Adaptive capacity of Dwarf Chameleon (*Bradypodion*) thermoregulation in a changing environment

By

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DECLARATION

I, Azraa Ebrahim, declare that “Adaptive capacity of Dwarf Chameleon (Bradypodion) thermoregulation in a changing environment” is my own work, that has not been submitted for any degree or examination at any other university, and that all the sources I have used or quoted have been indicated and acknowledged by means of complete reference.

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ABSTRACT

We are currently undergoing a period of rapid climate change and habitat transformation resulting from growing urbanisation. Species with low dispersal abilities cannot track suitable climate and must adapt to the new environmental conditions if they are to persist. Studying how species have responded to past and ongoing climatic changes can provide insight on their potential adaptive capacity and may be used to predict future responses to similar changes. The overall aim of this study was to assess the adaptive capacity of Dwarf Chameleon (*Bradypodion*) thermal biology on two timescales. Firstly, I compared the vertical thermal profiles between the sites of three forest species (*B. damaranum*, *B. setaroi*, and *B. thamnobates*) and two fynbos/grassland (*B. barbatulum* and *B. melanocephalum*) species. Environmental temperatures were measured using arrays of iButtons set up at 1-m intervals for a 10 m vertical profile. For the grassland/fynbos habitats, an additional array of iButtons was set up at 0.2-m intervals for a 1 m vertical profile. The hypothesis was that forest habitats would have more moderate temperatures (5 – 35 °C) than grasslands or fynbos, and that forests would have a distinct thermocline from the ground up. As a result, I hypothesised that forest species would have different selected temperatures (T_{sel}) to fynbos and grassland species. To measure T_{sel}, chameleons were placed in a thermal gradient and body temperatures were measured in one-hour intervals for a six-hour period. Environmental temperatures for *B. barbatulum* and *B. setaroi* were similar. All the other sites had differences in their thermal profiles at each height interval. T_{sel} was also similar for *B. barbatulum* and *B. setaroi*, as well as *B. damaranum* and *B. thamnobates*. T_{sel} differed between all other species. Secondly, adaptive capacity over a short time scale was evaluated by comparing environmental temperatures and chameleon T_{sel} in natural and urban sites for four species of *Bradypodion*. For all species, there were environmental temperature differences between natural and urban sites. Only *B. thamnobates* showed a difference in T_{sel} between sites. Overall, the results from the study suggest that there is some adaptive capacity of the thermal biology of *Bradypodion*. While behaviour can often mitigate the effects of environmental changes, it may become too costly where these differences are too great, and species may undergo physiological adaptation.

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T_b	Body temperature
T_{set}	Set point range
T_{sel}	Selected body temperature
CT_{max}	Critical thermal maximum
CT_{min}	Critical thermal minimum

CHAPTER 1

GENERAL INTRODUCTION

1.1 Adaptation to a changing environment

Adaptation to a changing environment has been shown to have taken place throughout the evolutionary history of species (Grant *et al.,* 2004; Seehausen, 2004; Losos & Ricklefs, 2009; Mahler *et al.,* 2013; Higham *et al.,* 2015; Albertson & Pauers, 2018; Donihue *et al.,* 2018). Due to the different degrees of phenotypic plasticity and heritability of traits, species have different capacities to adapt to environmental changes. Adaptation is a crucial aspect of persisting in the face of habitat change and environmental pressures (Stuart *et al.,* 2014). Species can respond to habitat or climatic changes by either shifting to more suitable habitat, adapting to the changes or facing the risk of extinction (Sinervo *et al.,* 2010). Adaptations are driven by selective pressures, and where selective pressures vary across habitats, differences in the morphology or physiology of organisms between these habitats may be the result (Grant *et al.,* 2004; Higham *et al.,* 2015; Munoz *et al.,* 2015).

Not all species evolve at the same rate, as the rate depends on the intensity and magnitude of environmental fluctuations, as well as resource availability in the environment, existing genetic variation in the population, generation time, and strength of selection (Parsons, 1994). There is a growing body of evidence which suggests that evolution can be rapid given strong selective pressures (Darwin, 1859; Reznick *et al.,* 2019; Bonnet *et al.*, 2022). Abrupt environmental changes caused by extreme events such as volcanism, meteor strikes, and hurricanes can be drivers of rapid evolutionary change (Grant *et al.,* 2017; Donihue *et al.,* 2018), but other less sudden factors can similarly produce rapid adaptive responses. There are numerous examples of species shifting phenotypes over relatively few generations when exposed to new, often anthropogenically transformed, environments (Kettlewell, 1955; Reznick *et al.*, 1977; Losos *et al.*, 1997). Rapid phenotypic plasticity has implications for conservation biology, as some species could possibly have the ability to rapidly adapt to human-transformed environments and this would mitigate extinction risks (Grant *et al.,* 2017; Reznick *et al.,* 2019).

1.2 Adaptation of thermal biology

For reptiles, environmental temperature is an important selective driver because it influences performance and physiological functions (Huey, 1982; Segall *et al.,* 2013; Gangloff &

Telemeco, 2018). Because reptiles are ectotherms, they are reliant on heat exchange with the environment allowing them to regulate their body temperature provided that environmental temperatures facilitate the achievement of optimal temperatures (Huey, 1982). Neurological signals elicit adjustments in the physiology and behaviour so that body temperature (T_b) can be maintained within a specific range (Alexander, 1996; Currin & Alexander, 1999). Reptiles have a range of body temperatures that are optimal for growth and maintenance, reproduction, and feeding, and they will thermoregulate to keep their body temperature within this optimal range (Bogert, 1949). It is therefore imperative for reptiles to have physiological and behavioural responses that allow them to carefully thermoregulate and respond to environmental temperatures to keep T_bs within an optimal range.

One of the challenges of measuring thermoregulation is that it is often difficult to know whether reptiles are making thermally motivated choices (Currin & Alexander, 1999). Preferred temperatures are also difficult to estimate as the measured temperature could be the result of a cost/benefit compromise due to ecological constraints on an organism (Huey & Slatkin, 1976). According to Hertz *et al.* (1993), an assessment of temperature regulation requires the following data streams: measures of T_b for the study species, the null distribution of T_b, and the target temperatures of the species, which is represented by the "set-point range" (T_{set}). The null distributions of T_bs are estimated using physical models, such as hollow copper tubes that respond rapidly to changes in environmental temperatures (operative temperature; Bakken, 1992). The temperature of the model represents the potential T_bs that a relatively small ectotherm could achieve, and this is known as operative temperature (Hertz *et al.,* 1993). Selected or preferred temperatures (T_{sel}) can be measured under laboratory conditions, for example by using thermal gradients, and are the best estimate of T_{set} (Hutchison & Dupre, 1992; Hertz *et al.,* 1993; Segall *et al.,* 2013). Operative temperatures therefore represent the range of temperatures that could potentially be achieved by an organism, while T_{set} represents the range of temperatures that a thermoregulating organism chooses.

Thermal regimes differ across habitats, and reptiles occurring in these habitats are adapted to cope with these thermal environments. Phenotypic variation together with selective pressures have presumably enabled different species or different populations to adapt to temperature regimes associated with different elevations and latitudes (Crawford *et al.,* 1999). For example, the cold-adapted *Vipera berus* has been reported to have a higher standard metabolic rate than *Vipera aspis*, a south-European species, and has the ability to thermoregulate more efficiently to keep T_b within the selected range for extended periods of time in cold climates (Lourdais, 2013). Cold-adapted species also adjust the time spent basking, body posture, and space use to meet thermal requirements (Aguado & Braña, 2014). On the other hand, desert-adapted reptiles adjust activity patterns and habitat selection to cope with extremely high temperatures (Lara-Reséndiz *et al.,* 2015). It is therefore expected that species occurring in habitats with contrasting thermal conditions would exhibit different behavioural and physiological responses to environmental temperatures to maintain optimal temperatures for a given trait.

1.3 Adaptation in the context of urbanisation

The compounded effects of urbanisation and climate change are greatly contributing to habitat loss and alteration, as well as increases in temperature and aridity (IPCC, 2014). While South Africa is predicted to experience an increase in temperatures of 3–7 °C (Boko *et al.,* 2007), urban environments present a set of conditions which differ from the conditions experienced by animals in natural environments. Particularly, urban environments generally have higher average temperatures and lower humidity than non-urban areas (Forman, 2014; Winchell *et al.,* 2016). These differences in environmental temperatures might result in thermoregulatory challenges for urban reptiles. The thermal differences associated with transformed habitats could influence thermoregulatory behaviour, and potentially result in a shift in selected body temperatures (T_{sel}) (Besson & Cree, 2010; Winchell *et al.,* 2016; Munoz & Losos, 2017). For example, some reptiles might adjust the amount of time spent basking and shade-seeking, which could have consequences for the amount of time an individual spends on activities such as foraging, mating, maintenance and growth (Sinervo *et al.,* 2010).

Rapid adaptation (<100 years) to the urban environment has been observed in some reptile species, where differences have been found between populations occurring in natural and anthropogenically transformed areas (Ditchkoff *et al.,* 2006; Winchell *et al.,* 2016; Winchell *et al.,* 2017; Thawley, 2019). Such adaptation has been studied extensively in *Anolis* lizards, with differences in morphology, behaviour, and habitat selection observed between populations in natural and transformed habitats (Winchell *et al.,* 2016, Winchell *et al.,* 2017). Because small, non-vagile reptiles are unlikely to quickly shift their range if suitable habitat is lost, physiological and behavioural flexibility becomes important (Clusella-Trullas &

Chown, 2014). While many species are considered at risk of extinction due to habitat loss, their capacity to adapt to environmental change is not commonly considered when predicting extinction risk and may result in over estimating extinction risk.

1.4 Study species

Dwarf Chameleons (*Bradypodion*) are small, mostly arboreal lizards that are near-endemic to South Africa. It is thought that the genus has undergone species-radiation since the Miocene climatic optimum and during the Late Pliocene in response to changes in habitat and creation of ecological opportunity (Tolley *et al.,* 2008). Ancestral habitat for *Bradypodion* was likely closed canopy forest and as forest became fragmented during the aridification of Africa in the Miocene, this resulted in diversification, with several species and ecomorphs becoming open-habitat specialists (Tolley *et al.,* 2008). Because some species of *Bradypodion* have adapted to novel, natural environments while others have remained in the natural forest, this allows for a comparison of the thermoregulatory responses of chameleons occurring in closed-canopy forests to species occurring in more arid open habitats like fynbos.

Some species of *Bradypodion* have populations occurring in both natural and anthropogenically transformed habitats. Since anthropogenically transformed habitats have less canopy cover and an increase in artificial surfaces in comparison to natural habitat, they might have differences in their thermal profile in comparison to natural environments (Kalnay & Cai, 2003; Zhou *et al.*, 2004; Foley *et al.*, 2005; Brazel *et al.*, 2007; Imhoff *et al.*, 2010). As a result of these habitat differences, populations in natural and transformed environments might undergo changes to their thermoregulatory behaviour or undergo physiological adaptation to the new conditions.

Five species of dwarf chameleon were investigated in this study. I included two sister species: *Bradypodion barbatulum* and *B. damaranum,* which occur in fynbos and forest habitats respectively, allowing for comparison of temperature preferences between a species from fynbos with the related species from forest and shows adaptation over a time-period ~ 5 million years. Similarly, the forest species *B. thamnobates* and the closely related grassland species, *B, melanocephalum*, were included. *Bradypodion setaroi*, another forest species which is more distantly related to the other species, was also included. With the exception of *Bradypodion barbatulum*, the remaining species occur in both natural and anthropogenically transformed habitats, which allowed me to investigate whether these species have rapidly adapted to recently (< 100 years) transformed habitats environments.

1.5 Problem statement

Urbanisation and climate change are expected to cause rapid changes to the thermal environments of many habitats (Boko *et al.,* 2007; Forman, 2014; Winchell *et al.,* 2016). While reptiles would be vulnerable to changes in temperature, there are mechanisms to consider which might buffer the effects of changing temperature, such as behavioural thermoregulation and historical responses to climatic variations (Labra *et al.,* 2009; Grigg & Buckley, 2013). However, little is known about the adaptive capacity of the thermal biology in *Bradypodion*. Therefore, the aim of the study is to assess whether *Bradypodion* species adapt to different thermal environments over long (~5 million years ago) and shorter (~100 years) temporal scales, by assessing the adaptive capacity of both temperature preferences and behavioural thermoregulation.

1.6 Overarching aim

I aimed to compare the thermal environments occupied by five species of *Bradypodion* and compare selected body temperatures (T_{sel}) between populations from different habitats. I hypothesised that open habitats such as fynbos and grassland will have more extreme temperature ranges than forests due to the relatively low availability of shade in open habitats. If species from similar habitats (e.g., forest, fynbos/grassland) have similar thermal environments, I expected that they would have similar T_{sel}. Moreover, if the fynbos and grassland habitats have radically different thermal environments to the forest habitat, I expected the species occurring in these habitats to have different T_{sel} to forest-dwelling species. Furthermore, I predicted that populations in anthropogenically transformed habitats will experience higher temperatures than in their natural counterparts, and that these populations may have undergone corresponding changes in their T_{sel} that may be adaptive.

CHAPTER 2

THERMAL ADAPTATION ON AN EVOLUTIONARY TIMESCALE

Abstract

Changes in habitat and climate may have influences on the thermal environment and this may potentially impact many species. For species with low dispersal ability that cannot track suitable climate, it is important to consider their ability to adapt to these changes, as well as to consider the microhabitat available to them which may allow them to persist. The aim of this study was to compare the habitat thermal profiles of five species of *Bradypodion* and assess the adaptive capacity of selected body temperature (T_{sel}) within this genus. It was hypothesised that the habitat of the three forest species (*Bradypodion damaranum*, *B. setaroi*, and *B. thamnobates*) would have moderate temperature ranges (5 – 35 °C) due to the relatively abundant canopy cover, as well as a gradual temperature gradient from the ground to the canopy. The fynbos and grassland habitats of *B. barbatulum* and *B. melanocephalum* on the other hand, were expected to have a more extreme temperature range and a less pronounced or absent temperature gradient. It was expected that T_{sel} for each species would reflect the environmental temperature ranges of their respective habitats. To assess the range of environmental temperatures available to chameleons, iButtons were used as physical models, set up as an array at 1-m intervals along a 10 m vertical profile from the ground up in habitats with high canopy. In the fynbos and grassland habitats, vertical arrays were set up from the ground to the maximum vegetation height, plus an additional set of iButtons were set up at 0.2-m intervals along a 1 m vertical profile. To measure T_{sel}, chameleons were placed in a thermal gradient (20 – 45 °C) and cloacal temperatures were measured once every hour over a six-hour period. The results revealed differences in environmental temperatures for all habitats, except between the habitats of *B. barbatulum* and *B. setaroi,* which had similar thermal environments. T_{sel} differed between all species except between *B. barbatulum* and *B. setaroi*, and between *B. damaranum* and *B. thamnobates*. The results suggest that T_{sel} may be flexible, and that chameleon thermal biology may adapt to changes in environmental temperatures.

2.1 Introduction

Climatic change can result in habitat changes and the effect on some species can be to diversify and/or shift into novel environments (Deepak & Karanth, 2018). Climate change has an effect on ectotherm species, as they rely heavily on heat exchange with the external environment to keep body temperature within ranges that are optimal for performance and physiological function (Huey, 1982; Segall *et al.,* 2013; Gangloff & Telemeco, 2018). While some species have morphologically and physiologically adapted to the new environmental conditions posed by a new habitat type (Grant *et al.,* 2004; Seehausen, 2004; Losos & Ricklefs, 2009; Mahler *et al.,* 2013; Higham *et al.,* 2015; Albertson & Pauers, 2018; Donihue *et al.,* 2018), it is important to note that many species have gone extinct as a result of these changes (Wiens *et al.*, 2019). We are currently experiencing a period of rapid climate and habitat change (Foley *et al*., 2005; Ahmed *et al.*, 2013), and it is important to assess how species have responded in the past so that we may make predictions on how they might respond to current and future changes. Therefore, comparisons of thermal biology of reptile species across different clades and from diverse habitats could provide insights on the adaptive potential of these species. For example, do all forest species show similar T_{sel} regardless of ancestry, and are their thermal habitats similar? Conversely, does habitat structure (e.g., forests versus open habitats such as fynbos and grassland) have an impact on thermal conditions in these habitats and if so, do species that occur in those habitats have different T_{sel}s regardless of ancestry?

Models predicting species responses to climate change often focus on ranges being affected by using forecasted climatic conditions to predict where suitable niches may exist in the future. This approach poses two major issues. Firstly, it does not take into account the capacity for species to adapt and secondly, broad-scale climatic measures may not be appropriate for defining the climatic niche of many species. Many species of reptiles may be unable to track climatic changes due to their low dispersal ability, or due to biotic or abiotic barriers (Petford and Alexander, 2021). In these cases, it is important to consider whether a species could modify thermal tolerances by phenotypic plasticity or adaptation. Average annual air temperature and similar measures are often used as the independent variables in niche models. These broad-scale (spatial and temporal) measures may not be good environmental variables to use for reptile responses, as they have little physiological significance (Mitchell *et al*., 2018). It is more important to take into consideration the range of temperatures available to an organism, and realistic measures representing body temperatures (T_b) an organism could attain (Carroll *et al.*, 2016; Mitchell *et al.*, 2018). It is suggested that spatial and temporal temperature variation, rather than mean temperature, is a better predictor of thermal trait responses (Clusella-Trullas *et al.*, 2011). Shady areas within an environment could act as a buffer to higher temperatures in the environment, while exposed areas can pose a risk for individuals under extreme conditions. Therefore, to assess how species may respond to climate change, it is essential to consider their capacity to adapt as well as the microclimate conditions in which they occur.

In order to model potential niche shifts in reptiles or assess their adaptive capacity to climatic change, it is important to consider the evolutionary lability of thermal traits. The original view of thermal biology adaptation is that thermal traits are slow to evolve since they require multiple co-evolved changes, and the findings of some studies support this view (Bogert, 1949; Van Damme *et al.*, 1990; Grigg & Buckley, 2013). Rather than adapting their thermal biology to new conditions, it has been assumed that ectotherms can adjust their thermoregulatory behaviour to buffer for changes in environmental temperatures (Caldwell et al., 2017). Furthermore, the low heritability of thermal traits is considered another contributing factor to the slow evolution of thermal traits (Logan *et al.*, 2018). However, while individuals can increase the time spent shuttling between warm and cool environments to keep their T_b within optimal ranges, this can be energetically expensive and put individuals at a higher risk of predation if time spent shuttling is dramatically increased (Huey & Slatkin, 1976). Recent studies have found labile responses of thermal traits to environmental changes for some species (Scheers & Van Damme, 2002; Logan *et al.*, 2014; Gilbert & Miles, 2017; Gilbert & Miles, 2019). Perhaps the potential for phenotypic or genotypic evolutionary responses of thermal traits is variable and dependent on the taxon.

Bradypodion (Dwarf Chameleons) are an interesting group of species for investigating thermal adaptation, as their diversification is strongly linked to habitat changes resulting from global climate change (Tolley *et al.*, 2008). It is thought that forest is the ancestral habitat type for the genus, but several species have invaded other habitat types such as grasslands and fynbos (Tolley *et al.*, 2008). Several species have undergone phenotypic adaptations to these novel habitats (Herrel *et al.*, 2011; Hopkins & Tolley, 2011, da Silva & Tolley 2013, Higham *et al.*, 2015, Tolley *et al.*, 2019), but little is known about the evolutionary lability of their thermal physiology. Due to the strong structural differences in vegetation and substrate in these habitat types, there could potentially be strong differences in the thermal environment.

My study focuses on the differences in the thermal biology of species occupying different habitat types. The aim was to compare the thermal environment and selected body temperatures (T_{sel}) of five species of *Bradypodion* that occur in two radically different habitat types, canopied forests (*B. damaranum*, *B. setaroi*, *B. thamnobates*) and open canopy shrubby/grassy vegetation (*B. barbatulum*, *B. melanocephalum*). This comparison allowed me to assess whether species in similar habitats show a similar range of T_{sel} and if so, does that pattern correspond to thermal environmental differences between habitats. This was achieved by measuring and analysing the differences in the vertical thermal profile between open and closed habitats and investigating how species are adapted to their respective thermal environments. I expected open habitats such as fynbos and grasslands to have more extreme temperature ranges than forest due to the relatively low availability of shade in open habitats, and the capacity of forests to be thermally buffered. If there is adaptation to specific thermal environments, I anticipate the three forest species to have similar T_{sel}s, but this also assumes that their thermal environments have similar temperature ranges available. Similarly, if the two species from open habitats are within similar thermal environments to each other, they would be predicted to have similar T_{sel}. Given that the two habitats are different in terms of vegetation structure, it is likely that forest will differ substantially in terms of thermal habitat from fynbos/grassland.

2.2 Study species

In this study, I collected data from five species of *Bradypodion* which included two sets of closely related species: *Bradypodion damaranum* (Knysna Dwarf Chameleon), and *Bradypodion barbatulum* (Tolley *et al.*, 2022). These are sister species but have notable different morphologies and habitat (Tolley *et al.*, 2022). *Bradypodion damaranum* occurs in remnant patches of Afrotemperate forest in Western Cape Province, South Africa (Tilbury, 2018). *Bradypodion barbatulum*, (Beardless Dwarf Chameleon), occurs in montane fynbos in the Kouga Mountains and the northern slopes of the Tsitsikamma Mountains (Tolley & Burger, 2007) (Fig. S1).

Similarly, *B. melanocephalum* (Gray, 1865) (Durban Dwarf Chameleon) and *B. thamnobates* (Raw, 1976) (Natal Midlands Dwarf Chameleon) are sister species with different morphologies and habitat types (Tolley *et al*., 2004, 2008). *Bradypodion melanocephalum* occurs in KwaZulu-Natal, with disjunct populations in grassland/forest ecotones, coastal wetlands, and reed beds (Raw, 1976; Tilbury, 2018). *Bradypodion thamnobates*, on the other hand, is restricted to the KwaZulu-Natal Midlands, and is restricted to Afrotemperate forests, although much of the landscape is highly degraded and some individuals also occupy degraded vegetation in peri-urban gardens and roadsides (Fig. S1).

I also included a more distantly related forest species, *Bradypodion setaroi* (Raw, 1976) (Setaro's Dwarf Chameleon) in my analysis. This species occurs in primary coastal dune forest along the KwaZulu-Natal coastline and has been recorded from slightly degraded habitats (Tilbury, 2018) (Fig. S1).

2.3 Methods

2.3.1 Field sampling

Chameleons were located at night using torchlight, which makes them easily visible. Each captured chameleon was placed in a uniquely numbered cloth bag. GPS coordinates were recorded at the point of capture and the perch where the chameleon was found was tagged so that chameleons could be returned to the exact capture site after measurement. The captured chameleons were transferred to 5 L plastic containers and kept at room temperature overnight. Each container was provided with enough vegetation for the chameleons to perch on. Chameleons were each returned to their point of capture and released within 24 hours.

2.3.2 Measuring operative temperatures

Operative temperatures in each of the five field sites were recorded directly using temperature sensitive data loggers (iButtons: Maxim Integrated). Physical models could not be used as data were downloaded every few days, which would necessitate disassembling many models multiple times throughout the study period and would not have been logistically feasible. iButtons were programmed using 1-Wire software (Maxim Integrated) to record temperatures at 15-minute intervals for a minimum of 24 hours from midnight to midnight. For each field site (per species), between two to four arrays of iButtons were suspended as vertical transects to cover as much accessible habitat of chameleons (max. 10 m height) as possible. The iButtons were spaced at one-meter intervals, from ground level to the maximum height of the array. For the grassland and fynbos sites, an additional array of iButtons was set up at 20-cm vertical intervals along a 1-m transect. This allowed for a record of the temperature profile of the proportion of the habitat available to the chameleons (grassland is generally <2 m high). Where logistically possible, iButton arrays were moved to different localities within the area every few days to capture temperature variations due to factors such as shade, proximity to water, or elevation. Temperature data were analysed using a multivariate analysis of variance (MANOVA), to test for differences in operative temperatures between the fields sites where chameleons occurred. The data were analysed for each corresponding metre along the profiles (i.e., up to eleven separate points along the profile) for the different field sties. Pairwise comparisons of the five field sites were carried out using Tukey's Honest Significant Difference (HSD) post hoc test.

2.3.3 Measuring T_{sel} (selected temperatures) - Temperature gradient

The thermal preferences of chameleons were measured following the methods used by Segall *et al.* (2013). The thermal gradient was established in a wooden box (1.26 x 0.73 x 0.38 m) with six lanes separated with wooden dividers. A dowel stick was positioned in each lane for chameleons to walk along. Five heat-generating bulbs (Eurolux G230 M-infrared 275 W) were suspended above the box at one end to provide a source of heat, adjusting their height to produce a thermal gradient of 20 to 45 °C horizontally across the lanes. Chameleons could move freely along the dowel to select T_b, which was considered the T_{sel}. Chameleons were placed in the lanes in the morning and left for at least an hour to acclimate. Body temperatures were then measured for each chameleon every hour for a maximum of five hours by inserting a lubricated thermocouple approximately 4 mm into the cloaca. Data were collected from five species of *Bradypodion* (*B. barbatulum*: females n = 12, males n = 9; *B. damaranum*: females n = 18, males n = 13; *B. melanocephalum*: females n = 10, males n = 13; *B. setaroi*: females n = 17, males n = 15; *B. thamnobates*: females n = 12, males n = 15).

Because weather appeared to have an effect on selected body temperature (field observation), weather on each day was recorded as either "sunny," "cloudy," "overcast," or "rainy". Differences between species for T_{sel} were analysed using a nested analysis of variance (ANOVA) design, with T_{sel} as the response variable, snout-vent length (SVL) as a covariate to correct for body size, and weather and sex as random factors. A backwards stepwise regression approach was used to achieve the most parsimonious model. Since SVL and sex had no effect on the T_{sel} for any species, they were then removed from the model and the analysis was re-run. Pairwise differences between species were assessed using Tukey's

Honest Significant Difference (HSD) post hoc test. Significant levels were set at 5% and all data were analysed using RStudio (version 1.2.1335).

2.4 Results

2.4.1 Environmental temperatures

Operative temperatures were significantly different between the study sites (MANOVA; $F_{4,5789} = 133.21$, $P < 0.001$). In addition, at each corresponding metre of the vertical profile, there were significant differences in environmental temperatures between study sites (Table S1). Post hoc pairwise comparisons showed significant differences between all study sites except for the sites where *B. barbatulum* and *B. setaroi* occur. The operative temperatures for *Bradypodion barbatulum* study site had the diel greatest range (Fig. 1, Table 1, Table S2), and *B. thamnobates* had the overall smallest temperature range. For all study sites, excluding that of *B. thamnobates*, average operative temperatures were between 20.0 and 25.0 °C. Average diel operative temperatures for *B. thamnobates* habitat (forest) were lower than for any other species (16.8 °C) (Table S3).

Night-time operative temperatures (from 16:01–09:00) for *Bradypodion barbatulum* field site were as low as 2.5 °C, bringing the diel average operative temperatures to 22.6 °C. However, the daytime operative temperatures averaged 33.6 °C but reached lows of 16.0 °C (Table 1). Daytime (from 09:01–16:00) temperature at ground level in this habitat was notably higher than those recorded from within the remainder of the vertical profile (Fig. 2).

Of the three forest study sites, the two Afrotemperate forest sites (*B. damaranum* and *B. thamnobates*) with a notably increasing thermocline along the vertical gradient with the forest floor often being several degrees cooler than the highest points measured (Figs 3 and 6). The coastal forest where *B. setaroi* occurs on the other hand did not exhibit an obvious thermal gradient in the vertical profile, with the exception of ground temperatures being cooler than the rest of the profile.

Both open habitats (fynbos and grassland) showed little temperature heterogeneity along the 10 m vertical profiles. However, the 1 m profile from fynbos (*B. barbatulum* habitat) showed a gradual decrease in temperatures from the ground up. This is the opposite trend to operative temperatures for grassland (*B. melanocephalum* habitat), which showed an overall increase in temperatures from the ground up (Fig. 4).

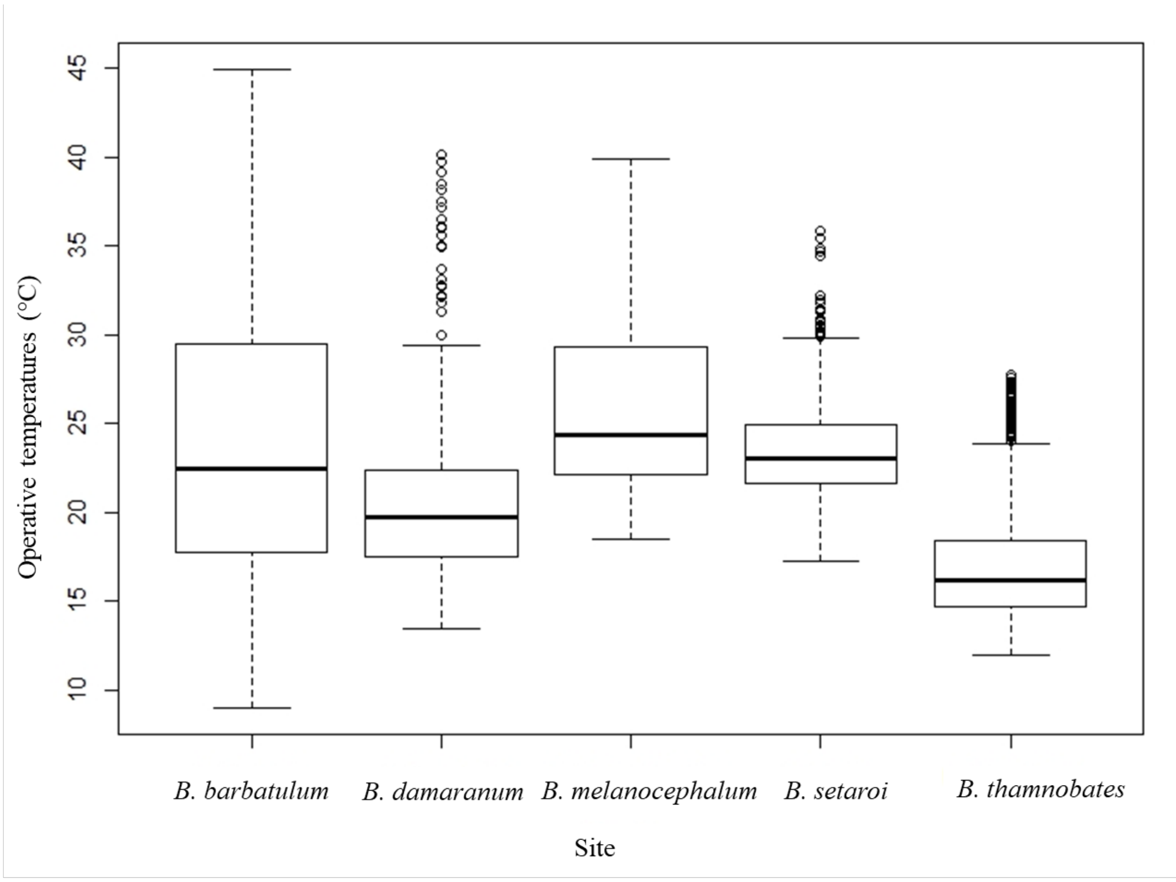

Figure 1. Range of operative temperatures (°C) for the field sites representing the habitats where five species of *Bradypodion* occur. Boxes show the range of temperatures from the 1st to 3rd percentiles, with the mean indicated by the line within the box. The whiskers show the range of values from min/max to the lower/upper percentiles, respectively. Outliers (values greater than 1.5 times the 75th or 25th interquartile range) are indicated by circles.

Table 1. Diel average, absolute minimum, and absolute maximum operative temperatures for the field sites representing the habitats where five species of *Bradypodion* occur. *Bradypodion damaranum*, *B. setaroi*, and *B. thamnobates* occur in forest habitats, while *B. barbatulum and B. melanocephalum* occur in fynbos and grassland habitats, respectively. All values are summarised across all intervals for the vertical profiles (per species' habitat) to provide an overview of the temperatures available in each habitat over the study period. Standard deviation of average temperatures across the entire period are presented. Minimum and average values for the daytime (09:00 – 16:00) were also included to provide an estimate of temperatures available when chameleons may be actively thermoregulating.

	B. barbatulum	*B. damaranum*	*B. setaroi*	*B. melanocephalum*	*B. thamnobates*
Min	2.5	13.0	16.5	16.1	11.5
Max	63.5	45.0	41.5	55.9	32.1
Ave	22.6	20.6	23.5	25.2	16.8
SD	8.3	4.0	2.8	4.9	3.1
Min (day)	16.0	17.0	20.1	23.1	12.5
Ave (day)	33.6	24.1	26.4	30.8	19.2

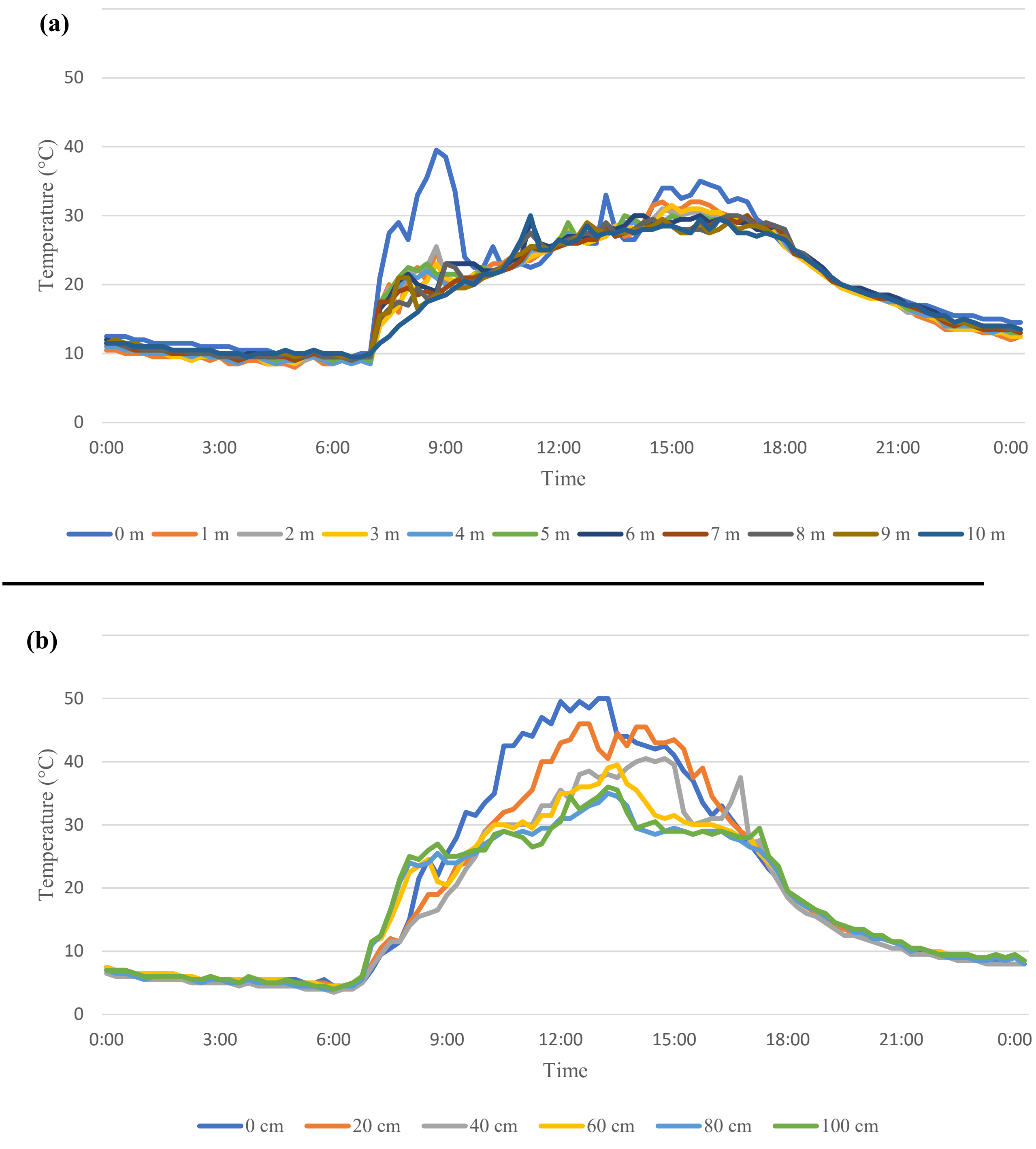

Figure 2. Diel operative temperatures recorded by iButton arrays at the study site representing fynbos habitat where *Bradypodion barbatulum* occurs, recorded on the 24th of February 2020 for (a) a 10 m vertical transect, at 1-m intervals, and (b) a 1-m vertical transect, at 0.2-m intervals. Both graphs have time in the x-axis and temperature in °C on the y-axis. Intervals are colour coded according to the key.

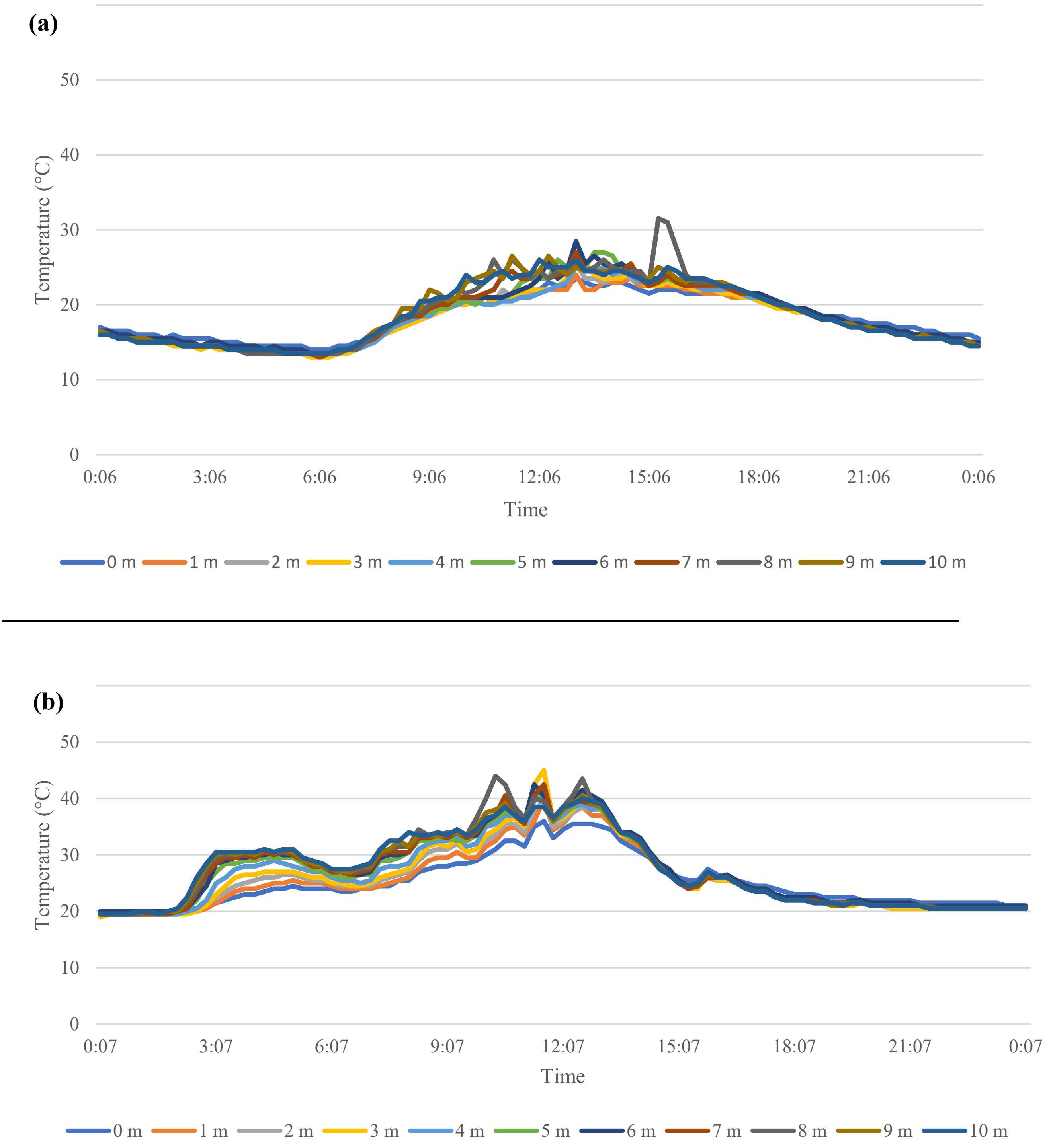

Figure 3. Diel operative temperatures recorded by two separate iButton arrays at the study site representing forest habitat where *Bradypodion damaranum* occurs, recorded on (a) the 21st of February 2020 and (b) 16th of February 2020, when strong berg winds and higher than average temperatures were observed. Temperatures were recorded along a 10 m vertical transect, at 1-m intervals. Both graphs have time in the x-axis and temperature in °C on the y-axis. Intervals are colour coded according to the key.

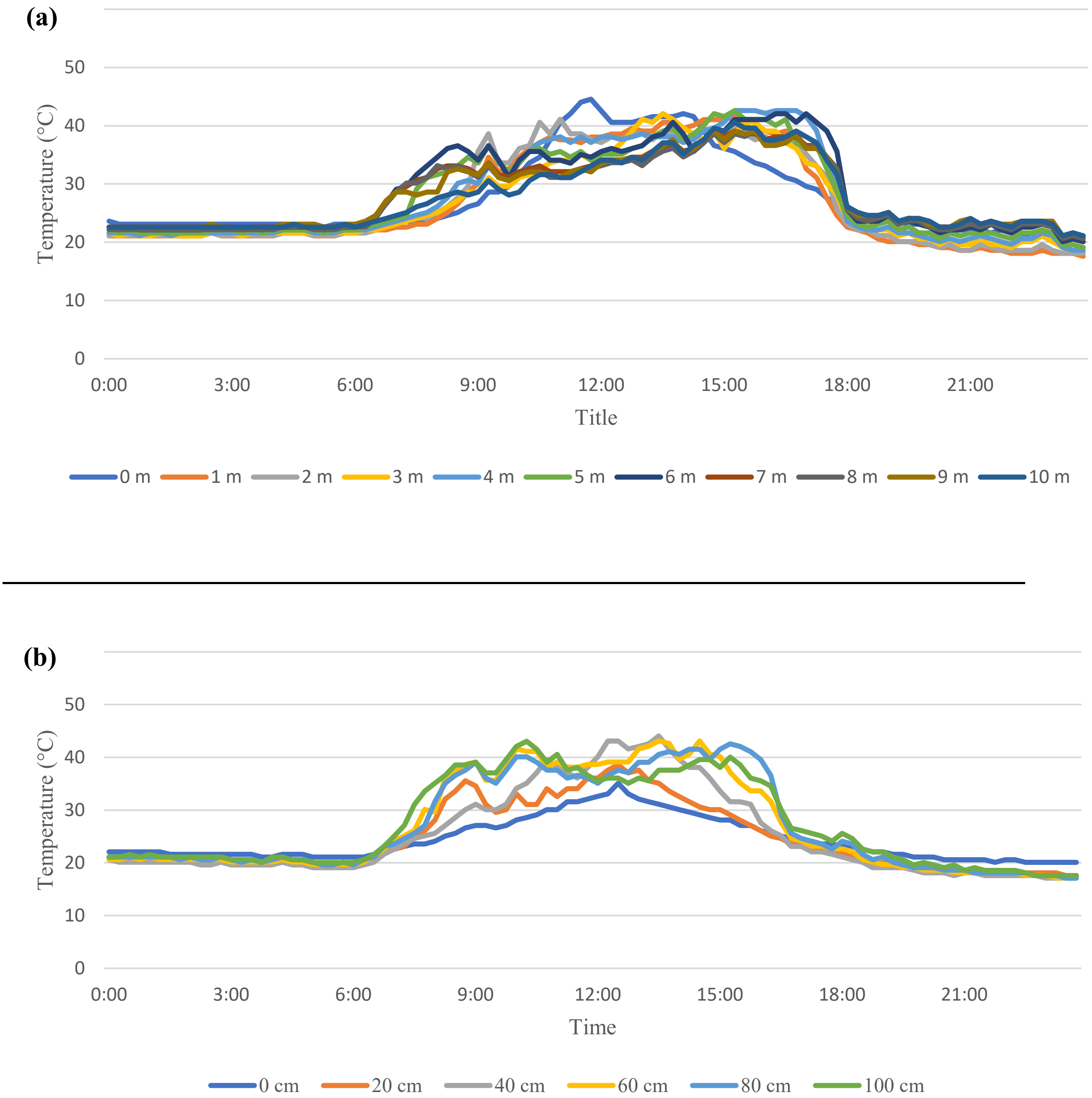

Figure 4. Diel operative temperatures at a study site representing (grassland habitat where *Bradypodion melanocephalum* occurs, recorded on the 10th of March 2021 for (a) a 10 m vertical transect, at 1 m intervals and (b) a 1 m vertical transect, at 0.2 m intervals. Both graphs have time on the x-axis and temperature in °C on the y-axis. Intervals are colour coded according to the key.

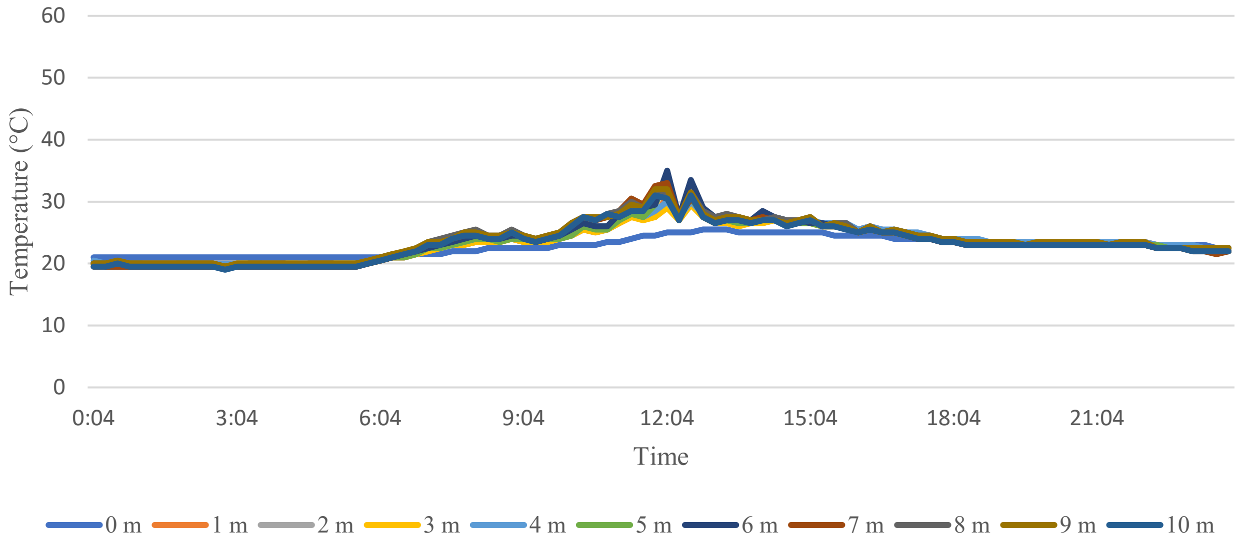

Figure 5. Diel operative temperatures recorded by an iButton array at the study site representing coastal forest where *Bradypodion setaroi* occurs, recorded on the 26th of November 2020. Temperatures were recorded along a 10 m vertical profile at 1-m increments. The graph shows time on the x-axis and temperature in °C on the y-axis. Intervals are colour coded according to the key.

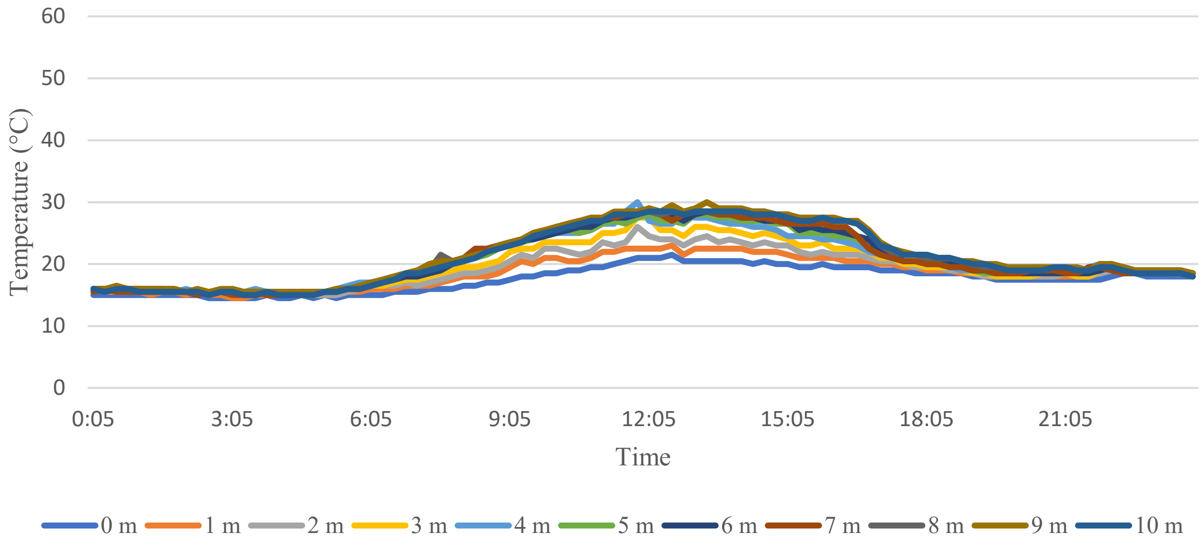

Figure 6. Diel operative temperatures recorded by an iButton array at the study site representing forest habitat where *Bradypodion thamnobates* occurs, recorded on the 18th of February 2021. Temperatures were recorded along a 10 m vertical profile at 1 m increments, over a 24-hour period. The line graph shows time on the x-axis and temperature in °C on the y-axis. Intervals are colour coded according to the key.

2.4.2 Selected temperatures

There were significant differences in T_{sel} between species (ANOVA, $F_{4,117} = 43.64$, $P < 0.001$), and weather was found to have a significant effect on T_{sel} (ANOVA, $F_{12,117} = 7.08$, $P < 0.001$), with chameleons selecting lower temperatures on rainy days. The pairwise post-hoc analysis revealed significant differences in T_{sel} between most species, with the exception of between *B. setaroi* and *B. barbatulum*, and *B. thamnobates* and *B. damaranum* (Table 2). *Bradypodion melanocephalum* had the highest T_{sel} (Fig. 7).

Table 2. Significance values of ANOVA pairwise comparisons of T_{sel} between five *Bradypodion* species. *p*-values are shown for each comparison, and significant comparisions are in bold font.

	B. barbatulum	*B. damaranum*	*B. melanocephalum*	*B. setaroi*	*B. thamnobates*
B. barbatulum	-	-	-	-	-
B. damaranum	**<0.001**	-	-	-	-
B. melanocephalum	**<0.001**	**<0.001**	-	-	-
B. setaroi	0.764	**<0.001**	**<0.001**	-	-
B. thamnobates	**0.004**	0.507	**<0.001**	**0.048**	-

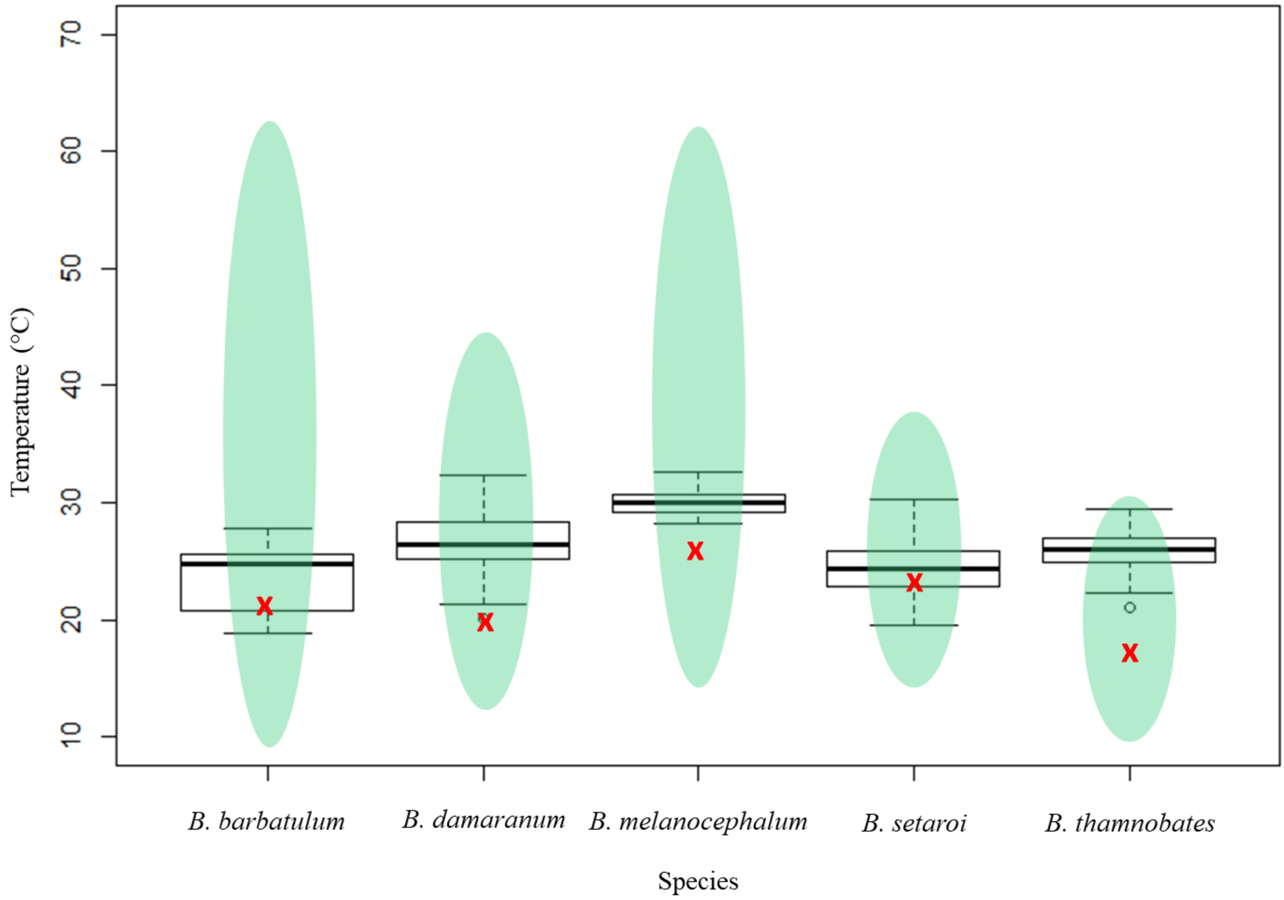

Figure 7. Box plots showing the range of selected body temperatures (°C) five species of *Bradypodion* measured in a thermal gradient (*B. barbatulum*: females n = 12, males n = 9; *B. damaranum*: females n = 18, males n = 13; *B. melanocephalum*: females n = 10, males n = 13; *B. setaroi*: females n = 17, males n = 15; *B. thamnobates*: females n = 12, males n = 15). Boxes show the range of temperatures from the 1st to 3rd percentiles, with the mean indicated by the line within the box. The whiskers show the range of values from min/max to the lower/upper percentiles, respectively. Outliers (values greater than 1.5 times the 75th or 25th interquartile range) are indicated by circles. The green shading shows the range of environmental temperatures available across a 10 m vertical profile for the habitat of each species, and the red crosses indicate the average environmental temperature.

2.5 Discussion

Dwarf chameleon species appear to show physiological adaptation to their environmental thermal niches. The two species which inhabit Afrotemperate forest (*B. damaranum* and *B. thamnobates*) show similar T_{sel}s. The forest species *B. setaroi* and fynbos species *B. barbatulum*, surprisingly, had similar T_{sel}s, as well as similar thermal environments. Although the means were not significantly different, the fynbos habitat where *B. barbatulum* occurs had high variability, with the most extreme maximum temperatures and a much wider range in temperatures than the other habitats. However, the forest habitat where *B. thamnobates* individuals were sampled had the smallest range in environmental temperatures and relatively low maximum temperatures compared to the operative temperatures recorded in the other habitats. The Afrotemperate forest sites also exhibited a wide range and incremental increase in temperatures across the vertical profile, while the coastal forest site did not. Open habitats (fynbos and grassland) showed relatively low spatial heterogeneity of environmental temperatures when looking at the 10 m profile, but if considering the 1-m profile, which is more representative of the thermal habitat that is actually available to chameleons, there was a distinct temperature gradient for both field sites.

Given that the T_{sel} values for these five species differ where there are differences in the thermal environment, it appears that environmental temperatures pose a selective pressure on T_{sel}. This could mean that where there are drastic differences in temperatures between habitats, there might be limits to the extent that behavioural thermoregulation can buffer the effects of extreme environmental temperatures, and species have to physiologically adapt in order to persist in novel environments. Therefore, lability of thermal traits might be a crucial characteristic for a species to persist given the prediction of rapid climatic change. However, it is important to note that during extreme weather events, the buffering effects of forest canopies may not be enough to shelter organisms from critical temperatures. Although the vertical temperature gradient for the *B. damaranum* site was maintained even during a heat wave, the minimum temperatures available in the environment were above 30 ºC at midday, which is above the selected temperatures of *B. damaranum*. If climate change increases the frequency and severity of extreme weather events, this could hinder the ability of chameleons to stay within optimal thermal ranges.

With the exception of *B. melanocephalum*, the T_{sel} of *Bradypodion* species in this study was lower than that reported in the literature for other *Bradypodion*, which is 29 – 30 (Segall *et al.*, 2013) and substantially lower than that of Chamaeleo from other regions in Africa (30 – 33 °C) (Bennett, 2004). *Bradypodion barbatulum* and *B. setaroi* have the lowest T_{sel}s, despite higher environmental temperatures being available to them. If a species is exposed to extreme maximum temperatures, there may be less risk in selecting lower temperatures to avoid staying at temperatures close to CT_{max} (Labra *et al.*, 2001), which may explain the low temperatures selected by *B. barbatulum*. For example, ground level temperatures in the fynbos habitat of *B. barbatulum* were recorded as high as 60 °C. High ground temperatures could mean that chameleons are isolated to specific trees or bushes for the hottest part of the day and cannot disperse over the ground to seek a cooler trees or bushes. This presents a limitation on the ability of a chameleon to behaviourally thermoregulate by shuttling between warmer and cooler areas in the environment.

Environmental temperatures were only measured during a small proportion of the year but to fully evaluate the selective pressure that environmental temperatures impose, it would be valuable to also consider the seasonality of environmental temperatures. The T_{sel} were measured in this study during late summer but may be instead linked to cooler environmental temperatures being available in winter months. However, the spatial heterogeneity of temperatures described for most of the habitats in this study highlight the importance of microclimate and display how only considering annual air temperatures for species distribution modelling may not be an adequate representation of temperature availability. In this study, I did not measure food availability or predator distribution. Since *Bradypodion* are selecting relatively low body temperatures, and low environmental temperatures are associated with the lower part of the canopy, these temperatures might not necessarily be optimal for body function but may be a trade-off for other selective pressures such as predator avoidance or resource exploitation.

The lability of a thermal trait reported in this study corroborate with findings of thermal adaptation in other species. Shifts in average Tb, Tsel, CTmax, as well as optimal performance temperatures have undergone selection due to anthropogenic changes or rapid climate changes (Scheers & Van Damme, 2002; Logan et al., 2014; Akashi et al., 2016; Gilbert & Miles, 2017; Gilbert & Miles, 2019). While these studies have reported evidence of adaptation of thermal traits, several studies support the original view of thermal trait conservatism (Van Damme *et al.*, 1990; Labra *et al.*, 2009; Grigg & Buckley, 2013). These contrasting findings could be a result of thermal trait lability being different across species, or studies may over-estimate temperature differences between habitats if only ambient average temperatures are considered.

This study provides evidence for the adaptive lability of thermal biology in the genus *Bradypodion*. While this may or may not hold true for other genera, this highlights the importance of both analysing the adaptive capacity of species, as well as considering the ability to adapt or lack thereof in species distribution modelling. Although the results suggest that species could potentially withstand the temperature changes associated with climate change, it is important to note that habitat fragmentation may still play an even bigger role in population declines. While it is unclear whether this change is plastic or evolutionary, there is evidence of lability of this thermal trait, contrary to the original view of ectotherm thermoregulation. This finding is especially important for species with low dispersal potential, as adaptive capacity can ensure species survival in changing environments.

CHAPTER 3

THERMAL ADAPTATION ON AN ECOLOGICAL TIMESCALE: RESPONSES TO URBANIZATION

Abstract

The reduction of vegetative cover and increase in artificial surfaces resulting from urbanisation can change local climates. Several species of *Bradypodion* occur in natural as well as anthropogenically transformed habitats, and it is unknown whether they have physiologically adapted to the changes presented by urbanisation. The aim of this study was to compare the environmental temperatures of natural and urban habitats, as well as compare the selected temperature (T_{sel}) of chameleon populations occurring in these habitats. It was hypothesised that for forest species (*B. damaranum*, *B. setaroi*, and *B. thamnobates*), urban areas would be warmer than their natural habitats due to the reduction of canopy cover. The grassland habitat of *B. melanocephalum* on the other hand was expected to be warmer on average and have a wider temperature range than its urban counterpart, due to urban gardens having relatively more canopy cover than the natural grassland habitat. Environmental temperatures were measured using iButtons set up at 1-m intervals along a 10 m vertical profile in each habitat type. In the grassland habitat, an additional set of iButtons was set up at 0.2-m intervals along a 1 m vertical profile. To measure T_{sel}, chameleons were placed in a thermal gradient and cloacal temperatures were measured once every hour over a six-hour period. The results showed that the forest habitats were cooler than their urban counterparts, while the grassland habitat was warmer than its urban counterpart. *Bradypodion thamnobates* was the only species to show a difference in T_{sel} between natural and urban populations, with the population in the transformed habitat selecting higher body temperatures. The results suggest that although *Bradypodion* species have the capacity for thermal adaptation, for some species behavioural thermoregulation may be an adequate buffer for the present-day differences in environmental temperatures between natural and transformed habitats.

3.1 Introduction

Land-use change and urbanisation result in changes to natural habitat and climate on various spatial and temporal scales (Findell *et al.*, 2007; Maheshwari *et al.*, 2020). These changes influence the distribution (Mallery *et al.*, 2007), abundance (Germaine & Wakeling, 2001; Ackley *et al.*, 2009), physiology (Chick *et al.*, 2018; Campbell-Staton *et al.*, 2020), and behaviour of animals and have an impact on extinction risk (Sinervo *et al.*, 2010; Atwell *et al.*, 2012). With urbanisation and global temperatures expected to continue to increase (United Nations, Department of Economic and Social Affairs, Population Division, 2015), it has become increasingly important to evaluate how species are responding to rapidly changing habitats. While rapid evolution of traits on a short timescale was initially thought an anomaly (e.g., peppered moths, Kettlewell, 1955), there is a growing body of evidence documenting changes in the physiology and morphology of species (Winchell *et al.*, 2016; French *et al.*, 2018; Baxter-Gilbert *et al.*, 2020). This is important to consider, as many models predicting species range shifts may underestimate the ability of species to adapt to these changing environments. The capacity to adapt to urban environments is not uniform across all species (Hamer & McDonnell, 2010), and it is important to document the mechanisms driving adaptation to rapidly changing habitats.

The rise in temperatures in the form of heat-islands due to urbanization is well-documented (Kalnay & Cai, 2003; Zhou *et al.*, 2004; Foley *et al.*, 2005; Brazel *et al.*, 2007; Imhoff *et al.*, 2010), but there remains a gap in our knowledge regarding temperature tolerance and adaptation in ectotherms. This deficiency is due mainly to poor resolution in the macroclimate data, as well as neglecting the capacity of thermal traits to adapt. Studies that predict changes to population distributions in responses to climate change neglect the potential for species to adapt to changes in temperature (Harte *et al.*, 2004). Studies have also used ambient temperature forecasts rather than considered microclimates and actual temperatures available. The way a species may respond to changes in temperature is difficult to assess since species might track favourable climate but may also adjust their behaviour to reach preferred temperatures or adapt their preferred temperatures.

Reptile performance and physiology is reliant on environmental temperatures and careful thermoregulation is necessary to keep body temperatures within optimal ranges (Huey, 1982). Thermal traits, such as selected and critical temperature limits are all under natural selection and may evolve at different rates (Munoz *et al.*, 2013). In general, it has been assumed that most of these traits evolve slowly, but recent studies have revealed cases where there has been more rapid evolution due to environmental changes (Leal & Gunderson, 2012; Bodensteiner *et al.*, 2020). Some species of lizards have undergone changes in Tb, Tsel, and CTmax on anthropogenic timescales (Scheers & Van Damme, 2002; Leal & Gunderson, 2012; Logan *et al*., 2014; Gilbert & Miles, 2017; Gilbert & Miles, 2019). Thermal interactions between animals and their environment are complex (Mitchell *et al.,* 2018), and behaviour plays an additional component on how species may adapt to rising temperatures. If overall temperatures are increasing, reptiles might adjust their behaviour to reach optimal temperatures and avoid reaching their critical limits. They do this by shuttling between shady and sunny areas and by adjusting body positions (Bogert; 1949; Huey, 1982; Andrews, 2008). Changes in the environmental temperatures available to reptiles could affect the effort an individual must expend on thermoregulation. For example, changing temperatures may result in individuals having to travel greater distances to find shade or sunlight and have impacts on activity patterns, mating opportunities, food availability and type, and predation risks (Huey & Slatkin, 1976).

Dwarf Chameleons (*Bradypodion*) are an ideal taxon for assessing how reptiles may adapt to rapidly changing environments. Much of the habitat occupied by *Bradypodion* (Dwarf Chameleons) has undergone some degree of transformation due to urban expansion. Some species of *Bradypodion* have managed to persist in transformed areas, such as urban gardens or road verges. These transformed areas are expected to have thermal profiles that are different to those of natural habitats as a result of the differences in canopy cover and height, as well as increased artificial surfaces which absorb more solar radiation and increase the local temperature (Brazel *et al.*, 2007; Imhoff *et al.*, 2010). Chameleons are active thermoregulators and they occupy a variety of habitats, each of which are likely to have unique thermal characteristics. It is therefore valuable to assess how various species respond to the effects of urbanisation, as the thermal profile changes between natural and transformed habitats may not be uniform, and species may have differences in their adaptive capacity for a given trait.

The primary aim of this study was to compare the selected body temperature (T_{sel}) between natural and urban populations of four species of *Bradypodion*. The hypothesis is that due to the differences in habitat structure (canopy cover, height, etc.), natural and urban populations would have differences in their thermal environments. I expected that if there were large temperature differences between the natural and urban thermal environments, populations in these environments would have different selected temperatures (T_{sel}). The more open grassland habitat might have wider temperature ranges than its urban counterpart, due to the relative increase of canopy cover in urban gardens and parks. Forest habitats, on the other hand, might suffer a reduction of canopy cover and shade availability due to urbanization. I therefore predicted that forest species were more likely to have differences in T_{sel} between natural and transformed environments, due to the assumption of a greater degree of differences in the thermal environment.

3.2 Methods

3.2.1 Study species and habitats

Bradypodion damaranum (Boulenger, 1887) (Knysna Dwarf Chameleon), occurs in remnant patches of Afrotemperate forest in the Knysna region in Western Cape Province, South Africa (Tolley & Burger, 2007; Tilbury, 2018) (Fig. S1). This species also occurs in urban gardens, parks and road verges (Tolley & Burger 2007). Natural populations were sampled in forest patches near the town of George, Western Cape Province, South Africa. The forest has high canopy crown (e.g., 30 m) with dense vegetation. Populations from transformed habitat were sampled in urban gardens and parks within the town of George. Here the canopy was much lower than in natural areas, with trees rarely exceeding 10 m. Vegetation was much patchier and had less canopy cover than in natural areas, and much of the area had exposed ground of different types (e.g., asphalt, grass, cobble/gravel).

Bradypodion setaroi (Raw, 1976) (Setaro's Dwarf Chameleon), occurs in trees and bushes and shrubs in coastal dune forests between Richards Bay and Kosi Bay, KwaZulu-Natal Province, South Africa (Tilbury, 2018) (Fig. S1). The vegetation is very dense with a high canopy in natural areas (e.g., 20 m canopy crown). For this species, there was a less stark difference in the vegetation structure between natural and transformed habitat types. Unlike the transformed areas in George, the urban study site (St. Lucia) consisted of very dense, usually natural vegetation within the town itself, and the vegetation was relatively continuous with the natural forest.

Bradypodion thamnobates (Raw, 1976) (Natal Midlands Dwarf Chameleon) is found in naturally fragmented indigenous forest patches in the Midlands region of KwaZulu-Natal

Province, South Africa (Fig. S1). Natural populations were sampled in tall, thick canopy vegetation in the indigenous forests. In contrast, populations from transformed habitat were sampled along road verges and in some gardens. This habitat had sparse canopy cover, consisted of grasses, bushes, and some trees, and was rarely taller than 2 m.

Bradypodion melanocephalum (Gray, 1865) (Durban Dwarf Chameleon) occurs along the coastal margin and inland up to about 50 km in KwaZulu-Natal Province, South Africa, with populations in natural grassland vegetation, forest ecotones, coastal wetlands, and reed beds (Tilbury, 2018; Raw, 1976) (Fig. S1). Chameleons from natural habitats were sampled in a remnant natural grassland patch consisting of tall grasses (<2 m), and scattered bushes. Transformed populations were sampled in the adjacent urban gardens and road verges, where vegetation structure was quite different to that in natural grassland. Urban gardens had trees and bushes which provided more shade and taller canopies than the grasslands where natural populations were found.

3.2.2 Field sampling

Chameleons were located at night using torchlight, which makes them easily visible. Each captured chameleon was placed in a uniquely numbered cloth bag. GPS coordinates were recorded at the point of capture and the perch where the chameleon was found was tagged so that chameleons could be returned to the exact capture site after measurement. The captured chameleons were transferred to 5 L plastic containers and kept at room temperature overnight. Each container was provided with enough vegetation for the chameleons to perch on. Chameleons were each returned to their point of capture and released within 24 hours. A relatively even sex split was sampled, and no juveniles or gravid females were included in the study.

3.2.3 Measuring environmental temperatures (operative temperatures)

Operative temperatures were recorded in each of the four field sites using iButtons (Maxim Integrated). iButtons were used as they were considered to have the thermal inertia representative of operative temperatures available to dwarf chameleons. iButtons were programmed using 1-Wire software (Maxim Integrated) to record temperatures at 15-minute intervals for a minimum of 24 hours from midnight to midnight. For each field site (per species), between two to four arrays of iButtons were suspended as vertical transects to cover as much accessible habitat of chameleons (max. 10 m height) as possible. The iButtons were spaced at one-meter intervals, from ground level to the maximum height of the array. Where logistically possible, iButton arrays were moved to different localities within the study sites every few days to record temperature variations due to factors such as shade, proximity to water, or elevation. Environmental temperatures for the habitats occupied by *B. damaranum*, *B. thamnobates*, and *B. melanocephalum* were recorded in late summer, while environmental temperatures for habitat occupied by *B. setaroi* was recorded in early summer. For each habitat, temperature data were analysed using a multivariate analysis of variance (MANOVA), to test for differences in operative temperatures between the natural and urban field sites where chameleons occurred. The analysis was run for the dataset consisting of all data points recorded for all vertical profiles including data collected over the entire diel period. The data were analysed for each corresponding metre along the profiles (i.e., for each of the eleven separate points along the profile) to assess differences between the two habitat types. Pairwise comparisons of the natural and sites were carried out using Tukey's Honest Significant Difference (HSD) post hoc test.

3.2.4 Measuring T_{sel}

The thermal preferences of chameleons were measured following the methods used by Segall *et al.* (2013). The thermal gradient was established in a wooden box (1.26 x 0.73 x 0.38 m) with six lanes separated with wooden dividers. A dowel stick was positioned in each lane for chameleons to walk along. Five heat-generating bulbs (Eurolux G230 M-infrared 275 W) were suspended above the box at one end to provide a source of heat, adjusting their height to produce a thermal gradient of 20 to 45 °C. Chameleons could move freely along the dowel to select T_b. Chameleons were placed in the lanes in the morning and left for at least an hour to acclimate. Body temperatures then were measured for each chameleon every hour for a maximum of five hours by inserting a lubricated thermocouple approximately 4 mm into the cloaca.

Because weather might have an effect on T_{sel}, weather on each day was recorded as either "sunny," "cloudy," "overcast," or "rainy". Differences between natural and urban populations of each species for T_{sel} were analysed using a nested analysis of covariance (ANCOVA) design, with T_{sel} as the response variable, snout-vent length (SVL) as a covariate to correct for body size, and weather and sex as random factors. A backwards stepwise regression approach was used to achieve the most parsimonious model and the analysis was re-run. Since SVL and sex had no effect on the T_{sel} for any species, they were then removed from the model. Pairwise differences between species were assessed using Tukey's Honest Significant Difference (HSD) post hoc test.

3.3 Results

3.3.1 Environmental temperatures

All three natural forest habitats were cooler than their urban counterparts throughout majority of the vertical profile, while the grassland habitat occupied by *B. melanocephalum* was warmer than its urban counterpart (Table 3, Fig. 8). The urban habitat occupied by *B. damaranum* had more rapid temperature fluctuations than the natural habitat across a 24-hour period (Fig. 9). The same trend was observed in the habitat occupied by *B. setaroi* (Fig. 10). The forest habitat occupied by *B. thamnobates* had spatial heterogeneity of temperatures, with ground temperatures being the coolest, and temperatures gradually increasing further up the canopy. There was no clear temperature gradient in the urban habitat (Fig. 11). Similarly, the grassland habitat occupied by *B. melanocephalum* had a wider range in temperatures across the vertical profile than the urban habitat (Fig. 12).

Table 3. Significance values of pairwise comparisons of environmental temperatures for habitats of four *Bradypodion* species, between natural and urban environments. Comparisons were made at 1-m intervals across 10 m vertical profiles. *F*-values and corresponding *p*-values are shown for each comparison, and significant comparisions are in bold font.

	B. thamnobates		*B. setaroi*		*B. damaranum*		*B. melanocephalum*	
Metre	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value
0	60.243	**<0.001**	29.074	**<0.001**	98.796	**<0.001**	196.76	**<0.001**
1	39.918	**<0.001**	12.709	**<0.001**	77.748	**<0.001**	68.546	**<0.001**
2	8.4558	**0.004**	32.315	**<0.001**	82.412	**<0.001**	41.961	**<0.001**
3	20.316	**<0.001**	51.645	**<0.001**	53.804	**<0.001**	27.122	**<0.001**
4	3.2809	**0.07**	13.448	**<0.001**	56.413	**<0.001**	29.891	**<0.001**
5	13.878	**<0.001**	17.806	**<0.001**	32.159	**<0.001**	68.876	**<0.001**
6	31.157	**<0.001**	40.047	**<0.001**	29.767	**<0.001**	112.57	**<0.001**
7	26.942	**<0.001**	1.9152	0.167	32.103	**<0.001**	107.42	**<0.001**
8	0.0404	0.841	20.479	**<0.001**	27.675	**<0.001**	31.267	**<0.001**
9	0.3155	0.574	0.8355	0.361	30.684	**<0.001**	72.45	**<0.001**
10	28.518	**<0.001**	0.9872	0.321	25.159	**<0.001**	43.239	**<0.001**

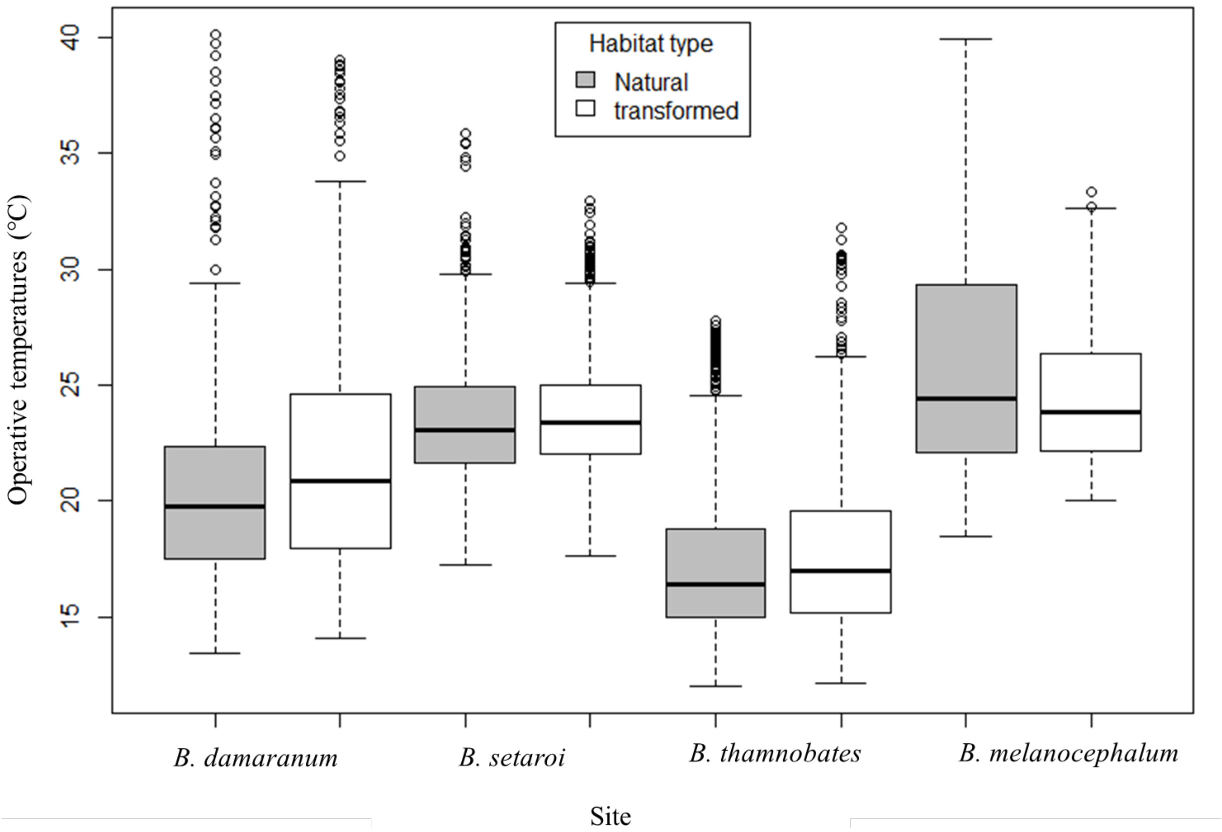

Figure 8. Range of operative temperatures (°C) for the natural and transformed habitats where four species of *Bradypodion* occur. *Bradypodion damaranum*, *B. setaroi*, and *B. thamnobates* naturally occur in forests, and *B. melanocephalum* naturally occurs in grassland. Transformed habitats were urban gardens/parks and road verges. Boxes show the range of temperatures from the 1st to 3rd percentiles, with the mean indicated by the line within the box. The whiskers show the range of values from min/max to the lower/upper percentiles, respectively. Outliers (values greater than 1.5 times the 75th or 25th interquartile range) are indicated by circles. Boxes representing temperatures for the natural habitats are filled in grey and transformed habitats in white.

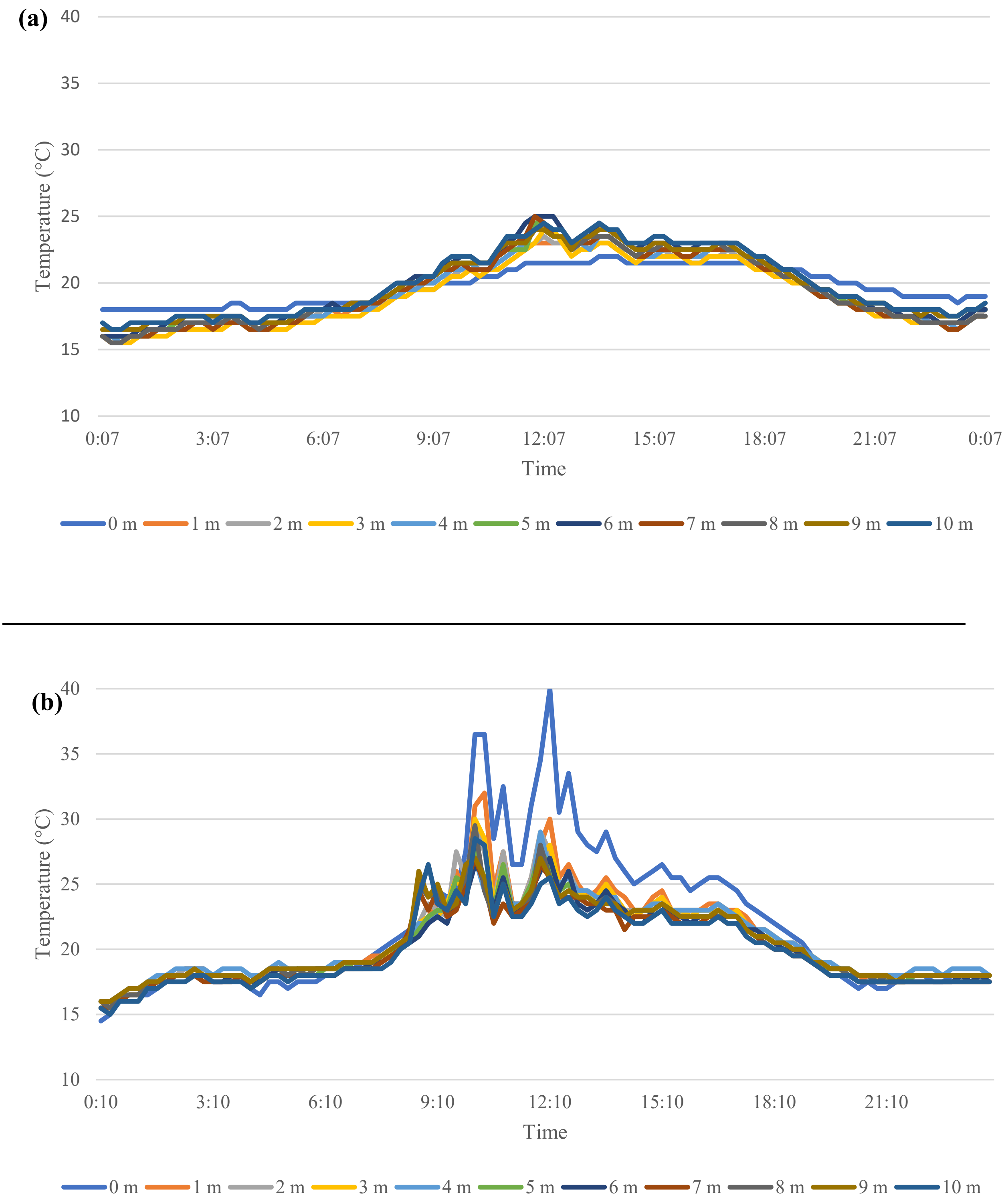

Figure 9. Diel operative temperatures for (a) natural forest habitat and (b) urban habitat where *Bradypodion damaranum* occurs, recorded on the 18th of February 2020. Temperatures were recorded along a 10 m vertical profile at 1-m increments. The graph shows time on the x-axis and temperature in °C on the y-axis. Intervals are colour coded according to the key.

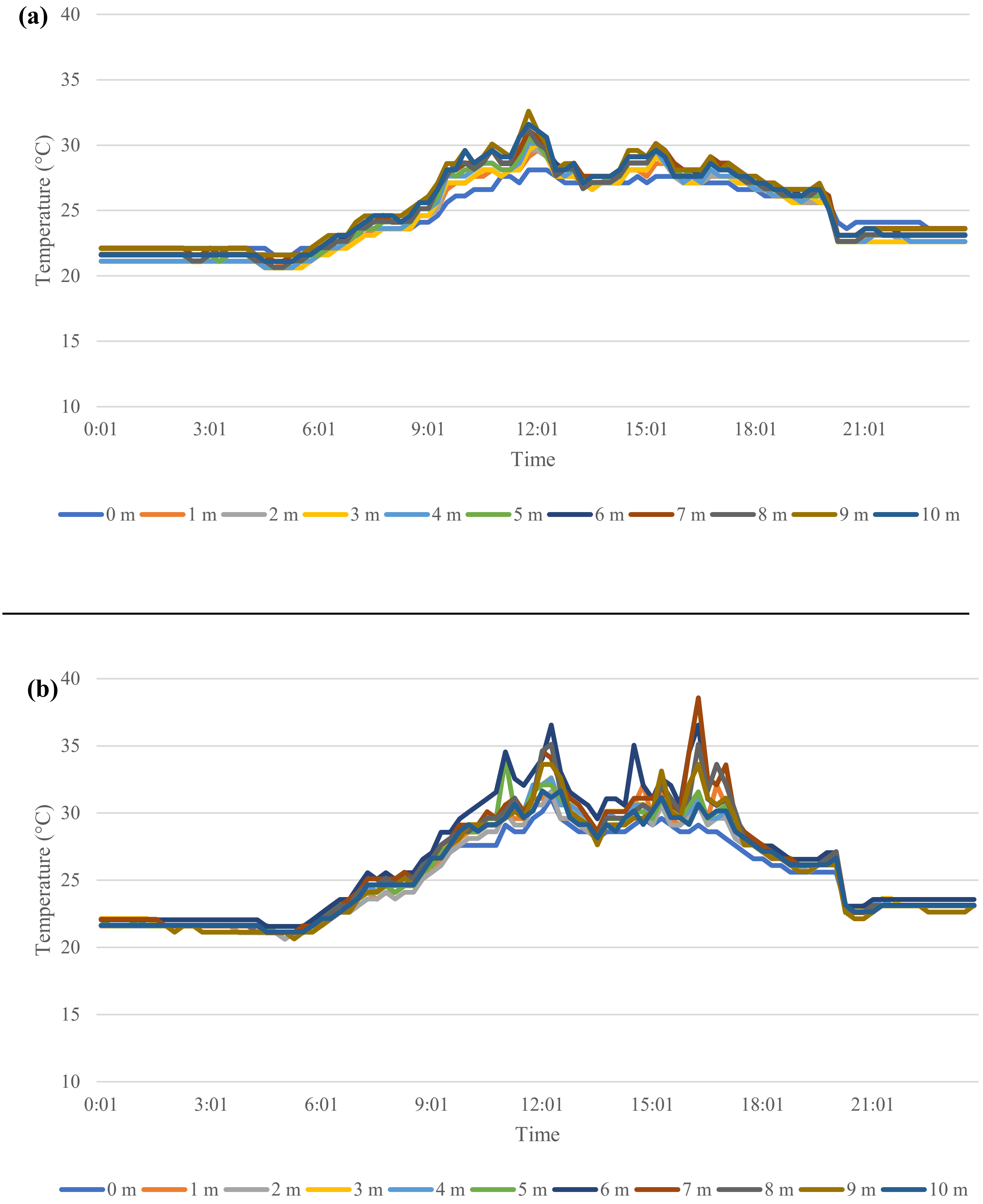

Figure 10. Diel operative temperatures for (a) natural forest habitat and (b) urban habitat where *Bradypodion setaroi* occurs, recorded on the 23rd of November 2020. Temperatures were recorded along a 10 m vertical profile at 1-m increments. The graph shows time on the x-axis and temperature in °C on the y-axis. Intervals are colour coded according to the key.

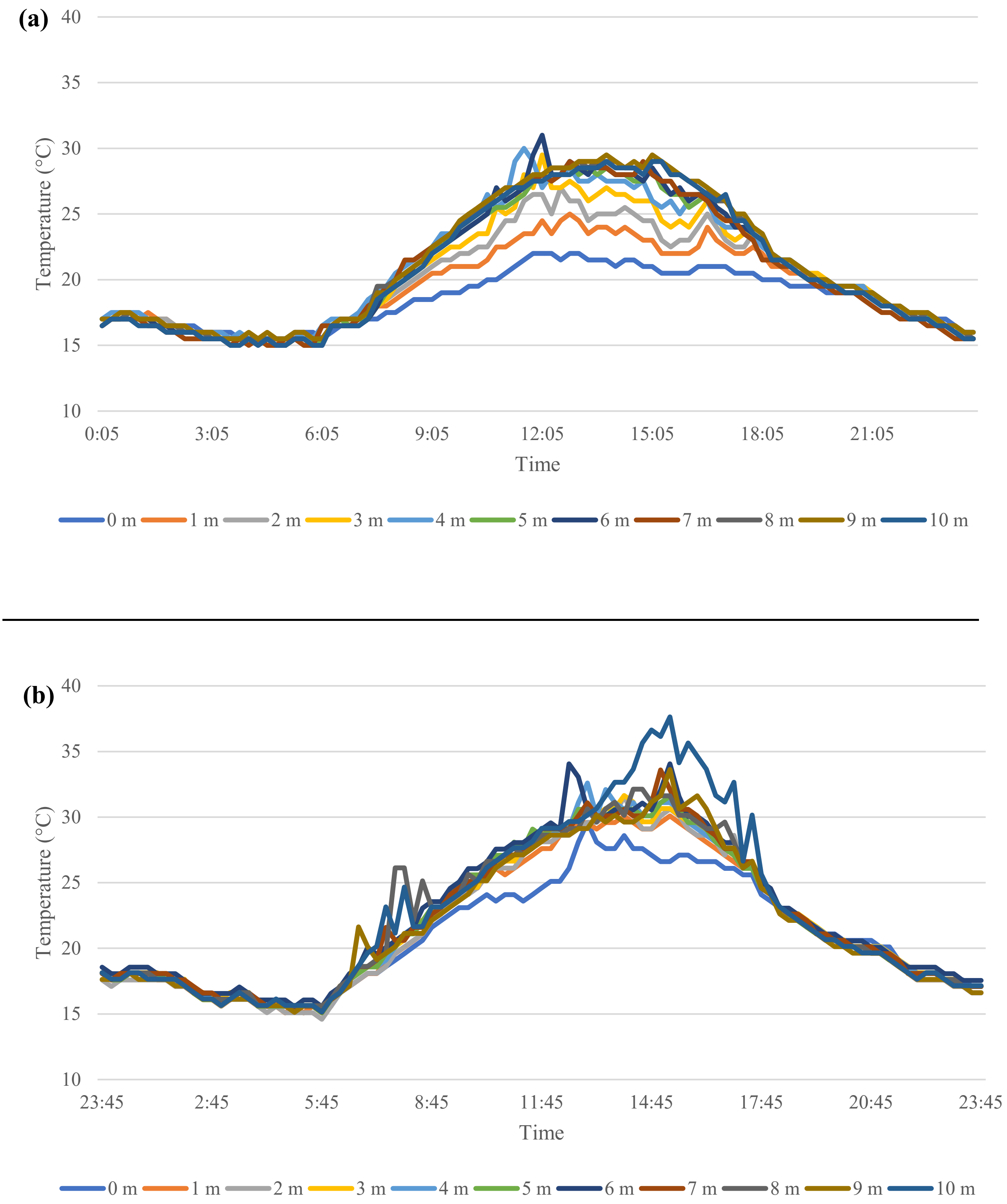

Figure 11. Diel operative temperatures for (a) natural forest habitat and (b) urban habitat where *Bradypodion thamnobates* occurs, recorded on the 20th of February 2021. Temperatures were recorded along a 10 m vertical profile at 1-m increments. The graph shows time on the x-axis and temperature in °C on the y-axis. Intervals are colour coded according to the key.

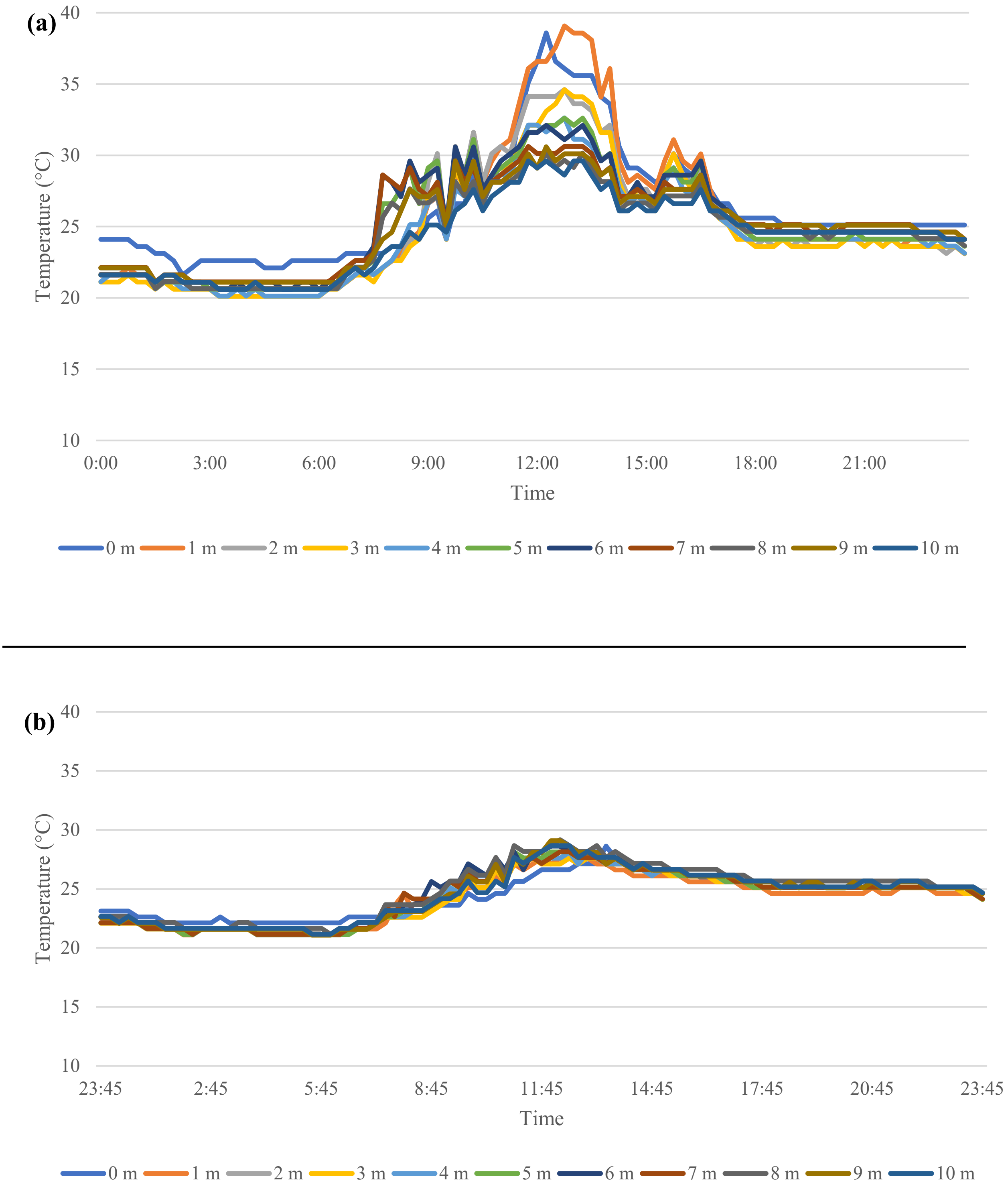

Figure 12. Diel operative temperatures for (a) natural grassland habitat and (b) urban habitat where *Bradypodion melanocephalum* occurs, recorded on the 12th of March 2021. Temperatures were recorded along a 10 m vertical profile at 1-m increments. The graph shows time on the x-axis and temperature in °C on the y-axis. Intervals are colour coded according to the key.

3.3.2 Selected temperatures

Bradypodion thamnobates (natural habitat: females n = 12, males n = 15; transformed habitat: females n = 13, males n = 14) was the only species that exhibited a difference in T_{sel} between urban and natural populations (ANOVA, $F_{1,47} = 4.060$, $P = 0.049$). While there was no difference in T_{sel} between urban and natural populations of *Bradypodion damaranum* (natural habitat: females n = 18, males n =13; transformed habitat: females n = 19, males n = 14) (ANOVA, $F_{1,56} = 0.597$, $P = 0.433$), weather had an effect on T_{sel} (ANOVA, $F_{6,56} = 7.447$, $P < 0.001$), with lower temperatures being chosen under rainy conditions. T_{sel} did not differ between natural and urban populations of *Bradypodion setaroi* (natural habitat: females n = 17, males n = 15; transformed habitat: females n = 17, males n = 17) (ANOVA, $F_{1,58} = 3.077$, $P = 0.084$) and *B. melanocephalum* (natural habitat: females n = 10, males n = 13; transformed habitat: females n = 10, males n = 11) (ANOVA, $F_{1,38} = 0.878$, $P = 0.355$).

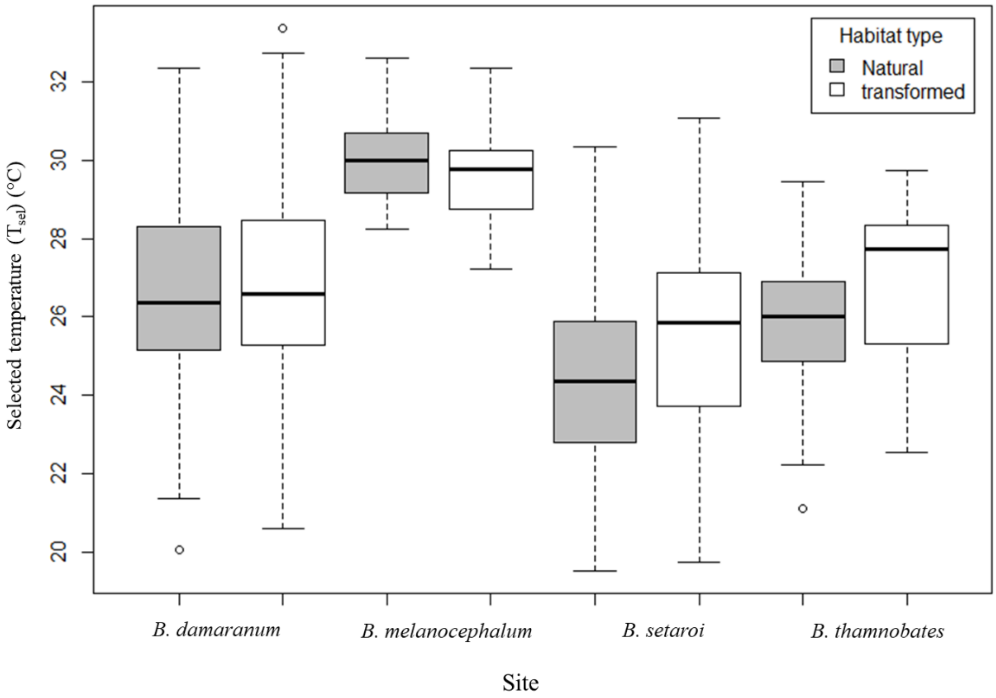

Figure 13. Range of selected body temperatures (°C) at natural and urban sites for four species of *Bradypodion* measured in a thermal gradient. Boxes show the range of temperatures from the 1st to 3rd percentiles, with the mean indicated by the line within the box. The whiskers show the range of values from min/max to the lower/upper percentiles, respectively. Outliers (values greater than 1.5 times the 75th or 25th interquartile range) are indicated by circles.

3.4 Discussion

The difference in T_{sel} between urban and natural populations of *B. thamnobates* suggests that dwarf chameleons have some capacity to rapidly adjust their thermal physiology in response to changing environments. The thermal environments of all four species in this study differed between natural and transformed sites. As expected, the forest sites (of *B. damaranum*, *B. setaroi*, and *B. thamnobates*) were cooler than their urban counterparts, while the natural grassland site where *B. melanocephalum* occurs was warmer than its urban counterpart. From the vertical profiles, it appears that the urban sites of *B. damaranum* and *B. setaroi* had greater thermal heterogeneity than the corresponding natural sites. On the other hand, the urban sites of *B. thamnobates* and *B. melanocephalum* had less thermal heterogeneity than the natural sites.

Given that behavioural thermoregulation can act as a buffer for temperature change (Bogert, 1949), the same principal can be applied to more local environmental changes resulting from habitat changes and urbanisation. This could explain why despite all species having environmental differences between urban and natural sites, not all of them had a corresponding shift in T_{sel} between sites. In addition to behaviour buffering the effects of environmental temperature changes, chameleons can undergo rapid colour change which may further help buffer environmental changes by altering the absorptance of solar energy (Smith *et al.*, 2016). Anecdotal evidence from the field suggests that some colour changes may be thermally motivated. For example, on days where sunlight was patchy and limited, chameleons would often turn darker hues and bask in patches of sunlight. Similarly, several other species not included in this chapter, *B. barbatulum B. occidentale,* and *B. pumilum* have been observed to turn white during very hot conditions (i.e., approaching 40 °C) under which they could not find shade (pers. obs.; K.A. Tolley pers. comm.).

Despite the differences in environmental temperatures between sites, some species of chameleons can stay within optimal temperature ranges, and this is presumably done by shuttling between warmer and cooler areas within their habitat. However, if warm and cool sites are very far apart or there is little spatial heterogeneity of temperatures, behavioural thermoregulation can become costly (Huey & Slatkin, 1976). Perhaps species only experience physiological changes when behavioural thermoregulation is too costly a means to achieve preferred body temperatures. The reduction of spatial heterogeneity of environmental temperatures for the urban sites of *B. thamnobates* is likely a contributor to this species undergoing a change in T_{sel}. In cases where thermal heterogeneity is decreasing and temperatures are increasing, chameleons would be unable to find refuge from dangerously high temperatures. Therefore, there is additional selective pressure for *B. thamnobates* to undergo physiological adaptation in urban spaces, but none for *B. melanocephalum* in urban spaces given they are cooler than the natural grassland. While behavioural thermoregulation is an efficient buffer to climatic changes, it can only be beneficial when habitats have adequate thermal heterogeneity. Maintaining this heterogeneity in urban environments could be a crucial step to ensuring ectotherm persistence.

A potential drawback of this study is that environmental temperatures were only measured during one season and are not representative of temperatures available to chameleons during colder months. This also meant I could not assess whether seasonality affects T_{sel}. Temperatures were only measured for 10 m in forests, and the forest canopy was often higher than that. Chameleons may be exploiting thermal environments which were not measured in this study. Additionally, the scope of this study did not include assessing whether physiological changes are evolutionarily plastic. However, the study design presents many advantages. Rather than considering just the average temperatures of a habitat, temperatures available throughout a vertical profile were considered. This provides a better representative of the microclimate available to chameleons and allows me to infer how it may promote or hinder the ability of chameleons to behaviourally thermoregulate by shuttling between warmer and cooler areas. Additionally, this study includes data on the habitat and physiology of several species within the genus. This highlighted how it is inaccurate to assume all urban areas will be warmer than natural areas, as the structural differences in vegetation and shade availability between habitats is more important to consider. Secondly, it is evident that not all species may respond the same way.

There are very few studies done on reptile thermal adaptation to urbanisation. However, in corroboration with the findings in this study, Campbell-Staton (2020) compared the environmental temperatures and species responses between natural and urban habitats and found that urban habitats were significantly warmer than natural sites, and that lizards in the urban environments occupied warmer localities than lizards in forest sites. Similarly, recently introduced (~35 years) populations of *Anolis cristatellus* experienced cooler temperatures than in their native ranges and had corresponding lower critical thermal minima (CT_{min}) (Leal & Gunderson, 2012).

The results of this study suggest that the capacity of *Bradypodion* to undergo thermal adaptation in response to environmental changes may be limited on a short (~100 years) timescale. However, it is important to consider that behavioural thermoregulation might enable some species of chameleons to comfortably stay within their optimal ranges and preventing the need for physiological adaptation. Although the presence of thermal adaptation in only one of the four species in this study could be attributed to the effects of behavioural thermoregulation or perhaps to the severity of the habitat transformation, there is the possibility that thermal adaptation for most species could be too slow to keep up with rapid climatic and habitat changes. It is evident that if it is assumed that thermal traits of all ectotherms cannot evolve, we may be inaccurately predicting how species may respond to climatic and environmental changes. What is a concern, regardless of the adaptive capacity of *Bradypodion*, is that urbanisation in some cases may lead to the loss of thermal habitat heterogeneity, which could make species more vulnerable to changes in climate (Neel *et al.*, 2020). Even if the overall average temperatures of urban areas increase, maintaining urban habitats with structural complexity that provide thermal heterogeneity could ensure that chameleons have a range of temperatures to shuttle between and stay within optimal temperature ranges. While chameleons can use behavioural thermoregulation to buffer climatic changes, as well as potentially adapt to changes, it is crucial to ensure that the thermal quality of their environments is preserved to ensure their survival.

CHAPTER 4

GENERAL CONCLUSION

This main aim of this study was to assess the evolutionary capacity and adaptability of the thermoregulatory response in the genus *Bradypodion* on both evolutionary and ecologically relevant timescales. This was achieved by comparing the thermal environments of different habitats and sites within these habitats and then assessing whether populations in these habitats or sites have undergone changes in their selected temperature (T_{sel}). Firstly, comparisons were made between forest habitats and more open habitats such as grasslands and fynbos. The expectation was that chameleons occurring in these respective habitats would be exposed to different thermal environments, and as a result, display differences in their thermal physiology. Secondly, for species which occur in urban areas, the thermal environment was compared between natural environments and their anthropogenically transformed counterparts. It was predicted that forest habitats would have more shade and spatial thermal heterogeneity than their urban counterparts, while grassland habitats would have less shade than in urban areas and that temperatures in natural grassland habitats would be warmer and more extreme.

On both timescales, there was evidence of thermal physiology adjustments in response to environmental changes. This finding has an impact on the way we approach predictive models of species distribution changes, as species may adapt to temperature changes rather than only track suitable temperature ranges. For a genus such as *Bradypodion* where dispersal capacity is low, the ability to adapt to environmental changes is a crucial aspect of persisting in this rapidly changing period. The adaptive capacity of thermal traits has been a contentious topic in the scientific community for many decades, with many studies reporting evidence that thermal traits are not evolutionary labile. However, this study as well as several others suggest that the hypothesis that thermal trait evolution is static cannot simply apply to all reptiles, as changes in thermal biology on an anthropogenic timescale were reported (Scheers & Van Damme, 2002; Leal & Gunderson, 2012; Logan *et al*., 2014; Akashi et al., 2016; Gilbert & Miles, 2017; Gilbert & Miles, 2019; Campbell-Staton, 2020). Whether species have the capacity to undergo thermal adaptation may depend on the inherent biology of the taxon, or the nature of the environment that it occurs in. Before predictions can be made on whether a taxon can rapidly adapt to environmental changes, it is necessary to assess whether the taxon has shown historical lability of the traits in question, as well as assess the thermal quality of its environment.

The study also brings to light the importance of thermal heterogeneity in habitats. For the three species that did not show a change in T_{sel} on an ecological timescale, it is likely that the vegetation in the urban space is intact enough to allow them to shuttle between warmer and cooler areas to efficiently thermoregulate. In contrast, *B. thamnobates* occurs in a severely altered urban habitat and it is likely that thermal heterogeneity has been decreased enough that individuals cannot seek refuge from critical temperatures. Assuming that the capacity to behaviourally thermoregulate has been compromised due to the lack of suitable microclimates, the response has been to shift T_{sel} to a value that is more frequently attainable in the environment. Maintaining structurally complex habitats with thermal heterogeneity will play a key role in ensuring the persistence of ectotherms, as thermal homogeneity significantly increases the risk of local extirpation (Neel *et al*., 2021).

The effects of urbanisation and global climatic change are expected to intensify (United Nations, Department of Economic and Social Affairs, Population Division, 2015), and while several studies have looked at morphological adaptations to these issues (Herrell *et al*., 2011; Hopkins & Tolley, 2011; Winchell *et al*., 2016), our knowledge on thermal adaptations on a short timescale is relatively sparse (Leal & Gunderson, 2012; Winchell *et al*., 2017; Campbell-Staton, 2020). In groups such as chameleons, which are often habitat specialists – they are facing threats of habitat reduction and the compounded effects of changes in their thermal environment. It is becoming increasingly important to understand how these groups may respond to rapid changes, in order to implement the best possible conservation strategies.

In summary, the persistence of species in novel thermal environments is dependent on the lability of the thermal physiology, as well as the quality of the thermal habitat of the species. There are two major factors that need to be considered in assessing the vulnerability of species to rapid environmental changes. Firstly, the historical adaptation or lack thereof of thermal traits may give some indication as to whether there is any potential for a trait to undergo adaptation on a short timescale. Secondly, coarse environmental data does not provide an adequate measure of how at risk a species may be. Average environmental temperatures do not provide details such as environmental heterogeneity and may result in over or under estimating differences between environments. Methodology to improve the mapping of microclimatic data will be crucial for assessing species vulnerability (e.g. Kearney *et al.,* 2020). To better predict species responses to changes, the inherent physiological lability as well as habitat quality will need to be taken into consideration.

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Table S1. Significance values of pairwise comparisons of environmental temperatures for sampling sites of five *Bradypodion* species. Comparisons were made at 1-m intervals across 10 m vertical profiles. F values and corresponding p values are shown for each comparison, and significant comparisions are in bold font.

Metre	Habitat		*p*-value	Metre	Habitat		*p*-value
0	*B. barbatulum*	*B. damaranum*	**<0.001**	6	*B. barbatulum*	*B. damaranum*	**<0.001**
		B. melanocephalum	**<0.001**			*B. melanocephalum*	**<0.001**
		B. setaroi	**<0.001**			*B. setaroi*	0.894
		B. thamnobates	**<0.001**			*B. thamnobates*	**<0.001**
	B. damaranum	*B. barbatulum*	**<0.001**		*B. damaranum*	*B. barbatulum*	**<0.001**
		B. melanocephalum	**<0.001**			*B. melanocephalum*	**<0.001**
		B. setaroi	**<0.001**			*B. setaroi*	**<0.001**
		B. thamnobates	**<0.001**			*B. thamnobates*	**<0.001**
	B. melanocephalum	*B. barbatulum*	**<0.001**		*B. melanocephalum*	*B. barbatulum*	**<0.001**
		B. damaranum	**<0.001**			*B. damaranum*	**<0.001**
		B. setaroi	**<0.001**			*B. setaroi*	**<0.001**
		B. thamnobates	**<0.001**			*B. thamnobates*	**<0.001**
	B. setaroi	*B. barbatulum*	**<0.001**		*B. setaroi*	*B. barbatulum*	0.894
		B. damaranum	**<0.001**			*B. damaranum*	**<0.001**
		B. melanocephalum	**<0.001**			*B. melanocephalum*	**<0.001**
		B. thamnobates	**<0.001**			*B. thamnobates*	**<0.001**
	B. thamnobates	*B. barbatulum*	**<0.001**		*B. thamnobates*	*B. barbatulum*	**<0.001**
		B. damaranum	**<0.001**			*B. damaranum*	**<0.001**
		B. melanocephalum	**<0.001**			*B. melanocephalum*	**<0.001**
		B. setaroi	**<0.001**			*B. setaroi*	**<0.001**
1	*B. barbatulum*	*B. damaranum*	**<0.001**	7	*B. barbatulum*	*B. damaranum*	**<0.001**
		B. melanocephalum	**<0.001**			*B. melanocephalum*	**<0.001**
		B. setaroi	**<0.001**			*B. setaroi*	0.118
		B. thamnobates	**<0.001**			*B. thamnobates*	**<0.001**
	B. damaranum	*B. barbatulum*	**<0.001**		*B. damaranum*	*B. barbatulum*	**<0.001**
		B. melanocephalum	**<0.001**			*B. melanocephalum*	**<0.001**
		B. setaroi	**<0.001**			*B. setaroi*	**<0.001**
		B. thamnobates	**<0.001**			*B. thamnobates*	**<0.001**
	B. melanocephalum	*B. barbatulum*	**<0.001**		*B. melanocephalum*	*B. barbatulum*	**<0.001**
		B. damaranum	**<0.001**			*B. damaranum*	**<0.001**
		B. setaroi	**<0.001**			*B. setaroi*	**<0.001**
		B. thamnobates	**<0.001**			*B. thamnobates*	**<0.001**
	B. setaroi	*B. barbatulum*	**<0.001**		*B. setaroi*	*B. barbatulum*	0.118
		B. damaranum	**<0.001**			*B. damaranum*	**<0.001**
		B. melanocephalum	**<0.001**			*B. melanocephalum*	**<0.001**
		B. thamnobates	**<0.001**			*B. thamnobates*	**<0.001**
	B. thamnobates	*B. barbatulum*	**<0.001**		*B. thamnobates*	*B. barbatulum*	**<0.001**
		B. damaranum	**<0.001**			*B. damaranum*	**<0.001**
		B. melanocephalum	**<0.001**			*B. melanocephalum*	**<0.001**
		B. setaroi	**<0.001**			*B. setaroi*	**<0.001**
2	*B. barbatulum*	*B. damaranum*	**<0.001**	8	*B. barbatulum*	*B. damaranum*	**<0.001**
		B. melanocephalum	**<0.001**			*B. melanocephalum*	**<0.001**
		B. setaroi	**0.003**			*B. setaroi*	0.081
		B. thamnobates	**<0.001**			*B. thamnobates*	**<0.001**
	B. damaranum	*B. barbatulum*	**<0.001**		*B. damaranum*	*B. barbatulum*	**<0.001**
		B. melanocephalum	**<0.001**			*B. melanocephalum*	**<0.001**
		B. setaroi	**<0.001**			*B. setaroi*	**<0.001**
		B. thamnobates	**<0.001**			*B. thamnobates*	**<0.001**
	B. melanocephalum	*B. barbatulum*	**<0.001**		*B. melanocephalum*	*B. barbatulum*	**<0.001**
		B. damaranum	**<0.001**			*B. damaranum*	**<0.001**
		B. setaroi	**<0.001**			*B. setaroi*	**<0.001**
		B. thamnobates	**<0.001**			*B. thamnobates*	**<0.001**
	B. setaroi	*B. barbatulum*	**0.003**		*B. setaroi*	*B. barbatulum*	0.081
		B. damaranum	**<0.001**			*B. damaranum*	**<0.001**
		B. melanocephalum	**<0.001**			*B. melanocephalum*	**<0.001**
		B. thamnobates	**<0.001**			*B. thamnobates*	**<0.001**
	B. thamnobates	*B. barbatulum*	**<0.001**		*B. thamnobates*	*B. barbatulum*	**<0.001**

		B. damaranum	**<0.001**			*B. damaranum*	**<0.001**
		B. melanocephalum	**<0.001**			*B. melanocephalum*	**<0.001**
		B. setaroi	**<0.001**			*B. setaroi*	**<0.001**
3	*B. barbatulum*	*B. damaranum*	**<0.001**	9	*B. barbatulum*	*B. damaranum*	**<0.001**
		B. melanocephalum	**<0.001**			*B. melanocephalum*	**<0.001**
		B. setaroi	0.181			*B. setaroi*	**0.008**
		B. thamnobates	**<0.001**			*B. thamnobates*	**<0.001**
	B. damaranum	*B. barbatulum*	**<0.001**		*B. damaranum*	*B. barbatulum*	**<0.001**
		B. melanocephalum	**<0.001**			*B. melanocephalum*	**<0.001**
		B. setaroi	**<0.001**			*B. setaroi*	**<0.001**
		B. thamnobates	**<0.001**			*B. thamnobates*	**<0.001**
	B. melanocephalum	*B. barbatulum*	**<0.001**		*B. melanocephalum*	*B. barbatulum*	**<0.001**
		B. damaranum	**<0.001**			*B. damaranum*	**<0.001**
		B. setaroi	**<0.001**			*B. setaroi*	**<0.001**
		B. thamnobates	**<0.001**			*B. thamnobates*	**<0.001**
	B. setaroi	*B. barbatulum*	0.181		*B. setaroi*	*B. barbatulum*	**0.008**
		B. damaranum	**<0.001**			*B. damaranum*	**<0.001**
		B. melanocephalum	**<0.001**			*B. melanocephalum*	**<0.001**
		B. thamnobates	**<0.001**			*B. thamnobates*	**<0.001**
	B. thamnobates	*B. barbatulum*	**<0.001**		*B. thamnobates*	*B. barbatulum*	**<0.001**
		B. damaranum	**<0.001**			*B. damaranum*	**<0.001**
		B. melanocephalum	**<0.001**			*B. melanocephalum*	**<0.001**
		B. setaroi	**<0.001**			*B. setaroi*	**<0.001**
4	*B. barbatulum*	*B. damaranum*	**<0.001**	10	*B. barbatulum*	*B. damaranum*	**<0.001**
		B. melanocephalum	**<0.001**			*B. melanocephalum*	**<0.001**
		B. setaroi	0.915			*B. setaroi*	0.499
		B. thamnobates	**<0.001**			*B. thamnobates*	**<0.001**
	B. damaranum	*B. barbatulum*	**<0.001**		*B. damaranum*	*B. barbatulum*	**<0.001**
		B. melanocephalum	**<0.001**			*B. melanocephalum*	**<0.001**
		B. setaroi	**<0.001**			*B. setaroi*	**<0.001**
		B. thamnobates	**<0.001**			*B. thamnobates*	**<0.001**
	B. melanocephalum	*B. barbatulum*	**<0.001**		*B. melanocephalum*	*B. barbatulum*	**<0.001**
		B. damaranum	**<0.001**			*B. damaranum*	**<0.001**
		B. setaroi	**<0.001**			*B. setaroi*	**<0.001**
		B. thamnobates	**<0.001**			*B. thamnobates*	**<0.001**
	B. setaroi	*B. barbatulum*	0.915		*B. setaroi*	*B. barbatulum*	0.499
		B. damaranum	**<0.001**			*B. damaranum*	**<0.001**
		B. melanocephalum	**<0.001**			*B. melanocephalum*	**<0.001**
		B. thamnobates	**<0.001**			*B. thamnobates*	**<0.001**
	B. thamnobates	*B. barbatulum*	**<0.001**		*B. thamnobates*	*B. barbatulum*	**<0.001**
		B. damaranum	**<0.001**			*B. damaranum*	**<0.001**
		B. melanocephalum	**<0.001**			*B. melanocephalum*	**<0.001**
		B. setaroi	**<0.001**			*B. setaroi*	**<0.001**
5	*B. barbatulum*	*B. damaranum*	**<0.001**				
		B. melanocephalum	**<0.001**				
		B. setaroi	0.539				
		B. thamnobates	**<0.001**				
	B. damaranum	*B. barbatulum*	**<0.001**				
		B. melanocephalum	**<0.001**				
		B. setaroi	**<0.001**				
		B. thamnobates	**<0.001**				
	B. melanocephalum	*B. barbatulum*	**<0.001**				
		B. damaranum	**<0.001**				
		B. setaroi	**<0.001**				
		B. thamnobates	**<0.001**				
	B. setaroi	*B. barbatulum*	0.539				
		B. damaranum	**<0.001**				
		B. melanocephalum	**<0.001**				
		B. thamnobates	**<0.001**				
	B. thamnobates	*B. barbatulum*	**<0.001**				
		B. damaranum	**<0.001**				
		B. melanocephalum	**<0.001**				
		B. setaroi	**<0.001**				

Table S2. Significance values of ANOVA pairwise comparisons of environmental temperature ranges between the habitats of five *Bradypodion* species. *p*-values are shown for each comparison, and significant comparisions are in bold font.

	B. barbatulum	*B. damaranum*	*B. melanocephalum*	*B. setaroi*	*B. thamnobates*
B. barbatulum	-	-	-	-	-
B. damaranum	**<0.001**	-	-	-	-
B. melanocephalum	**<0.001**	**<0.001**	-	-	-
B. setaroi	**<0.001**	0.879	**<0.001**	-	-
B. thamnobates	**<0.001**	0.697	**<0.001**	0.997	-

Table S3. Significance values of ANOVA pairwise comparisons of environmental temperature averages between the habitats of five *Bradypodion* species. *p*-values are shown for each comparison, and significant comparisions are in bold font.

	B. barbatulum	*B. damaranum*	*B. melanocephalum*	*B. setaroi*	*B. thamnobates*
B. barbatulum	-	-	-	-	-
B. damaranum	**<0.001**	-	-	-	-
B. melanocephalum	0.888	**<0.001**	-	-	-
B. setaroi	0.159	**0.005**	0.066	-	-
B. thamnobates	**<0.001**	**0.003**	**<0.001**	**<0.001**	-

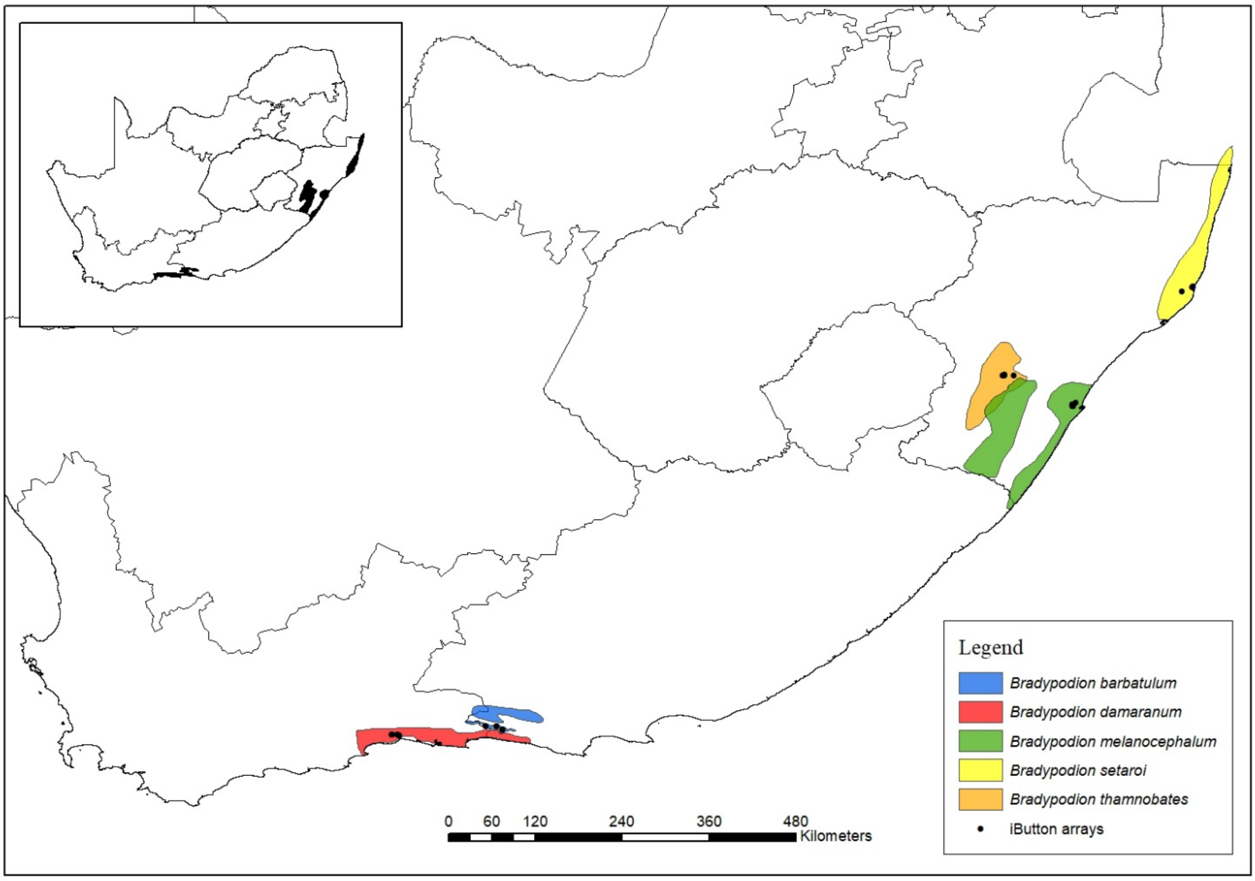

Figure S1. Species distribution map for five species of *Bradypodion*. Black dots represent where environmental temperatures were recorded (Tolley *et al*. 2022).