

**Heat Hardening and the Influence of Thermal Heterogeneity of  
Habitat on Aboveground Activity in Spiny-tailed Lizard (*Saara  
hardwickii*)**

by

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**in**

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Under the supervision of

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**June, 2025**



## DECLARATION

I hereby declare that the work conducted under the thesis entitled “Heat Hardening and the Influence of Thermal Heterogeneity of Habitat on Aboveground Activity in Spiny-tailed Lizard (*Saara hardwickii*)”, is a record of original and independent research work done by me and subsequently submitted for the award of the degree of Master’s in Wildlife Science at the Academy of Scientific and Innovative Research. This research work has been carried out under the guidance and supervision of Dr. Sutirtha Dutta, Scientist - E, and co-supervision of Mr. Varun Kher, Scientist - C of Wildlife Institute of India, Dehradun. The work has not formed the basis for the award of any other degree, diploma, or any other qualification. I also declare that the thesis embodies my own work, analysis, observation, understanding and the particulars given in it are true to the best of my knowledge.

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## CERTIFICATE

This is to certify that the thesis by Chirag Vassa entitled "Heat Hardening and the Influence of Thermal Heterogeneity of Habitat on Aboveground Activity in Spiny-tailed Lizard (*Saara hardwickii*)" is an original and independent research work submitted to the Academy of Scientific and Innovative Research, for the award of the degree of Master's in Wildlife Science.

Chirag Vassa has put one semester of research work embodied in this thesis under my guidance and supervision. The work presented in this thesis has not been submitted to any other University or Institute for the award of any degree, diploma or distinction.

  
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## Summary

Human induced climate change has challenged the persistence of many organisms at different biotic levels and alter their interaction with the environment. Ectotherms are particularly vulnerable to anthropogenic climate change due to their reliance on external environment for various life history traits. Thermoregulatory behaviour is often used by many ectotherms especially reptiles to regulate their body temperature and will be crucial in imminent future with intensification of climate change. Although behavioural thermoregulation is widely studied, but studies looking at influence of thermal heterogeneity of a habitat on aboveground activity is limited.

Moreover, with escalation in global warming ectotherms might have to rely on another mechanism in addition to thermoregulatory behaviour to combat the effect. One such physiological mechanism is heat hardening a quick response to thermal tolerance that temporarily enhances thermal tolerance, allowing lizards to withstand greater temperatures for a short period. Despite its importance very limited data is available for heat hardening capacity in reptiles.

This study examined the influence of thermal heterogeneity of a habitat on aboveground activity in spiny-tailed lizard (*Saara hardwickii*) and assessed their heat hardening capacity. Additionally, trade-off hypothesis was also tested which has been recently proposed to explain the dynamics of thermal tolerance plasticity.

I found positive influence of habitat heterogeneity on aboveground activity of the lizard. However, individuals in both the conditions spent similar amount of time in different behaviours. With respect to phenotypic plasticity i.e. heat hardening, I did not find any evidence of thermal tolerance plasticity during my experimental trials but few individuals did show

increased thermal tolerance implying intraspecific variation. Additionally, the results from heat hardening experiments found no support for trade-off hypothesis.

My study underscores the importance of habitat heterogeneity for species like spiny-tailed lizard which thrives in extreme temperatures. Furthermore, Lack of phenotypic plasticity in thermal tolerance increases it's risks to overheating and dependency on behavioural thermoregulation.

## Glossary

| Abbreviation        | Term                            | Explanation   |
|---------------------|---------------------------------|---|
| $T_b$               | Body temperature                | The temperature of lizard's body  |
| $T_e$               | Operative temperature           | Temperatures of an inanimate object which is in thermal equilibrium with the environment, resembling the size, shape and radiative properties of animal, when placed in the same environment. |
| $VT_{max}$          | Voluntary maximum temperature   | The upper $T_b$ at which the lizard retreats to its refugia to prevent further heating  |
| $CT_{max}$          | Critical thermal maxima         | The upper $T_b$ at which the lizard loses neuromuscular coordination  |
| $B_{95}$            | 95% Thermal performance breadth | The range of $T_b$ at which the performance is greater than or equal to 95% of $V_{max}$  |
| $T_{set}/ T_{pref}$ | Preferred temperature           | Range of body temperature selected/preferred by lizards   |

## Table of Contents

|   |           |
|---|-----------|
| <b>1. Introduction:</b> .....   | <b>1</b>  |
| <b>2. Study Area:</b> .....   | <b>9</b>  |
| <b>3. Materials and Methods:</b> .....                                | <b>11</b> |
| <b>3.1 Selection of observational plots and reconnaissance:</b> ..... | <b>11</b> |
| <b>3.2 Study Design:</b> .....  | <b>13</b> |
| <b>3.3 Field Methods:</b> .....                                       | <b>14</b> |
| 3.3.1 <i>Field Active Body Temperature (T<sub>b</sub>)</i> .....      | 14        |
| 3.3.2 <i>Operative Temperatures (T<sub>e</sub>)</i> .....             | 15        |
| 3.3.3 <i>Quantifying boldness</i> .....                               | 15        |
| 3.3.4 <i>Focal Animal Sampling</i> .....                              | 15        |
| <b>3.4 Laboratory Methods:</b> .....                                  | <b>16</b> |
| 3.4.1 <i>Housing Conditions</i> .....                                 | 16        |
| 3.4.2 <i>Data Collection</i> .....                                    | 17        |
| <b>3.5 Analytical Methods:</b> .....                                  | <b>18</b> |
| 3.5.1 <i>Habitat Variables</i> .....                                  | 18        |
| 3.5.2 <i>Field body temperature and Morphology</i> .....              | 18        |
| 3.5.3 <i>Aboveground activity</i> .....                               | 19        |
| 3.5.4 <i>Time allocation in different behaviours</i> .....            | 19        |
| 3.5.5 <i>Aggression and Shuttling frequency</i> .....                 | 19        |
| 3.5.6 <i>Heating rates, mass and sex differences</i> .....            | 20        |
| 3.5.7 <i>Temporal dependence and heat hardening</i> .....             | 20        |
| <b>4. Results:</b> .....  | <b>21</b> |
| 4.1.1 <i>Habitat Variables</i> .....                                  | 21        |
| 4.1.2 <i>Field body temperature and Morphology</i> .....              | 22        |
| 4.1.3 <i>Aboveground activity</i> .....                               | 24        |
| 4.1.4 <i>Time allocation in different behaviours</i> .....            | 27        |
| 4.1.5 <i>Aggression and Shuttling frequency</i> .....                 | 28        |
| 4.1.6 <i>Heating rates, mass and sex differences</i> .....            | 29        |
| 4.1.7 <i>Temporal dependence and heat hardening capacity</i> .....    | 30        |
| <b>5. Discussion</b> .....  | <b>32</b> |
| <b>6. References</b> .....  | <b>37</b> |

## List of Figures

| Figures  | Page no. |
|--|----------|
| <b>Figure 1:</b> Map of the study area for spiny-tailed lizard behaviour near Ramdevra village in Rajasthan (2025).  | 10       |
| <b>Figure 2:</b> (A) Homogeneous and (B) Heterogeneous habitat   | 12       |
| <b>Figure 3:</b> Photograph of a spiny-tailed lizard attached with temperature datalogger  | 14       |
| <b>Figure 4:</b> Average daily temperatures of field Tb of the lizards (N=3) and biophysical models in different microhabitats (N=4) during April.   | 22       |
| <b>Figure 5:</b> Relationship between log-transformed mass and snout-vent length   | 24       |
| <b>Figure 6:</b> Comparison of mean (SE) proportion of time spent by focal individuals (n = 6 per plot) in aboveground activity between heterogeneous and homogeneous conditions   | 25       |
| <b>Figure 7:</b> Model-estimated mean (SE) proportion of time spent in aboveground activity by Spiny tailed lizard against hours of the day between thermally homogeneous and heterogeneous conditions in Ramdevra during 2025 | 26       |
| <b>Figure 8:</b> Comparison of mean (SE) proportion of time spent by focal individuals in aboveground activity between high and moderate thermal stress  | 27       |
| <b>Figure 9:</b> Time allocation in different behaviours between homogeneous and heterogeneous conditions  | 28       |
| <b>Figure 10:</b> Predicted aggression counts between conditions   | 29       |
| <b>Figure 11:</b> Boxplot showing basal and final CT <sub>max</sub> for all individuals of each time interval  | 30       |
| <b>Figure 12:</b> Boxplot showing heat hardening ( $\Delta$ CT <sub>max</sub> ) across treatments  | 31       |

## List of Tables

| Tables   | Page no. |
|--|----------|
| <b>Table 1:</b> Mean of different habitat variables with standard deviation  | 21       |
| <b>Table 2:</b> Morphological data of spiny-tailed lizard (n=25) in Ramdevra during 2025   | 23       |
| <b>Table 3:</b> Model statistics of different behaviours   | 27       |
| <b>Table 4:</b> Mean and standard deviation of CT <sub>max</sub> values of Spiny-tailed lizard ( <i>Saara hardwickii</i> ) with standard error in parentheses. | 31       |

# **1. Introduction:**

## **1.1 Climate change as a driver of biodiversity loss**

Anthropogenic activities have accelerated at an unprecedented rate making a global impact on biodiversity, ultimately questioning the integrity of ecosystem against ever-changing environment (Oliver et al., 2015; Keith et al., 2023). Climate change is considered as one of the major drivers of biodiversity loss that has been documented to cause changes in community composition, phenology, range shifts of species, and disruption of an array of ecosystem, adversely impacting different ecological hierarchies (Walther et al., 2002; Bellard et al., 2012). Several other drivers have also been a contributor of biodiversity loss (Christian, 2023; Jaureguiberry et al., 2022), but human induced climate change has attracted the most attention leading to skewed research on its role in biodiversity loss (Mazor et al., 2018; Caro et al., 2022).

Drivers of biodiversity loss are complex and can have interactive effects exacerbating the global crisis of species extinction (Brook et al., 2008). Several studies have explored the interactions between climate change and land-use change, suggesting compounded impact of these two drivers on species' persistence (Schweiger et al., 2010; Oliver & Morecroft, 2014; Guo et al., 2018; Nunez & Alkemade, 2021; Santos et al., 2021). For instance, Mantyka-pringle et al., (2012) found that the effect of habitat fragmentation and loss were greatest in regions with highest maximum temperature, whereas, a review by Davison et al., (2021) found taxonomic and geographic biases in land use change studies and an underrepresentation of hot, cold arid and warm tropical regions. Our comprehension of the interaction between drivers of biodiversity loss, and their underlying mechanisms, are still primitive and the above-mentioned biases can further dampen our efforts to decipher such complexity effectively (Schweiger et

al., 2010; Mantyka-pringle et al., 2012; Oliver & Morecroft, 2014; Davison et al., 2021; Nunez & Alkemade, 2021; Santos et al., 2021).

## 1.2 Ectotherm vulnerability to climate change

Ectotherms are most sensitive to climatic variability as they rely heavily on ambient temperature for thermoregulation and other biological processes, thus changes in temperature can have a direct impact on their physiology and behaviour (Huey, 1982; Paaijmans et al., 2013; Rohr & Palmer, 2013). Behavioural thermoregulation is a key adaptation to optimise this dependency and offset the effect of variability in temperatures and use suboptimal conditions effectively (Huey, 1982; Huey & Berrigan, 2001). Notably, behavioural thermoregulation plays a crucial role in mitigating the effects of thermal changes by enabling ectotherms to regulate their body temperatures that is achieved by adjusting their activity patterns and strategically using sunny and shaded areas to maintain optimal thermal conditions in variable environments (Caldwell et al., 2017; Kearney et al., 2009). However, despite being crucial for survival, behavioural thermoregulation may restrict rather than drive the evolution of traits for thermal tolerance, known as “Bogert Effect” which might be the case for the spiny-tailed lizard *Saara hardwickii* – a lizard inhabiting hot deserts in India (Bogert, 1949; Tatu et al., 2024). The Bogert effect also depends on thermal heterogeneity of the environment i.e., the variability in ambient temperatures across space and time (Leal & Gunderson, 2012).

Uncertainty in environmental conditions and increased frequency of heat waves in future due to climate change may have a strong negative influence on evaporative water loss and metabolic activity (Huey et al., 2012; Stillman, 2019). It will be more troublesome for species such as the spiny-tailed lizard which survives in extreme summers via thermoregulation

and by hibernating through extreme winters. Changes in ambient temperature during hibernation can result in increased metabolic requirements resulting from cycles of rewarming and metabolic depression (Reading, 2007; Blaustein et al., 2010; Hayden Bofill & Blom, 2024).

Moreover, many ectotherms are seasonal breeders which are in sync with the environmental stimuli but climate induced shifts can disrupt this sync and can alter reproductive output and reduce fitness (Blaustein et al., 2010). Additionally, limited dispersal ability of many reptiles and amphibians owing to their reliance on external conditions for various life-history needs, hinders their capacity to traverse across unsuitable habitats, restricting them to patches that are climatically suitable (Hayden Bofill & Blom, 2024). This specialization may lead to higher dependency on local conditions and hasten the risk of extinction (Böhm et al., 2016). While global warming has been associated with faster growth rate in early life stages of ectotherms, such changes are often negatively correlated with longevity in many taxa (Comfort, 1963; Altwegg & Reyer, 2003; Lee & Roh, 2010; Burraco et al., 2020).

### **1.3 Importance of thermal heterogeneity**

Given these vulnerabilities, habitat characteristics that mediate thermal exposure become very critical for buffering ectotherms against climate change. Thermal refugia plays a critical role in the wellbeing of ectotherms. In heterogeneous habitat, diverse microhabitats can create thermal mosaics allowing organisms to shuttle between sunny and shaded areas for effective behavioural thermoregulation ultimately enhancing physiological performance, survival and fitness (Huey, 1991; Hertz et al., 1993; Sears & Angilletta, 2015). Microclimatic variation reduces thermal stress and expands activity window, allowing organism to forage,

mate and perform other fitness enhancing behaviours (Kearney et al., 2009; Scheffers et al., 2014; Basson et al., 2017; Sunday et al., 2019). Structural complexity of a habitat can be a good proxy for thermal heterogeneity as different microhabitats diversify the thermal conditions (Milling et al., 2018; Gaudenti et al., 2021).

#### **1.4 Woody Encroachment in Open Natural Ecosystem (ONEs)**

An ubiquitous land-use transformation that the Open Natural Ecosystems (desert, grassland, savannah and other tropical open habitats) are facing worldwide, and in India, is woody encroachment, a phenomenon where trees and shrubs takeover these habitats (Ratajczak et al., 2012). Such habitat change can be driven by multiple factors such as increased atmospheric CO<sub>2</sub>, climate change, over-grazing and altered fire regimes (Ratajczak et al., 2012). Globally, ONEs occupy more than 40% of terrestrial landscape but are subject to drastic changes like loss of herbaceous cover, change in soil moisture and nutrient dynamics due to overexploitation for economic pursuits (Briggs, 2005; Hoekstra et al., 2005). Woody encroachment may or may not benefit ectotherms. On one hand, the spread of woody plants can increase habitat heterogeneity and potentially provide thermal refugia for many species. On the other hand, it can also reduce the availability of basking sites essential for thermoregulation, heterogeneity and potentially provide thermal refugia for many species. On the other hand, it can also reduce the availability of basking sites essential for thermoregulation, or restrict visibility requiring more time allocation to vigilance against predators. Thus, the effect of woody encroachment can interact with global warming to influence the behavioural decisions and have implications on thermoregulation of the spiny-tailed lizard and other ectotherms inhabiting India's ONEs (Shoemaker & Gibbs, 2010).

## 1.5 Heat Hardening

As temperature is becoming more extreme and variable, ectotherms might have to rely on additional mechanisms to compensate for this variability. Heat hardening, a form of thermal tolerance plasticity, can rapidly enhance thermal tolerance within minutes to hours when exposed to thermal stress (Bowler, 2005). It serves as a primary defence against heat stress and contributes to increased fitness (Phillips et al., 2016; Deery et al., 2021). Heat stress triggers production and expression of heat-shock proteins (HSPs) as a stress response, which protects other cellular proteins from denaturing at high temperature and has been shown to have positive association with ambient temperature, such that species thriving in warmer areas exhibit higher concentration of HSP (Ulmasov et al., 1992; Zatssepina et al., 2000; Horowitz, 2001; Sørensen et al., 2003; White et al., 2024). Species that show thermal tolerance plasticity are supposedly more resilient to high temperature, although this phenotypic plasticity may not fully buffer against extreme temperatures (Huey et al., 2012; Rohr & Palmer, 2013; Gunderson et al., 2017; Morley et al., 2019).

Evidence for heat hardening is very limited, known only for six species out of approximately 7800 described lizard species (Phillips et al., 2016; Gilbert & Miles, 2019; Deery et al., 2021; Lapwong et al., 2021; White et al., 2024; Uetz et al., 2025). Deery et al., (2021) studied heat hardening for a pair of *Anolis* lizards (*Anolis sagrei* and *Anolis carolinensis*) and found positive relationship of thermal tolerance in *A. carolinensis* after exposing it to sublethal temperatures. However, *A. sagrei* did not show respond to heat shocks treatments, indicating that not all lizards exhibit heat hardening capacity. Phillips et al., (2016) conducted the first population level study on heat hardening capacity and revealed that there are indeed geographical variances caused by variation in temperature range that the population is exposed to. Gilbert & Miles, (2019) conducted heat hardening experiment on ornate tree lizard

(*Urosaurus ornatus*) to explore population level differences and consequences of heat hardening on other phenotypic traits that showed reduction in performance capacity and preferred body temperature as well as shifts in phenotypic traits after exposure to sub-lethal temperature; questioning the efficacy of heat hardening. Lapwong et al., (2021) compared heat hardening capacity of native and introduced population of Asian house gecko and found no difference in heat hardening capacity between populations. White et al., (2024) measured heat hardening capacity of *Eublepharis macularius* and found thermal tolerance peak at 6h which has been found in many other studies as well.

Gunderson & Stillman, (2015) and Barley et al., (2021) have argued that thermal tolerance of ectotherms has limited potential of protecting them against rapid climate change. Stillman, (2003) indicated that species that have already adapted to high temperatures, often exhibit limited plasticity, increasing their vulnerability to extreme heat events. Recently, the trade-off hypothesis (TOH) has been proposed to explain the relationship between basal thermal tolerance and thermal tolerance plasticity (van Heerwaarden & Kellermann, 2020). This hypothesis posits that species adapted to high temperatures, having higher thermal tolerance have limited potential of improving their thermal tolerance via phenotypic plasticity (van Heerwaarden & Kellermann, 2020). TOH has been debated, as not all species show trade-off between basal  $CT_{max}$  and  $CT_{max}$  difference and that this support may be due to statistical artifact (Deery et al., 2021; Gunderson, 2023).

Understanding the response of ectotherms to climate change requires an integrated view of their behaviour and physiology. Given the above premises and research gaps, my study explores the effect of habitat heterogeneity (owing to woody encroachment) on aboveground activity, heat hardening capacity and test trade-off hypothesis (TOH) with spiny-tailed lizard as the model organism.

## 1.6 Study Species

The spiny-tailed lizard (*Saara hardwickii*) is a burrow living thermophile found in the Thar Desert and adjoining semiarid regions of Rajasthan and Gujarat in India (Daniel 2002). Adults are herbivorous mainly feeding on grass but juveniles additionally feed on insects (Dutta & Jhala, 2007). An ecologically important species in the landscape, they contribute to nutrient cycling and act as a vital prey species for many desert predators. Despite its ecological significance, study on the species is scarce, with only a few studies addressing its ecological aspects (Dutta & Jhala, 2007; Ramesh & Ishwar, 2008; Ramesh & Sankaran, 2013; Das et al., 2013; Dutta & Jhala, 2014). The species shows bimodal activity pattern in warmer months and uses burrow to evade predators and to take refuge (Ramesh & Ishwar, 2008).

The population of the spiny-tailed lizard was historically distributed from Baluchistan in Pakistan to Uttar Pradesh in India, but has declined drastically owing to factors like poaching and habitat loss, causing the current population to be fragmented (Ramesh & Ishwar, 2008). Currently, the species is listed as “Vulnerable” by IUCN and is a schedule I species under Indian Protection Act, 1972 (Vyas et al., 2022).

## 1.7 Study Objectives

### 1.7.1 Objectives

1. To examine the effect of habitat heterogeneity (as a proxy for thermal heterogeneity) on their aboveground activity and behaviour of spiny-tailed lizard
2. To measure the heat hardening capacity of spiny-tailed lizard and test the trade-off hypothesis

### ***1.7.2 Research Questions***

A. How does habitat heterogeneity (as a proxy for thermally heterogeneity) affect aboveground activity, and behaviour of spiny-tailed lizard?

B. Whether spiny-tailed lizard exhibits heat-hardening?

### ***1.7.3 Hypothesis & predictions with justification:***

#### ***Objective 1:***

**Hypothesis:** Thermally heterogeneous habitat facilitates aboveground activity

**Prediction:** Individuals in (thermally) homogeneous habitat will have lower aboveground activity than individuals in (thermally) heterogeneous habitat.

**Justification:** Lizards use behavioural thermoregulation, such as shuttling between thermally stable burrows, shade and open ground, as an adaptive strategy to cope with thermal extremes and regulate their body temperature. By comparing aboveground activity in different thermal landscapes, I will address whether habitat heterogeneity has an effect on aboveground activity and aid in fitness enhancing behaviours like foraging.

#### ***Objective 2:***

**Hypothesis:** Organisms that are adapted to higher temperatures will show limited plasticity in their thermal tolerance referred to as “trade-off hypothesis” (van Heerwaarden & Kellermann 2020)

**Prediction:** Individuals with higher basal thermal tolerance will show lower plasticity in their thermal tolerance

**Justification:** Thermal tolerance limits are key to understanding how animals might cope with increasing environmental temperatures. Predictions of future activity time or distribution of species under anthropogenic climate change do not factor in phenotypic plasticity for majority of species/studies, including spiny tailed lizard (Tatu et al., 2024). Evaluating their heat-hardening capacity and factors influencing it will be critical in predicting their survival in environments with varying thermal stress.

## **2. Study Area:**

The study was conducted in 100-hectare grazing plot near Ramdevra village in Jaisalmer district of Rajasthan (Fig.1). The region experiences harsh cold winter with temperature dropping to 5°C, and dry hot summer with temperature reaching up to 50 °C. The region is characterized by low rainfall of 100-200 mm annually, scant and scattered vegetation. The ground vegetation was dominated by *Dactyloctenium sp.* and other major vegetation included *Cassia angustifolia*, *Ziziphus sp.*, *Calotropis procera*, *Fagonia indica*, *Capparis decidua* and an invasive, *Prosopis juliflora*.



**Figure 1:** Map of the study area for spiny-tailed lizard behaviour near Ramdevra village in Rajasthan (2025). Red polygons depict homogeneous plots and green polygons depicts heterogeneous plots where study was conducted. The larger red triangular polygon was surveyed during reconnaissance.

Despite its proximity to the village, the study area had mammalian species like *Gazella bennettii*, *Boselaphus tragocamelu* and *Herpestes edwardsii* that were frequently sighted during sampling. *Falco jugger* (Lagger Falcon), *Circus aeruginosus* (Marsh Harrier), *Aquila nipalensis* (Steppe Eagle) and *Tachyspiza badia* (Shikra) were observed predated on *Saara hardwickii* (spiny-tailed lizard). Other herpetofaunal that were encountered during sampling are *Eryx johnii*, *Echis carinatus sochureki*, *Varanus griseus*, *Ophisops sp.* and *Trapelus agilis*.

### **3. Materials and Methods:**

This study was aimed to understand the effect of habitat (and thermal) heterogeneity on the aboveground activity of spiny-tailed lizard and to examine whether the species exhibits heat hardening, a rapid short-term increase in thermal tolerance measured using lab-based experiment in controlled environment. To achieve this, sampling sites were selected to control for confounding factors (other than thermal heterogeneity), habitat variables (shrub density, grass cover and lizard burrow density) were quantified, aboveground activity of lizards were measured using focal animal sampling of marked individuals, and temperature was recorded using various loggers. Physiological variables like mass (g) and snout-vent-length (SVL, cm), which may have an impact on aboveground activity, were recorded and controlled for sampled lizards. In addition, we scored boldness as response to handling. We also measured heat hardening capacity of the study species after the focal observations were done, wherein the marked lizards were recaptured by noose and thermal tolerance trials were conducted to measure critical thermal maximum ( $CT_{max}$ ) in a controlled environment and safe manner.

#### **3.1 Selection of observational plots and reconnaissance:**

##### *3.1.1 Reconnaissance survey*

The reconnaissance was carried out in the month of February 2025 (Fig 1). My first objective required selecting plots with varying level of habitat heterogeneity (based on shrub count) but similar grass cover (proxy for food availability), burrow density, and other confounding factors. To achieve this, I walked zigzag transects of varying lengths in a 100 ha grazing area near the field station, and tagged all spiny-tailed lizard burrows. The Burrow locations were mapped to find clusters of similar sized burrows (~ 4-6 cm) using ArcGIS 10.7. From literature and reconnaissance, it was established that the species stays close to its burrow and only in rare cases venture (beyond >50 m). Hence, I delineated 50 x 50 m square plots

(n=4), two each with negligible shrub cover (representing homogeneous condition) and moderate shrub cover (heterogeneous condition) (Fig. 2).

**A****B**

**Figure 2:** (A) Homogeneous and (B) Heterogeneous habitat

The plots were physically delineated on ground, and habitat variables were quantified therein (see below), to ascertain if these plots strong difference in structural heterogeneity, while ensuring that other confounding variables were similar. I also observed the spiny-tailed lizard behaviour for several days prior to data collection, to sampling methodology and to habituate the lizards to our presence.

### 3.1.2 Selection of plots

I selected two plots with minimal shrub/tree cover (structurally homogenous) and two plots with good shrub/tree cover, to represent the two conditions – thermally homogeneous and heterogeneous habitats. I used the number of shrubs as a proxy for habitat heterogeneity and selected two plots each representing homogeneous and heterogeneous conditions. Grass height and cover were systematically sampled in 1 m square quadrats at 10 m interval from the nearest neighbour (n = 16) in each plot, by measuring the grass height using a scale and ocularly

assessing the percentage grass cover. All plots were within 200m distance from other plots. This small distance between plots were kept to reduce other confounding factors that can influence the species' behaviour such as predation risk and/or general temperature difference.

## **3.2 Study Design:**

### **3.2.1 Objective I**

In each of the four plots of 50 m square plots, two in homogeneous and two in heterogeneous condition, six individual lizards were captured using lasso and marked for behavioural sampling. Each plot was visited six times during data collection, wherein visits were interspersed among plots (conditions), and a team of three observers recorded the behaviour of marked lizards using focal animal sampling (two per observer) from 07:00 am to 18:30 pm on these days. A focal session was for one hour with 10 minutes break between two consecutive sessions, to avoid observer fatigue.

### **3.2.2 Objective II**

I conducted trials on recaptured lizards to test their thermal tolerance in controlled environment. Prior to the start of each experiment, mass of each lizard was recorded using digital scale. Starting temperature can influence  $CT_{max}$  values; to prevent this from biasing results, I ensured that the cloacal temperatures was approximately 30 °C ( $\pm 0.5$  °C) before commencing the experiment. If the cloacal temperature was lower or higher than this, the lizards was either placed under 150W infrared lamp or in a pre-cooled box to achieve the desired body temperature (Terblanche et al., 2007). Lizards were tested one at a time, wherein a k-type thermocouple connected to temperature datalogger (Amprobe model TMD-50) was inserted approximately 1 cm inside cloaca and secured with the help of medical tape (Deery et al., 2021). To measure  $CT_{max}$ , lizards were placed in metal container and the 150W infrared

lamp was placed 40 cm above it resulting in heating rate of  $1\text{ }^{\circ}\text{C min}^{-1}$  (Tatu et al., 2024). After concluding the  $CT_{\text{max}}$  trials, lizards were transferred to pre-cooled chamber aiding in lowering their body temperature. All lizards were safely released back to their burrows immediately after the trials.

### 3.3 Field Methods:

#### 3.3.1 Field Active Body Temperature ( $T_b$ )

Lizards (N=4) were captured by noose near their burrows and were marked with non-toxic temporary coloured marker. Their mass (using digital scale), snout-vent length (SVL,  $\pm 0.1\text{ cm}$ ), sex and gravidity (using abdominal palpations) were recorded. Temperature data loggers (Maxim integrated ibutton DS-1921G F5, accuracy  $\pm 0.5\text{ }^{\circ}\text{C}$ ) was stitched to cotton harness and attached to lizard such that only the sensing surface was in contact with the lizard's skin (Tatu et al., 2024) (Fig.3). Datalogger was set to record lizard skin temperatures every 30 minutes between March and April. The combined weight of ibutton with harness was  $<6\text{ g}$  ( $< 5\%$  of the body weight) and allowed free movement. Lizard skin temperature was used as a proxy for core temperature as previous research on this species has validated this alternative and have found a strong relationship between skin and cloacal temperatures (Tatu et al., 2024).



**Figure 3:** Photograph of a spiny-tailed lizard attached with temperature datalogger

### 3.3.2 *Operative Temperatures (Te)*

Biophysical models made of copper pipes, depicting morphological characteristics and radiative properties of the study species were used to measure the temperatures of different microhabitats that a lizard would experience in absence of behavioural thermoregulation (Bakken, 1992; Dzialowski, 2005; Bakken & Angilletta, 2014). Temperature data loggers (Maxim integrated ibutton DS-1921G F5, accuracy  $\pm 0.5$  °C) were fixed in the middle of the pipe ensuring that it did not come in contact with the inner walls of the pipe and was set to record operative temperatures every 30 minutes during the month of April and May. These models were deployed in three different microhabitats Open (N=1), Burrow (N=1) and plant shade (N=2). To calibrate the copper model, we compared cloacal temperatures recorded using k-type thermocouple connected to temperature logger (Amprobe model TMD-50) with the temperature of copper model under different temperatures using 150W infrared bulb (Tatu et al., 2024). Additionally, another datalogger (Testo 174H, accuracy  $\pm 0.5$  °C) was placed at the height of approximately 1.5m under the tree shade to record ambient temperature every 15 minutes between the month of March and May.

### 3.3.3 *Quantifying boldness*

Once we captured the individuals, the boldness was scored on the basis of how much they struggle while being measured. The scoring was between 0-2, with 0 corresponding to remaining still, 1 being infrequent to frequent wriggling; and 2 being violent struggling, hissing and throat inflation and tail flicking (Ward-Fear et al., 2018).

### 3.3.4 *Focal Animal Sampling*

Lizards (N=6) were captured by noose near their burrows from each plot and marked with temporary coloured marker for individual identification. A team of three observers

observed the tagged individuals following the same standardized methodology from separate hides using binoculars from 07:00 to 18:30 during 22<sup>nd</sup> March to 16<sup>th</sup> April 2025. The marked individuals were continuously monitored and their behaviours were recorded till they were out-of-sight following focal animal sampling method (Altmann, 1974). The behaviours were divided into *states* and *events*. State corresponds to behaviours which are not instantaneous and recorded as the duration of behaviour or percent of time spent, whereas, event is an onset of behaviour which are instantaneous and recorded as frequency (Altmann, 1974). The behaviours that we considered as states are as follows: Basking (Exposing body to heat source like solar radiation to regulate body temperature while being immobile), Territorial (Aggression between conspecifics), Vigilance (Raising the first half of the body and scanning the surrounding), Foraging (actively moving to acquire food) and Moving (Short sprints either to evade predators or to forage). States like vigilance and moving were recorded only after the threshold of 3 seconds. Apart from this, head bobbing (Repeated up and down motion of head), tail flicking, shuttling (moving between sunny and shaded area to regulate body temperature), biting and chasing (aggressive behaviour in which one lizard is chased by another conspecific) were recorded as events.

### **3.4 Laboratory Methods:**

#### *3.4.1 Housing Conditions*

Total 25 lizards (14 males and 11 females) were recaptured and brought to the field station after the completion of focal sampling. The lizards were housed in individual terraria (94×55×40 cm) that had a 150W heating lamp and refugia made of rock. They were not fed prior to the trial to prevent any influence of feeding on CT<sub>max</sub> measurements (White et al. 2024).

The trials were carried out in a cool room (24 °C) between 08:00 and 19:00 after acclimation for 24 h (Deery et al., 2021; Tatu et al., 2024). Capture was staggered such that lizards spent no more than 2-3 days in captivity. After that lizards were released back safely to their burrows in the wild, without any mortality. Only adults within the size range of 14.5 to 20.2 cm SVL were used, as individuals of different life-stages may affect measurements of critical thermal maximum ( $CT_{max}$ ) (Camacho & Rusch, 2017; Telemeco & Gangloff, 2021).

### 3.4.2 Data Collection

Critical thermal maximum ( $CT_{max}$ ), or temperature at which an animal loses its neuromuscular coordination and cannot tolerate any higher temperature, is a metric of thermal tolerance (Lutterschmidt & Hutchison, 1997a, 1997b). To examine the heat hardening capacity of lizards, I subjected them to gradual increase in temperature by placing them in metal container and 150W infrared lamp was placed 40 cm above it resulting in heating rate of approximately  $1\text{ }^{\circ}\text{C min}^{-1}$  (since studies have shown that differences in heating rate might affect  $CT_{max}$  values, see Rezende et al., 2011; Ribeiro et al., 2012) and measured their  $CT_{max}$  twice: once following the first trial, or the basal  $CT_{max}$  at 0 hours, and then upon repeating the trial after a set interval, or the final  $CT_{max}$  (White et al., 2024). Each time, the ambient temperature was increased and loss of righting response for 10 sec. was considered as  $CT_{max}$ , by checking the righting response every minute. (Huey & Stevenson, 1979; Deery et al., 2021; White et al., 2024).

Each of the 25 individuals were randomly assigned to one of the set time intervals, ranging from 2 hours, 4 hours to 11 hours after initial  $CT_{max}$  measurement trial. Thus, all individuals were tested at 0 hours (basal  $CT_{max}$ ) and then either at 2h, 4h and 11h after 0h (final  $CT_{max}$ ), The 2h interval treatment included 5 males and 4 females, the 4h interval included 4 males and 4 females, and the 11h interval included 5 males and 3 females.

### 3.5 Analytical Methods:

#### Objective I:

##### 3.5.1 *Habitat Variables*

To test if there was any statistical difference in grass cover between conditions, I used Welch t-test using “t.test” function from *stats* package, after testing the assumption of unequal variances using “leveneTest” function from *car* package.

##### 3.5.2 *Field body temperature and Morphology*

Linear models were used to test for the effect of sex on morphology (i.e. mass, SVL and BCI) and, boldness, and to examine the relationship between mass and SVL. Mass and SVL were log-transformed prior to fitting the model. Linear mixed effect model was used to test the effect of open temperature on field active body temperature. Variability in mass and SVL was incorporated using body condition index (BCI). The BCI was calculated using below mentioned formula:

$$BCI = M_i * [(SVL_0)/(SVL_i)]^{b_{SMA}}$$

Where  $M_i$  and  $SVL_i$  are the mass and SVL of the individual respectively,  $SVL_0$  is the mean SVL of the population, and  $b_{SMA}$  is the standardized major axis slope from the regression of natural log of mass on natural log of SVL for the population (Peig & Green, 2009; Cecchetto et al., 2020; Tatu et al., 2024). The  $b_{SMA}$  was calculated using the “lmodel” function from the package called “lmodel2” in R (R Core Team 2024).

### 3.5.3 *Aboveground activity*

I computed the proportion of time spent in aboveground activity (hereafter activity rate). Thereafter, I determined if activity rate differed between conditions (thermally homogeneous vs heterogeneous), using linear mixed effect model with activity rate as the response variable, condition, BCI, sex, and boldness as predictors/ fixed effects, and identity of individual lizards as a random effect. Next, I modelled activity rate against time of the day (hours), to determine daily activity patterns using linear mixed effect model. Further, I classified the focal session into high and moderate thermal stress periods around a threshold temperature of 46 °C. Using linear mixed effect model, I examined the effect of moderate vs high thermal stress along with condition (fixed effects) on activity rate, with individual identity as a random effect. I chose this temperature threshold because it is the temperature at which lizards retreat to refugia ( $VT_{max}$ ), which is ecologically relevant.

### 3.5.4 *Time allocation in different behaviours*

Mixed effect models were used to assess the difference in time spent in different behaviours (proportion of aboveground time allocated to different behaviours). Each behaviour was modelled separately against condition with sex, BCI, boldness as fixed effect predictors and individual identity as random effect.

### 3.5.5 *Aggression and Shuttling frequency*

Frequency of occurrences of events except shuttling was pooled as aggressive events and generalized linear mixed model (GLMM) with poisson family was used to determine whether the frequency of aggression differed significantly between condition and sexes with BCI, boldness as covariates and individual ID as a random effect. Similarly, we ran two

separate GLMM with poisson family to determine whether condition and time of the day had a significant effect on to shuttling frequency and Individual ID was taken as a random effect.

## **Objective II:**

### *3.5.6 Heating rates, mass and sex differences*

I used a paired, two-sample t-test to test the difference between basal and final heating rates. Similarly, I performed paired, two-sample t-test to test if mass differed before basal and final CT<sub>max</sub> trials. I used linear models (LMs) to test the influence of sex on body mass measured before basal and final CT max and on SVL before basal CT<sub>max</sub> using “lm” function from the *stats* package.

### *3.5.7 Temporal dependence and heat hardening*

Generalized Linear Model (GLMs) were used to examine the effects of treatment groups, sex, SVL, and mass on basal CT<sub>max</sub>, final CT<sub>max</sub> and  $\Delta$  CT<sub>max</sub>. While performing GLM for final CT<sub>max</sub>, I used mass that was measured before final CTmax instead of the mass measured before basal CT<sub>max</sub>. All GLMs were performed using “glm” function from *stats* package.

To test for trade-off hypothesis, a Pearson product-moment correlation analysis was conducted for all individuals within and across all time intervals using “cor.test” function from *stats* package.

All statistical analyses were performed using the software Rstudio (v. 4.4.2, R Core Team 2024).

## 4. Results:

### Objective I:

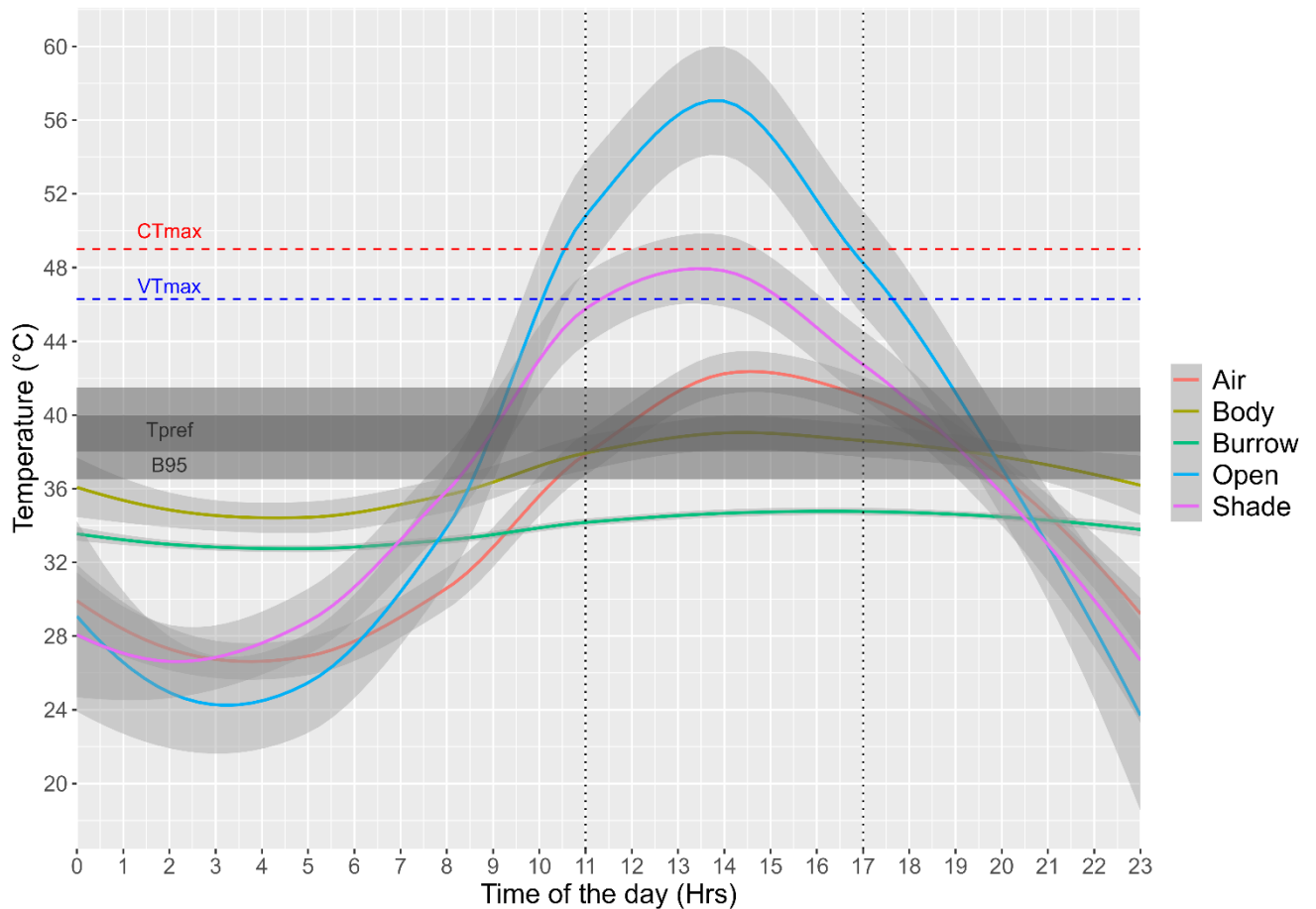
#### 4.1.1 Habitat Variables

Shrub count differed between the two conditions, with much lower shrub count in homogeneous sites than in heterogeneous sites. Burrow count in heterogeneous was slightly higher than in homogeneous sites. Grass cover also did not differ between the conditions ( $t = 0.24$ ,  $df = 1.99$ ,  $p = 0.83$ ). The above results revalidated that the sampling sites were similar in terms of food availability and competition, but they differed in terms of structural heterogeneity – one with shade shrubs, and another without (Table 1).

**Table 1:** Mean of different habitat variables with standard deviation

| Plot ID | Condition     | Grass height | Grass cover (%) | Burrow density | Shrub density |
|---------|---------------|--------------|-----------------|----------------|---------------|
| He1     | Heterogeneous | 5.63±2.2     | 46.25±28.62     | 52             | 76            |
| He2     | Heterogeneous | 11.46±4.05   | 27.81±21.11     | 54             | 86            |
| Ho1     | Homogeneous   | 7.9±1.6      | 42.81± 27.44    | 35             | 18            |
| Ho2     | Homogeneous   | 10.35±3.94   | 25±15.81        | 44             | 3             |

Air temperature and operative temperature data in different microhabitats showed that temperature under shade shrubs was lower than open ground during mid-day, and this led to more thermal heterogeneity (intra-plot variation and availability of ‘thermal refuges’) in the ‘heterogeneous’ condition, than in the ‘homogeneous’ condition (Fig 4).



**Figure 4:** Average daily temperatures of field  $T_b$  of the lizards ( $N=3$ ) and biophysical models in different microhabitats ( $N=4$ ) during April.

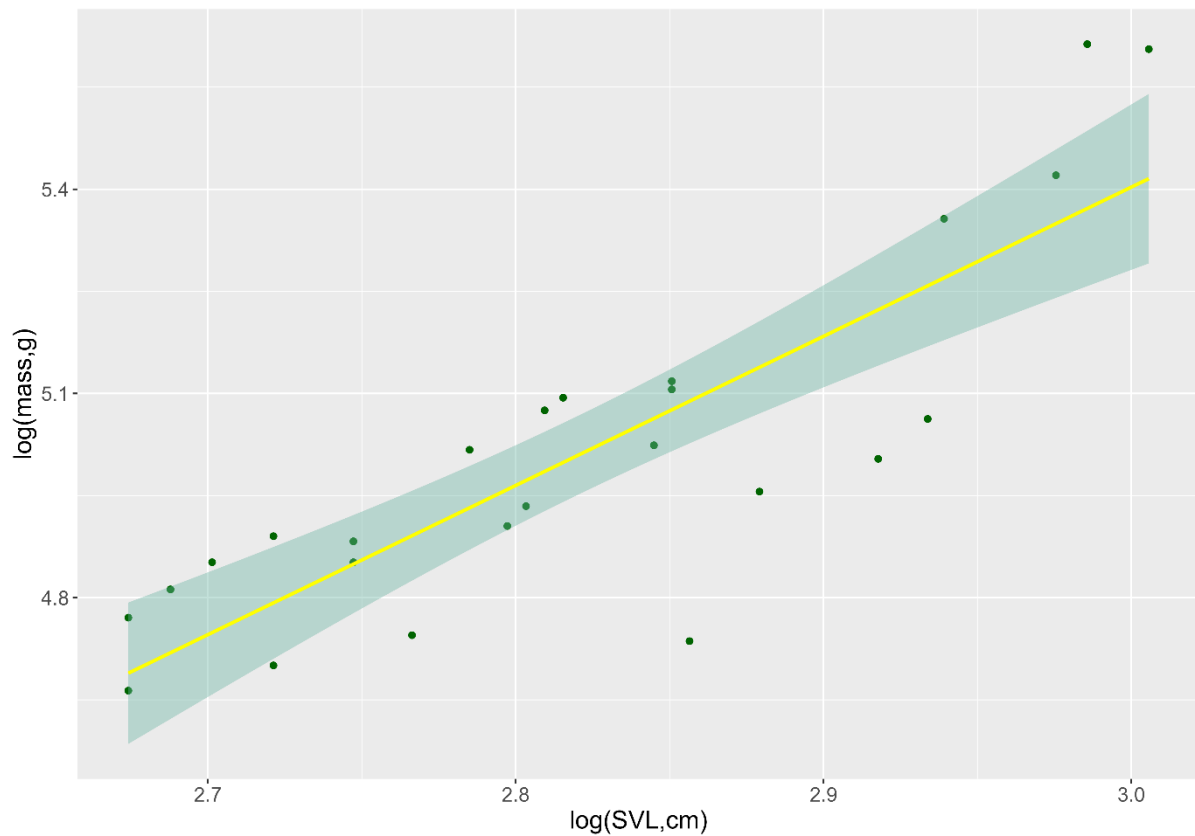
#### 4.1.2 Field body temperature and Morphology

Lizard body temperature was strongly and positively associated with open temperature ( $\beta = 0.083$ ,  $T_{2865} = 22.11$ ,  $SE = 0.0037$ ,  $P = <0.001$ ). There was no effect of sex on log transformed snout-vent length ( $\beta = 0.03$ ,  $R^2 = 0.02$ ,  $F_{23} = 0.654$ ,  $P = 0.42$ ), log transformed body mass ( $\beta = 0.09$ ,  $R^2 = 0.03$ ,  $F_{23} = 0.833$ ,  $P = 0.37$ ) and body condition index ( $\beta = 4.3$ ,  $R^2 =$

0.01,  $F_{23} = 0.278$ ,  $P = 0.603$ ). Log-transformed body mass and log-transformed snout-vent length were strongly and positively associated ( $\beta = 2.19$ ,  $R^2 = 0.718$ ,  $F_{23} = 58.63$ ,  $P = <0.001$ ) (Fig 5). Boldness did not differ between sexes ( $\beta = 0.44$ ,  $R^2 = 0.08$ ,  $F_{23} = 2.01$ ,  $P = 0.16$ ).

**Table 2:** Morphological data of spiny-tailed lizard (n=25) in Ramdevra during 2025

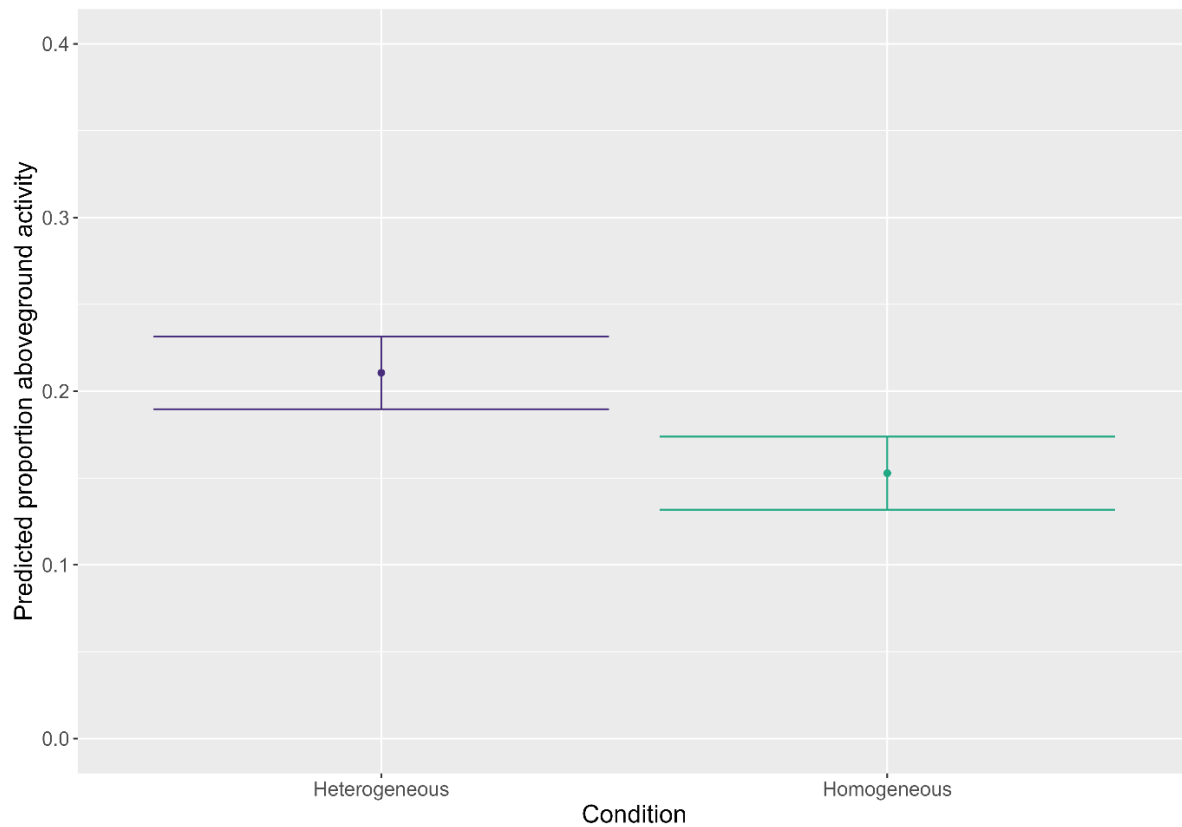
| Sr. no. | Lizard_ID | Plot_ID | Sex    | SVL (cm) | Mass (g) | Boldness | BCI    |
|---------|-----------|---------|--------|----------|----------|----------|--------|
| 1       | LHo1_1    | Ho1     | Female | 18.5     | 149      | 1        | 121.35 |
| 2       | LHo1_2    | Ho1     | Male   | 18.8     | 158      | 1        | 124.21 |
| 3       | LHo1_3    | Ho1     | Male   | 17.4     | 114      | 2        | 106.25 |
| 4       | LHo1_4    | Ho1     | Female | 14.9     | 128      | 0        | 167.82 |
| 5       | LHo1_5    | Ho1     | Female | 15.6     | 128      | 2        | 151.69 |
| 6       | LHo1_6    | Ho1     | Male   | 16.2     | 151      | 2        | 164.69 |
| 7       | LHe1_1    | He1     | Male   | 17.3     | 165      | 1        | 155.75 |
| 8       | LHe1_2    | He1     | Female | 17.2     | 152      | 2        | 145.32 |
| 9       | LHe1_4    | He1     | Female | 15.2     | 133      | 2        | 166.89 |
| 10      | LHe1_5    | He1     | Female | 16.5     | 139      | 0        | 145.61 |
| 11      | LHe1_6    | He1     | Male   | 19.8     | 274      | 1        | 192.19 |
| 12      | LHe1_3    | He1     | Male   | 14.5     | 118      | 2        | 164.25 |
| 13      | LHe1_7    | He1     | Male   | 16.6     | 160      | 1        | 165.39 |
| 14      | LHo2_1    | Ho2     | Female | 17.3     | 167      | 0        | 157.63 |
| 15      | LHo2_2    | Ho2     | Male   | 19.6     | 226      | 1        | 162.10 |
| 16      | LHo2_3    | Ho2     | Female | 14.7     | 123      | 0        | 166.13 |
| 17      | LHo2_4    | Ho2     | Male   | 16.4     | 135      | 1        | 143.32 |
| 18      | LHo2_5    | Ho2     | Male   | 15.9     | 115      | 0        | 130.69 |
| 19      | LHo2_6    | Ho2     | Female | 17.8     | 142      | 0        | 125.89 |
| 20      | LHe2_1    | He2     | Male   | 16.7     | 163      | 1        | 166.28 |
| 21      | LHe2_2    | He2     | Male   | 14.5     | 106      | 2        | 147.55 |
| 22      | LHe2_3    | He2     | Male   | 20.2     | 272      | 2        | 182.57 |
| 23      | LHe2_4    | He2     | Female | 15.2     | 110      | 1        | 138.03 |
| 24      | LHe2_5    | He2     | Male   | 15.6     | 132      | 2        | 156.43 |
| 25      | LHe2_6    | He2     | Female | 18.9     | 212      | 2        | 164.72 |



**Figure 5:** Relationship between log-transformed mass and snout-vent length

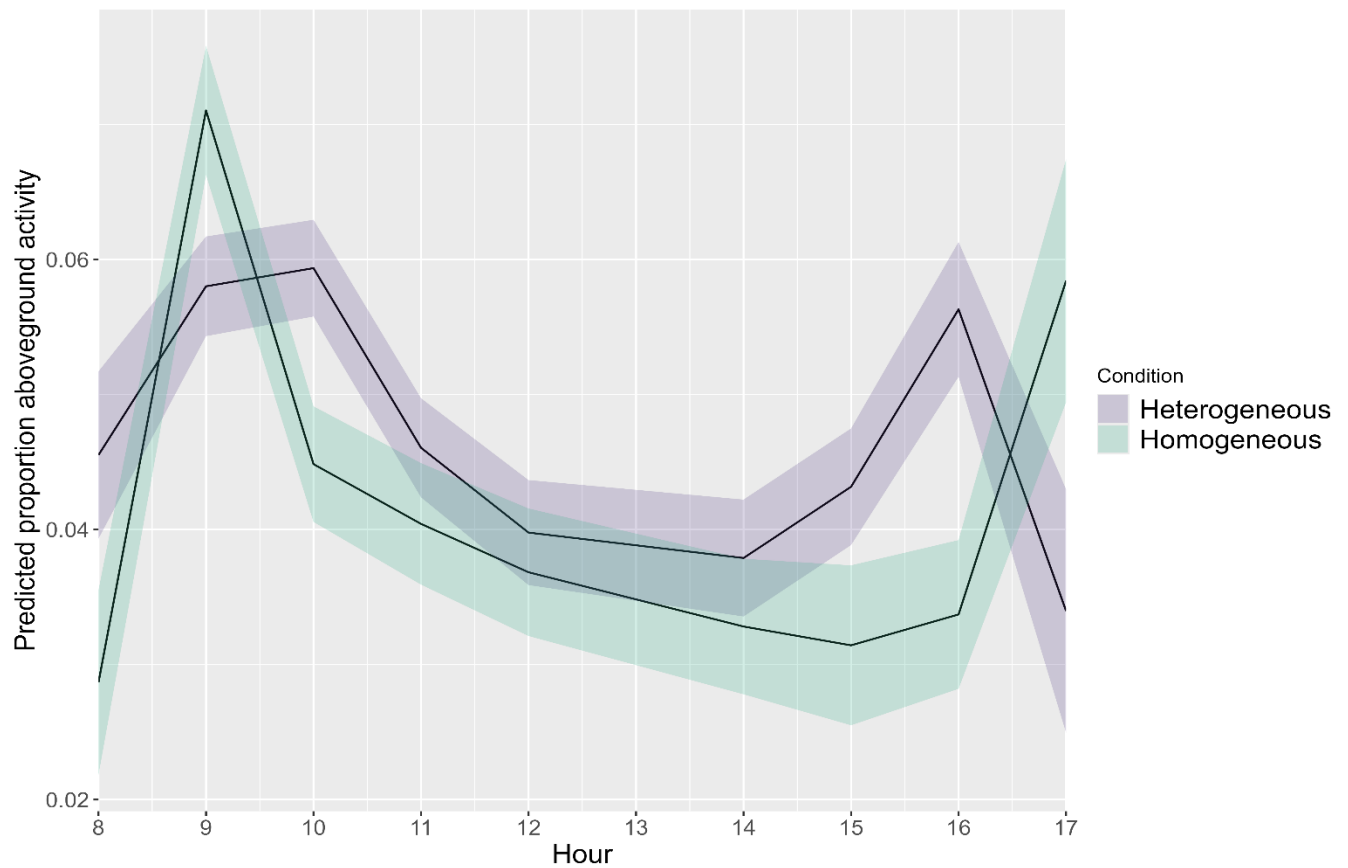
#### 4.1.3 Aboveground activity

Linear mixed effect model explaining the proportion of time spent by focal lizards in aboveground activity (hereafter, activity rate) was lower in homogeneous (than heterogeneous) condition ( $\beta = -0.05$ ,  $T_{19} = -2.25$ ,  $SE = 0.025$ ,  $P = 0.035$ ) (Fig. 6). Activity rate was positively associated with boldness of the individual ( $\beta = 0.04$ ,  $T_{19} = 2.9$ ,  $SE = 0.01$ ,  $P = 0.009$ ). Whereas, sex ( $\beta = -0.005$ ,  $T_{19} = -0.22$ ,  $SE = 0.023$ ,  $P = 0.825$ ) and body condition index ( $\beta = 0.0005$ ,  $T_{19} = 1.16$ ,  $SE = 0.0005$ ,  $P = 0.258$ ) did not influence activity rate.



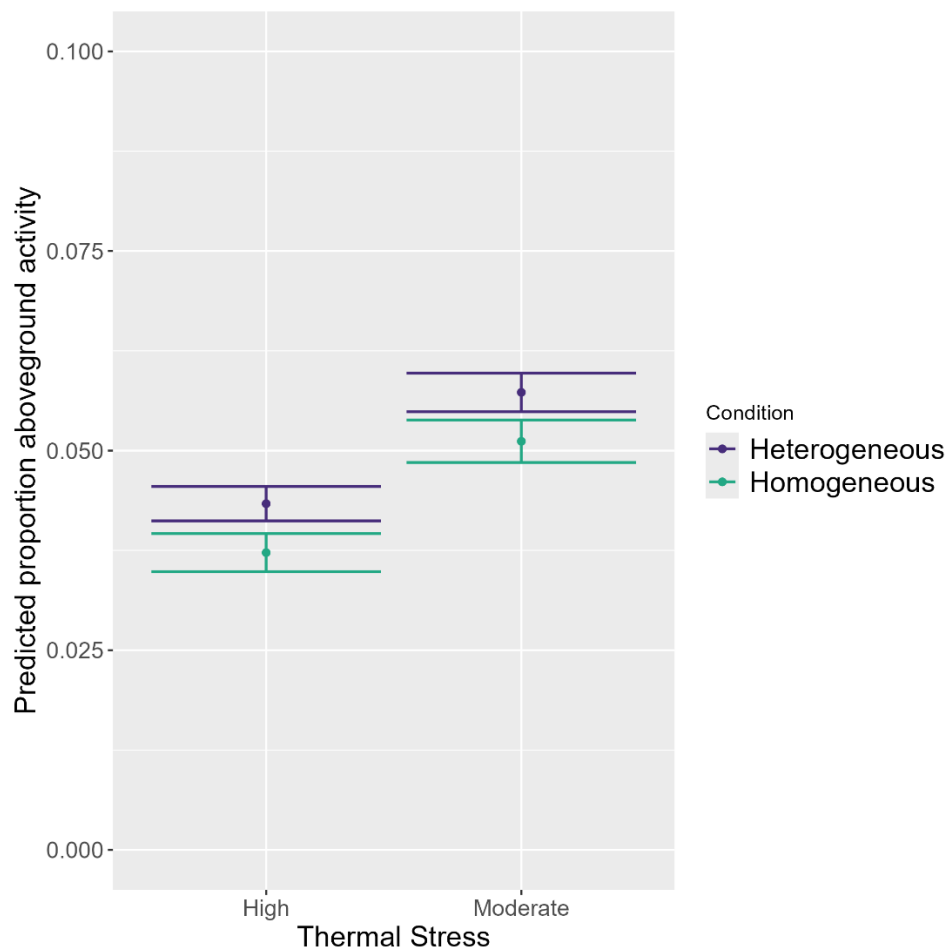
**Figure 6:** Comparison of mean (SE) proportion of time spent by focal individuals ( $n = 6$  per plot) in aboveground activity between heterogeneous ( $n = 2$ ) and homogeneous conditions ( $n = 2$ )

Above ground activity of lizards were largely bimodal, but with finer differences between conditions: proportion of time spent in aboveground activity was more restricted in homogeneous plots, peaking at 9 h and 17 h, whereas activity was more spread out in heterogeneous plots with relatively higher activity rate at 9, 10 and 16 (Fig 7).



**Figure 7:** Model-estimated mean (SE) proportion of time spent in aboveground activity by Spiny tailed lizard against hours of the day between thermally homogeneous and heterogeneous conditions in Ramdevra during 2025

Examination of activity rates between daily hours with moderate (36-45 °C) and high (> 46 °C) thermal stress levels showed small but significant reduction of the proportion of time spent in aboveground activity during the periods of high thermal stress (than moderate thermal stress -  $\beta = 0.01$ ,  $T_{549,9} = 6.16$ ,  $SE = 0.002$ ,  $P = <0.001$ ) but not between conditions ( $\beta = -0.006$ ,  $T_{20,6} = -2.05$ ,  $SE = 0.002$ ,  $P = 0.053$ ) (Fig 8).



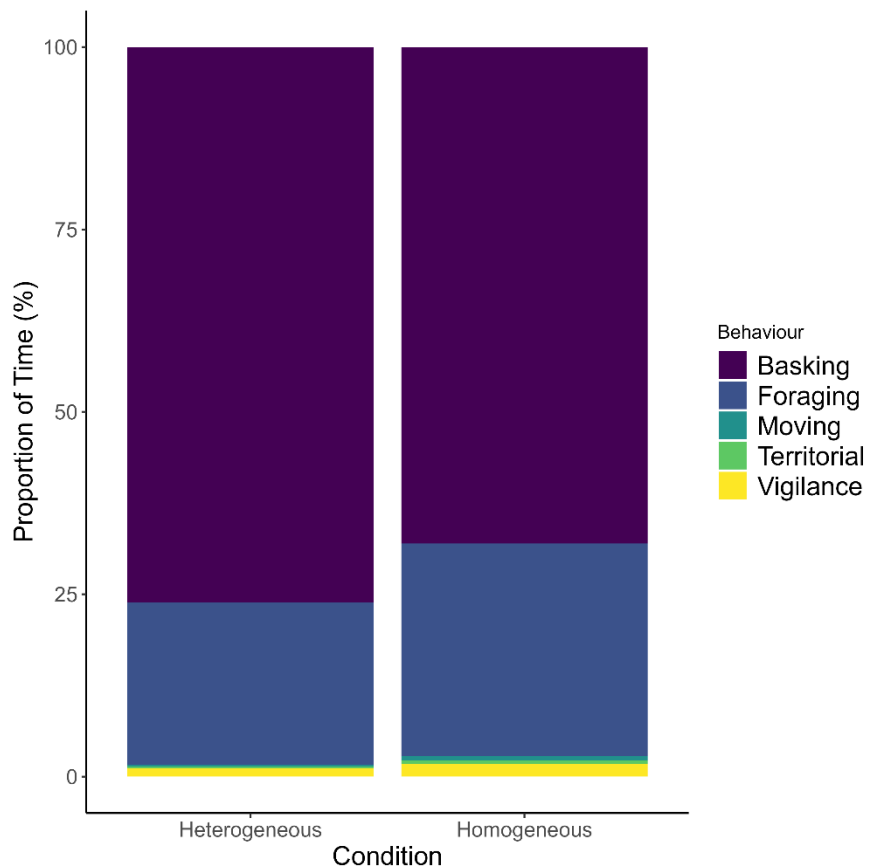
**Figure 8:** Comparison of mean (SE) proportion of time spent by focal individuals in aboveground activity between high and moderate thermal stress

#### 4.1.4 Time allocation in different behaviours

Major behaviours such as basking, territorial, foraging, vigilance, and moving did not differ between the heterogeneous and homogeneous conditions (Fig 9) (Table 3).

**Table 3:** Model statistics of different behaviours

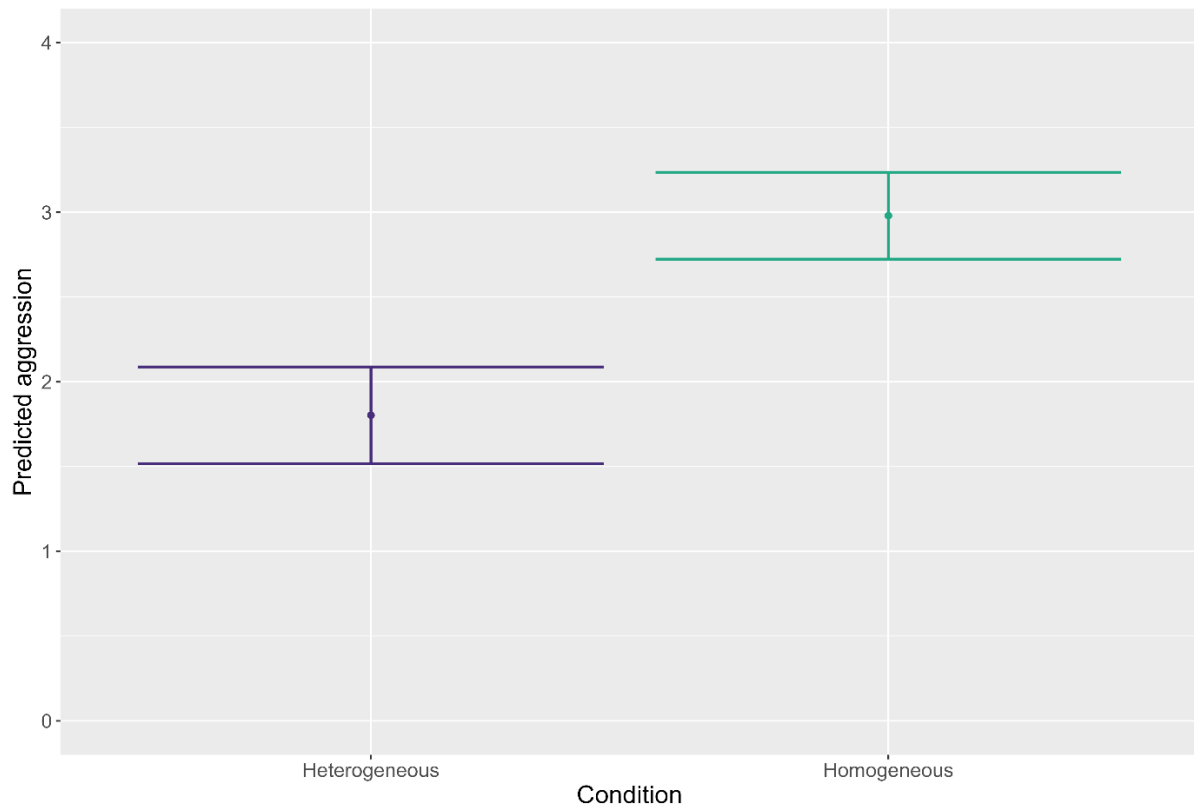
| States      | $\beta$ | $T$   | $SE$   | $P$  |
|-------------|---------|-------|--------|------|
| Basking     | -0.48   | -1.91 | 0.25   | 0.07 |
| Territorial | 0.007   | 1.7   | 0.0042 | 0.1  |
| Foraging    | -0.01   | 0.16  | 0.069  | 0.87 |
| Vigilance   | 0.004   | 0.48  | 0.0094 | 0.63 |
| Moving      | 0.002   | 0.38  | 0.004  | 0.7  |



**Figure 9:** Time allocation in different behaviours between conditions

#### 4.1.5 Aggression and Shuttling frequency

Aggression rate was more under homogeneous condition ( $\beta = 0.5, z = 2.21, SE = 0.2, P = 0.026$ ) and for males than females ( $\beta = 0.54, z = 2.05, SE = 0.26, P = 0.04$ ) (Fig 10). However, boldness and body condition index did not exert any influence on aggression rate ( $P > 0.05$ ). Individuals in homogeneous condition showed higher shuttling frequency than those in heterogeneous condition ( $\beta = 3.45, z = 6.99, SE = 0.4, P = < 0.001$ ). Time of the day did not influence shuttling frequency.



**Figure 10:** Comparison of mean (SE) aggression counts by focal individuals between heterogeneous and homogeneous conditions

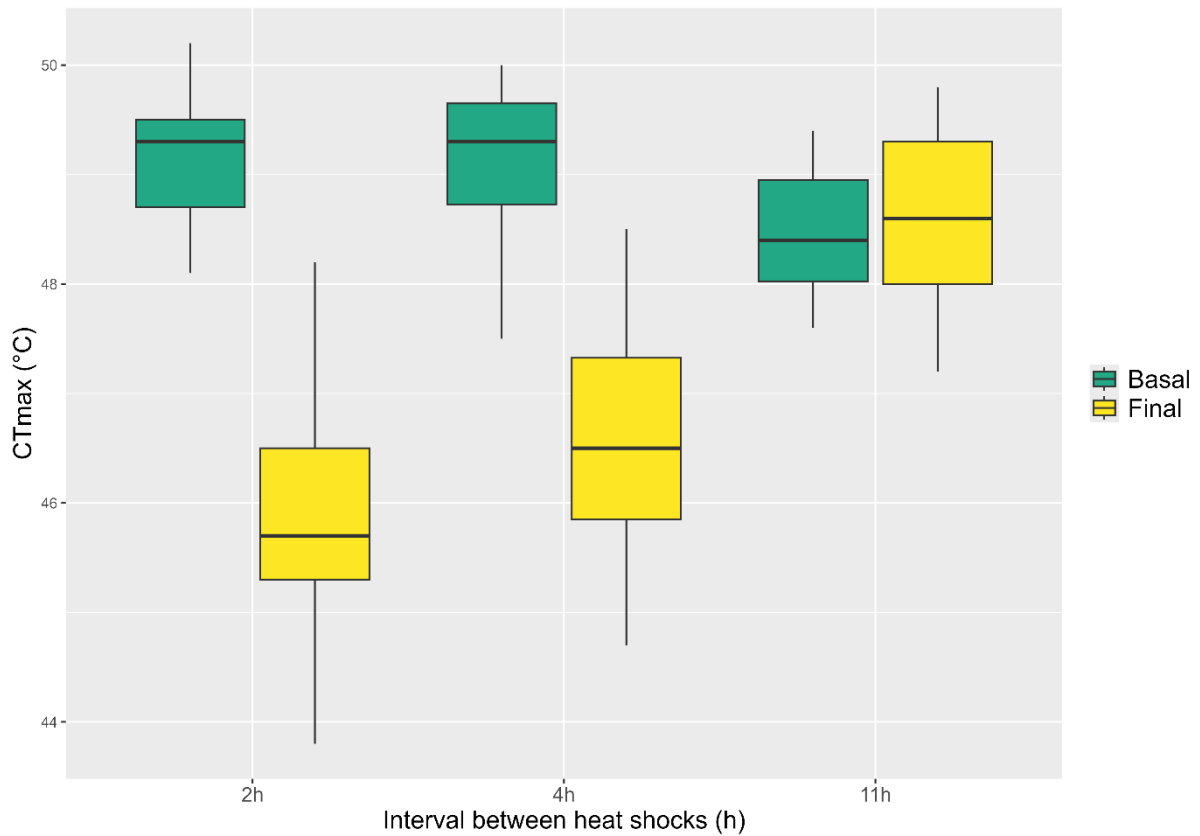
## Objective II:

### 4.1.6 Heating rates, mass and sex differences

There was no difference between basal and final heating rates (increment in body temperature per unit time) for lizards used in  $CT_{max}$  trials (t-value = -0.65, df = 24,  $P = 0.52$ , mean difference = -0.02, 95% CI = (-0.083, -0.043)). There was a small but significant difference in body mass before (mean = 154.84 g) and after (152.78 g) the trials (t-value = 7.24, df = 24,  $P < 0.001$ , mean difference = 2.16, 95% CI = (1.54, 2.78)). However, there was no difference in body mass between sexes measured before ( $\beta = 18.87$ ,  $R^2 = 0.04$ ,  $F_{23} = 1.11$ ,  $P = 0.304$ ) or after the trials ( $\beta = 20.05$ ,  $R^2 = 0.05$ ,  $F_{23} = 1.24$ ,  $P = 0.28$ ), according to the linear

model results. The above results indicated that all confounding factors were controlled for during the  $CT_{max}$  trials.

#### 4.1.7 Temporal dependence and heat hardening capacity



**Figure 11:** Boxplot showing basal and final  $CT_{max}$  for all individuals of each time interval treatment.

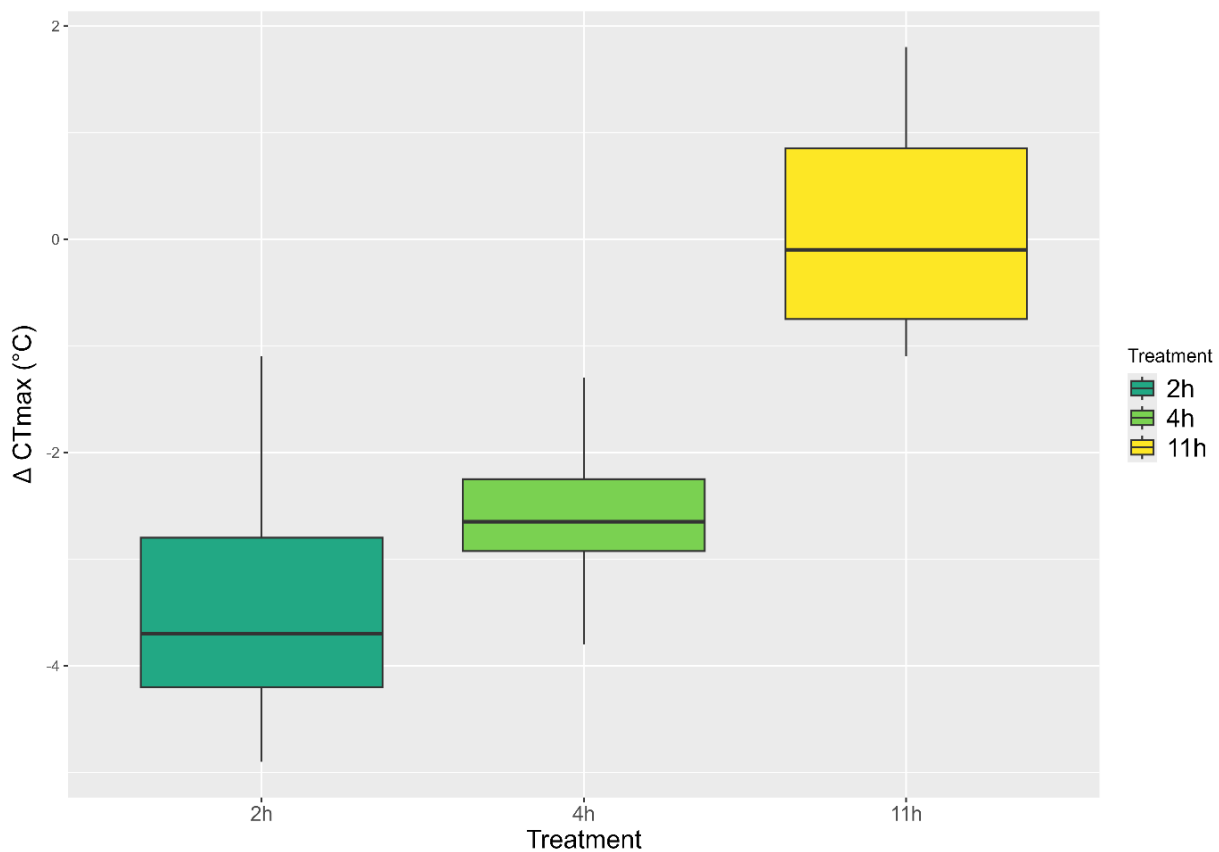
Generalized linear model did not show any effect of sex ( $\beta = -0.07$ ,  $T_{19} = -0.25$ ,  $P = 0.8$ ), snout-vent length ( $\beta = 0.21$ ,  $T_{19} = 1.23$ ,  $P = 0.23$ ) or mass ( $\beta = -0.005$ ,  $t_{19} = -0.85$ ,  $P = 0.4$ ) on basal  $CT_{max}$ . Even for final  $CT_{max}$ , there was no effect of sex ( $\beta = 0.22$ ,  $T_{16} = 0.46$ ,  $P = 0.64$ ), snout-vent length ( $\beta = 0.06$ ,  $T_{16} = 0.25$ ,  $P = 0.79$ ) or mass ( $\beta = -0.01$ ,  $t_{16} = -1.004$ ,  $P = 0.33$ ). Moreover, there was no effect of 2h and 4h treatments and their interaction with basal  $CT_{max}$  ( $P > 0.05$ ). However, Basal  $CT_{max}$  of the 11h treatment group differed from other treatments ( $\beta = -0.85$ ,  $T_{19} = -2.35$ ,  $P = 0.031$ ). Similarly, there was no effect of sex ( $\beta = 0.22$ ,  $T_{16} = 0.46$ ,  $P = 0.64$ ), SVL ( $\beta = 0.06$ ,  $T_{16} = 0.25$ ,  $P = 0.79$ ), mass ( $\beta = -0.01$ ,  $T_{16} = -1.004$ ,  $P = 0.33$ ), basal CT

max ( $\beta = -0.27$ ,  $T_{16} = -0.48$ ,  $P = 0.63$ ), treatment groups and their interaction ( $P > 0.05$ ) on  $\Delta CT_{max}$ .

Mean basal  $CT_{max}$  pooled across all treatments was  $48.94\text{ }^{\circ}\text{C}$  (Table 4). Mean final  $CT_{max}$  were  $45.85\text{ }^{\circ}\text{C}$  and  $46.48\text{ }^{\circ}\text{C}$  for 2h and 4h treatment groups respectively (Fig. 11). Unlike 2h and 4h treatments where  $\Delta CT_{max}$  (basal – final  $CT_{max}$ ) was consistently negative (mean =  $-3.3\text{ }^{\circ}\text{C}$  for 2h and  $-2.61\text{ }^{\circ}\text{C}$  for 4h), 11 h treatment had similar basal and final  $CT_{max}$  (Fig. 12).

**Table 4:** Mean and standard deviation of  $CT_{max}$  values of Spiny-tailed lizard (*Saara hardwickii*) with standard error in parentheses.

| Time Interval | Mean $CT_{max}$ ( $^{\circ}\text{C}$ ) | <i>n</i> |
|---------------|--|----------|
| 0h            | $48.95 \pm 0.76$ (0.15)                | 25       |
| 2h            | $45.85 \pm 1.21$ (0.40)                | 9        |
| 4h            | $46.48 \pm 1.29$ (0.45)                | 8        |
| 11h           | $48.57 \pm 1.04$ (0.36)                | 8        |



**Figure 12:** Boxplot showing heat hardening ( $\Delta CT_{max}$ ) across treatments.

In 11h treatment, three out of eight individuals showed increased thermal tolerance after the trial, however, the average final  $CT_{max}$  (48.57 °C) was slightly but not significantly higher than the basal average for 11h treatment (mean = 48.46 °C) (Fig. 11). Product-moment correlation showed no significant correlation between basal and  $\Delta CT_{max}$  for 2h ( $r_7 = -0.23$ ,  $P = 0.535$ ), 4h ( $r_6 = 0.34$ ,  $P = 0.399$ ) and 11h ( $r_6 = -0.44$ ,  $P = 0.274$ ). Since there was no significant correlation, I reject trade-off hypothesis which postulates that individuals with higher basal temperature will have less flexibility to increase thermal tolerance.

## 5. Discussion

### 5.1 Aboveground activity

Ectotherms such as spiny-tailed lizard, depend heavily on environmental temperatures to regulate their body temperature. Therefore, the availability of suitable microclimates is critical for their daily activities, especially species that occupy arid regions. In this study, I examined how habitat heterogeneity influences aboveground activity in spiny-tailed lizards. We found that lizards in heterogeneous habitats exhibited significantly greater aboveground activity compared to homogeneous habitats. This aligns with other studies which found similar results (Gaudenti et al., 2021).

On the contrary, while the aboveground activity increased in heterogeneous habitats, the relative time allocation in different behaviours such as foraging, vigilance, moving, and basking remained very similar between the two conditions. This indicates that heterogeneity in habitat did not induce any behavioural shifts in individuals rather it may have provided an expanded activity window with suitable thermal requirements (Kearney et al., 2009). Specifically, the basking rate showed marginal differences with individuals in heterogeneous conditions showing higher basking rates than individuals in homogeneous conditions.

In homogeneous conditions, lizards showed higher shuttling frequency indicating that individuals may have spent high energy to maintain body temperature and may lead to higher predation risk. However, due to a lack of temperature data, we could not ascertain whether there are any differences in the degree of thermoregulation of individuals between conditions. Future studies should incorporate quantifying behavioural thermoregulation when studying the influence of habitat heterogeneity.

These findings have major implications for woody encroachment, a phenomenon where the open natural grasslands are transformed into woodlands. At first glance, this increase in vegetation may look like habitat heterogeneity but it may not always provide functional benefit. Woody encroachment spreads unpalatable, woody plants often invasives like *Prosopis juliflora* leading to closed canopies, reduced visibility, and less variability in temperatures. It may reduce basking sites and limit lizard movement. Fine-scale patchiness with interspersed shrubs and trees can create an appropriate microhabitat for lizards to behaviourally thermoregulate. In contrast, woody encroachment resulting from land-use change, overgrazing, and fire suppression may lead to creating pseudo heterogeneity that may lack functional benefits.

## 5.2 Heat Hardening capacity

In addition to showing behavioural plasticity, Lizards may also rely on physiological plasticity to combat future climate change. Climate change induced global warming are adversely impacting the fitness of many species through myriad pathways, and ectotherms of hot deserts may be particularly vulnerable to this phenomenon. Previous studies on thermal ecology of spiny-tailed lizard have shown that spiny-tailed lizard are vulnerable to climate change with reduction in overall activity rate and increased dependency on burrows in future (Tatu et al., 2024). Heat waves are going to get more harsh and frequent in imminent future and thermal tolerance plasticity has a potential to buffer elevated temperatures caused by heatwaves benefitting ectotherms especially for species that inhabit arid regions (Vose et al., 2005; Rohini et al., 2019; Stillman, 2019). I studied heat hardening capacity of the spiny-tailed lizard (*Saara hardwickii*) and investigated the trade-off hypothesis which has been recently proposed to explain the variation in thermal tolerance plasticity among species (van Heerwaarden & Kellermann, 2020). I found that exposing spiny-tailed lizard to sub-lethal temperatures can

reduce their thermal tolerance ability, if these heating episodes occur in short intervals. This would mean that, if lizards are exposed to high temperatures during their initial foraging bout, the subsequent activity bouts of the day will be shorter due to reduction in thermal tolerance. However, with sufficient recovery period (~ 11h), lizards reacquired their original thermal tolerance, but did not surpass (Fig. 11).

Body mass decreased significantly between two consecutive heat shocks indicating that rapid increase in diurnal temperature in future may reduce body conditions of organisms through heat stress. Unlike other studies with similar time intervals that found plasticity in thermal tolerance (Gilbert & Miles, 2019; Deery et al., 2021; White et al., 2024), we found heat hardening only for a few individuals (N = 3) and only in the 11h treatment however, the average plasticity in this treatment was still below the average basal CTmax (Table 4). Thus, no evidence of heat hardening for *Saara hardwickii* was detected under these experimental conditions. A study on a pair of *Anolis* lizards by Deery et al., (2021) found inter species differences with one showing highest plasticity and another showing no plasticity at all. Absence of heat hardening capacity in species may lead to investing more effort in behavioural thermoregulation which may be the case for my study species as well (Deery et al., 2021). Lack of heat hardening capacity can increase the risk of heat exposure and reduce the performance capability (Deery et al., 2021). Nonetheless, the evidence of heat hardening in some individuals implied intraspecific variation in this trait that may experience strong selection pressure in the wake of warmer environments.

There might also be a possibility of variation in heat hardening capacity among local populations of *Saara hardwickii* owing to the large variation in temperature across its wide distribution range. Philips et al. (2016) found interpopulation differences of heat hardening capacity in *Lampropholis coggeri*. Future studies should look at inter population differences in heat hardening capacity in *Saara hardwickii*.

Trade-off hypothesis, rapid thermal tolerance plasticity induced by heat stress was recently proposed to explain the variation in thermal tolerance plasticity. Support for this hypothesis varies across taxa, For instance, multiple studies on *Acartia tonsa* (Crustacea), found evidence in support of the trade-off hypothesis (Gunderson, 2023), whereas studies on *Gasterosteus aculeatus* and *Danio rerio* did not find any support for it (Gunderson, 2023). However, studies on reptiles are limited despite being one of the diverse taxa and majority of the studies do not find support for this hypothesis except two studies (Phillips et al., 2016; Gunderson, 2023; Lapwong et al., 2021).

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