an increased risk of overheating when physiological functions are not acclimated to cope with warmer temperatures. Being too cold is typically less acutely critical for tropical species than being too hot, likely leading to selection for behaviours that reduce rather than increase body temperature because lethal operative temperatures are reached in full sun across much of the day (Vickers et al. 2011) and because increased desiccation rates at high temperatures may be a more limiting factor (Chapter III). Species from colder climates, on the other hand, may experience a benefit from behaviourally increasing body temperatures and a low risk of overheating, especially in the morning, as they can remain in full sun without reaching dangerously high temperatures most of the time (Kearney et al. 2009, Vickers et al. 2011). Consequently, tropical species may be surprisingly good at behaviourally buffering the effects of climate change (Sunday et al. 2014, Buckley et al. 2015) if suitable microhabitats for thermoregulation remain available. In fact, it has previously been suggested that vulnerability to climate change may be greater for mid-latitude species than for tropical species, because of predicted reductions in cloud cover and increases in radiation (Clusella-Trullas et al. 2011) and vulnerability would be exacerbated if the behavioural thermoregulation of mid-latitude species already places them at higher temperatures.

Previously, latitudinal trends in PBTs have been shown to be inconsistent among studies, and are often obscured by strong phylogenetic signal or differences in ecology and basking behaviour (Clusella-Trullas and Chown 2014). In contradiction to this, the incorporation of diurnal patterns in this study resulted in the detection of clear increases in PBT with time of day as well as differential diurnal trends in species from different latitudes and, additionally, diurnal and latitudinal patterns in SPR. Neither the interaction between diel rhythms and latitude nor the detected latitudinal and diurnal trends in SPR have, to my knowledge, previously been observed. In addition, and in contrast with previous studies (Huey et al. 2009, Clusella-Trullas et al. 2011, Clusella-Trullas and Chown 2014), phylogenetic signal was extremely low for PBT (λ=0.043) and SPR (λ= 0.011). My results suggest that PBT and SPR were evolutionarily plastic and adaptive among species from this taxon, although conserved within species. Strong diurnal trends in PBT are likely to obscure latitudinal patterns and should therefore be accounted for in comparative studies. High variation in thermoregulatory behaviour among individuals of the same species, as observed here, may additionally obscure trends when sample sizes are low.

Standard Metabolic Rate

Our prediction that species and populations extending into colder climates (higher latitudes) would exhibit raised metabolic rates and higher acclimation potential of metabolic rates to compensate for lower average and minimum temperatures, respectively, was met. Interspecifically, larger acclimation potential in metabolism resulted in higher cold acclimated metabolic rates in high latitude species, despite absence of the trend in warm acclimated SMR

(**Fig.4.6**). Intraspecifically, high latitude populations had both, a higher hot and cold acclimated metabolic rates as well as greater acclimation potential (**Fig.4.7**). The strong trends observed among, as well as within, species suggest ongoing adaptation and, consequently, high relevance of metabolic rates for the distributions of species from this taxon. At low latitudes, annual temperature variability is low and mean temperatures (which are indicative of refuge temperatures experienced during inactivity; Kearney et al. 2012) are higher. Under these circumstances high standard metabolic rates are detrimental because energy expenditure during inactivity cannot be reduced by selecting cold temperatures, thus energy balance can be negatively affected (Dillon et al. 2010). At high latitudes, however, increased metabolic rates are essential to increase potential activity time in colder seasons. Although inverse acclimation may additionally be favourable to conserve energy in times of inactivity (Tsuji 1988), I did not expect my acclimation regime (18-22°C) to present a strong enough cue to elicit brumation and, accordingly, lizards remained active and feeding with no inverse acclimation responses observed. Lastly, observed acclimation effects were stronger for larger animals, most likely due to increased thermal inertia (Stevenson 1985), which creates a pressure to function well at low body temperatures, because prolonged basking is required to reach activity temperatures (Pincheira-Donoso et al. 2008).

Overall, the trends observed within and among species suggest that thermal effects on energy budgets over species' distributions impose strong selection on metabolic rates and that, in turn, metabolic rates may, therefore, be highly limiting to potential distributions. Thus, metabolic rates may be of greater relevance for how species are affected by climate change than the more commonly assessed traits of thermal performance and tolerances (Dillon et al. 2010). The notion that metabolic rates and energy expenditure are determinants of potential range extents is not novel (Kearney and Porter 2004, Kearney et al. 2009) but is underappreciated. Specifically, high current metabolic rates combined with high, predicted increases in temperatures during periods of inactivity may indicate vulnerability. Because hot acclimated metabolic rates are similar across latitudes (**Fig.4.6**), but predicted future temperature increases are greater at high latitudes (IPCC 2013), species from colder climates may be more vulnerable than tropical species. Intraspecifically, this is exacerbated by higher warm acclimated metabolic rates in high latitude populations (**Fig.4.7**), potentially leading to unfavourably high energy expenditure in a warming environment (Dillon et al. 2010) and differential intraspecific extinction risk unless potential activity time, resource availability and resource acquisition can also increase.

Water Loss Rate

While water loss rates of reptiles are sometimes measured in conjunction with metabolic rates to estimate energy and water balance for species' distribution models (Kearney and Porter

2004), only few studies have addressed inter- or intraspecific differences in water loss in reptile species or populations occupying climatically different habitats (Snyder 1975, Hillman et al. 1979, Dunson and Bramham 1981, Neilson 2002), despite water loss being recognized to have a strong effect on the distributions of other dry-skinned ectotherms such as insects (Kellermann et al. 2009, Chown et al. 2011). Consequently, our knowledge of the influence of water loss rates on potential distributions in dry-skinned ectotherms is very limited (Kearney et al. 2013). Considering that the clade I examined has a rainforest origin (Couper et al. 2005, Dolman and Hugall 2008), it is very likely that range expansions from tropical rainforests into drier and climatically more variable habitats would have required not only adaptations in thermal tolerances but also desiccation resistance. In accordance with this, my results show that water loss rates vary with latitude and, furthermore, acclimate to different temperatures. While we know acclimation of water loss rates to different humidity regimes occurs in some lizards (Hillman et al. 1979, Kobayashi et al. 1983, Kattan and Lillywhite 1989), acclimation of water loss to different temperatures has, to my knowledge, not been observed except in some birds (Williams and Tieleman 2000) and insects (Terblanche et al. 2006). My results on temperature dependence of acclimation responses, however, need to be treated with caution, since vapour pressure deficit changes with temperature even if relative humidity is kept similar and acclimation responses to temperature and hydric conditions are, therefore, hard to distinguish.

Interestingly, water loss rates of cold acclimated animals decreased slightly towards the tropics, while those of hot acclimated animals increased, and thus water loss rates were higher for hot acclimated animals in the tropics and for cold acclimated animals in temperate regions (**Fig.4.8**). The most likely reason for these trends is that, across the Australian East Coast, hot temperatures are associated with dry seasons at high latitudes, whereas they are associated with wet seasons at tropical latitudes (http://www.bom.gov.au/climate/data/). Desiccation risk, is therefore likely highest in the cold season in the tropics but in the warm season at high latitudes.

The relatively strong influence of the random effect in my analysis of water loss suggests that, while latitude is a significant predictor of water loss rates when accounting for acclimation regime, it may not be a very good proxy for the actual climate patterns driving trends in water loss rates. Vapour pressure deficit, precipitation, seasonality, and other variables affecting water balance, have more complex spatial and temporal gradients than do temperature (Williams et al. 2010). Similarly, different locations within a given tropical region vary more with respect to relative humidity, precipitation, and seasonality at any given latitude (for example in savannah or rainforest) and between seasons (in the dry and wet seasons) than at high latitudes (Williams et al. 2010). Complex geographic patterns in hydric conditions, combined with the interspecific variability observed here, suggests that further clarification of the exact climate variables driving the differential evolution of water loss rates is essential to our understanding of how climate change will impact on species' distributions, because climate change is likely to cause changes in precipitation as well as drought frequency and intensity and the size and direction of such changes are far less understood than impacts on temperatures (IPCC 2013). Species with higher water loss rates, or those already near their desiccation threshold may be more strongly affected, and may be of conservation concern. Because desiccation is actively avoided through behavioural hydroregulation (Chapter III), drier conditions may lead to further reductions in activity times in addition to those resulting from thermoregulatory behaviour and make consequences of increased metabolic rates during inactivity even more severe. Future research should therefore address the potential importance of desiccation resistance and behavioural avoidance of desiccation (Chapter III) on activity times and distribution limits in dimensions other than latitude.

Conclusion

The macrophysiological approach of this study combined with the broad range of traits examined within and among closely related species with similar ecologies using identical methodology, allowed me to detect fine detail in latitudinal trends. A lack of comprehensive studies with this approach on a sufficient number of different model taxa and a current focus on insects as such model taxa (Hoffmann et al. 2002, Kellermann et al. 2012, Overgaard et al. 2014) constitutes a major knowledge gap in the generality of our understanding of determinants of species' range limits, and differential vulnerability to climate change (Calosi et al. 2008, Calosi et al. 2010, Clusella-Trullas and Chown 2014). My study provides extensive physiological baseline data on a reptile model taxon and, in combination with the ongoing phylogenetic and phylogeographic work on the same taxon (Stuart-Fox et al. 2002, Phillips et al. 2004, Couper et al. 2005, Dolman and Moritz 2006, Dolman and Hugall 2008, Donnellan et al. 2009, Potter et al. 2016), provides a potential basis for further research on differential evolution of physiological traits in ectotherms.

Differences in thermal tolerances of species from different latitudes with different diurnal or seasonal temperature variability (the "climatic variability hypothesis") is thought to be a major determinant of species' latitudinal range extents and vulnerability to climate change (Chapter I; Stevens 1989, Gaston et al. 1998, Gaston and Chown 1999, Calosi et al. 2010, Pintor et al. 2015). It is well known, however, that latitudinal differences in temperature ranges are mostly driven by a gradient in minimum rather than maximum temperatures (Müller 1982, Stevens 1989) and this is reflected in stronger trends in lower than upper thermal tolerances across latitudes (Clusella-Trullas and Chown 2014; **Fig.4. 2** & **4.3** in this study). CT_{min} is, therefore, likely one of the most important traits limiting potential range sizes at high latitudes, while tropical range limits are likely to be influenced by other thermal traits, such as water loss rates, energy assimilation and expenditure (Christian et al. 1999, Dillon et al. 2010). CT_{max} may limit species' ranges, but more likely with respect to differences in exposure of different species, rather than as a direct measure of tolerance of maximum air temperatures. Detrimental sub-lethal effects of high temperatures are likely to manifest through increased metabolic rates and desiccation and may explain apparent underfilling of species potential ranges according to their physiological tolerances (Sunday et al. 2012). It has furthermore been suggested that high latitude limits to species' ranges are determined by physical stressors, while low latitude limits are set by biotic interactions (Brown et al. 1996) and this would be in agreements with stronger geographic variation in traits facilitating cold adaptation, although this possibility is very poorly studied and needs to be further explored.

Lastly, much of the vulnerability to changes in future climates will depend on species' potential to alter activity time or range positions in the novel environments created by climate change (Williams and Jackson 2007, Williams et al. 2007). These subtle impacts lie outside our current knowledge and currently can only be estimated using mechanistic models of species' distributions based on detailed physiological traits (Kearney and Porter 2004). In conclusion, despite recent advances in the field of macrophysiology, there is much residual uncertainty about factors defining species' spatial and temporal range limits, and determinants of differential vulnerability to climate change. My study provides substantial novel insights into the relevant patterns and the traits that may have the greatest potential to limit species' distributions, but much more is to be learnt about the drivers of current and future species' range limits.

-Chapter V-

Revisiting Latitudinal Clines in Performance Parameters within Thermoregulatory Boundaries of Rainbow Skinks (*Carlia* and *Lygisaurus* spp.)

Abstract

The dependence of fitness on temperature in ectotherms can be quantified using thermal performance curves. Parameters extracted from thermal performance curves are widely used to estimate differences among species' resilience to climatic variability and, consequently, to climate change. Species from tropical latitudes, in particular, often have narrower performance curves, are adapted to thermally stable environments, and are thought to be more vulnerable to climate change. Most ectotherms, however, thermoregulate behaviourally, and are only active in a narrow window of temperatures close to their thermal optimum. The temperature window voluntarily chosen for activity periods, and performance within this temperature range, are likely be much better estimates of resilience to environmental changes than performance at temperatures organisms usually avoid. Past research, however, has focused mostly on organismal performance at thermal extremes. Our understanding of interactions between species' thermoregulatory behaviour and the temperature dependence of their performance is very limited. I conducted a comprehensive study of thirteen species of rainbow skinks to (i) assess the influence of latitude, body size and acclimation on performance curves, (ii) review whether commonly used metrics of vulnerability to climate change are appropriate estimates of heat sensitivity, and (iii) develop new metrics that further our understanding of how thermoregulatory behaviour buffers vulnerability. I found that, although there was latitudinal variation in the shape of performance curves, there was little latitudinal difference in heat sensitivity, and precise thermoregulation prevented exposure to detrimental temperature extremes. Body size was the best predictor of most interspecific differences in physiological and behavioural heat sensitivity. Vulnerability to climate change is likely to be influenced by changes in habitat complexity and exposure, that affect the potential for successful thermoregulation and limit potential activity times, rather than by physiological heat sensitivity. Thus, species' "behavioural" microhabitat, rather than their physiological heat tolerance needs to be quantified when assessing vulnerability to climate change.

Introduction

The differential evolution of species' abilities to deal with variation in climate conditions has been discussed extensively in the scientific literature. Assessing how sensitive species' fitness is to climatic variability and climatic extremes, and how this influences their distributions, is central to our understanding of geographic patterns in species diversity, range limits, plasticity, and adaptability. It helps us classify species into specialists and generalists (Gvoždík and Van Damme 2008, Angilletta 2009), determine extents of fundamental niche overlap (Broennimann et al. 2012), clarify the ecological and evolutionary responses to climate gradients (Stevens 1989, Overgaard et al. 2011), and predict differential vulnerability of species to climate change (Deutsch et al. 2008, Huey et al. 2009).

Species from climatically more stable habitats, such as those in the tropics (Müller 1982), are often proposed to have narrower thermal tolerances, smaller range extents across geographic gradients in climatic conditions, and greater sensitivity to climatic variability (Stevens 1989). Ectotherms, which have a limited capacity to thermoregulate physiologically, may be especially susceptible to such evolutionary pressures of climate variability. Combining the previous two generalities has led to the common conclusion that tropical ectotherms must be more sensitive to future climate change, especially because mean temperatures are already close to their upper thermal tolerances here (Deutsch et al. 2008, Huey et al. 2009). There is evidence that tropical species have narrower climatic tolerances than their temperate counterparts (Calosi et al. 2008, Deutsch et al. 2008, Calosi et al. 2010). Despite this, there are also suggestions in the literature that species from mid-latitudes are most at risk (Dillon et al. 2010, Clusella-Trullas et al. 2011), because tropical areas will experience relatively less warming, and more rainfall in the future, whereas temperatures at mid-latitudes will increase more and will be accompanied by reduced precipitation (higher desiccation risk) and less cloud cover (higher exposure to radiation; Zhou et al. 2009, Dillon et al. 2010, Clusella-Trullas et al. 2011, IPCC 2013). In addition, ectotherms from habitats with more cloud cover, such as tropical rainforests, currently thermoregulate at lower temperatures relative to ambient air temperatures (Clusella-Trullas et al. 2011), and may therefore successfully buffer climatic extremes through behavioural thermoregulation (Sunday et al. 2014, Buckley et al. 2015). Consequently, differential vulnerability of species to climate change may result from complex interactions of thermal tolerance, behavioural avoidance of extremes, habitat exposure, and the magnitude of predicted changes in climatic conditions.

Within species, the thermal dependence of performance is described by an asymmetric curve that increases slowly from critical thermal minimum (CT_{min}) to the thermal optimum (T_{opt}) and then, at higher temperatures, rapidly decreases towards the critical thermal maximum (CT_{max}; Huey and Stevenson 1979, Huey and Kingsolver 1993, Deutsch et al. 2008, Angilletta

2009, Vasseur et al. 2014). Two parameters commonly derived from the thermal performance curve are the thermal tolerance breadth and thermal performance breadth. The thermal tolerance breadth is the absolute tolerance range of an organism and is estimated by the difference between CT_{max} and CT_{min}. The thermal performance breadth is the breadth of the performance curve at an arbitrary proportion (often 80%) of maximum performance (Angilletta 2009) and quantifies the range of temperatures over which organisms can be active without risking a “significant” decrease in fitness. These two metrics typically assess species’ sensitivity to variability. In addition to tolerance breadth and performance breadth, the thermal safety margin and the warming tolerance have been proposed as metrics to measure vulnerability to climate warming (Deutsch et al. 2008). The ‘thermal safety margin’ is defined as the temperature increase a species can tolerate without experiencing a decrease in performance, and is calculated by taking the difference between T_{opt} and mean habitat temperatures (Deutsch et al. 2008), although some studies calculate it as the difference between CT_{max} and maximum habitat temperatures (Kellermann et al. 2012). The ‘warming tolerance’ is the maximum non-lethal temperature increase, defined as the difference between mean habitat temperature and CT_{max} (Deutsch et al. 2008). These two metrics and their variations assess species’ risk of experiencing unfavourably high temperatures. While tolerance and performance breadths measure performance in relation to thermal variation overall, the thermal safety margin and warming tolerance estimate risk associated with environmental heating.

There are several shortcomings of these measures of thermal sensitivity. Firstly, thermal tolerance breadth and performance breadth mostly express tolerance to cold rather than hot conditions. This is because CT_{min} varies more, geographically, than CTmax because minimum temperatures vary more than maximum temperatures (Overgaard et al. 2011, Araújo et al. 2013) and because active avoidance of exposure to high, often lethal temperatures reduces selection pressures on CT_{max} (Chapter IV; Bogert 1949, Marais and Chown 2008). For the same reasons, interspecific differences in performance breadths are likely to be influenced more by the improved performance of some species at low temperatures than by increases in performance of some species at high temperatures. Secondly, thermal safety margins and warming tolerance express organisms’ risk of overheating according to current ambient air temperatures (Deutsch et al. 2008). In reality, air temperatures, and especially mean air temperatures, do not accurately predict operative temperatures of ectotherms (Dillon et al. 2010, Clusella-Trullas et al. 2011, Sunday et al. 2014) because they do not account for the impact of radiation, evaporative cooling, thermal habitat complexity (i.e. availability of microhabitats with different amounts of thermal buffering) or, most importantly, behavioural thermoregulation (Kearney et al. 2009).

Although measures of thermal sensitivity are useful when comparing the evolution of thermal physiology among species, their relevance as main proxies for the assessment of differential vulnerability to climate change is questionable. Most vertebrate ectotherms, such as many lizards (Angilletta 2009, Clusella-Trullas et al. 2011, Clusella-Trullas and Chown 2014), thermoregulate behaviourally and select preferred body temperatures (PBTs) that maximise performance while minimizing risks associated with environmental temperature fluctuations (Cerdá et al. 1998). PBTs chosen during activity are typically slightly below T_{opt}, because the risk of reduction in performance, particularly by reaching lethal temperatures, is greater above than below T_{opt} (Huey and Kingsolver 1993, Huey et al. 2012). When temperatures preferred for activity cannot be achieved, lizards will retreat to thermally buffered microhabitats, generally far below T_{opt}, to avoid thermal extremes and to minimize unnecessary energy expenditure (Regal 1966, Christian et al. 1999, Kearney et al. 2013). This means that vulnerability of ectotherms to climatic variability greatly depends on (i) how much the performance is reduced during activity (i.e. precision of thermoregulation around T_{opt}), (ii) how well they avoid lethal temperatures (i.e. how far from lethal temperatures they thermoregulate), and (iii) how much activity time is reduced (i.e. for how long thermally suitable microhabitats are available to allow for thermoregulation within PBT limits).

Metrics of species' vulnerability to climate change should, therefore, incorporate thermoregulatory behaviour into analyses of performance curves. Especially because thermoregulatory behaviour should theoretically coevolve to match and optimize performance (Angilletta et al. 2002), it is not always coadapted to thermal optima (Huey and Bennett 1987). Interspecific differences in how thermoregulation interacts with performance parameters may, consequently, be crucial with respect to how performance during activity periods is affected by changes in available climatic conditions. Especially in precise thermoregulators, tolerance breadth should only be of relevance where avoidance of extremes is not always possible (e.g. in habitats with little thermal heterogeneity). With respect to performance breadth, the commonly used threshold of 80 % of maximum performance is an arbitrary figure. Although two species with different performance breadths can theoretically utilize different ranges of temperatures without risking a decrease in fitness, it is not known how much of a reduction in performance different species will voluntarily accept. The thermal performance breadth is therefore of limited ecological relevance. If, however, we are interested in comparing relative fitness decrements as we move away from the optimum temperature (i.e. the thermal sensitivity of performance), it may be better to quantify how much performance is reduced within the range of selected body temperatures of an organisms (selected heat or cold sensitivity) or, if PBTs are unknown, how much performance is reduced in response to a certain standardized deviation in temperature from the optimum (e.g., 5°C lower or higher; standardized heat or cold sensitivity of performance). Surprisingly, it is currently unknown how much of a decrement in performance is tolerated by ectotherms before ceasing activity.

To estimate the risk of overheating associated with climate change, measures of how far below T_{opt} and CT_{max} species thermoregulate (behavioural thermal safety margin and behavioural warming tolerance), rather than how far current air temperatures lie below these parameters, become valuable. This would express the degree of 'heat caution' that species exercise. The more precise thermoregulatory behaviour is, and the more heat cautious organisms are, the less likely are they to allow overheating, reducing the effect of short term temperature increases on fitness and mortality risk (Vasseur et al. 2014). Lastly, how much vulnerability is affected by reductions in activity time with increases in air temperature depends greatly on habitat exposure and complexity as well as on diurnal and seasonal variability in temperatures. Such analyses, however, require much more detailed knowledge of habitat climate and habitat usage as well as on how much time a particular organism requires for sufficient energy assimilation.

I described latitudinal patterns in performance of thirteen species of small, lizards in the monophyletic clade incorporating the genera *Carlia* and *Lygisaurus*. Climatic variability has strongly influenced the evolutionary physiology of this group (Chapter I & IV; Pintor et al. 2015), making it an ideal model system in which to examine latitudinal variation in physiological tolerances and performance parameters. I examined inter- and intraspecific latitudinal variation in the shape of performance curves, quantified latitudinal trends in some of the metrics used in the literature to assess species' vulnerability to climate change and suggested new metrics to estimate thermoregulatory precision and heat caution, or degree of high-temperature avoidance by combining data on thermoregulatory behaviour (Chapter IV) with data on thermal dependence of performance. I hypothesised that, while there would be geographic variation in performance parameters, and narrower thermal tolerances in tropical species, thermal vulnerability would not change much with latitude, because there would be coevolution of precise and cautious thermoregulatory behaviour with physiological tolerances. Under this conceptual framework, I expected to find that tropical species would be less (rather than more – Deutsch et al. 2008, Huey et al. 2009) vulnerable to climate change, because the environment they use already selects for avoidance of high temperatures.

Methods

Collection, husbandry and acclimation procedures were as outlined in Chapter IV. The genera *Carlia* (nine species) and *Lygisaurus* (four species) are mostly leaf-litter-dwelling skinks, with extreme differences in latitudinal range sizes and positions. The species sampled were *Carlia dogare*, *C. jarnoldae*, *C. longipes*, *C. munda*, *C. rubrigularis*, *C. schmeltzii*, *C. storri*, *C. tetradactyla*, *C. vivax*, *Lygisaurus aeratus*, *L. foliorum*, *L. laevis* and *L. rococo*. All these species are distributed along the Australian East Coast, where a clear latitudinal gradients in mean, minimum, and maximum temperatures, as well as in temperature variability occurs (Chapter I; Pintor et al. 2015). Species that extend inland (across longitudinal environmental gradients) were excluded (except for *Carlia munda*, which is distributed along the east coast but also reaches much more western longitudes in the tropics) to reduce the potential effects of multidirectional climate gradients. Individuals of each species were collected from several locations across their latitudinal distribution and returned to the laboratory for acclimation. The number of populations collected per species varied. Highly restricted species were collected from only one location, more wide ranging species were collected from up to three locations across their latitudinal distribution. Generally, an attempt was made to collect more widely distributed species from the centre of their range as well as from both latitudinal extremes within their range (see Appendix IV). Population effects were assessed based on the deviation of sampling locations from the average location of the species' range, i.e. as an additional continuous predictor of traits modifying average species trends as described by species' range position (latitudinal midpoint), rather than a categorical description of population ID. Up to 30 individuals were collected at each location.

Animals were housed in 30cm (D) x 20cm (W) x 10cm (H) plastic containers with mesh lid on a substrate of potting mix and leaf litter and were fed crickets and a calcium and vitamin D_3 supplement. Water was provided *ad libitum*. UV light was provides one day per week (Reptile One UVB 10.0 Fluorescent Light Tube). Half of the individuals from each population were acclimated to a hot (28-32°C) and half to a cold temperature regime (18-22°C). *Carlia longipes* requires approximately sixteen weeks to complete cold acclimation of CT_{min} (Chapter II; Pintor et al. 2016) even though hot acclimation of CT_{max} is relatively rapid in many species (approximately 24-48h; Hutchison and Maness 1979). Animals were, therefore, acclimated for at least 16 weeks before experiments. Light/dark cycle was kept constant at 12h:12h.

Preferred body temperatures increase linearly with time of day in *Carlia* and *Lygisaurus*, especially in tropical species (Chapter IV) but do not vary with acclimation temperature. Set point ranges (SPRs, defined as the interquartile range of temperatures selected by individuals) also vary with time of day and acclimation regime. Median preferred body temperatures (PBT) of individuals, and upper and lower quartiles of temperatures selected by each individual across time of day (recorded in 60 sec intervals over a two-hour period resulting in 120 records per individual), were obtained from an earlier study (Chapter IV).

Linear regressions were fitted separately to each species' PBT, as well as to upper and lower limits of their SPR across time of day. Several parameters were extracted from these regressions. The first parameter was the "PBT range" (the range between lowest and highest PBT selected by each species across their activity period), calculated as the difference between each species' predicted PBT at 8:00 and 17:00. The second parameter was the total SPR of each species, the range between the lower quartile of selected temperatures at the time of day when PBT was lowest (8:00) and the upper quartile of selected temperatures at the time of day when PBT was highest (17:00). Because SPR was broader for hot-acclimated animals (Chapter IV), only hot-acclimated individuals were used to calculate this parameter, to reflect the greatest average temperature range voluntarily selected by different species across acclimation states and time of day.

I used endurance as a measure of functional performance capacity because it has been shown to be heritable (Sorci et al. 1995), affects survival (Irschick et al. 2008), is subject to natural selection in lizards (Irschick et al. 2008), can predict dominance in male-male combat (Robson and Miles 2000, Perry et al. 2004), and is correlated to the percentage of time spent moving as well as movement distance in the field (Garland 1999), indicating that it is an ecologically relevant performance trait. Endurance (in seconds) was determined in a circular racetrack at 16, 22, 26, 28, 30, 32, 34 and 36°C. Each individual was encouraged to run without interruption until exhaustion, which was the point at which individuals stopped moving forward and lost their righting response. If animals shed their skin or physical fitness seemed compromised on the day of the experiment, the experiment was repeated up to twice, on other days, and the highest value obtained was used in analyses. Half of each population of lizards was tested at progressively increasing temperatures, while the other half was tested at progressively decreasing temperatures to eliminate any differential effects of the order of temperatures used on acclimation state or on physical fitness. Values for critical thermal minimum and critical thermal maximum of individuals were obtained from a previous study (Chapter IV) and were used to anchor thermal performance curves, i.e. to provide values for the temperatures at which performance was zero. For this, CT_{min} and CT_{max} were converted to an endurance of zero seconds at the temperature at which loss of righting had occurred in experiments on critical thermal limits.

Latitudinal Midpoint of species' ranges and latitudinal range extents were calculated for each species from occurrence data from the Atlas of Living Australia (2014). Museum records were compared with distribution maps in the "Complete Guide to Reptiles of Australia" (Wilson and Swan 2008) and outliers (single occurrence records far outside the distribution) excluded. I also excluded records that were no longer relevant because of recent division of species into geographically distinct species. Latitudinal Midpoints were the mid-point between maximum and minimum latitudinal occurrences. These were expressed as negative values because they are in the southern hemisphere. Thus, less negative or "larger" values reflect more tropical locations. Latitudinal range extents were the difference between maximum and minimum latitude of occurrence. Deviations of individuals' capture locations from the species' midpoints (population deviations) were calculated as the difference, in latitudinal degrees, between the collection latitude of individuals and the midpoint of species' ranges. Deviations from midpoints that were directed north towards the tropics were expressed as positive values, deviations towards high latitudes as negative values.

There has been some uncertainty as to which function best describes the temperature dependence of performance and how to decide on a function of best fit for analysis of performance curves (Angilletta 2006). For my purposes, I determined the function of best fit (based on χ^2) out of 208 functions in LabFit (Silva and da Silva 2011). A cubic function fit the data the best and was, therefore, used in all further analyses, which were performed using R (R Core Team (2014).

To quantify differences in temperature dependence of performance, I used linear mixed models (function lme; package nlme; Pinheiro et al. 2013). The initial model included the cubic expression for temperature as well as fixed terms for body mass, acclimation regime, latitudinal midpoint of species' ranges, population deviation from species' midpoint, latitudinal range extent and the interaction term of each of these with temperature. Stepwise backward selection using AIC values was used to exclude terms. The final model was that with the lowest AIC. I decided *a priori* to include the random effect of individual nested within species, as multiple values were included per species and repeated measures were performed per individual. This approach addresses some of the previously identified issues when comparing performance curves along the vertical (differences in absolute performance), horizontal (shifts in performance and thermal optimum across temperatures), and with respect to specialist and generalist trade-offs (changes in breadth with changes in height), that have previously been addressed suing the Template Mode of Variation Method (Izem and Kingsolver 2005, Angilletta 2006), because the random effect allowed for inter- as well as intraspecific variability in performance curve predictors beyond the fixed effects of the model. Additionally, specific performance curve parameters such as thermal optimum and maximum endurance were also compared by fitting separate curves to each species and comparing these parameters in siolation from other changes in the shape of performance curves. To determine the extent of variation explained by the random effects in each model, I used intraclass correlation coefficients (ICC; Goldstein 1986, Burton et al. 1998). An ICC below 0.5 indicated low residual variation explained by species or individual. In addition, to assess the improvement attributed to the random effect, I compared partial pseudo-R^2 of the fixed effects to the pseudo-R^2 of the final model (Nakagawa and Schielzeth 2013). Once the final model of best fit were found, they were refitted using standardized dependent and independent variables to obtain standardized correlation coefficients to aid with comparison of effect size. Both, unstandardized and standardized coefficients are reported in the results.

Table 5.1 Descriptions of parameters describing interspecific differences in performance curve attributes and thermal sensitivity

Parameter	Description
T_{opt}	The thermal optimum, i.e. temperature at which performance was the highest for either cold and for hot acclimated animals
Maximum Endurance	The maximum endurance of hot acclimated animals (which consistently performed better than cold acclimated animals)
CT_{min}	The minimum temperature tolerated by cold acclimated animals, i.e. the lower temperature threshold at which righting response was lost
CT_{max}	The maximum temperature tolerated by hot acclimated animals, i.e. the upper temperature threshold at which righting response was lost
Thermal tolerance	The difference between cold acclimated CT_{min} and hot acclimated CT_{max}
Standardized cold sensitivity	The percentage of maximum performance of cold acclimated animals that could be achieved at 5°C below their cold acclimated T_{opt}
Standardized heat sensitivity	The percentage of maximum performance of hot acclimated animals that could be achieved at 5°C above their hot acclimated T_{opt}
Selected cold sensitivity in PBT range	The percentage of maximum performance of cold acclimated animals that could be achieved at the lower end of their preferred body temperature range
Selected heat sensitivity in PBT range	The percentage of maximum performance of hot acclimated animals that could be achieved at the upper end of their preferred body temperature range
Accepted cold sensitivity in SPR	The percentage of maximum performance of cold acclimated animals that could be achieved at the lower end of their set point range
Accepted heat sensitivity in SPR	The percentage of maximum performance of hot acclimated animals that could be achieved at the upper end of their set point range
Behavioural thermal safety margin	A measure describing how far away from the hot acclimated T_{opt} animals thermoregulated (using either the lower or upper end of their range of preferred temperatures as the two possible “extremes”): this measure estimates how likely animals are to be exposed to unfavourably high temperatures that decrease performance based on their thermoregulatory behaviour
Behavioural warming tolerance	A measure describing how far away from the hot acclimated CT_{max} animals thermoregulated (using either the lower or upper end of their range of preferred temperatures as the two possible “extremes”): this measure estimates the risk of animals are to be exposed to detrimentally high temperatures that cause loss of righting response based on their thermoregulatory behaviour

To assess variation in thermal optima, precision of thermoregulation and vulnerability to temperature variability (especially at warmer temperatures), I extracted several parameters from performance curves and combine them with results from PBT analyses. I fitted separate mixed effects models to endurance of individuals from each acclimation regime (warm and cold) in each species. Data for different populations within species was combined, as there was no change in the shape of performance curves across temperature. Because I used repeated measures on individuals, I *a priori* included the random effect of individual ID in these models. Parameters were then extracted for hot and cold acclimation for each species. The parameters extracted and used for further analyses (see **Table 5.1**) were (i) the thermal optimum (T_{opt}) for hot and cold acclimated animals, (ii) the hot acclimated maximum endurance, (iii) the cold acclimated CT_{min} and hot acclimated CT_{max}, and (iv) the overall thermal tolerance (the difference between CT_{min} and CT_{max}). Additionally, I calculated the following novel parameters: (v) the "behavioural thermal safety margin" of all species at the lower and upper end of their PBT range (T_{opt} of hot acclimated animals minus lower and upper limit of PBT range), (vi) the "behavioural warming tolerance" (CT_{max} of hot acclimated animals minus lower and upper limit of PBT range), and (vii) the standardized heat and cold sensitivity of performance (the percentage that performance decreased to if temperature increased or decreased by 5 °C from T_{opt}). To relate this sensitivity to thermoregulatory behaviour, I furthermore calculated the (viii) selected heat and cold sensitivity (the percentage that performance decreased towards the extreme points of the PBT range) and the vi) accepted heat and cold sensitivity (the percentage that performance decreased towards the extreme points of the SPR). For all measures of heat sensitivity data from hot acclimated animals was used, and for those of cold sensitivity the data was from cold acclimated animals. I correlated these parameters to the latitudinal midpoint of species' range positions, their range extent and their mean body mass using multiple linear regressions, for which terms were excluded stepwise by backward selection based on F-tests.

Results

Endurance increased gradually with temperature from critical thermal minimum to optimum endurance, and subsequently decreased more abruptly towards the critical thermal maximum. The final model of best fit describing endurance included, in addition to the cubic expression for the temperature, the significant main terms of (log transformed) body mass, latitudinal range position, population deviation from range position, and acclimation regime, as well as significant interactions of temperature with log transformed body mass, latitudinal midpoint and acclimation regime (**Table 5.2; Fig.5.1 & 5.2**).

Table 5.2 Correlation coefficients of fixed terms included in the final model describing endurance

Term (n= 2758; 291 individuals; 13 species)	Estimate	SE	St. Estimate
Intercept	12.44	13.90	0.53
Temperature [°C]	-12.34	0.73	0.63
Temperature^2 [°C]	0.92	0.03	-0.67
Temperature^3 [°C]	-0.016	0.0004	-0.26
Log transformed body mass [g]	8.76	3.16	-0.046
Acclimation regime[hot or cold] (H)	-13.22	3.18	0.045
Latitudinal range midpoint [°S]	-1.53	0.65	-0.28
Population deviation from midpoint [latitudinal °]	-1.28	0.35	-0.11
Temperature [°C] * log transformed body mass [g]	-0.42	0.06	-0.063
Temperature [°C] * acclimation regime[hot or cold] (H)	0.56	0.09	0.11
Temperature [°C] * latitudinal range midpoint [°S]	-0.039	0.009	-0.039

Note: More negative latitudinal midpoints represent higher latitudes. A positive correlation with latitude therefore represents an increase in the dependent variable towards the tropics.

Tropical species had a lower endurance, on average, than temperate species and this was more notable at low than at high temperatures (**Fig. 5.1**). Populations from relatively lower latitudes within species' ranges also had lower endurance than high latitude populations, but this relationship did not vary with temperature. Smaller animals consistently outperformed large animals except at very low temperatures (**Fig. 5.2**). Hot acclimated animals had a lower endurance than cold acclimated animals at low temperatures. However, at temperatures above 24°C, hot acclimated individuals performed increasingly better, with a higher overall maximum performance and a higher optimum temperature than cold acclimated individuals (**Fig. 5.1** and **5.2**). An intermediate amount of variability in endurance was accounted for by the nested random effect of species and individual (intra-class correlation coefficient = 0.45), but 66% out of the overall pseudo R^2 of 81% was explained by the fixed effects, indicating a low influence of the random variation between species and individuals on the overall fit of the model.

Multiple linear regressions on the parameters extracted from performance curves revealed that body mass was, in most cases, a significant predictor of how sensitive species were to temperature increases (e.g. based on thermal safety margins, warming tolerance or heat tolerance), while species' latitudinal range position was not (**Table 5.3**).

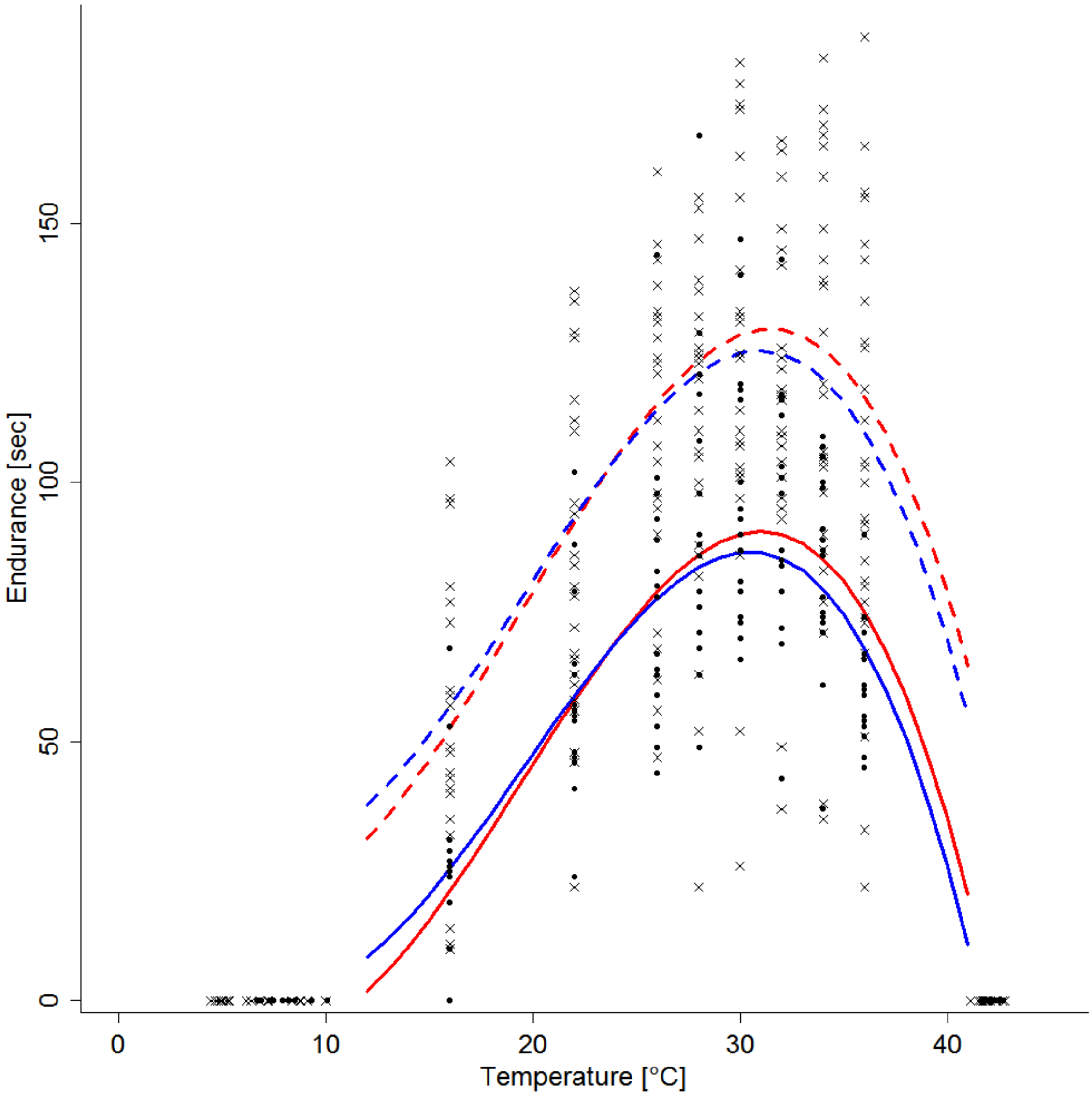


Fig. 5.1 Performance data of a low latitude (*Carlia rubrigularis*; solid circles) and high latitude (*Carlia tetradactyla*; crosses) species of similar body mass. Predictor lines are for hot (red) and cold (blue) acclimated performance of a species of 2.98g body mass occurring at -17.52 °S (mean body mass and latitudinal midpoint of the range of *C. rubrigularis*; solid lines) and one of 3.44g body mass occurring at -31.92 °S (mean body mass and latitudinal midpoint of the range of *C. tetradactyla*; dashed lines). Performance was higher for high latitude species, especially at cold temperatures.

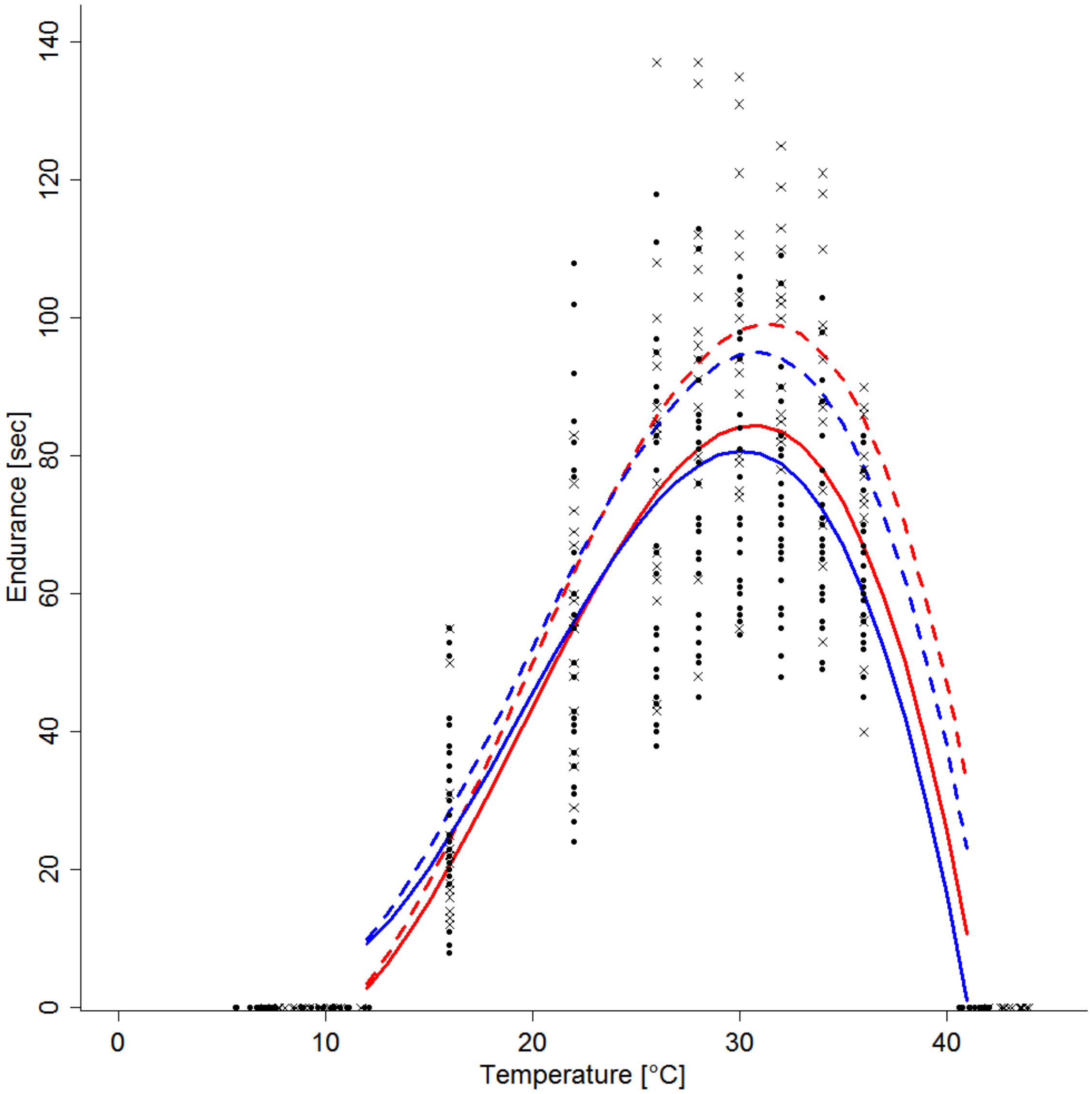


Fig. 5.2 Performance data for a large (*Carlia longipes*; solid circles) and small (*Carlia munda*; crosses) species from relatively low latitudes. Predictor lines are for hot (red) and cold (blue) acclimated performance of a species of 6.51g body mass occurring at -16.53 °S (mean body mass and latitudinal midpoint of the range of *C. longipes*; solid lines) and a species of 1.49g body mass occurring at -19.51 °S (mean body mass and latitudinal midpoint of the range of *C. munda*; dashed lines). Performance was higher for smaller species, except for at low temperatures.

Table 5.3 Summary of significant predictors of performance parameters and temperature sensitivity.

Parameter (n=13)	Mean value	Main predictor	coefficient
T_{opt} (cold acclimated)	30.6 °C	Body mass	-0.3
T_{opt} (hot acclimated)	31.6 °C	Body mass	-0.3
Maximum endurance	95.3 sec	Latitude	-3.1
CT_{min}	7.3 °C	Latitude	0.1
CT_{max}	42.6 °C	Body mass	-0.2
Thermal tolerance	35.3 °C	Latitude	-0.1
Standardized cold sensitivity	87.8 %	NA	NA
Standardized heat sensitivity	81.7 %	NA	NA
Selected cold sensitivity in PBT range	98.8 %	NA	NA
Selected heat sensitivity in PBT range	95.5 %	Body mass	-1.4
Accepted cold sensitivity in SPR	95.5 %	NA	NA
Accepted heat sensitivity in SPR	90.4 %	Body Mass	-1.7
Behavioural thermal safety margin (8:00)	0.4 °C	NA	NA
Behavioural thermal safety margin (17:00)	-2.4 °C	Body mass	-0.4
Behavioural warming tolerance (8:00)	11.3 °C	NA	NA
Behavioural warming tolerance (17:00)	8.6 °C	Body Mass	-0.4

Species' thermal optimum (T_{opt}) differed significantly between acclimation regimes (paired t-test, $t_{(12)}$=6.54, p<0.0001) and was higher for hot (mean = 31.59°C, min=30.76, max=32.77) than cold (mean=30.63°C, min=29.24, max=31.96) acclimated animals. For both hot and cold acclimation, larger species had significantly lower T_{opt} (**Fig. 5.3**; multiple linear regression; hot acclimation: 32.16 - 0.25*body mass, $F_{(1,11)}$=8.87, p=0.013, R^2=0.40; cold acclimation: 31.21 - 0.26 *body mass, $F_{(1,11)}$=6.53, p=0.027, R^2=0.32). Latitudinal range midpoint and range size were not significant predictors of T_{opt}.

Maximum endurance (endurance at T_{opt}; mean=98 sec, min=69 sec, max=139 sec) was significantly correlated with latitude (**Fig. 5.4**; 38.28-3.14*latitudinal range midpoint, $F_{(1,11)}$=10.51, p=0.008, R^2=0.44) and was lower for more tropical species. Mean maximum endurance ranged from 84 (northernmost species) to 139 seconds (southernmost species).

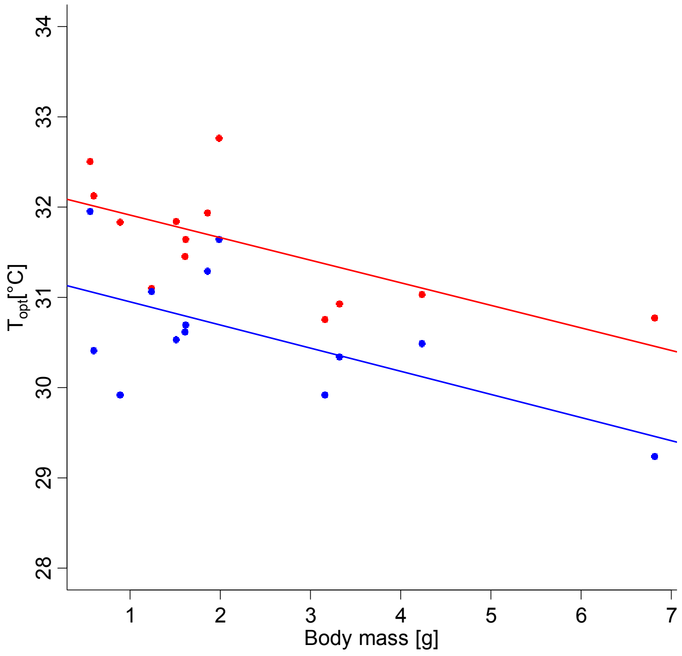


Fig. 5.3 Linear regression on body mass dependence of hot (red) and cold (blue) acclimated thermal optimum of 13 skink species. Larger species performed better at lower temperatures at both acclimation states.

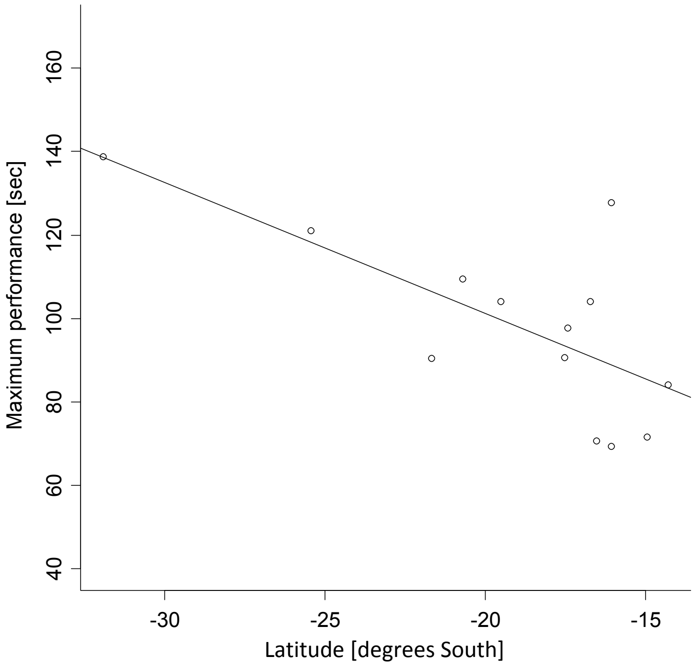


Fig. 5.4 Linear regression on latitudinal differences in maximum performance of 13 skink species. High latitude species had a longer endurance than low latitude species.

Species specific cold acclimated CT_{min} was significantly correlated with latitude (9.66 + 0.12*latitude, $F_{(1,11)}$=25.48, p<0.0005, R^2=0.67; see Chapter IV). Hot acclimated CT_{max} was only correlated with body mass (43.09 – 0.24*body mass, $F_{(1,11)}$=12.48, p=0.005, R^2=0.49; see Chapter IV). The resulting thermal tolerance of species (mean hot acclimated CT_{max} minus mean cold acclimated CT_{min}) was significantly correlated with latitude (33.37 – 0.098*latitude, $F_{(1,11)}$=11.53, p=0.006, R^2=0.47).

Standardized heat sensitivity of performance was greater, i.e. resulted in lower performance (mean=81.7%, min=79.3%, max=84.2%) than cold sensitivity (mean=87.8%, min=86.2%, max=89.5%) due to the asymmetrical shape of performance curves (paired t-test, $t_{(12)}$=-15.20, p<0.0001). However, there was little variation among species, and neither heat nor cold sensitivity was significantly correlated with latitude, range size or body mass. Within the 10°C of T_{opt}, none of the species experienced a performance decrement to less than 79% of optimum performance.

PBTs increase with time of day such that, on average, they are below T_{opt} in the morning and above T_{opt} in the afternoon (see **Fig. 5.5**). The total PBT range of each species over the day was small, on average only 2.8°C (min=0.4, max=5.8). Thus, selected heat sensitivity of performance (decreases in performance towards the upper limit of the PBT range; mean=95.5%, min=90.7%, max=100.0%) as well as selected cold sensitivity (mean=98.8%, min=94.8%, max=100.0%) were small. The decrease in performance between T_{opt} and the upper end of the PBT range was significantly greater than that to the lower end (paired t-test, $t_{(12)}$=-4.05, p=0.002). Despite the low variability in the data, the selected heat sensitivity was significantly correlated with body mass (98.74 – 1.42*body mass, $F_{(1,11)}$=41.82, p<0.0001, R^2=0.77; **Fig. 5.6**), in that larger species experienced a greater decrease in performance by selecting warm PBTs than smaller species. Selected cold sensitivity was not influenced significantly by any of the tested covariates.

Accepted heat and cold sensitivity within the total daily range of temperatures to which species will voluntarily expose themselves across time of day (SPR; mean=5.9°C, min=1.6, max=12.3), resulted in decreases in performance above T_{opt} (mean=90.4%, min=81.2%, max=97.2%) and below T_{opt} (mean=95.9%, min=72.0%, max=100.0%) that were still relatively small and, on average, did not differ significantly in either direction (paired t-test, $t_{(12)}$=-1.84, p=0.09). Accepted heat sensitivity was, again, significantly correlated with body mass (94.30 – 1.71*body mass, $F_{(1,11)}$=7.68, p=0.02, R^2=0.36; **Fig. 5.6**) but not with latitudinal covariates, while accepted cold sensitivity was not significantly correlated with any covariates.

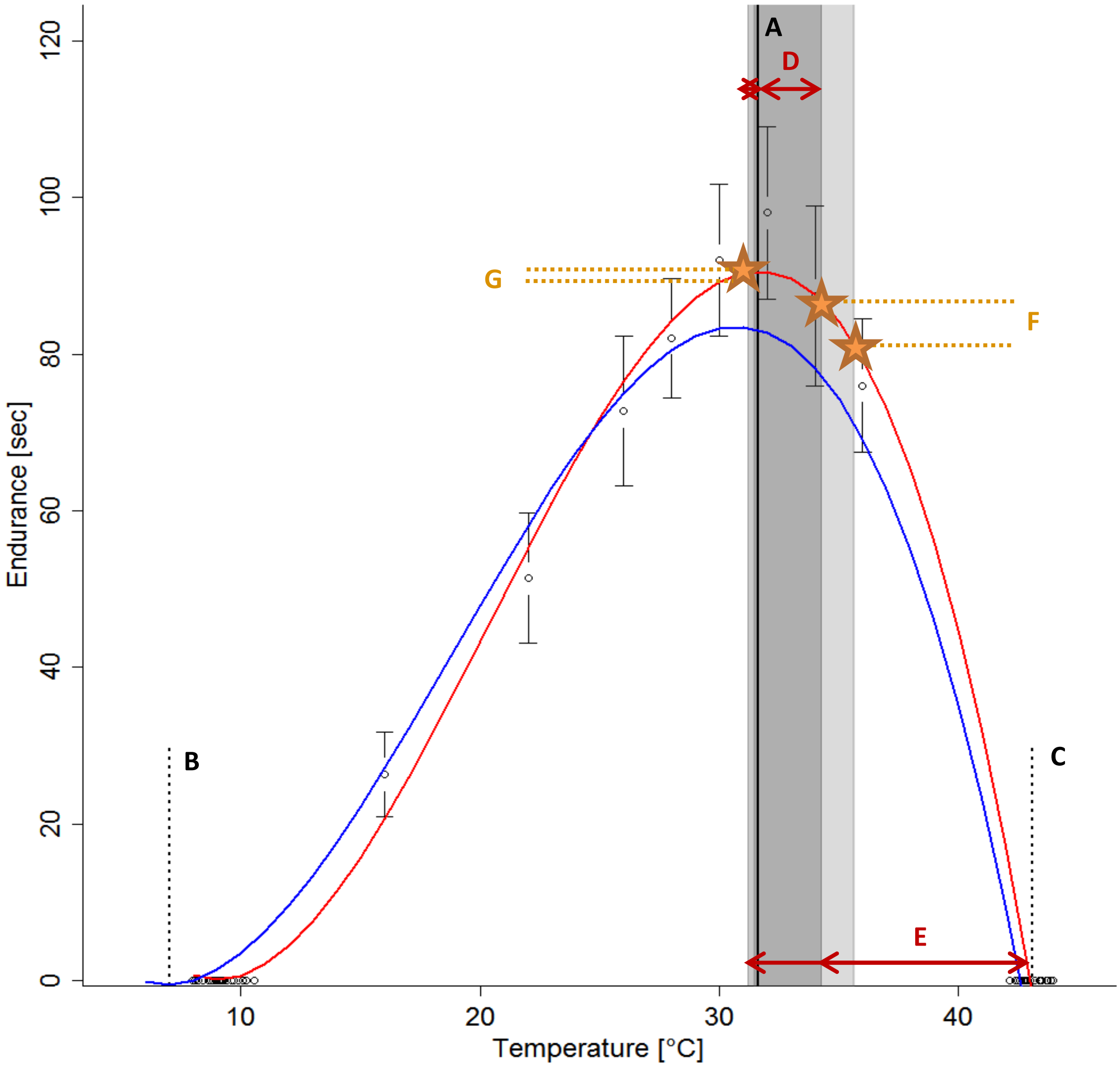


Fig. 5.5 Temperature dependence of hot (red; data shown as error bars) and cold (blue; data not shown) acclimated endurance of *Carlia vivax*. The following parameters discussed in Table 5.2 and other parts of this chapter are show: The range of mean preferred body temperatures (dark shading) and set point range (light shading) across time of day; T_{opt} (“A”; vertical solid line); cold acclimated CT_{min} (“B”) and hot acclimated CT_{max} (“C”; vertical dotted lines); minimum and maximum behavioural thermal safety margin (“D”) and warming tolerance (“E”); the selected (within PBT range) and accepted (within SPR range) heat (“F”) and cold sensitivity (“G”) of performance. See Table 5.1 for further descriptions of parameters. As in this example, most species thermoregulated above T_{opt} (negative behavioural thermal safety margin) for most of the day but stayed well below CT_{max} (positive warming tolerance). Hot acclimation lead to increased performance above 24 °C, while cold acclimation was only beneficial for performance under 24 °C but detrimental to performance at all other temperatures.

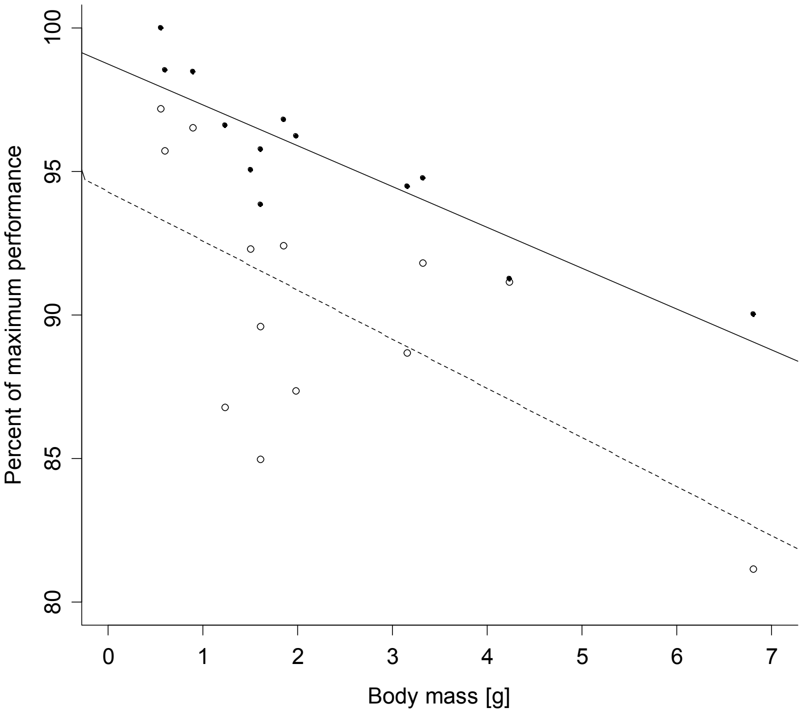


Fig. 5.6 Linear regression on selected heat sensitivity within PBT range (filled circles, solid line) and SPR range (empty circles, dotted line) for species of different body mass. Larger species voluntarily exposed themselves to greater reductions in performance at high temperatures.

Overall, species specific performance never fell below 90% within species-specific PBT ranges, and never fell below 70% within species-specific SPR. Thermoregulation occurred around T_{opt} and was often above Topt for much of the day (see *Carlia vivax* in **Fig. 5.3**) rather than limited to temperatures below T_{opt}.

The behavioural thermal safety margin (as opposed to the thermal safety margin based on ambient temperatures; Deutsch et al. 2008), was significantly larger in the morning (lower limit of PBT range) than in the afternoon (upper limit of PBT range; paired t-test, $t_{(12)}=7.38$, $p<0.0001$). In the morning, species thermoregulated on average 0.36 °C below T_{opt}, although some already had negative safety margins at this time of day (min= -1.88, max= 3.68). In the afternoon, all species thermoregulated above T_{opt} and had a negative thermal safety margin of, on average, -2.39 (min=-3.69, max=-0.16). In the morning, when tropical species exhibit lower PBTs than temperate species (Chapter IV), the thermal safety margin trended to be greater for species from temperate areas (**Fig.5.7**; 3.24 + 0.15*latitude, $F_{(1,11)}=4.45$, $p=0.059$, $R^2=0.22$). In the afternoon, when preferred body temperatures of species from different latitudes were similar (Chapter IV), the thermal safety margin was significantly correlated only with body mass (**Fig. 5.8 A**; -1.49 - 0.40 *body mass, $F_{(1,11)}=13.57$, $p<0.005$, $R^2=0.51$). Larger species thermoregulated further above their T_{opt} (i.e. had more negative thermal safety margins) than small species. Similarly, behavioural warming tolerance (as opposed to standard warming tolerance; Deutsch et al. 2008), was greater in the morning (mean=11.33°C, min=9.22°C, max=14.13°C) than in the afternoon (mean=8.58°C, min=6.95°C, max=10.67°C; paired t-test, $t_{(12)}$=7.38.85, p<0.0001). In the morning, warming tolerance was not correlated significantly with any covariates. However, in the afternoon it was correlated with body mass (**Fig. 5.8 B**; 9.45 - 0.39*body mass, $F_{(1,11)}$ =16.67, p=0.002, R^2=0.57). Larger species had lower warming tolerances and accepted exposure to temperatures closer to their CT_{max} than small species.

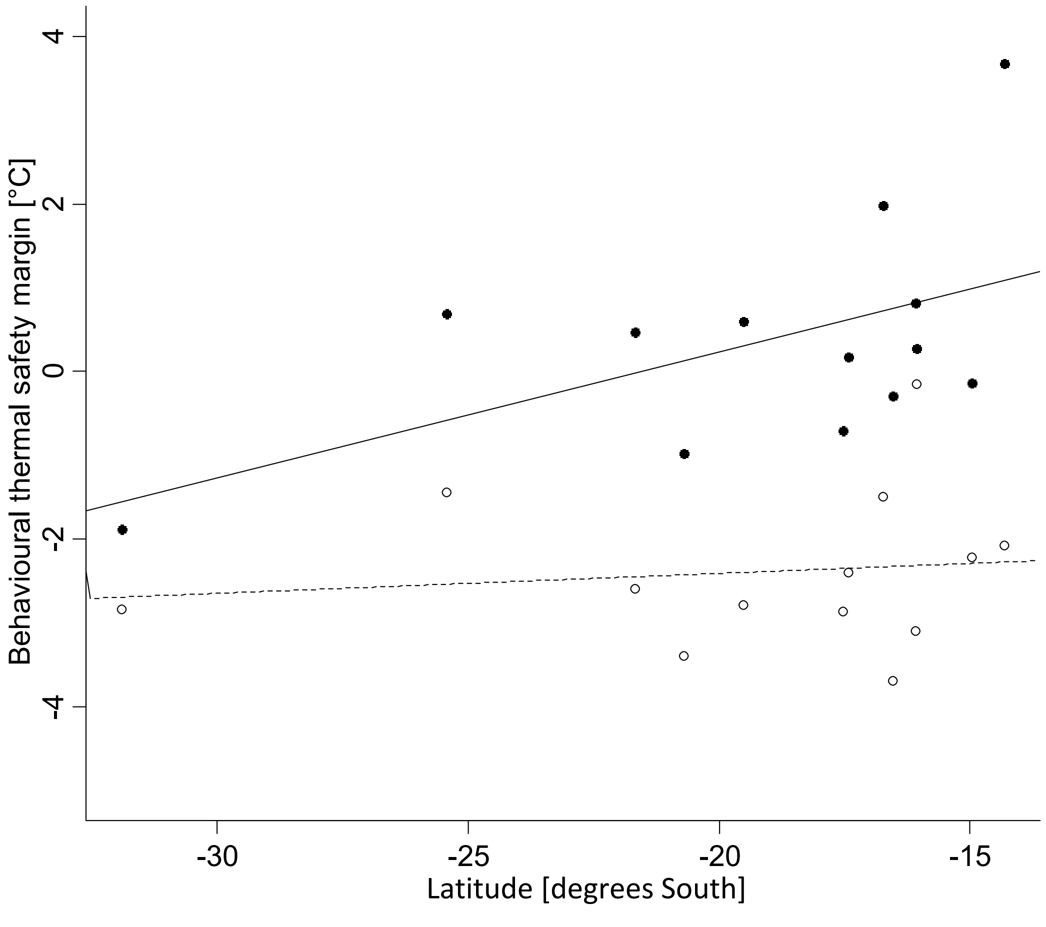
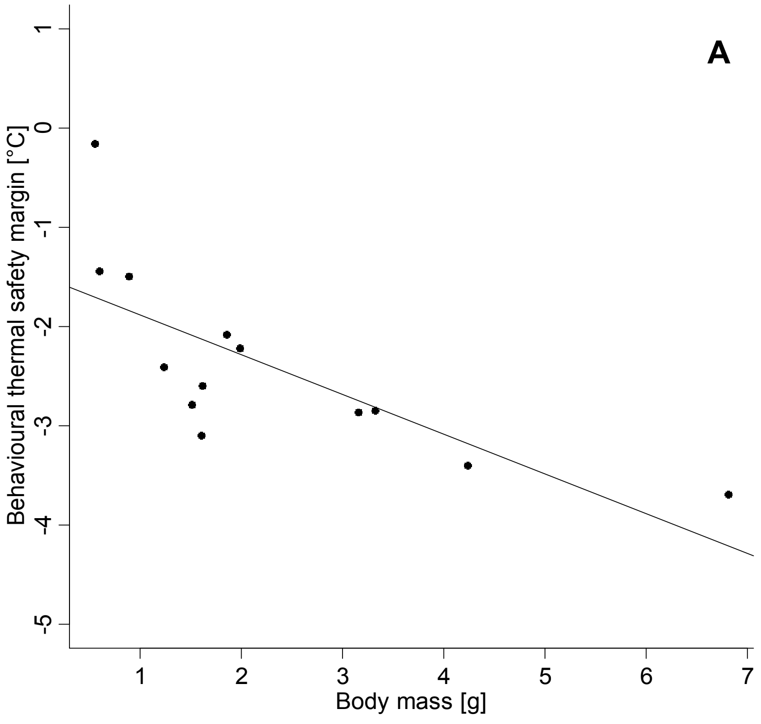


Fig. 5.7 Linear regression on behavioural thermal safety margins of species from different latitudes. There was no significant relationship between thermal safety margin and latitude in the afternoon (empty circles, dotted line; $F_{(1,11)}$ =0.17; p=0.69). In the morning (filled circles, solid line), thermal safety margins of species were greater at lower latitudes, i.e. more tropical species thermoregulated further below T_{opt}. However, this trend was marginally insignificant ($F_{(1,11)}$ =4.45; p=0.059).



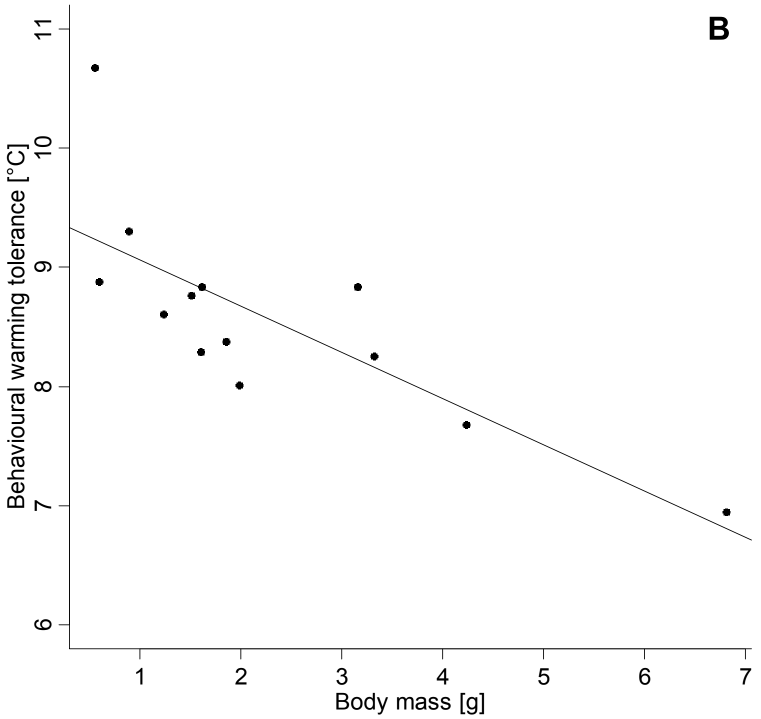


Fig. 5.8 Linear regression on behavioural thermal safety margin (**A**) and warming tolerance (**B**) for species of different body mass at the upper end of their PBT range. Larger species thermoregulated further above their T_{opt} and closer to their CT_{max}.

Discussion

Tropical species had narrower thermal tolerances than temperate species, because of their higher critical thermal minima (Chapter IV). Tropical species also had narrower performance curves than temperate species, because their performance decreased more rapidly at low temperatures. But, neither optimum temperature, CT_{max} (Chapter IV), thermal sensitivity of performance, nor species' tendency to expose themselves to detrimentally high temperatures varied with latitude, indicating that tropical and temperate species had similar abilities to cope physiologically and behaviourally with hot temperatures. All species thermoregulated very precisely within their optimum temperature range. Whether species were tropical or temperate in origin was not related to their vulnerability to thermal variability, except at very low temperatures, when temperate species fared better. Body mass did, however, influence interspecific differences in thermal sensitivity significantly; larger-bodied animals were physiologically less tolerant of hot temperatures (they had lower CT_{max}), they performed better at lower temperatures (they had lower T_{opt}), they voluntarily exposed themselves to greater decrements in performance at the upper end of their preferred body temperature and set point ranges, and thermoregulated further above their optimum temperature and closer to their upper thermal threshold. At low temperatures, thermal sensitivity appeared to be determined by adaptation to colder, temperate environments, whereas at hot temperatures it was more strongly influenced by body size. Greater thermal inertia of large animals (Stevenson 1985, Blackburn et al. 1999, Pincheira-Donoso et al. 2008) may, therefore, reduce selection pressures on heat tolerance and heat caution because the risk of sudden overheating is lower and because similar basking periods would result in lower body temperatures than in small animals (Pincheira-Donoso et al. 2008).

Importantly, my results confirm that thermoregulatory behaviour has a critical mitigating effect on vulnerability to dangerously high temperatures (Sunday et al. 2014, Buckley et al. 2015) in species from all latitudes, as long as suitable habitats for thermoregulation (including refuges) are available. Tropical species tended to have larger thermal safety margins in the morning (although this trend was marginally insignificant at the study sample size of thirteen species), suggesting that they are behaviourally more likely to avoid high temperatures even though they are not more sensitive to high temperatures. Tropical species experienced reduced performance in the morning by selecting temperatures below T_{opt}, while any performance reductions in the afternoon were caused by selecting temperatures above T_{opt}. They, therefore, did not experience a greater performance reduction in their PBT range overall than did temperate species but expanded the window of temperatures exploited throughout the day by thermoregulating around T_{opt} rather than consistently above T_{opt} (as seen in temperate species). Thus, they appear to more effectively avoid short term increases in temperature throughout much of the day, while nevertheless exploiting a broader thermal activity window. This may be related to latitudinal differences in exposure to radiation, which can lead to increases in operative body temperature far above air temperature (Sunday et al. 2014). Given that predicted increases in temperature in the tropics are likely to be small, and coupled with increased cloud cover, leading to decreased future exposure to thermal radiation (Zhou et al. 2009, Clusella-Trullas et al. 2011, IPCC 2013), it seems likely that the climate space for continued efficient thermoregulation in tropical ectotherm species will remain available unless changes in vegetation cover lead to a reduction in the availability of shades microenvironments (Kearney et al. 2009).

I observed a decrease in endurance in tropical species and populations at all temperatures, but especially at low temperatures (**Fig. 5.1**). I expected the performance of tropical species to be reduced at lower temperatures, because they are adapted to warmer temperature regimes. Narrower thermal tolerances in tropical species, however, were driven entirely by changes in the shape of the performance curve at low temperatures, indicating that there was no difference the tolerance to, or fitness at, high temperatures between tropical and temperate species. The performance of tropical species did not decrease more than that of temperate species at 5°C above and below T_{opt}, and always remained above 80% of maximum performance. The standardized heat and cold sensitivity of performance at 5°C above or below T_{opt} reveals that substantial deviation in temperature from T_{opt} can be tolerated without reducing performance more than the amount usually deemed acceptable by the 80% limit used to measure performance breadth (Angilletta 2009). Interestingly, all species thermoregulated above their T_{opt} for most of the day (see **Fig. 5.3**), but experienced only a small decrease in performance when active around the upper (about 10% on average) or lower (about 5% on average) end of their set point ranges, which, again, did not correlate with latitude. Sensitivity to thermal variability of different species, whether as a standardized measure or based on their thermoregulatory behaviour, consequently, did not vary with latitude. When increased performance breadth is found in temperate species, it is, therefore, most likely driven by increased performance in cold temperatures, but does not affect sensitivity to heat or temperature-dependent performance in the range of temperatures in which animals are active.

The higher overall endurance of temperate species, combined with their broader thermal tolerances (due to lower CT_{min}) suggests that there is no trade-off between performance amplitude and width, and supports the alternative hypothesis that species with broader performance curves also have the highest performance (Huey and Hertz 1984). Interestingly, I observed a trend for increased performance in temperate groups both among species, but also among populations within species (which also have lower CT_{min} at higher latitudes; Chapter IV), indicating that the selection pressures producing these patterns may be strong and ongoing. Trade-offs between traits, such as sprint speed and endurance, may reduce our ability to detect trade-offs between amplitude and width in any given performance trait (Huey and Hertz 1984, Goodman et al. 2007) and this may have occurred in my study. Thus if reductions in endurance were accompanied by increases in sprint speed, overall fitness may remain the same. In this respect, endurance may, for example, be more important to species that must cross open habitat, whereas sprint speed may be more relevant in species in closed habitats where only short distances have to be crossed between refuges (Arendt 2009). Further clarification of such inter-trait performance trade-offs would be of great relevance to clarify the evolutionary drivers behind latitude-dependent variation in average performance, and its relevance to vulnerability to climate change.

Similar to the cold adaptation in temperate species, cold acclimation only increased endurance at temperatures below species' set point range (below 24°C). Hot acclimation, however, increased endurance at temperatures in the entire activity temperature range and raised T_{opt}. Thus, from the point of view of acclimation, there was a trade-off between amplitude and width of performance curves. Cold acclimation decreased performance but enabled activity at suboptimal temperatures, and is probably advantageous only when these cannot be avoided. Indeed, cold acclimation does not occur in the wild in low latitude *Carlia* species unless individuals are forced into a low temperature regime for long periods (Chapter II; Pintor et al. 2016). Within an individual's life time, trade-offs between performance width and amplitude in endurance do therefore occur, while across populations or species, trade-offs among performance traits may be more common (Huey and Hertz 1984, Goodman et al. 2007).

Body mass had a surprisingly large effect on performance (physiological temperature sensitivity), and on the tendency to be active at high temperatures (behavioural temperature sensitivity). Smaller animals outperformed large ones at higher temperatures (**Fig. 5.2** & **5.4**), endured higher maximum temperatures (Chapter IV), had higher thermal optima (**Fig. 5.3**), experienced less of a decrease in performance at the hot end of their preferred body temperature and set point range (**Fig. 5.6**), and avoided exposure to detrimental temperatures above T_{opt} or close to CT_{max} more strongly (**Fig. 5.7**). These effects of body size, combined with the trend for increased performance and higher T_{opt} in hot-acclimated animals support the hypothesis that "hotter is better", i.e. that hot adapted (or acclimated) species with higher T_{opt} also perform better overall (Angilletta et al. 2010). However, the main driver for hot adaptation appears to be body size, rather than latitude. Larger animals are both more likely to thermoregulate close to CT_{max}, and simultaneously less heat tolerant than smaller species, probably because they simply don't have to be as cautious of potential overheating. Greater thermal inertia should provide a buffer for short term temperature fluctuations in larger animals (Stevenson 1985, Blackburn et al. 1999, Pincheira-Donoso et al. 2008) that probably allows them to be active at higher temperatures without risking overheating. Similarly, large animals may not need much tolerance for extreme temperatures because they have a longer timeframe in which to correct accidental exposure before morality occurs. Longer basking times required to achieve high body temperatures (Pincheira-Donoso et al. 2008) may make lower T_{opt} favourable. Last but not least, lower predation risk at larger body size (Arendt 2009) may decrease the disadvantages associated with voluntary decreases in performance. Body size affects many life history (Blueweiss et al. 1978, Angilletta et al. 2004) and thermal traits (Andrews and Pough 1985, Gillooly et al. 2001, Dell et al. 2011), both of which may vary in space along climate gradients (Tsuji 1988, Angilletta et al. 2004). Latitudinal trends in body mass (Partridge and Coyne 1997, Pincheira-Donoso et al. 2008) may be confounding variables if ignored in analyses of physiological differences across latitudes. Disentangling the direct effects of temperature on body mass and physiology, as well as their interactions, is potentially of great interest to further our understanding of biogeographic patterns.

Our results suggest that, although I observed effects of latitude on the shape of performance curves, traits defining the evolutionary responses of species' behaviour and physiology to high temperatures are more strongly influenced by body size than by any of the other covariates I examined, at least for this clade. High intraspecific variability of physiological traits in this clade indicate that the lack of latitudinal variation in performance parameters is not caused by evolutionary conservatism (Chapter IV). Other variables, such as exposure to radiation or habitat complexity may be better predictors of differences in performance among species than latitude and should be examined further, because they impact directly on the effectiveness of thermoregulation. I tested hypotheses about latitudinal variation in thermal safety margins and warming tolerances using preferred body temperature rather than habitat temperatures as my standard for comparison, because preferred body temperatures are more representative of the thermal habitat species experience than mean environmental temperatures. Further clarification of how potential activity times change with climate warming, given the actual habitat temperatures available for thermoregulation, would further elucidate differential impacts of climate change on fitness of species from different habitats (Kearney et al. 2009, Kearney 2013).

I have introduced several novel metrics of species' susceptibility to thermal variability. I proposed a standardized heat and cold sensitivity measure (instead of using the 80% performance breadth), as well as a measures of the decrease in performance to which individuals will voluntarily expose themselves within their preferred body temperature range and set point range (selected and accepted thermal sensitivity). Lastly, I proposed a behavioural thermal safety margin and warming tolerance to estimate risk of overheating based on thermoregulatory behaviour rather than mean habitat conditions. In conclusion, my results show that although species use a large range of habitats over the latitudinal climate gradient (Stevens

1989), the behaviourally selected thermal niche of species is very narrow and does not vary much with latitude. The implications are that range extensions across the latitudinal gradient are most likely facilitated by tolerance to low temperatures and increased maximum endurance rather than by differential potential to exploit warm regions through reduced sensitivity to hot air temperatures. In this respect the “behavioural” microhabitat to which organisms expose themselves differs greatly from the physical microhabitat in which they occur. The incorporation of differential capacities to behaviourally buffer environmental variability is therefore a crucial component for assessing species’ vulnerability to climate change.

-Chapter VI-

Which Physiological Traits Best Describe Ectotherm Resilience to Environmental Extremes and Climate Change?

Abstract

To quantify differential sensitivity of organisms to climatic variability, and future climate change, it is critical to identify the physiological variables that limit species' distributions. Physiological traits that may improve resilience to climatic extremes, rather than those that maximize fitness at average conditions, are of greatest interest in this respect. Despite substantial advances in the fields of macrophysiology and species' distribution modelling, we still struggle to identify key traits that determine resilience and range boundaries. Here, I establish which, out of an extensive set of physiological traits best determined how far species' ranges could extend into extremes in thermal and hydric conditions, using a clade of thirteen closely related species of small ectotherms (lizards) from Eastern Australia. Traits allowing species to extend further into colder and drier conditions limited their geographic distributions the most, most notably through high tolerance of cold, metabolic compensation at low temperatures, and decreased water loss. Physiological tolerance of high temperatures did not predict maximum air temperatures species could extend into within their geographic range, but did predict occurrence in habitats with higher maximum solar radiation. Exposure to radiation may therefore be more limiting, or more difficult to compensate for through behavioural thermoregulation, than exposure to high ambient air temperatures. Ectotherm species most at risk to climate warming are likely to be those that occur in dry, high radiation (open) habitats, or those that will experience the most change in dryness or radiation. Large-scale assessments of species' climatic sensitivity should examine water loss rates and critical thermal minimum temperatures to further test the potential of these traits to limit species' current distributions and to predict range expansions and contractions resulting from climate change at broader geographic and taxonomic scales.

Introduction

Biologists have studied biogeographical patterns in physiological, anatomical, behavioural, and dispersal traits of organisms for centuries, trying to elucidate how species evolve in response to geographic variation in biotic and abiotic factors, and how these traits, in turn, limit abundance and distribution (Bergmann 1848, Wallace 1860, Bartholomew 1958, Brown 1984, Stevens 1989, Huey and Bennett 1990, Gaston 2003, Holt 2003 , Clusella-Trullas and Chown 2014, Sunday et al. 2014). The differential potential of species to deal with geographic and temporal variations in climate, and especially in temperature, has become a topic of great relevance in the face of anthropogenic climate change (Kearney and Porter 2004, Deutsch et al. 2008, Sunday et al. 2014). Despite increasing scientific interest in the topic, however, many of the proposed factors that limit species' distributions remain equivocal (Gaston 2003, Calosi et al. 2010, Pintor et al. 2015), and questions regarding the extent to which distributions can be appropriately estimated based on different niche concepts and using different sets of climate variables are increasingly being recognized as fundamental to our ability to estimate climate change impacts on species' ranges (Soberón 2007).

The climatic variability hypothesis (CVH) is a concept central to our understanding of species' range limits, and predicts that species exposed to greater temperature variability in their ancestral habitat will evolve broader thermal tolerances and consequently have larger range extents along natural geographic gradients in temperature (Chapter I; Stevens 1989, Gaston et al. 1998, Pintor et al. 2015). This implies that the main factor limiting species' distributions are environmental tolerances, which has encouraged the notion that measures of physiological critical thermal limits can be used to estimate species' vulnerability to predicted increases in (mean) temperatures, and determine their overall potential to extend into thermally extreme habitats (Deutsch et al. 2008, Overgaard et al. 2014). While thermal limits definitely limit potential species' distributions in some respects, a number of points need to be considered when inferring species' likely geographic limits based on their physiological tolerances.

Firstly, species' exposure to current conditions within their range needs to be described in adequate and physiologically relevant terms. Because mean environmental temperatures are predicted to increase with climate change, they are often used to estimate the impact on species with different physiological tolerances (Deutsch et al. 2008). However, mean temperatures are not very useful for estimating extremes (i.e., maximum or minimum environmental exposure) within species' habitats (**Fig. 6.1 A**; Dillon et al. 2010). Also, latitudinal differences in mean temperatures are mostly driven by geographic variation in minimum, rather than maximum temperatures (Müller 1982). Consequently, geographic differences in species' physiological tolerances are largely caused by differences in cold tolerance rather than heat tolerance

(Clusella-Trullas et al. 2011). Absolute minimum and maximum temperatures within a species' current range are, therefore, better descriptors of environmental exposure than mean temperatures and are, consequently, most likely better predictors of required physiological tolerances under current and future climates. Species may, furthermore, occasionally be recorded in "sinks", which are areas that individuals occasionally disperse into from nearby "sources" (in which population growth is positive) but where populations cannot persist (Pulliam 2000). When using occurrence records to estimate environmental exposures, a standardized method should therefore be used to exclude extreme values with very low frequency (see "sinks" in **Fig. 6.1**) to avoid overestimating environmental exposures based on a few outliers.

Secondly, a distinction has to be made between environmental and physiological tolerances. Environmental tolerances can be estimated from climate within species' current distributions and describe the range of climatic conditions that an organism can exist under as a combined result of its behaviour and physiology. Physiological tolerances only describe the absolute temperature thresholds an organism can physiologically tolerate in the absence of behavioural corrections and do not necessarily represent environmental tolerances accurately. Thermal performance curves for most physiological processes typically rise slowly from a critical thermal minimum (CT_{min}) to a thermal optimum (T_{opt}), followed by a more abrupt decrease towards a critical thermal maximum (CT_{max}; **Fig. 6.1 B**; Huey and Stevenson 1979, Angilletta 2006, Deutsch et al. 2008). The difference between CT_{max} and CT_{min} is the physiological tolerance breadth. Mobile ectotherms have developed mechanisms to avoid detrimental extremes in climatic conditions towards the ends of the physiological tolerance breadth through thermoregulation (Buckley et al. 2015) because fitness is greatly reduced at extremes (Huey and Slatkin 1976), and risk of accidental exposure to lethal extremes is high (Vickers et al. 2011). In fact, ectotherms tend to thermoregulate behaviourally within a narrow range of selected temperatures (T_{sel}) around their T_{opt} (**Fig. 6.1 B**; Hertz et al. 1993, Vickers et al. 2011). Behavioural thermoregulation can, therefore, enable ectotherms to occur in environmental conditions outside their physiological tolerance range if thermally buffered microhabitats for activity as well as for inactivity (refuges) are available (Sunday et al. 2014, Buckley et al. 2015). Consequently, even though critical thermal limits may broadly predict environmental tolerance limits, the two are not interchangeable: environmental tolerance estimated from current occurrence should generally be much broader than physiological tolerances and neither of the two necessarily predict vulnerability to thermal extremes (**Fig. 6.1 B**; Sunday et al. 2014, Buckley et al. 2015). I, therefore, define environmental tolerance as the general climate conditions that species can persist under as suggested by their occurrence records, and emphasize the need to clearly distinguish them from the physiological tolerances, which are the thresholds in body temperature, hydration states or other properties of the organism that can be survived. Of course, environmental tolerance can in this context only be an approximation of actual environmental tolerance because dispersal limitations and biotic interactions may inherently be included in the distributions described by occurrence records.

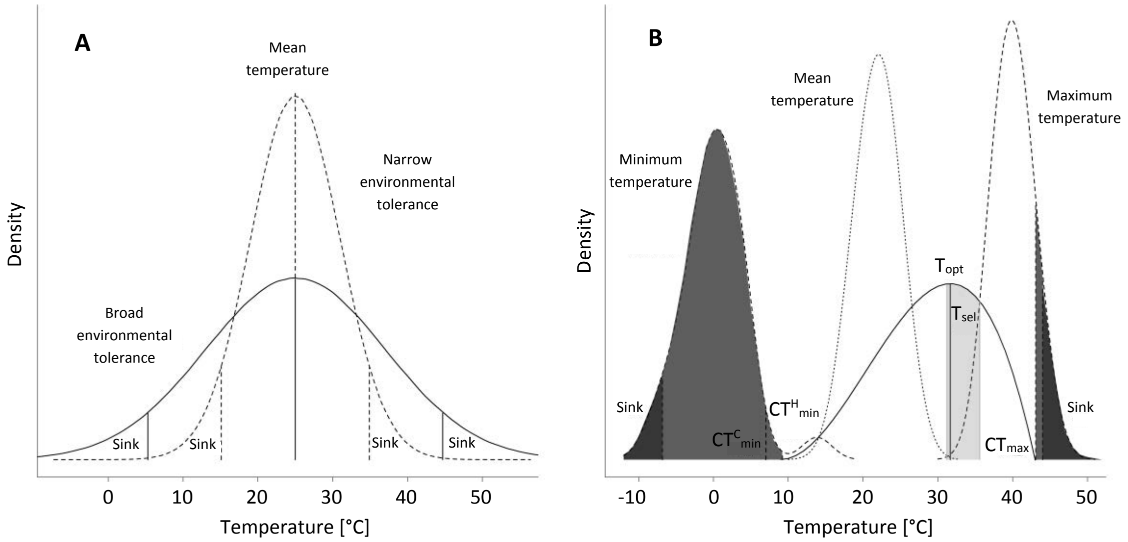


Fig. 6.1 (**A**) Species occurring at the same mean temperature can occur in environments with vastly different thermal variability, meaning that mean temperatures are an inappropriate measure of the environmental tolerance of species. This applies to both, mean temperature and (temporal) variability within any one habitat, as well as mean temperature and (spatial) variability within a geographical range. The range of experienced conditions may therefore be of greater physiological relevance than mean conditions. Similarly, species can often be found in marginal sink habitat, where long term survival is not possible. Rare extreme values of conditions recorded for species based on their collection sites of museum records should, therefore, not be included in estimates of their environmental tolerance (**B**) Example of how measures of absolute minimum and absolute maximum but not mean temperatures can function as proxies for temperature regimes a species (here *Carlia vivax*; see Chapter V) is exposed to throughout its distribution and how physiological tolerance relates to these proxies of environmental tolerance. The density distributions of minimum and maximum environmental temperatures can be compared (but are not necessarily equivalent) to a species' heat and cold tolerance, as it may exert selection pressures on these traits. Mean temperatures within a species' range, however, have no physiological representation that they may exert selection pressures on, because they do not summarize climatic extremes or represent available temperatures during animal's activity periods appropriately. For *Carlia vivax*, for example, temperatures selected for activity as well as optimum temperatures are far above mean environmental temperatures, indicating that mean temperatures are usually lower than the thermal conditions they are adapted to. Selection pressures on these activity related traits are much more likely to stem from actual frequency distributions of daytime temperatures (i.e. the frequency of different temperatures available to animals during their activity period), rather than the statistical mean of conditions.

Additionally, distributions can be limited by other physiological processes and environmental stressors other than simply via tolerance of temperature extremes. Lethal operative temperatures may be reached as a result of exposure to radiation rather than ambient air temperature (Sunday et al. 2014). Sub-lethal temperature increases and dry conditions can lead to high water loss that may limit animals potential to be active (Chapter III) or even limit distributions through differences in physiologically tolerated desiccation thresholds. Warm temperatures, especially during inactivity, may also increase metabolic rates, and therefore energy expenditure, beyond what is sustainable in available foraging time and rates of energy acquisition (Tsuji 1988, Christian et al. 1999, Dillon et al. 2010, Sunday et al. 2014). At the other extreme, low temperatures may only be tolerated if metabolic rate can be increased sufficiently to allow activity and growth during the cold season (metabolic compensation; Tsuji 1988). Similarly, precision of thermoregulation may influence maximum potential activity times and the risk of overheating in extreme environments (Chapter IV & V). However, it is rarely acknowledged that climate variables other than air temperature (e.g. aridity or solar radiation; Dmi'el et al. 1997, Sunday et al. 2014) and physiological traits other than thermal tolerances (Kearney and Porter 2004, Dillon et al. 2010) may limit species' distributions.

Lastly, we have to distinguish between two questions: (i) how does climate influence the differential evolution of physiological traits? And (ii) which traits are the most limiting to organisms geographically and climatically? The first question is relevant to evolutionary physiology, while the second is more important for predictions of environmental and geographical limits to species' distributions. For example, high temperature environments may favour low metabolic rates to reduce energy expenditure, high critical thermal maxima to reduce the risk of overheating, high critical thermal minima because no cold tolerance is required, low water loss rates because of high desiccation risk, and high optimum temperatures to reduce the need for thermoregulation to achieve high performance. However, these traits may not necessarily be equally geographically limiting. Mechanistic models of species' distributions have recently advanced our understanding of physiologically limiting traits (Kearney and Porter 2004). However, many studies focus more on trait evolution rather than on the potential for these traits to limit distributions (Clusella-Trullas et al. 2011, Clusella-Trullas and Chown 2014). It is, therefore, important to note that not all traits that evolve in response to climatic pressures are necessarily geographically limiting. Because traits that limit species now are likely to affect how potential distributions will change in the future, establishing a clear understanding of what limits species' ranges the most is of utmost importance for our ability to predict impacts of climate change.

We should also distinguish between climatic variables that are physiologically limiting and those that are both physiologically and spatially limiting. Even if exposure to a stressor, such as high temperatures, has detrimental physiological consequences, it will not limit distributions differentially unless it (i) varies across geographic space and (ii) correlates with interspecific differences in physiological, behavioural or other intrinsic traits. High temperatures may be one of the most physiologically limiting climatic extremes (Vickers et al. 2011, Kearney et al. 2013). However, species' range limits and their vulnerability to climate change will only be affected differentially if climate variables that affect operative temperatures (e.g. maximum air temperatures, radiation or exposure) vary geographically and if species can tolerate this geographic variation to different degrees. Consequently, a physiological or behavioural trait may only function as a predictor of how different species will deal with climate change in different ways, if it predicts well how far different species' ranges can currently extend into relevant climatic extremes.

Here I examine a range of physiological traits as potential predictors of how far species from a clade of small ectotherms (Scincidae; genera *Carlia* and *Lygisaurus*) from Eastern Australia can extend into current climatic extremes. I assess the potential for extremes of temperature, solar radiation, relative humidity and vapour pressure deficit to limit species geographically and determine whether (i) species with high tolerance for local environmental conditions extend over a broader geographical range in environmental conditions, (ii) whether environmental tolerances are correlated with, but greater than, physiological thermal tolerances. I furthermore test the hypotheses that (iii) cold tolerances predict species' potential to extend their ranges into cold climates, (iv) while upper thermal limits are less geographically limiting with respect to maximum air temperatures tolerated by different species. I predict that (v) heat tolerance limits species more by allowing for differential tolerance of high solar radiation levels and (iv) reduced water loss rates allow species to extend further into extremes in hydric conditions and are of greater importance for dry-skinned ectotherms than often assumed. Based on my results, I propose which physiological traits are most limiting to ectotherms now, and in the future, and which traits predicting sensitivity to climatic extremes may be easily collected for other organisms, to best assess vulnerability of ectotherms to climate change at broader geographic and taxonomic scales.

Methods

Thirteen species of small, mostly leaf-litter dwelling scincid lizards from a monophyletic clade comprised of the genera *Carlia* (nine species: *Carlia dogare*, *C. jarnoldae*, *C. longipes*, *C. munda*, *C. rubrigularis*, *C. schmeltzii*, *C. storri*, *C. tetradactyla*, and *C. vivax*) and *Lygisaurus* (four species: *Lygisaurus aeratus*, *L. foliorum*, *L. laevis* and *L. rococo*; Dolman and Hugall 2008, Wilson and Swan 2008) were used for this study. The included species exhibit extreme differences in range size and position and are all distributed along the Australian East Coast and to moderate extents inland, except for *Carlia munda*, which occurs both along the east coast and westward in the tropics. The Climatic Variability Hypothesis which influences range size distributions across climatic gradients globally (Stevens 1989, Gaston et al. 1998), applies to this clade, i.e. species with more temperate range positions experience more variable habitats and have wider latitudinal distributions (Chapter I; Pintor et al. 2015). This clade, therefore, provides an ideal model to examine physiological predictors of interspecific differences in tolerances to climatic extremes.

Body mass, metabolic rate, water loss rate, preferred body temperatures, set point range, critical thermal limits, thermal optimum, maximum endurance, cold- and heat-sensitivity of performance (performance reduction at 5°C below or above thermal optimum), behavioural warming tolerance (difference between preferred and optimum body temperature) and behavioural thermal safety margin (difference between preferred body temperature and CT_{max}) for individuals from several populations of the different species were collated from previous research (Chapter IV & V) and the existing data was summarized to obtain species averages for each trait. For traits that acclimate to differences in temperature, separate values for hot- and cold-acclimated individuals were used, as was the difference of the two (i.e. acclimation potential of the trait). For preferred body temperatures, which vary with time of day (Chapter IV), I used minimum (morning) and maximum (afternoon) values, as well as lower (morning) and upper (afternoon) limits of set point range (range of accepted body temperatures around median preferred body temperature). For metabolic rates, I used cold acclimated basal metabolic rate at 20°C and hot acclimated metabolic rate at 30°C.

Latitudinal and longitudinal coordinates of occurrence points for each species were downloaded from the Atlas of Living Australia (http://spatial.ala.org.au/, 10/07/2014). Museum Records were compared with distribution maps in the "Complete Guide to Reptiles of Australia" (Wilson and Swan 2008) and definite outliers (single occurrence records far outside the known distribution) excluded. For each occurrence record, values of several climate layers were downloaded directly through the Atlas of living Australia. The layers used were absolute maximum temperatures (Williams et al. 2010), absolute minimum temperatures (Williams et al. 2010), annual mean temperatures (Bio01; Hijmans et al. 2005); see http://www.worldclim.org/bioclim), radiation in the highest and lowest period (Bio21; Bio22; Hijmans et al. 2005); see http://www.worldclim.org/bioclim), annual mean radiation (Bio20; Hijmans et al. 2005); see http://www.worldclim.org/bioclim), vapour pressure deficit (VPD) in the driest and wettest month (Williams et al. 2010), annual mean VPD (Williams et al. 2010), relative humidity (RH) in the driest and wettest month (Williams et al. 2010) and annual mean RH (Williams et al. 2010).

Table 6.1 Summary of traits used as potential predictors of tolerance to environmental extremes

Trait	Trait summary
Body mass	Average body mass [g]
MR^C	Body mass adjusted, cold acclimated metabolic rate at 20 °C [µl/min*g]
MR^H	Body mass adjusted, hot acclimated metabolic rate at 30 °C [µl/min*g]
WLR^H	Hot acclimated water loss at 30 °C, over 24h [% of body mass]
WLR^C	Cold acclimated water loss at 30 °C, over 24h [% of body mass]
CT_{min}^C	Cold acclimated critical thermal minimum [°C]
CT_{min}^H	Hot acclimated critical thermal minimum [°C]
CT_{min}^{AP}	Acclimation potential of critical thermal minimum [°C]
CT_{max}^H	Hot acclimated critical thermal maximum [°C]
CT_{max}^C	Cold acclimated critical thermal maximum [°C]
CT_{max}^{AP}	Acclimation potential of critical thermal maximum [°C]
Thermal tolerance	Difference between CT_{max}^H and CT_{min}^C [°C]
Thermal tolerance^{AP}	Difference between total thermal tolerance and thermal tolerance based on CT_{max}^C and CT_{min}^H
Maximum endurance	Average endurance at thermal optimum [sec]
T_{opt}^C	Cold acclimated thermal optimum, i.e. the temperature at which endurance is highest [°C]
T_{opt}^H	Hot acclimated thermal optimum, i.e. the temperature at which endurance is highest [°C]
T_{opt}^{AP}	Acclimation potential of thermal optimum, i.e. the temperature at which endurance is highest [°C]
$T_{sel\ min}$	Minimum median selected body temperature (8 am) [°C]
$T_{sel\ max}$	Maximum median selected body temperature (5 pm) [°C]
$T_{sel\ range}$	Range of selected body temperatures ($T_{sel\ max}$ - $T_{sel\ min}$) [°C]
$SPR_{lower\ limit}$	Lower limit of set point range (8 am) [°C]
$SPR_{upper\ limit}$	Upper limit of set point range (5 pm) [°C]
SPR_{range}	Set point range ($SPR_{upper\ limit}$ - $SPR_{lower\ limit}$) [°C]
Safety margin $_{max}$	Maximum thermal safety margin (T_{opt}^H - $T_{sel\ min}$)
Safety margin $_{min}$	Minimum thermal safety margin (T_{opt}^H - $T_{sel\ max}$)
Warming tolerance $_{max}$	Maximum warming tolerance (CT_{max}^H - $T_{sel\ min}$)
Warming tolerance $_{min}$	Minimum warming tolerance (CT_{max}^H - $T_{sel\ max}$)
Cold Sensitivity	Decrease in endurance from maximum endurance 5 °C below T_{opt}^C [%]
Heat Sensitivity	Decrease in endurance from maximum endurance 5 °C above T_{opt}^H [%]
Heat Sensitivity PBT	Decrease in endurance from maximum endurance at $T_{sel\ max}$ [%]
Heat Sensitivity SPR	Decrease in endurance from maximum endurance at $SPR_{upper\ limit}$ [%]

The potential of climatic variation to limit species geographically in terms of latitude and longitude, was assessed using Spearman’s rank correlation coefficients. Values from longitudes less than 142° (i.e., occurrence records of *Carlia munda* across northern Australia) were excluded for these assessments because they disguised relevant patterns occurring over Eastern Australia, to which all other species were restricted. These records were, nevertheless, used to describe climate in the range of *Carlia munda,* as outlined below.

We determined the climate extremes experienced by each species within their range, the average conditions in which they occurred, and the greatest level of variability they experience within their range for temperature, radiation, VPD and RH. Solar radiation was used as an alternative measure of overheating risk, in addition to the commonly used measure of maximum air temperature, because ectotherm operative body temperature is greatly affected by this climate variable (Sunday et al. 2014). VPD and RH were used as two different variables affecting hydration. Rare, extreme climatic conditions may characterise sink areas, where a species cannot persist. Because such outliers have strong effects on statistical analyses, the 95th or 5th quantile of climatic extremes were used, instead of absolute maximum and minimum values. For example, the 95th quantile of absolute maximum temperatures and the 5th quantile of absolute minimum temperatures for each species were used. For mean climate variables, I used both the 5th and 95th quantile to estimate the extremes of mean conditions. To estimate central tendency of climate conditions, I used median values of mean climate variables within ranges. To estimate the environmental tolerance breadth of species for different climate variables, the range of conditions between the 5th and 95th quantiles was used. For example, the absolute range of temperatures to which species were exposed across their range, was the difference between the 95th quantile of absolute maximum temperatures and the 5th quantile of absolute minimum temperatures recorded across their occurrence points, while the range of mean temperatures across their geographic range was the difference between the 95th and 5th quantiles of mean temperatures.

All data exploration, analyses, and model validations were performed in R (R Core Team (2014). Using the R package nlme (Pinheiro et al. 2013), I fitted separate generalized least squares models to predict each of the summarized climate variables with each of the summarized physiological traits (see **Table 6.1** for a summary of traits used as potential predictors). Models for each climate variable were ranked according to their AIC values to find the single physiological trait that best predicted interspecific differences in each environmental variable. All significant predictors where assessed for collinearity. The best significant predictors (i.e. those with the lowest AICs and a p-value above 0.05) that were not collinear with any previous, better predictors were determined. Significant predictors collinear with previous, better predictors were considered alternative predictors. Significant predictors not collinear with previous, better predictors were considered potential additional predictors. The most common best predictors for sets of similar climate variables and their practicality as easily collected measures of vulnerability to different climatic extremes were assessed.

Results

Along the Australian East Coast (latitude: -36.88° to -9.033°; longitude: 142.00° to 153.63°), minimum temperatures decreased at higher longitudes (i.e. towards the coast; **Fig. 6.2 A**; Spearman's rank correlation; ρ= -0.35, p < 0.0001) and more temperate latitudes (**Fig. 6.2 B**; ρ= 0.72, p < 0.0001). Maximum temperatures varied less along both dimensions but nevertheless increased significantly at higher longitudes (ρ= 0.15, p < 0.0001) and higher latitudes (ρ= -0.50, p < 0.0001). Like minimum temperatures, mean temperatures decreased with longitude and at high latitudes (ρ= -0.73 and p < 0.0001 for longitude; ρ= 0.83 and p < 0.0001for latitude). Thus there was greater overall temperature variability in coastal habitats and at temperate latitudes, mostly driven by trends in minimum temperatures. Minimum, mean and maximum radiation levels all decreased coastally (**Fig. 6.2 C**; ρ= -0.90, ρ= -0.76 and ρ= -0.67, respectively; p < 0.0001 for all) and increased at tropical latitudes (**Fig. 6.2 D**; ρ= 0.75, ρ= 0.54 and ρ= 0.54, respectively; p < 0.0001 for all), although maximum radiation also displayed a minor peak at very high latitudes (-30° to -35°). Thus, maximum radiation levels were higher in the tropics and further inland, even though maximum air temperatures were slightly lower at both of these locations. Trends in mean and minimum radiation levels, on the other hand, were similar to trends in minimum temperatures. Variability in radiation levels (i.e. difference between minimum and maximum) was relatively constant across longitude but increased at high latitudes, because of stronger decreases in minimum than maximum radiation levels.

Minimum, mean, and maximum vapour pressure deficit (VPD) decreased towards the coast (**Fig. 6.3 A**; Spearman's rank correlation; ρ= -0.59, ρ= -0.47, and ρ= -0.45; p <0.0001). Minimum and mean VPD increased in the tropics (**Fig. 6.3 B**; ρ= 0.43 and ρ= 0.15; p < 0.0001) but maximum VPD decreased in this direction (ρ= -0.069). However, variation in maximum VPD was substantial everywhere, and trends were not unidirectional (**Fig. 6.3 A** and **B**). Differences in VPD variability were, therefore, driven mostly by large differences in maximum VPD among habitats at any given longitude or latitude. Results for RH trends with longitude were similar to those for VPD: maximum, mean and minimum RH increased towards the coast (ρ= 0.18, ρ= 0.089 and ρ= 0.35; p < 0.0001) even though there was great variation of minimum

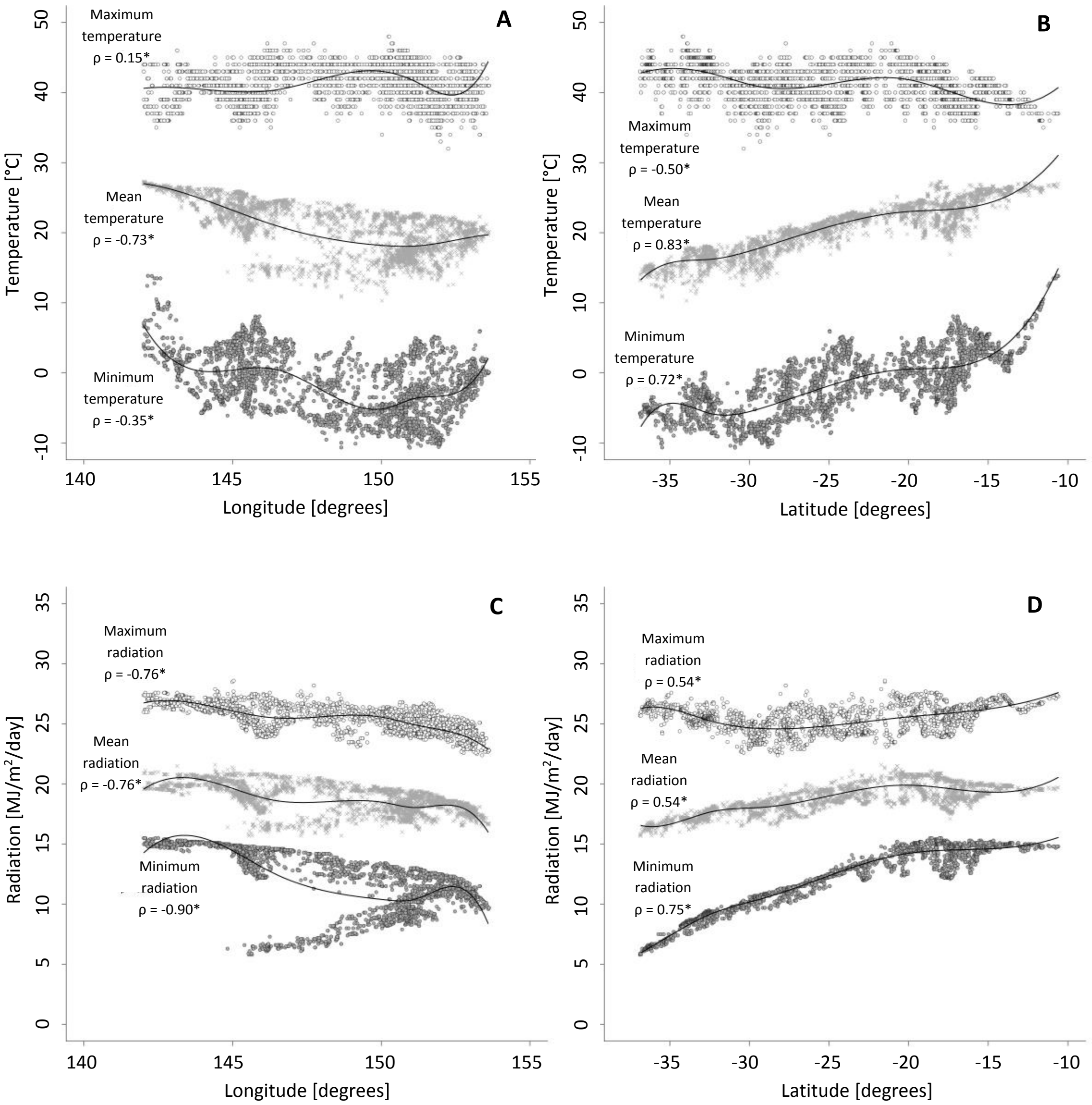


Fig. 6.2 longitudinal (**A** and **C**) and latitudinal (**B** and **D**) trends in minimum (dark grey filled circles), mean (grey crosses) and maximum (empty circles) temperature (**A** and **B**) and radiation (**C** and **D**) using values at all combined occurrence points for species used in this study. Cubic smoother splines were fitted to guide the reader's eye. Values for Spearman's rank correlation coefficient ρ are given in the figure and significance is marked with an asterisk.

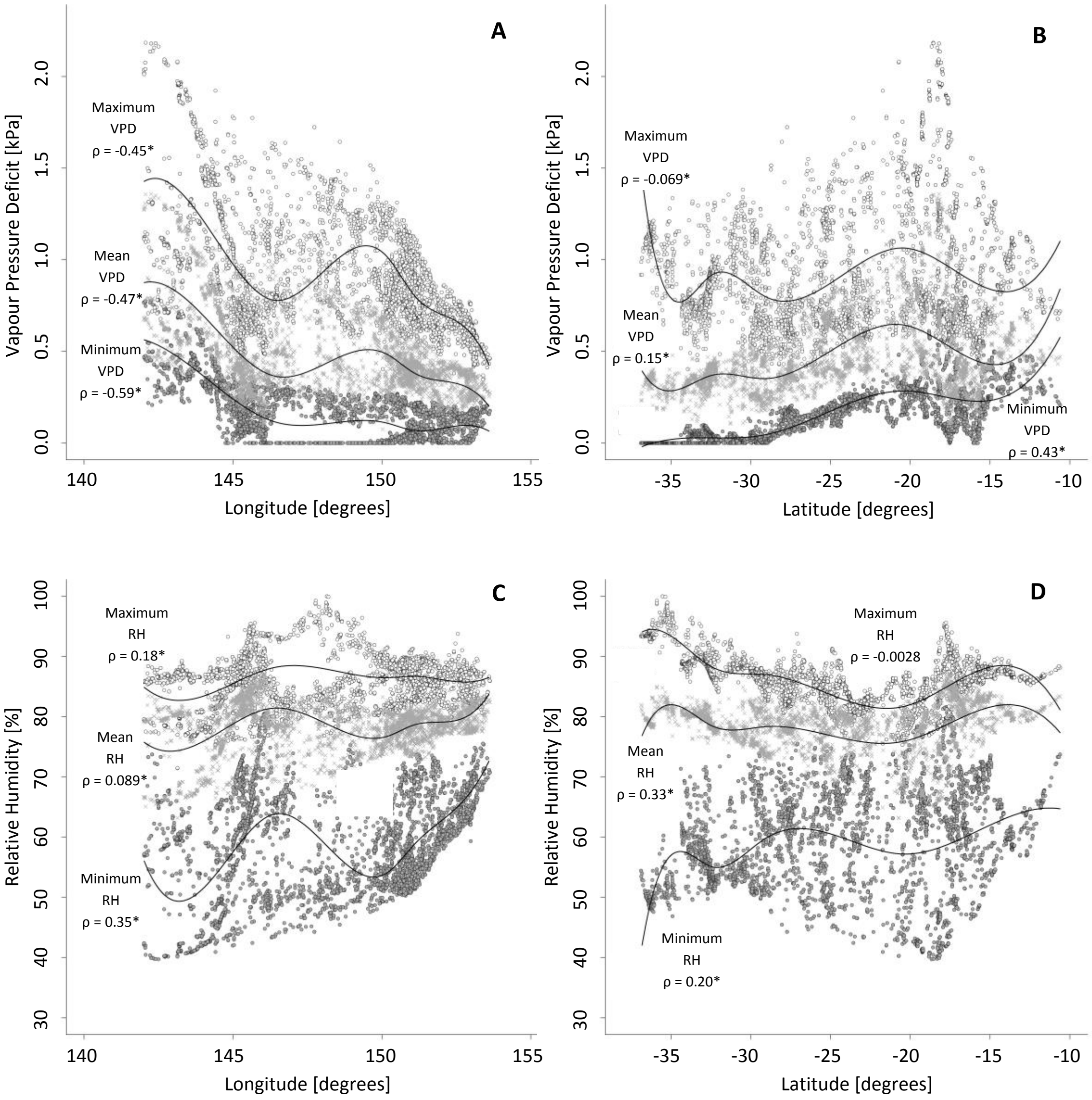


Fig. 6.3 longitudinal (**A** and **C**) and latitudinal (**B** and **D**) trends in minimum (dark grey filled circles), mean (grey crosses) and maximum (empty circles) vapour pressure deficit (VPD; **A** and **B**) and relative humidity (RH; **C** and **D**) using values at all combined occurrence points for species used in this study. Cubic smoother splines were fitted to guide the reader's eye. Values for Spearman's rank correlation coefficient ρ are given in the figure and significance is marked with an asterisk.

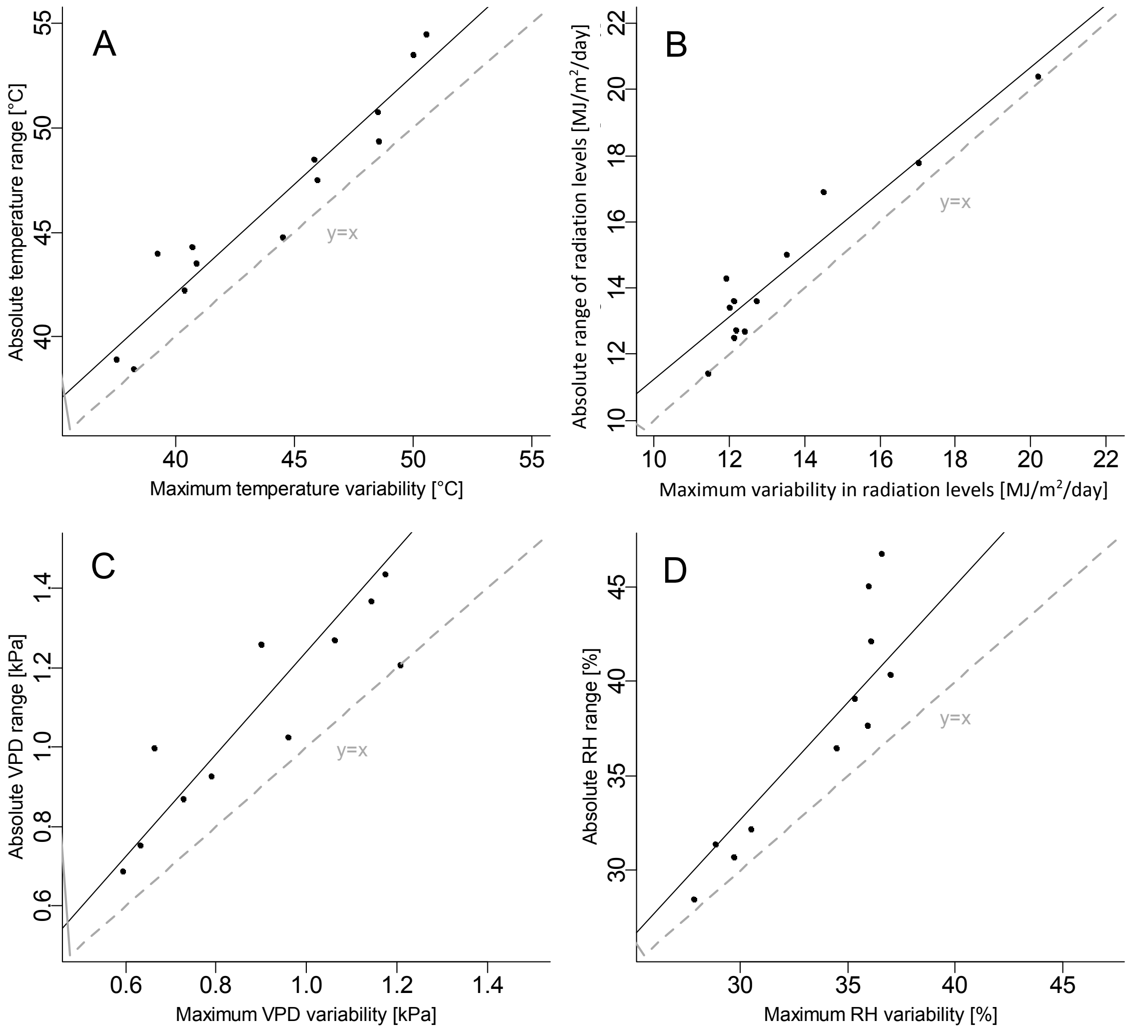


Fig. 6.4 Correlations between maximum variability at any one location within species' range and absolute range of conditions across their whole range for temperature (**A**), radiation (**B**), vapour pressure deficit (VPD; **C**) and relative humidity (RH; **D**). Absolute range of conditions across range was always greater than maximum local variability for all climate variables. Grey dashed lines show expected relationship if y=x.

RH at any given longitude, and even though trends in minimum RH were not unidirectional with longitudes. Maximum RH did not have a significant monotonic relationship with latitude (ρ= -0.0028, p = 0.77) but appeared highest at very low and very high latitudes. Mean RH increased, on average, slightly towards the tropics (ρ= 0.33, $p < 0.0001$) and minimum RH was, again, highly variable at any given latitude but, on average, increased towards the tropics (ρ= 0.20, $p < 0.0001$). Overall, VPD and RH indicated that "dryness" (maximum VPD and minimum RH) was more severe further inland, despite great variability at any given longitude. Latitudinal trends were less well defined. "Wetness" indicators (minimum VPD and maximum RH) were less variable and most extreme at the coast and in temperate regions. Overall, variability in hydric conditions appears to be driven more by high variability in "dryness".

As predicted by the environmental variability hypothesis, maximum temperature variability within species' ranges was significantly correlated with the absolute range of temperatures present across their whole distribution (**Fig. 6.4 A**; linear regression; $a = 0.34$, $b = 1.04$, $F_{1,11}=130.4$, $p < 0.0001$, $R^2=0.92$). The same was true for radiation (**Fig. 6.4 B**; $a = 1.83$, $b = 0.94$, $F_{1,11}=104$, $p<0.0001$, $R^2=0.90$), VPD (**Fig. 6.4 C**; $a = -0.048$, $b = 1.29$, $F_{1,11}= 52.97$, $p < 0.0001$, $R^2=0.81$) and RH (**Fig. 6.4 D**; $a = -4.50$, $b = 1.24$, $F_{1,11}= 56.85$, $p < 0.0001$, $R^2=0.82$). In all cases, the variability across species' whole ranges was greater than the maximum variability in any one location within their range (one sample t-test of the difference to a standard of zero; temperature: mean±sd $=2.28\pm1.42$, $t_{12}=5.77$, $p<0.0001$; radiation: mean±sd$=1.06\pm0.79$, $t_{12}=4.87$, $p<0.0005$; VPD: mean±sd$=0.29\pm0.17$, mean±sd$=3.91\pm3.06$, $t_{12}=4.85$, $p<0.0005$; RH: $t_{12}=4.60$, $p<0.001$).

Many species occurred in habitats with maximum temperatures higher than their upper critical thermal limits. Temperate species experienced maximum temperatures significantly further above their critical thermal limits than tropical species (**Fig. 6.5 A**; linear regression; $a = -4.52$, $b = -0.20$, $F_{1,11} = 23.36$, $p = 0.0005$), because maximum air temperatures are higher at temperate latitudes (**Fig. 6.2**), while CT_{max} does not vary with latitude (Chapter IV). Minimum temperatures were always lower than species' CT_{min}. Temperate species occurred at minimum temperatures further below their CT_{min} than tropical species (**Fig. 6.5 B**; linear regression; $a = -1.90$, $b = 0.37$, $F_{1,11} = 20.48$, $p = 0.0009$). Even though temperate rainbow skinks are more cold tolerant (Chapter IV), the decrease in temperature at high latitudes was steeper than the decrease in CT_{min}.

Hot and cold acclimated measures of many traits, including T_{opt}, CT_{max}, CT_{min}, water loss rate and metabolic rate, were collinear (Pearson's product moment correlation coefficient; $\rho \geq 0.6$). Hot acclimated traits were, therefore, often suitable alternate predictors for cold acclimated traits. There were several other notable collinearities. Species with higher preferred body temperatures had lower water loss rates ($\rho = -0.7$). Species with higher thermal optima were more cold sensitive (i.e. showed a greater decline in performance 5° below their optimum temperature; $\rho = 0.6$) but were more heat tolerant (i.e., had higher CT_{max}; $\rho = 0.7$). Species with higher hot-acclimated CT_{max} had higher CT_{max} acclimation potential ($\rho = 0.7$) and species with lower CT_{min} had higher CT_{min} acclimation potential ($\rho = -0.6$). Lower CT_{min}, but not higher CT_{max}, was collinear with absolute thermal tolerance breadth ($\rho = -0.7$). Lastly, species that showed characteristics often associated with adaptation to colder habitats, such as higher cold-acclimated metabolic rate (i.e., those that compensated more at cold temperatures), lower preferred body temperature (i.e., were active at colder temperatures), and lower CT_{min} (i.e., were more cold tolerant), had higher maximum endurance ($\rho = 0.7$, -0.6 and -0.6, respectively).

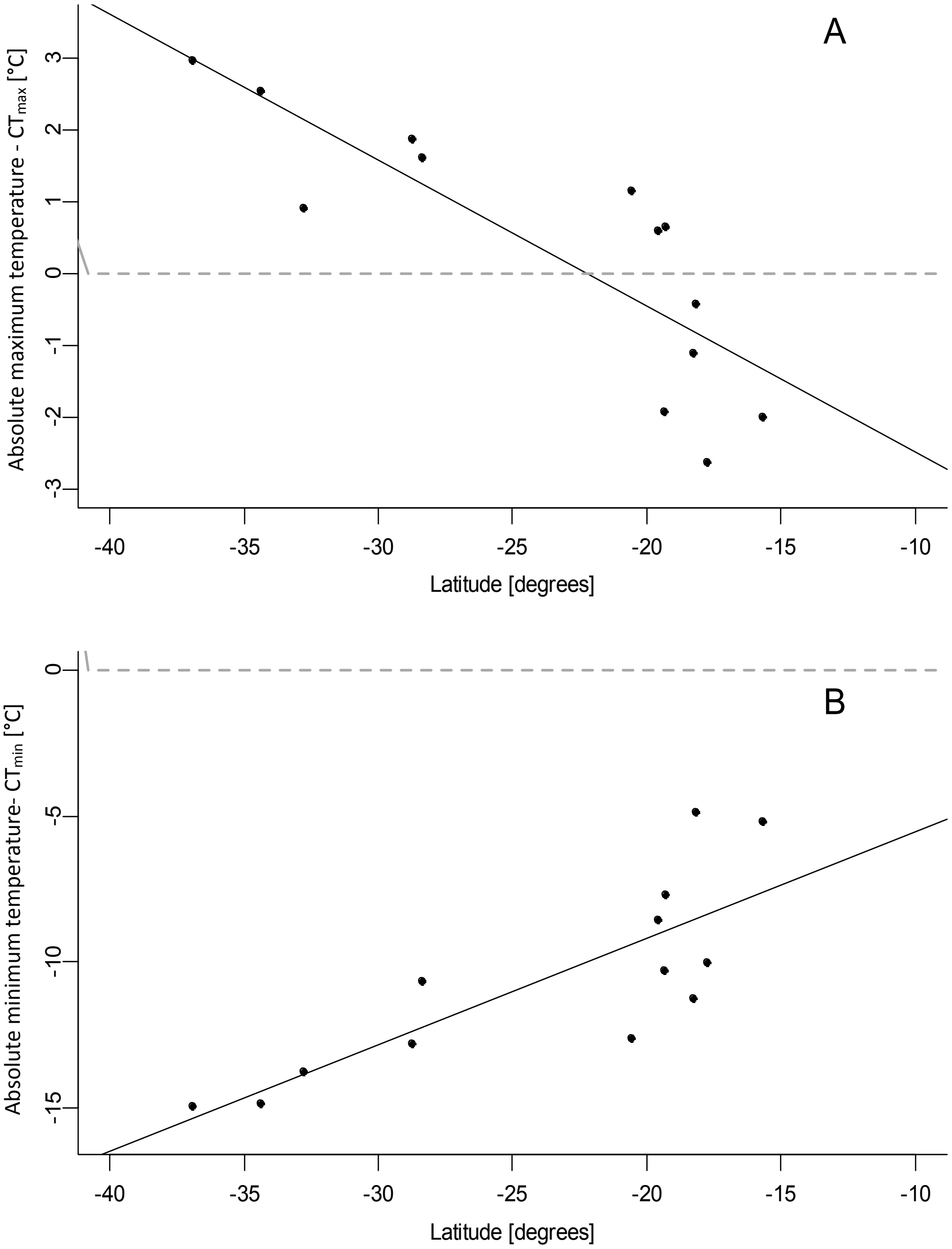


Fig. 6.5 Regression lines showing that species extending into more temperate habitats (i.e. into more temperate southernmost latitudes) experienced temperatures further above their CT_{max} (**A;** positive values) and further below their CT_{min} (**B;** more negative values). Dashed grey reference lines indicate a scenario in which there is no difference between experienced temperature extremes and thermal tolerance limits.

The traits that were the most common best single predictors of how far species' ranges extended into colder absolute (minimum) air temperatures or radiation levels as well as colder mean air temperatures or radiation levels, were cold acclimated CT_{min} (**Table 6.1**; **Fig. 6.6 A** and **B**) and cold acclimated metabolic rate (**Table 6.1**; **Fig. 6.6 E** and **F**). Species with higher cold acclimated metabolism and lower CT_{min} occurred in colder climates. Even though more cold tolerant species occurred in colder climates, minimum temperatures were substantially lower

Table 6.2. Best single physiological or behavioural predictors of different measures of maximum, minimum and mean temperature conditions species extend into within their ranges. Main significant predictors not collinear to any previous predictors (additional predictors) are in bold, those exhibiting collinearity with previous predictors (alternative predictors) are in normal font. Traits not significant but included in summary for comparison to predictors of other climate variables are shown in grey.*

Category	Environmental variable	Best single predictor	AIC	β	SE	p
Upper limits of temperature range	Absolute maximum temperature within range	**Thermal tolerance**	**53.02**	**1.63**	**0.69**	**0.04**
		CT_{max}^{H}	57.20	0.99	0.96	0.32
	Upper limit of mean annual temperatures across geographic range	**CT_{min}^{C}**	**50.29**	**2.78**	**0.58**	**<0.001**
		CT_{min}^{H}	57.05	2.63	0.86	0.01
		Warming tolerance$_{max}$	59.48	1.10	0.45	0.03
		Safety margin $_{max}$	**60.19**	**0.99**	**0.44**	**0.047**
	Maximum radiation within geographic range	**WLR^{C}**	**14.66**	**-0.25**	**0.067**	**0.004**
		WLR^{H}	18.72	-0.18	0.070	0.02
		CT_{max}^{H}	**19.65**	**0.53**	**0.23**	**0.04**
	Upper limit of mean annual radiation levels across geographic range	**CT_{min}^{C}**	**29.86**	**0.79**	**0.26**	**0.01**
		CT_{max}^{AP}	**31.49**	**1.82**	**0.71**	**0.03**
		CT_{max}^{H}	32.86	0.83	0.38	0.049
Lower limits of temperature range	Absolute minimum temperature within geographic range	**MR^{C}**	**69.18**	**-16.4**	**6.1**	**0.02**
		CT_{min}^{H}	**69.72**	**3.55**	**1.40**	**0.03**
		Maximum endurance	70.23	-0.097	0.041	0.04
		CT_{min}^{C}	70.47	2.92	1.26	0.04
	Lower limit of mean annual temperatures across geographic range	**CT_{min}^{C}**	**61.53**	**3.68**	**0.89**	**0.002**
		MR^{C}	**64.02**	**-17.6**	**5.0**	**0.005**
		CT_{min}^{H}	65.50	3.71	1.19	0.01
		Thermal tolerance	66.69	-3.26	1.16	0.02
		Maximum endurance	66.81	-0.099	0.036	0.02
		SPR $_{upper\ limit}$	68.81	2.42	1.08	0.046
	Minimum radiation within geographic range	**CT_{min}^{C}**	**43.23**	**3.26**	**0.44**	**<0.001**
		Thermal tolerance	58.59	-2.57	0.85	0.01
		MR^{C}	**60.14**	**-11.4**	**4.3**	**0.02**
		CT_{min}^{H}	58.59	2.35	1.01	0.04
	Lower limit of mean annual radiation levels across geographic range	**CT_{min}^{C}**	**28.96**	**1.37**	**0.25**	**<0.001**
		MR^{C}	**40.94**	**-4.6**	**2.1**	**0.048**
		Thermal tolerance	41.00	-0.95	0.43	0.049
		CT_{min}^{H}	42.44	088	0.49	0.10
Mean temperature conditions	Median mean annual temperature across geographic range	**CT_{min}^{C}**	**53.42**	**3.60**	**0.65**	**<0.001**
		CT_{min}^{H}	60.39	3.57	0.98	0.004
		Thermal tolerance	64.67	-2.73	1.08	0.03
		MR^{C}	**64.90**	**-12.9**	**5.2**	**0.03**
		Maximum endurance	65.38	-0.079	0.033	0.04
	Median mean radiation levels across geographic range	**CT_{min}^{C}**	**25.07**	**0.98**	**0.22**	**<0.001**
		CT_{min}^{H}	33.60	0.80	0.35	0.04

*Superscripts indicate whether the hot (H) or cold (C) acclimated trait or its acclimation potential (AP) is referred to.

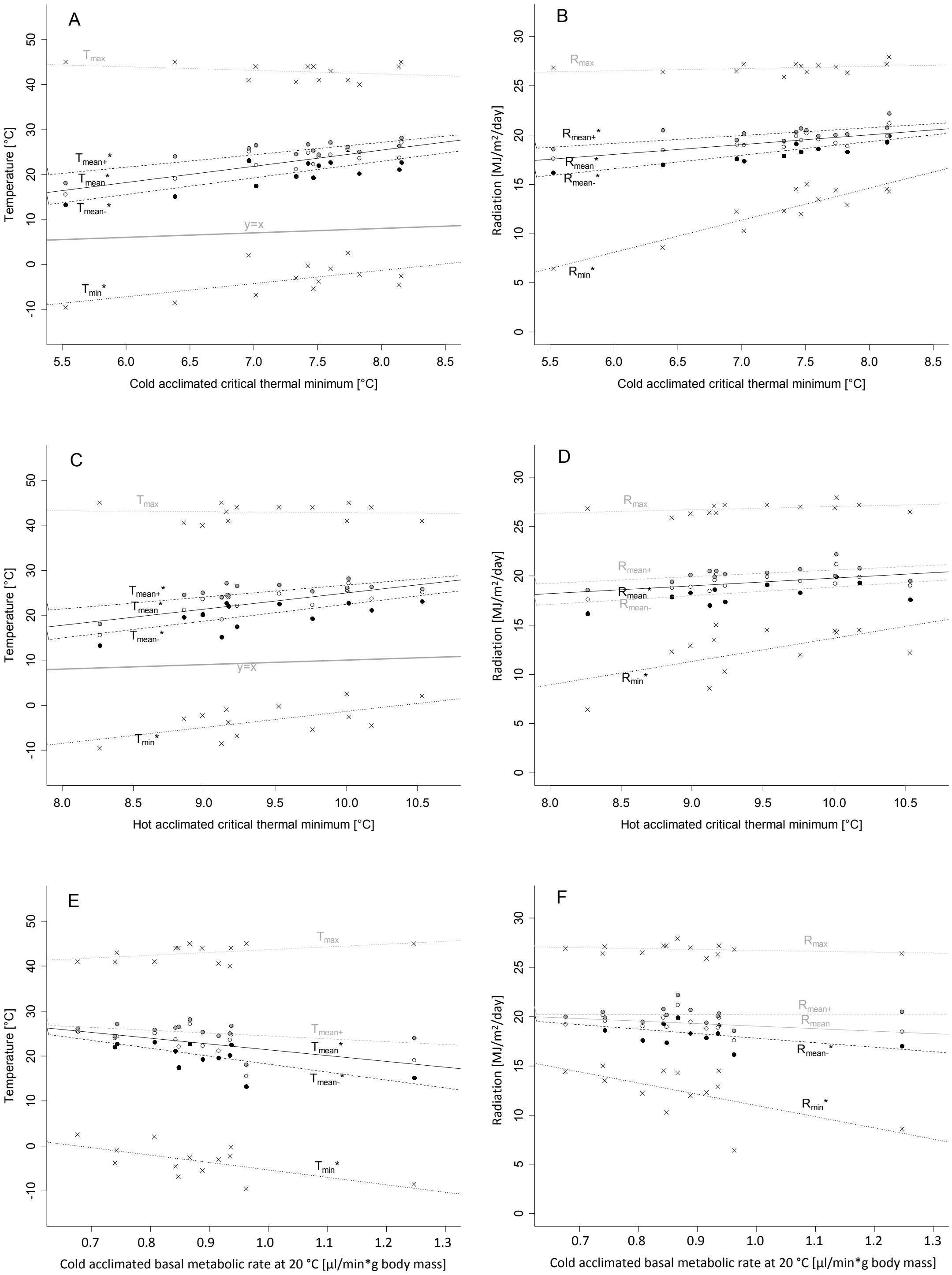


Fig. 6.6 Best single predictors of species' potential to extend into low temperatures (cold acclimated CT_{min}: **A** and **B**; cold acclimated metabolic rate at 20 °C: **E** and **F**) and their relationship with absolute minimum (T/R_{min}; lower dotted line), minimum mean (T/R_{mean-}; lower dashed line), mean (T/R_{mean}; solid line), maximum mean (T/R_{mean+}; upper dashed line) and absolute maximum (T/R_{max}; upper dotted line) temperature (**A**, **C** and **E**) and radiation (**B**, **D** and **F**). Relationships of climate variables with an alternative predictor (hot acclimated CT_{min}: **C** and **D**) are shown for comparison purposes. Insignificant relationships are shown in grey, significant ones in black (marked by asterisk). In **A** and **C**, grey y=x lines show expected relationship if CT_{min} were identical to minimum temperatures experienced within ranges.

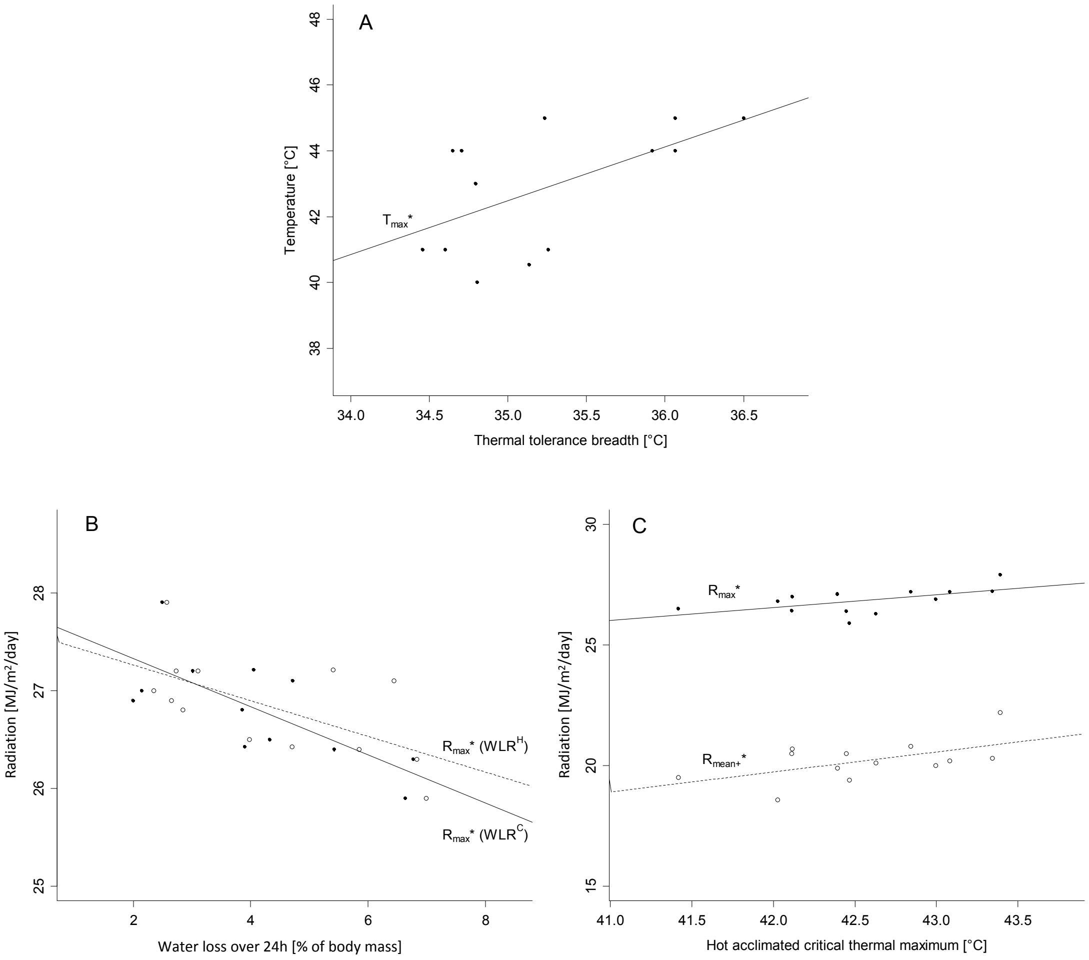


Fig. 6.7 Best single predictors of species' potential to extend into hot conditions as measured by maximum temperatures (T_{max}; **A**) and maximum solar radiation levels (R_{max}; **B**). These were thermal tolerance for T_{max} (**A**), and cold and hot acclimated water loss rates (WLR^C and WLR^H as alternative predictor) as well as hot acclimated CT_{max} for R_{max} (**B** and **C**). Upper mean radiation levels (R_{mean+}) were included in **C** for comparison purposes, as they were also significantly correlated to the best predictor of maximum radiation levels (R_{max}). Lines are linear regression predictor lines. Significance is marked by an asterisk.

than CT_{min} (thick grey lines for x=y in **Fig. 6.6 A** and **C**). Hot acclimated CT_{min} was a significant alternative predictor to cold acclimated CT_{min} in most cases (**Table 6.1**; **Fig. 6.6 C** and **D**).

No single trait consistently predicted how far species extended into hot climates when environmental exposure to heat was estimated using absolute maximum or maximum mean air temperatures or radiation levels within ranges. However, tolerance of higher absolute maximum air temperature was predicted well by absolute physiological thermal tolerance breadth (but not by CT_{max}; **Table 6.1**; **Fig. 6.7 A**) and environmental tolerance of higher maximum solar

Table 6.3. Best single physiological or behavioural predictors for different measures of maximum, minimum and mean hydric conditions species extend into within their ranges. Main significant predictors not collinear to any previous predictors (additional predictors) are in bold, significant predictors exhibiting collinearity with previous predictors (alternative predictors) are in normal font and non-significant predictors included for comparison purposes with predictors of similar environmental variables are shown in grey.*

category	Environmental variable	Best single predictor	AIC	β	SE	p
Maximum "Dryness"	Maximum VPD within geographic range	**WLR^{C}**	**10.48**	**-0.15**	**0.057**	**0.03**
		CT_{max}^{AP}	**11.03**	**0.78**	**0.32**	**0.03**
		CT_{max}^{H}	11.53	0.38	0.17	0.04
		WLR^{H}	12.05	-0.12	0.054	0.057
	Upper limit of mean annual VPD across geographic range	**CT_{max}^{H}**	**2.30**	**0.29**	**0.12**	**0.03**
		CT_{max}^{AP}	2.65	0.55	0.23	0.04
		WLR^{C}	3.30	-0.095	0.044	0.051
		WLR^{H}	5.41	-0.065	0.042	0.15
	Minimum RH within geographic range	**WLR^{C}**	**84.10**	**3.23**	**0.98**	**0.007**
		WLR^{H}	85.77	2.66	0.92	0.01
		CT_{max}^{AP}	**88.10**	**-14.16**	**6.24**	**0.04**
		CT_{max}^{H}	90.87	-4.99	3.49	0.18
	Lower limit of mean annual RH across geographic range	**CT_{max}^{AP}**	**80.15**	**-11.47**	**4.60**	**0.03**
		WLR^{C}	**80.26**	**2.07**	**0.84**	**0.03**
		WLR^{H}	80.81	1.77	0.76	0.04
		CT_{max}^{H}	83.26	-4.17	2.61	0.14
Maximum "Wetness"	Minimum VPD within geographic range	**MR^{C}**	**-30.06**	**-0.42**	**0.14**	**0.01**
		T_{opt}^{C}	**-26.73**	**0.066**	**0.029**	**0.046**
		CT_{min}^{C}	-26.46	0.065	0.030	0.053
	Lower limit of mean annual VPD across geographic range	T_{opt}^{AP}	-18.33	-0.12	0.056	0.059
		T_{opt}^{C}	-18.01	0.082	0.041	0.069
	Maximum RH within geographic range	**CT_{min}^{C}**	**64.70**	**-2.50**	**1.01**	**0.03**
		Thermal tolerance	66.27	2.35	1.14	0.064
		CT_{min}^{H}	66.66	-2.42	1.25	0.078
	Upper limit of mean annual RH across geographic range	**T_{opt}^{AP}**	**59.35**	**2.54**	**1.11**	**0.042**
Mean hydric conditions	Median mean annual VPD across geographic range	CT_{min}^{C}	-10.06	0.11	0.057	0.08
		WLR^{C}	-9.84	-0.049	0.026	0.090
	Median mean annual RH across geographic range	Heat Sensitivity	60.68	0.82	0.41	0.07
		T_{opt}^{AP}	61.40	2.15	1.20	0.10

*Superscripts indicate whether the hot (H) or cold (C) acclimated trait or its acclimation potential (AP) is referred to.

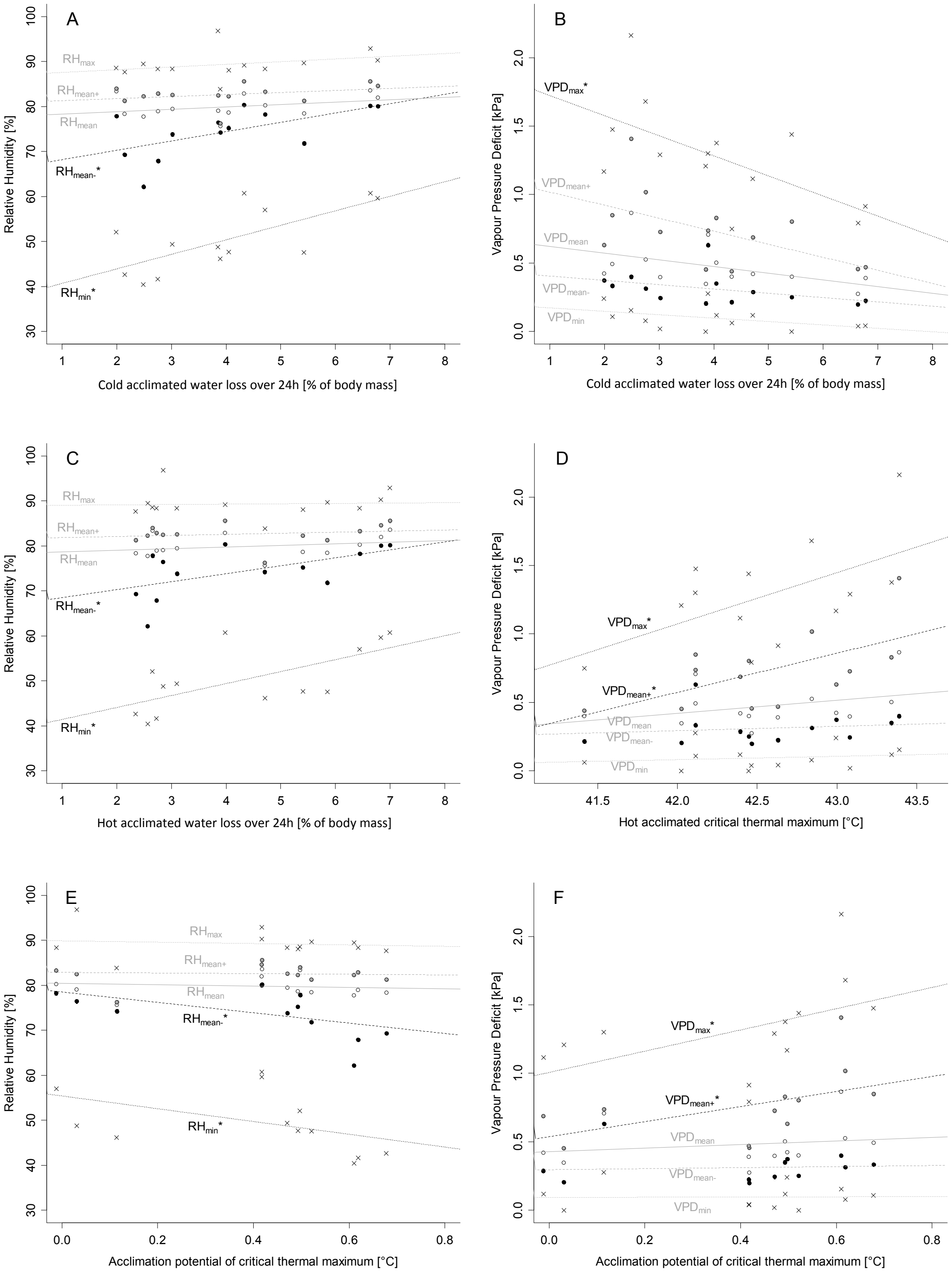


Fig. 6.8 Best single predictors of species' potential to extend into dry conditions (cold acclimated water loss: **A** and **B**; CT_{max} acclimation potential: **E** and **F**) and their relationship with absolute minimum (RH/VPD_{min}; lower dotted line), minimum mean (RH/VPD_{mean-}; lower dashed line), mean (RH/VPD_{mean}; solid line), maximum mean (RH/VPD_{mean+}; upper dashed line) and absolute maximum (RH/VPD_{max}; upper dotted line) relative humidity (RH: **A**, **C** and **E**) and vapour pressure deficit (VPD; **B**, **D** and **F**). Correlations of RH (**C**) and VPD (**D**) with the alternative predictors hot acclimated water loss (**C**) and CT_{max} (**D**) are shown for comparison purposes. Insignificant and significant predictor lines are shown in grey and black.

radiation was best predicted by water loss rates (hot or cold acclimated; **Fig. 6.7 B**) or, additionally, by hot acclimated $\mathrm{CT}_{\mathrm{max}}$ (**Table 6.1**; **Fig. 6.7 C**).

Species' potential to extend into drier habitats to different degrees, measured as the maximum VPD or minimum RH experienced within their range, was best predicted by cold acclimated water loss rates (**Table 6.2**; **Fig. 6.8 A** and **B**) and by the acclimation potential of their $\mathrm{CT}_{\mathrm{max}}$ (**Table 6.2**; **Fig. 6.8 E** and **F**). Species with lower water loss rate and greater acclimation potential of $\mathrm{CT}_{\mathrm{max}}$ extended into drier conditions. Hot acclimated water loss rate was a significant alternative predictor to cold acclimated water loss with respect to the lowest RH

Table 6.4. Best single physiological or behavioural predictors for different measures of temperature variability species extend into within their ranges. Main significant predictors not collinear to any previous predictors (additional predictors) are in bold, significant predictors exhibiting collinearity with previous predictors (alternative predictors) are in normal font and non-significant predictors included for comparison purposes with predictors of similar environmental variables are shown in grey.*

category	Environmental variable	Best single predictor	AIC	β	SE	p
Variability in temperature	Absolute temperature range across geographic range	**MR^{C}**	**77.44**	**22.5**	**8.4**	**0.02**
		Thermal tolerance	**77.64**	**4.63**	**1.77**	**0.02**
		Maximum endurance	77.77	0.14	0.054	0.03
	Mean temperature range across geographic range	**MR^{C}**	**55.16**	**11.4**	**3.6**	**0.009**
		MR^{H}	60.21	3.6	2.0	0.097
		Thermal tolerance	60.47	1.58	0.91	0.11
	Highest absolute temperature variability in any one location within geographic range	**Maximum endurance**	**77.00**	**0.12**	**0.053**	**0.049**
		MR^{C}	77.18	18.0	8.4	0.054
		Thermal tolerance	77.59	3.60	1.77	0.066
Variability in radiation	Absolute radiation range across geographic range	**$\mathrm{CT}_{\mathrm{min}}^{C}$**	**47.24**	**-3.02**	**0.51**	**<0.001**
		Thermal tolerance	56.11	2.68	0.77	0.005
		MR^{C}	**60.18**	**10.5**	**4.3**	**0.03**
		Maximum endurance	60.89	0.063	0.028	0.05
		$\mathrm{CT}_{\mathrm{min}}^{H}$	61.85	-2.02	1.04	0.077
	Mean radiation range across geographic range	**MR^{C}**	**27.47**	**4.6**	**1.2**	**0.003**
		Thermal tolerance^{AP}	33.44	0.79	0.36	0.053
		Thermal tolerance	34.32	0.64	0.33	0.082
	Highest absolute radiation variability in any one location within geographic range	**$\mathrm{CT}_{\mathrm{min}}^{C}$**	**48.20**	**-3.02**	**0.53**	**0.001**
		Thermal tolerance	54.13	2.89	0.72	0.002
		Maximum endurance	58.81	0.074	0.026	0.02
		MR^{C}	**60.29**	**10.7**	**4.4**	**0.03**
		$\mathrm{CT}_{\mathrm{min}}^{H}$	61.06	-2.25	1.01	0.046

*Superscripts indicate whether the hot (H) or cold (C) acclimated trait or its acclimation potential (AP) is referred to.

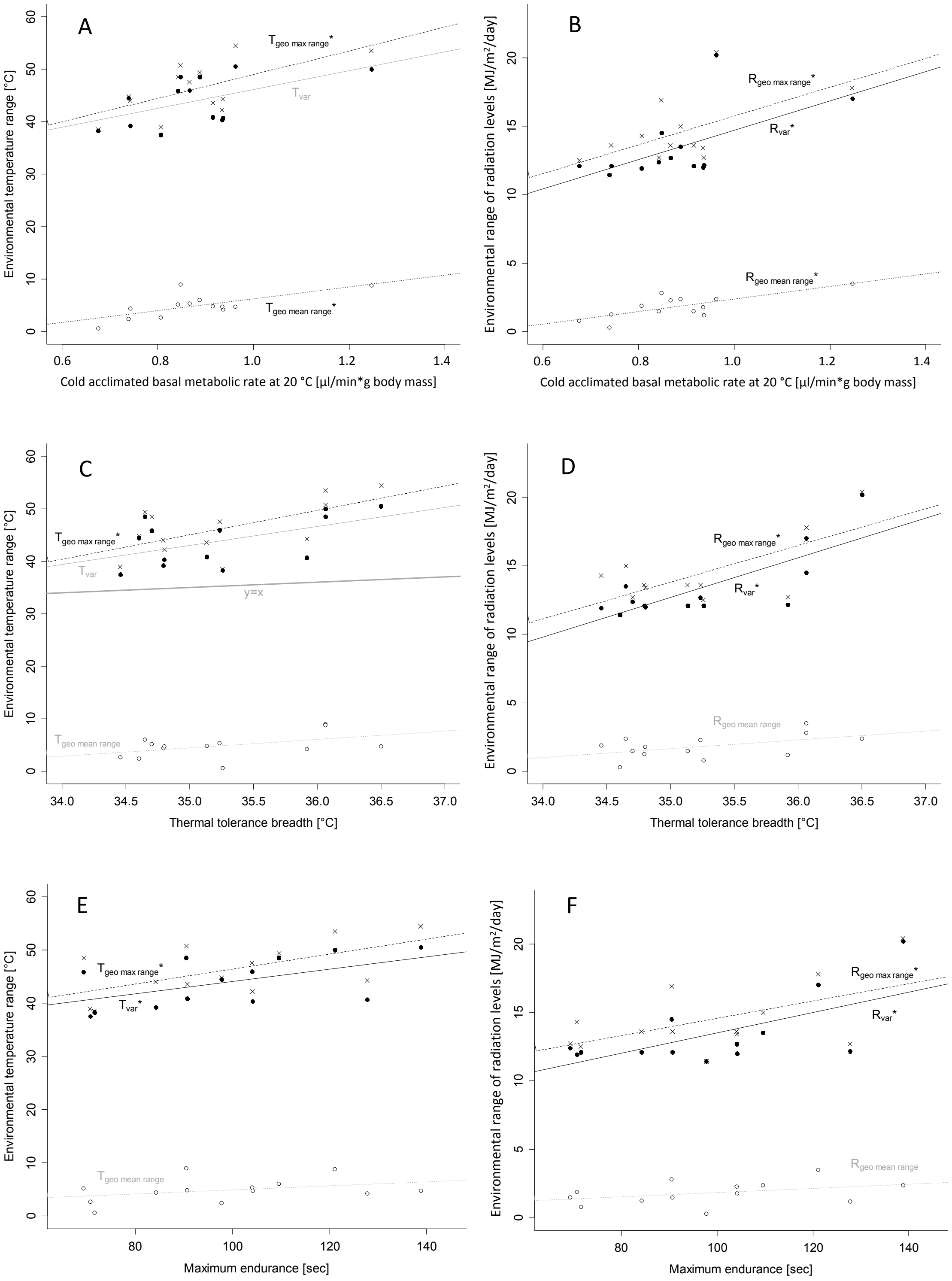


Fig. 6.9 Best single predictors of species' potential to occur into habitats with high thermal variability (cold acclimated metabolic rate: **A** & **B**; thermal tolerance breadth: **C** & **D**; maximum endurance: **E** & **F**) measured as variability in temperature (**A**, **C**, and **E**) and radiation (**B**, **D**, and **F**) and their relationship of predictors with absolute geographic variability ($T/R_{\text{geo max range}}$), geographic variability of mean conditions ($T/R_{\text{geo mean range}}$) and highest local variability within geographic ranges (T/R_{var}). Lines are linear regression predictor lines. Insignificant relationships are shown as grey, significant ones as black lines with asterisk.

(**Fig. 6.8 C**) but not the highest VPD species extended into (although only barely insignificant; **Table 6.2**). Hot acclimated CT_{max} was a significant alternative predictor to acclimation potential of CT_{max} for maximum VPD tolerated by species (although water loss was a better predictor) and was the best single predictor for upper limits of mean VPD. Overall, species with high tolerance to heat and high potential to acclimate tolerance to hot conditions extended into drier conditions (**Table 6.2**; **Fig. 6.8 D**). Mean hydric conditions that different species occurred in could not be predicted by any of the physiological traits included in this study (**Table 6.2**). Environmental tolerance of wet conditions was predicted by similar traits as environmental cold tolerance, namely cold acclimated metabolic rate and CT_{min}, but the best predictors were inconsistent across different variables associated with this climate dimension (**Table 6.2**).

We tested species' potential to deal with two different kinds of thermal variability in this study: absolute geographic variability (e.g. difference between hottest and coldest habitat: T_{geo}) and highest local variability (difference between highest and lowest temperature experienced in the most variable spot within a species' range; T_{var}). Greater T_{geo} tolerated was best predicted by higher cold acclimated metabolic rates (**Table 6.3**; **Fig. 6.9 A**), as well as by absolute thermal tolerance breadth (**Table 6.3**; **Fig. 6.9 B**). However, neither of these traits were significant predictors of maximum T_{var} species could tolerate within their range. The only significant predictor of T_{var} was maximum endurance, and this trait was also a significant alternative predictor of T_{geo} (**Table 6.3**; **Fig.9 C**), making it the only trait that predicted the degree to which different species could cope with different amounts of both types of thermal variability. Both, T_{geo} as well as T_{var} were consistently far higher than physiological thermal tolerance breadth (thick grey line for x=y in **Fig. 6.9 B**), i.e. environmental tolerances as estimated from occurrence records were greater than physiological tolerances.

Variability in radiation levels tolerated were best predicted by cold acclimated metabolic rate (**Fig. 6.9 B**) and CT_{min} (**Table 6.3**), while overall physiological thermal tolerance and maximum endurance were significant alternative predictors (**Fig. 6.9 D** & **F**).

The most common best predictor of any of the proxies used for thermal variability species can tolerate either at any given point within their range or across their whole range, both of which are mostly driven by variation in minimum temperatures and minimum radiation (Fig. 6.2), was cold acclimated metabolic rate, which was also one of the best predictors of environmental cold tolerance (**Table 6.1**; **Fig. 6.6 E** and **F**).

Variability in hydric conditions was mainly driven by variability in "dryness". The same traits that predicted how far species could extend into dry habitats, namely water loss rates and acclimation potential of CT_{max} (**Table 6.4**)., also predicted well how far they extended into habitats with more variable hydric conditions.

Table 6.5. Best single physiological or behavioural predictors for different measures of hydric variability species extend into within their ranges. Main significant predictors not collinear to any previous predictors (additional predictors) are in bold, significant predictors exhibiting collinearity with previous predictors (alternative predictors) are in normal font and non-significant predictors included for comparison purposes with predictors of similar environmental variables are shown in grey.*

category	Environmental variable	Best single predictor	AIC	β	SE	p
Variability in hydric conditions (VPD)	Absolute VPD range across geographic range	**CT$_{max}^{AP}$**	**10.06**	**0.77**	**0.31**	**0.03**
		CT$_{max}^{H}$	11.30	0.35	0.16	0.055
		WLR^{C}	11.72	-0.12	0.060	0.068
		WLR^{H}	12.00	-0.10	0.054	0.079
	Mean VPD range across geographic range	**CT$_{max}^{AP}$**	**-0.74**	**0.52**	**0.20**	**0.03**
		CT$_{max}^{H}$	-0.41	0.25	0.10	0.03
		WLR^{C}	2.71	-0.064	0.043	0.16
		WLR^{H}	3.21	-0.051	0.039	0.21
	Highest absolute VPD variability in any one location within geographic range	**WLR^{H}**	**0.47**	**-0.091**	**0.035**	**0.02**
		WLR^{C}	2.34	-0.089	0.042	0.058
Variability in hydric conditions (RH)	Absolute RH range across geographic range	**WLR^{H}**	**85.02**	**-2.58**	**0.90**	**0.02**
		WLR^{C}	86.72	-2.62	1.08	0.03
		CT$_{max}^{AP}$	88.22	12.65	6.27	0.069
	Mean RH range across geographic range	**CT$_{max}^{AP}$**	**76.85**	**10.81**	**4.05**	**0.02**
		WLR^{H}	78.77	-1.51	0.70	0.055
		WLR^{C}	79.34	-1.61	0.81	0.072
		CT$_{max}^{H}$	79.83	4.21	2.28	0.092
	Highest absolute RH variability in any one location within geographic range	**Maximum endurance**	**78.62**	**0.14**	**0.056**	**0.03**
		Thermal tolerance	78.67	4.56	1.84	0.03
		WLR^{H}	**78.82**	**-1.72**	**0.71**	**0.03**
		T$_{sel\ min}$	**79.33**	**2.38**	**1.03**	**0.04**
		WLR^{C}	80.80	-1.62	0.86	0.086

*Superscripts indicate whether the hot (H) or cold (C) acclimated trait or its acclimation potential (AP) is referred to.

Discussion

Because climate change will increase mean air temperatures across much of the globe (IPCC 2013), heat tolerances have recently received much attention with respect to species' vulnerability to climate change. However, maximum air temperatures vary little geographically when compared to minimum temperatures (**Fig. 6.2 A & B**). Furthermore, maximum temperatures decrease slightly in the tropics, tropical species are currently further below their CT_{max} because of this (**Fig. 6.5 A**), and CT_{max} is not a significant predictor of maximum air temperatures species are exposed to within their ranges (**Table 6.1**). CT_{max} is, however, a significant predictor of maximum radiation levels species currently tolerate. This suggests that, within this clade of diurnal ectotherms, (i) tropical species are under less current heat stress than temperate species, contradicting results of previous research (Deutsch et al. 2008) and (ii) heat stress caused by radiation limits species more, geographically, than do maximum air temperatures, and can be predicted using upper thermal limits. Furthermore, water loss rates can successfully predict the "dryness" of habitats that species can occupy (**Table 6.2**) and are, therefore, of underappreciated relevance with respect to the differential potential for dry-skinned ectotherm species to spread from coastal habitats into the arid interior of Australia.

Even though high temperatures may be physiologically more limiting to an ectothermic organism (Vickers et al. 2011), my results show that, across Eastern Australia, high temperatures (measured as air temperatures) are unlikely to be an important limiting factor, as they vary little compared to other climate variables (**Fig. 6.2A & B**). Furthermore, maximum air temperatures were lower in regions that are, on average, warmer (e.g., the tropics), contradicting the prediction that organisms from habitats with high mean temperatures are at higher risk of overheating (Deutsch et al. 2008). Maximum radiation, however, did increase in on-average warmer environments and showed clear latitudinal and longitudinal trends, which indicates that it may limit species physiologically and geographically more than high air temperatures. Nevertheless, the climate variables that varied the most, geographically, were measures that influenced minimum body temperatures (minimum air temperature and minimum radiation) and measures of dryness (minimum RH and maximum VPD; **Fig. 6.2 & 6.3**). These are, consequently, the variables that contributed the most to differences in environmental variability, and to mean conditions, and are the variables most likely to limit species' geographical ranges. Physiological differences allowing species to extend into these climatic extremes to different degrees, are therefore the ones of greatest interest to us, when trying to predict species' potential distributions and responses to climate change.

Our results suggest that species from more variable habitats occur over a broader range of climatic conditions, as predicted by the climatic variability hypothesis (**Fig. 6.4**). However, environmental variability is mostly driven by geographic variation in minimum temperatures and dryness (**Fig. 6.2 & 6.3**). Broad tolerance of organisms to environmental conditions is therefore of less relevance than their ability to cope with these particular extremes. This is confirmed by my finding that the main determinants of species' potential to extend into cold or dry conditions (**Table 6.1 & 6.2**; **Fig. 6.6 & 6.8**) were also good predictors of their ability to extend into habitats with high overall variability in temperature and dryness (**Table 6.3** & **6.4**; **Fig. 6.9**). Explicit tests of species' ability to cope with these particular extremes in climate are, therefore, of much greater interest than the overall breadth of conditions they can occur under. Additionally, the range of air temperatures or radiation levels in which species can occur across their range is significantly correlated to their physiological thermal tolerance breadth (**Fig. 6.9 B** & **D**). However, all species in this study used environments with extremes greater than their physiological thermal tolerances (**Fig. 6.5** & **6.9 B**). It is, therefore, clear that climatic extremes are successfully buffered by strong behavioural avoidance of detrimental conditions (Sunday et al. 2014, Buckley et al. 2015). The fact that species with higher maximum endurance occurred in more variable habitats with respect to variability in air temperature, radiation or relative humidity (**Table 6.3** & **6.4**; **Fig. 6.9**), suggests that increased ability to cope with variable climatic conditions may be related to greater dispersal abilities or smaller reductions in absolute rather than relative (compared to optimum) performance when exposed to fluctuating conditions. While physiological thermal tolerances can be used to predict environmental tolerances in some cases (e.g. **Fig. 6.6**), one consequently has to distinguish between the two. In fact, animals appear to avoid the greater extremes at higher latitudes more effectively. As a result, physiological tolerances increasingly underestimate current environmental tolerances in more variable habitats: even though high latitude species are physiologically more cold-tolerant (Chapter IV), environmental temperatures within their ranges also fall further below their tolerance limits than for tropical species (**Fig. 6.5 B**). Similarly, temperatures at high latitudes exceed temperate species' physiological limits, while air temperatures at low latitudes even remain below the CT_{max} of tropical species (**Fig. 6.5 A**). Because CT_{max} does not vary with latitude (Chapter IV), this translates into a greater need for temperate species to avoid high air temperature, contradicting predictions that tropical species are currently closer to their physiological limits (Deutsch et al. 2008) with respect to air temperatures.

While ambient air temperatures are likely to fluctuate slowly enough across time of day to allow animals to avoid them behaviourally (Buckley et al. 2015), above ground activity can easily lead to accidental, prolonged exposure to full sun, and consequently to a quick rise in body temperature: operative temperatures of ectotherms in most environments, therefore, exceed air temperatures (Vickers et al. 2011, Sunday et al. 2014). For small diurnal ectotherms with poor thermal buffering, sudden overheating through exposure to radiation is, consequently, an important threat in open (no shade), arid (less cloud cover) or other high-radiation or high-exposure habitats. Because radiation is higher in the tropics and in the arid interior of Eastern Australia (**Fig. 6.2 C** & **D**), being able to physiologically tolerate the potentially high body temperature caused by high radiation levels should limit species' distributions more than exposure to high air temperatures (**Table 6.1**, **Fig. 6.7 C**). Indeed, upper thermal limits from my study predicted maximum radiation levels species could occur at well, but failed to predict maximum air temperatures, confirming these expectations. Thermal tolerance breadth was the only significant single predictor of extension into environments of greater maximum air temperatures (**Fig. 6.7 A**): overall more generalist species extended into hotter habitats but not because of their physiological tolerance to high temperatures.

As suggested by my assessment of geographic variation in climatic conditions within the study region, physiological traits associated with tolerance of cold temperatures significantly predicted how far species could extend into colder climates. More specifically, lower CT_{min} as well as higher metabolic compensation at low temperatures appear to be the main traits that allow species to occur in colder climates (measured as low air temperatures or low radiation levels available for basking; **Table 6.1**, **Fig. 6.6**). Similarly, my expectation that species with lower water loss rates could use drier habitats was met (**Table 6.2**, **Fig. 6.8**). The limits that desiccation resistance may place on species' distributions and activity times is underappreciated for dry-skinned ectotherms, and requires urgent attention because climate change is likely to lead to substantial changes in precipitation, seasonality, drought frequency and aridity (Hughes 2003, Garnaut 2008, IPCC 2013). Increases in temperature alone can cause increased desiccation rates, even if other environmental conditions remain the same. Dehydration may, therefore, be of much greater relevance with respect to species' vulnerability to climate change than is tolerance of high air temperatures. This is supported by my additional observation that water loss rates are the best predictor of how far species extend into habitats with high radiation levels. Even though heat tolerance enables species to use habitats with higher radiation levels, increased water loss in these hot environments appears to be a stronger limiting factor. Another physiological trait that appeared to limit species' tolerance of dry environments was the acclimation potential of CT_{max}, despite a lack of significance of heat tolerance as a predictor in itself. This is possibly caused by correlation between habitat aridity and radiation exposure. If drier habitats also have higher radiation exposure because of reduced cloud and vegetation cover, the potential for upper thermal limits to acclimate is essential for survival if exposed ground needs to be traversed frequently. Exposure in this respect may relate to both the direct impact of radiation on an organism, as well as to the impact of radiation on the temperature of the traversed substrate. If hot acclimation facilitates short term survival in response to frequent high exposure, it would also explain why CT_{max} acclimates much more rapidly (Hutchison and Maness 1979) than CT_{min} (Chapter II; Pintor et al. 2016).

Interestingly, hot acclimated traits were often alternative predictors to cold acclimated traits (**Table 6.1** to **6.4**; **Fig. 6.6 C & D**; **Fig. 6.7 B**; **Fig. 6.8 C**). I therefore suggest that, because physiological tolerance limits cannot be used interchangeably with environmental tolerance limits even when fully acclimated, and because extensive acclimation in the laboratory is not always possible, data on hot acclimated water loss rates and hot acclimated tolerance to low temperatures traits may be used to predict species' differential sensitivity to climatic extremes, as long as acclimation state is similar across all individuals (e.g., all collected in the same season). When assessing species' differential sensitivities to climate variables on a large scale, hot (i.e. summer) acclimatised CT_{min} and water loss rates may, therefore, be the most easily collected, and most broadly applicable traits, as they can predict environmental tolerance of low temperatures (CT_{min}), minimum radiation (CT_{min}), maximum radiation (water loss) and maximum VPD or minimum RH (water loss). Measurements of these traits are also less risky to the wellbeing of study animals than measurements of CT_{max}. Predicting environmental tolerance to maximum air temperatures and "wetness" is less relevant.

Notably, many of the traits included in my analysis, such as preferred body temperatures, set point range, thermal optima, maximum performance, heat or cold sensitivity of performance, behavioural thermal safety margins and behavioural warming varied among the species in this study (Chapter IV & V). Despite this, these traits did not emerge as best predictors of species' potential to use climatic extremes. For example, temperate species tend to have higher minimum preferred body temperatures (Chapter IV), possibly because achieving high body temperatures is beneficial in colder environments. However, such behavioural traits did not predict the range of climatic extremes that could be tolerated by different species. Determining the traits that are the most limiting to species' environmental tolerance of extremes is crucial if we want to develop tools that help us assess species' climatic sensitivity. If we understand which physiological traits are currently most limiting to species in different dimensions of their niche, we can predict their potential to cope with climate change and focus conservation efforts on species that are near their physiological thresholds, or on habitats that will experience large changes in relevant climate variables. I have provided an initial assessment of which traits we should focus on, and which climate dimensions are currently most limiting, but further research is urgently needed to verify the potential of these traits to limit species' distributions across larger geographic and taxonomic scales.

Conclusion

In this thesis I have provided novel insight into fine scale patterns in thermal trait variation along natural climate gradients and determined which ones of the examined traits have the potential to be the most physiologically and geographically limiting. Most notably, the degree of compensation of metabolic rates and cold tolerances in colder climates as well as water loss rates and acclimation potential of heat tolerances in drier regions appear to restrict species the most in those two dimensions. Furthermore heat tolerance appears to enable species to extend into habitats with higher radiation levels, while air temperatures or tolerance thereof appear to be much less limiting geographically than often predicted. With respect to climate change, changes in radiation and moisture deficit may therefore be more threatening than rises in air temperature. Mean air temperature, particularly, is likely to have little predictive capacity in this context. If changing air temperatures have a detrimental effect, it is more likely to be due to reduced activity times (due to thermoregulation as well as hydroregulation) and because of increased metabolic rates (i.e. energy expenditure) than the simple tolerance of temperature *per se*. These factors, however, are more likely to be of concern in habitats where species currently already experience temperatures above their thermal tolerances and preferred body temperatures (i.e. at higher latitudes; see Chapter VI), or in habitats where moisture deficit is likely to either already be limiting (arid regions) or likely to change substantially. Notably, these predictions apply to ectotherms and cannot necessarily be transferred to endotherms, mostly because endotherms carry an additional thermal load resulting from their metabolic heat production (Speakman and Król 2010) and are, therefore, more sensitive to high ambient temperatures. The relevance of water loss rates with respect to water availability, however, is likely to be even more applicable to endotherms, because of their increased use of panting or sweating and, consequently water loss, at high temperatures (Robertshaw 2006).

In summary, several steps need to be taken to improve our approach to macrophysiology. We need to

(i) concentrate on the mechanisms underlying large-scale biogeographic patterns, rather than focussing on the presence or absence of the pattern itself.
(ii) develop standardized methodologies that increase our potential to use published data in meta-studies without distorting results,
(iii) acknowledge that environmental tolerances and physiological tolerances are not equivalent because of the intricate links between physiology and behaviour,
(iv) acknowledge the importance of water loss, even in dry-skinned ectotherms,

(v) consider the importance of physiological traits other than merely physiological thermal tolerances for species fitness in different climates, as well as incorporate temporal scales (acclimation and adaptation) into comparative physiological analyses,

(vi) carefully determine the most physiologically and geographically limiting climate variables and traits, when making predictions on species' vulnerability to climate change.

The direct implication resulting from this work is that large scale assessments of relevant, easily collected physiological traits such as water loss rates and critical thermal minima may provide detailed information on species' potential distributions now, and in the future, as long as acclimation state is standardized. Such studies should be conducted at broad geographic and taxonomic scales to improve our understanding of species' differential vulnerability to climate change.

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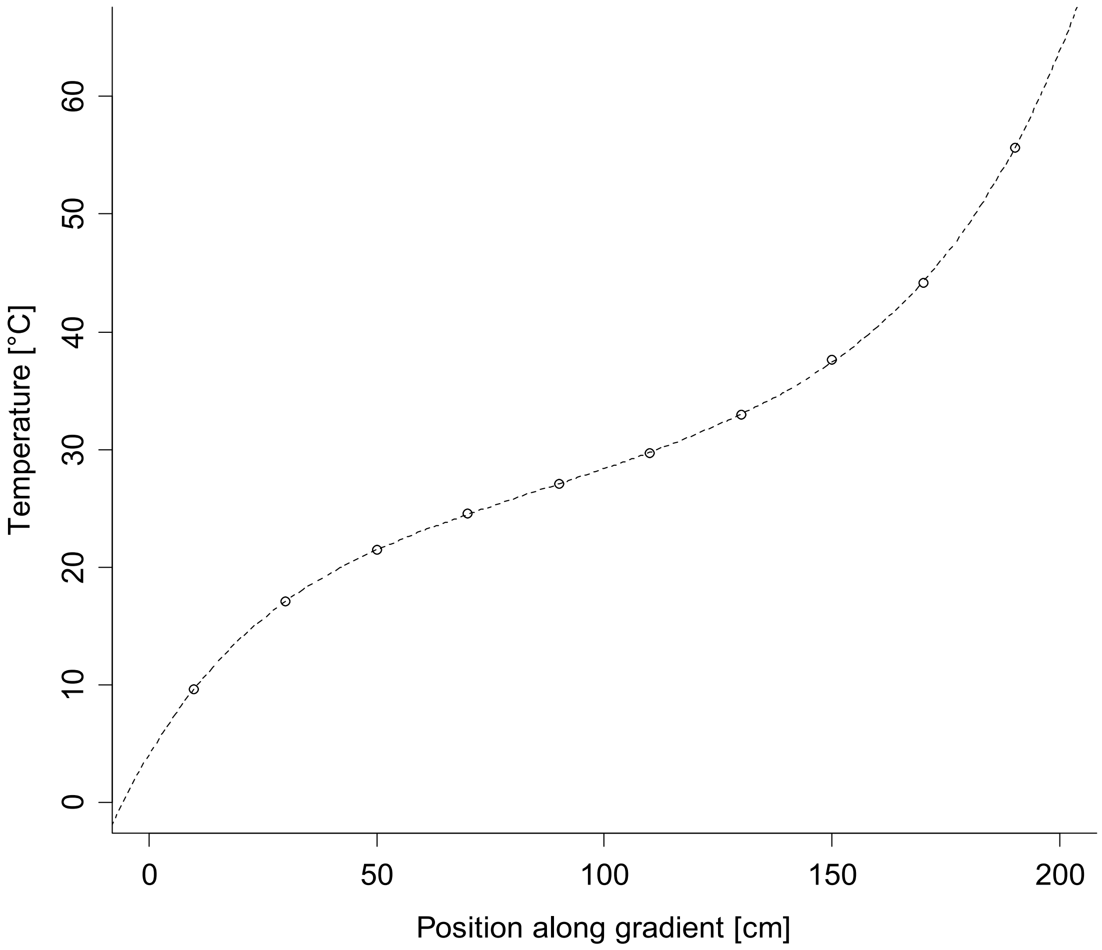
APPENDIX I:

Thermal Gradient Calibration Curve:

Figure of mean temperatures recorded along the thermal gradient used for experiments on lizards' preferred body temperature and 5th order polynomial calibration curve fitted to the data.



APPENDIX II

Publications:

The following manuscripts arising from work conducted as part of this thesis have been submitted for publication to date:

Accepted for publication:

Chapter I - Pintor, A.F.V., Schwarzkopf, L. & Krockenberger, A.K. (2015). Rapoport's Rule: do climatic variability gradients shape range extent? *Ecological Monographs* **85**(4): 643-659. Accessible at http://www.esajournals.org/doi/abs/10.1890/14-1510.1

Chapter II - Pintor, A.F.V., Schwarzkopf, L. & Krockenberger, A.K. (2016). Extensive Cold Acclimation Potential in a Restricted Tropical Lizard (*Carlia longipes*). PLOS ONE. **11**(3): e0150408. doi:10.1371/journal.pone.0150408.

Chapter III - Pintor, A.F.V., Schwarzkopf, L. & Krockenberger, A.K. (in press). Hydroregulation in a Tropical Dry-Skinned Ectotherm. Oecologia.

APPENDIX III

Data accessibility:

Data and supplementary material for the chapters included in this thesis can be accessed at the following locations.

Chapter I: http://dx.doi.org/10.4225/28/5536F28D28A5E

Chapter II: http://dx.doi.org/10.4225/28/55B58FDB5613E

Chapter III: http://dx.doi.org/10.4225/28/55B58C5D690A8

Chapter IV: http://dx.doi.org/10.4225/28/55B59232DCAF4

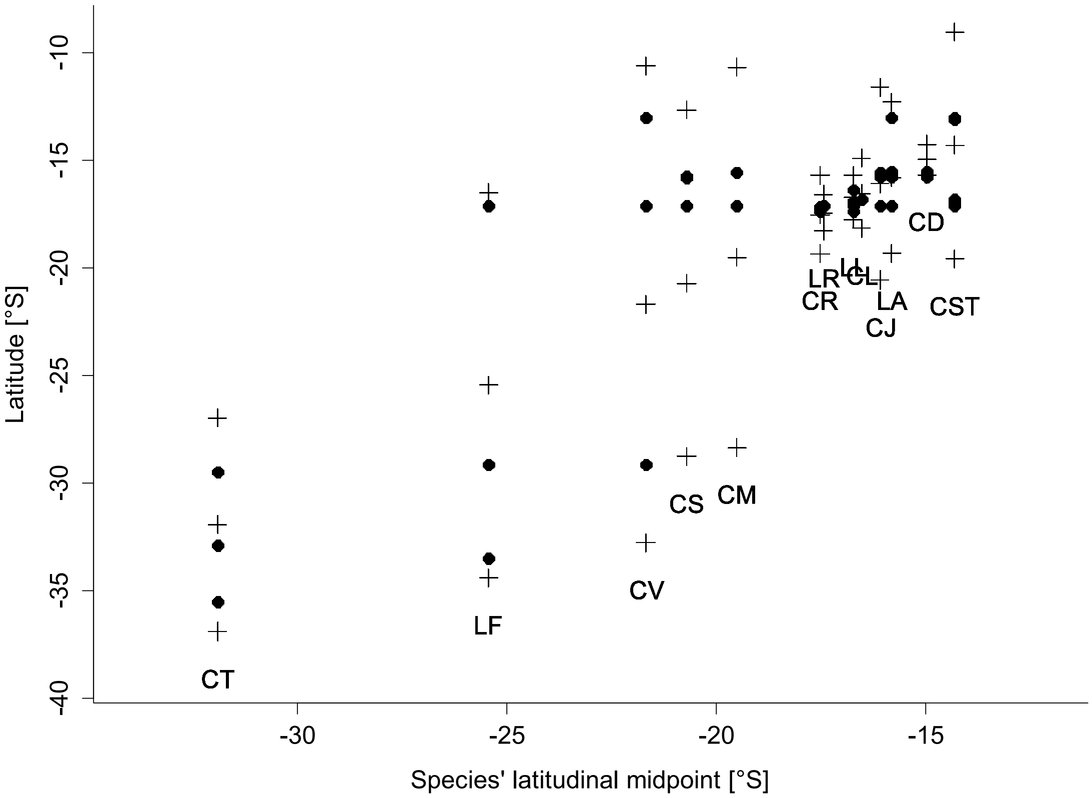
Chapter V: http://dx.doi.org/10.4225/28/55B59232DCAF4

Chapter VI: http://dx.doi.org/10.4225/28/55B58E6C46947

APPENDIX IV

Sampling latitudes for the 13 species included in this thesis:

Latitudinal position [°S] of species' sampling locations (●) and species' southernmost and northernmost recorded occurrence as well as range centre (+). Species IDs are shown as CT (*Carlia tetradactyla*), LF (*Lygisaurus foliorum*), CV (*Carlia vivax*), CS (*Carlia schmeltzii*), CM (*Carlia munda*), CR (*Carlia rubrigularis*), LR (*Lygisaurus rococo*), CL (*Carlia longipes*), LL (*Lygisaurus laevis*), CJ (*Carlia jarnoldae*), LA (*Lygisaurus aeratus*), CD (*Carlia dogare*), and CST (*Carlia storri*).



APPENDIX V

Example O_2, CO_2 and flow rate traces from respirometry experiments:

Example traces of baseline adjusted oxygen and carbon dioxide traces, as well as flow rate traces of seven sequentially measured chambers containing individuals of different size. Fluctuating traces indicating activity were discarded and repeated and often occurred outside the overnight resting periods of animals (i.e. before 6 pm or after 7 am). Y axes for oxygen and carbon dioxide traces show percent deviations from reference concentrations, the y axis for flow rates shows air flow in ml/min. X axes show time of day. Flow rates were similar for the different chambers but usually faster for reference measurements.