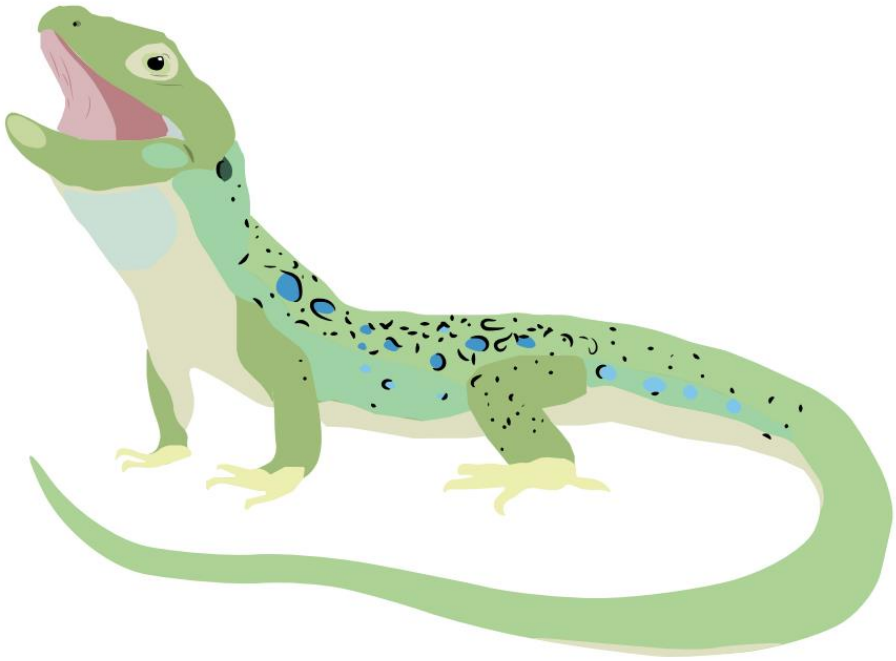


Understanding habitat use in green lizards: a mechanistic approach

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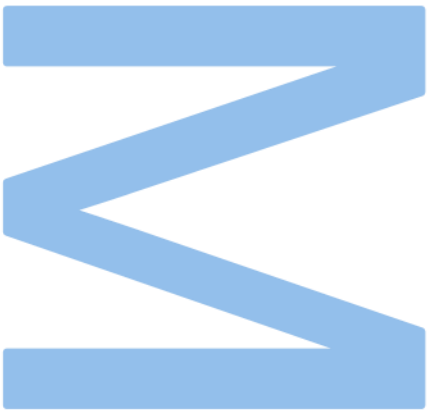
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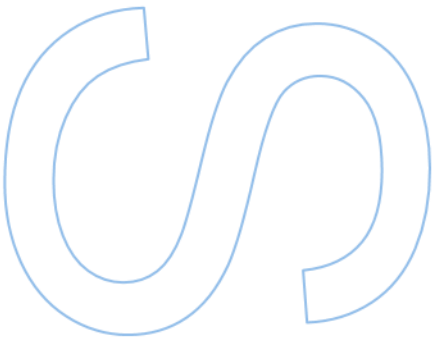
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Resumo

Avaliar o impacto das alterações climáticas relacionadas com actividade humana nas populações animais requer uma compreensão detalhada da forma como os organismos interagem com o seu ambiente biofísico. Os microhabitats que os animais seleccionam determinam as condições microclimáticas a que estão expostos, as quais, por sua vez, afectam as suas capacidades fisiológicas e desempenho ecológico. Os répteis, enquanto ectotérmicos, são particularmente vulneráveis às flutuações térmicas do seu ambiente, uma vez que a sua temperatura corporal está diretamente ligada à temperatura ambiente. Estas mesmas propriedades tornam os répteis excelentes candidatos para estudar as relações entre a variação fenotípica individual (por exemplo, preferências térmicas, comportamento termorregulador, tamanho do corpo), e o desempenho e distribuição dos animais no tempo e espaço.

Neste estudo, utilizámos os recentes avanços tecnológicos e metodológicos na área da ecologia para desvendar os mecanismos subjacentes às interacções animal-ambiente através de uma abordagem interdisciplinar. A actual miniaturização dos biologgers permite o seu uso em animais de pequeno porte e possibilita a quantificação em escala fina dos padrões de movimento e do desempenho locomotor. Além disso, a incorporação de parâmetros ecofisiológicos específicos nos modelos de nicho ecológico (ENMs) deu origem aos modelos de nicho mecanicistas (MNMs), que fornecem - entre outros - previsões exactas das temperaturas corporais (T_b) com base nas características biofísicas individuais e nos princípios termodinâmicos das trocas de calor no contexto do microclima do animal.

Neste caso, equipei lagartos machos adultos da espécie *Timon lepidus* com dispositivos externos de registo contendo acelerómetros tri-axiais e sensores de temperatura, humidade e luz. Posteriormente, libertei-os em mesocosmos individuais semi-naturais, onde podiam explorar livremente os microhabitats e microclimas disponíveis. Em primeiro lugar, analisei os dados obtidos dos biologgers para desenvolver um modelo de classificação não supervisionada do comportamento em grande escala (movimento vs. imobilidade) a partir de dados não tratados do acelerómetro, através de aprendizagem automática. A classificação dos períodos de movimento e imobilidade resultantes foram utilizados para descrever os padrões de movimento dos lagartos numa resolução temporal alta. Posteriormente, integrei os dados do biologger com os MNMs de balanço de calor para gerar previsões contínuas da temperatura corporal de todos os lagartos durante a sua estadia nos mesocosmos. O desempenho locomotor,

quantificado como aceleração, foi então mapeado através das temperaturas corporais estimadas para cada indivíduo para ajustar as curvas de desempenho térmico (TPCs), que descrevem a variação do desempenho individual em resposta à temperatura. Finalmente, utilizei os parâmetros térmicos derivados das previsões de temperatura corporal em escala fina e os TPCs (preferências térmicas, temperatura óptima, desempenho máximo, amplitude de desempenho) para explorar os efeitos da variação do tamanho do corpo no desempenho e a existência de de um trade-off evolutivo ('mais quente é melhor' e 'especialista-generalista') dentro da população.

A ausência de uma relação significativa entre o tamanho do corpo e os principais parâmetros térmicos sugere que a variação do tamanho do corpo na população de *T. lepidus* examinada não é suficiente para explicar as diferenças no desempenho locomotor. No entanto, relações mais subtis entre o tamanho do corpo e as preferências térmicas sugerem a existência de padrões mais complexos entre o balanço e sensibilidade térmica com o aumento do tamanho do corpo. Para além disso, encontrei uma correlação negativa significativa entre o desempenho máximo dos lagartos e a amplitude das suas temperaturas preferidas para locomoção, o que indica a existência de variação fenotípica intra-populacional nas estratégias de utilização do habitat térmico, confirmando o trade-off 'especialista-generalista'.

Os resultados deste estudo sublinham a importância de estudar os organismos num contexto natural e de e considerar padrões e mecanismos de escala fina ao explorar as relações complexas entre fisiologia térmica, desempenho e uso do habitat em ectotérmicos. Ao determinar como a temperatura corporal afecta o desempenho do organismo em diferentes indivíduos, este estudo fornece novo conhecimento sobre os factores e as restrições ao movimento dos animais, que por sua vez, influenciam outros aspectos da ecologia das espécies, como a utilização do habitat, a reprodução e fitness.

Palavras-chave: Microclima, microhabitat, ectotérmicos, escala fina, biologging, acelerómetro, padrões de atividade, comportamento de movimento, modelos de nicho mecanicistas, modelo biofísico, curva de desempenho térmico

Abstract

Evaluating the impacts of human-related climate change on animal populations requires a detailed understanding of how free-living organisms interact with their biophysical environments. The microhabitats that animals select determine the microclimatic conditions that they experience, which in turn affect their physiological capacities and ecological performance. Reptiles, as ectotherms, are particularly vulnerable to thermal fluctuations of their environment, as their body temperature is directly connected to ambient temperature. These same properties make reptiles excellent candidates for studying the links between individual variation in phenotypic traits (e.g. thermal preferences, thermoregulatory behaviour, body size) and animal performance and distribution through time and space.

In this study I took advantage of recent technological and methodological advances in ecological research to disentangle the mechanisms behind animal-environment interactions through an interdisciplinary approach. The ongoing miniaturization of biologgers allows their employment in small animals and enables the fine-scale quantification of movement patterns and locomotor performance. Furthermore, the incorporation of specific ecophysiological parameters into ecological niche models (ENMs) gave rise to the mechanistic niche models (MNMs), which provide – among others – accurate predictions of body temperatures (T_b) based on individual biophysical traits and on the thermodynamic principles of heat exchange in the context of the animal's microclimate.

Here, I equipped adult male green lizards of the species *Timon lepidus* with external logging devices containing tri-axial accelerometers and temperature, humidity, and light sensors. Then, I released them in individual semi-natural mesocosm enclosures, where they could freely exploit available microhabitats and microclimates. First, I analysed the data obtained from the animal biologgers to develop an unsupervised classification framework for coarse-scale behaviour (movement vs. immobile) from raw accelerometer data through machine learning. The resulting classified periods of movement and immobility were used to describe lizard movement patterns at a high temporal resolution. Subsequently, I integrated the biologger data with transient heat-budget MNMs to produce continuous body temperature predictions for all the lizards during their stay in the mesocosms. Locomotor performance, quantified as acceleration, was then mapped onto estimated body temperatures for each individual to fit thermal performance curves (TPCs), which describe individual performance variation in response to temperature. Finally, I used the thermal parameters derived from the fine-scale body temperature predictions

and the TPCs (thermal preferences, optimum temperature, maximum performance, performance breadth) to explore the effects of variation in body size on whole-organism performance and the existence of evolutionary trade-offs ('hotter-is-better' and 'specialist-generalist') within the population.

The lack of a significant relationship between body size and major thermal parameters suggested that body size variation within the examined *T. lepidus* population is not sufficient to explain differences in locomotor performance. However, more subtle relationships between body size and thermal preferences implied the existence of more complex patterns of heat balance and thermal sensitivity with increasing body size. Furthermore, I found a significant negative correlation between lizard maximum performance and their breadth of preferred temperatures for locomotion, which indicates the existence of intra-populational phenotypic variation in thermal habitat use strategies, confirming the 'specialist-generalist' trade-off.

The results of this study underline the importance of studying organisms in a natural context and considering fine-scale patterns and mechanisms when exploring the complex relationships between thermal physiology, performance and habitat use in ectotherms. By determining how body temperature affects whole-organism performance across different individuals, this study provides new insights into the drivers and constraints to animal movement, which in turn influence other aspects of species' ecology, such as habitat use, reproduction and overall fitness.

Keywords: Microclimate, microhabitat, ectotherms, fine-scale, biologging, accelerometer, activity patterns, movement behaviour, mechanistic niche modelling, biophysical model, thermal performance curve

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List of Abbreviations

AICc	Akaike Information Criterion (corrected for small sample size)
ASL	Above Sea Level
CICGE	Centro de Investigação em Ciências Geo-Espaciais
CLARA	Clustering Large Applications
CT_{max}	Critical Thermal Maximum
CT_{min}	Critical Thermal Minimum
CRP	Carolina Reyes-Puig
DBA	Dynamic Body Acceleration
ENM	Ecological Niche Model
GPS	Global Positioning System
MNM	Mechanistic Niche Model
NCEP	National Centers for Environmental Prediction
NLLS	Nonlinear Least-Squares Regression
NS	Neftalí Sillero
ODBA	Overall Dynamic Body Acceleration
PAM	Partitioning Around Medoids
Q_{sol}	Solar Radiation
r_{max}	Maximum Performance (rate)
RMSE	Root Mean Square Error
SD	Standard Deviation
SDM	Species Distribution Model
SVL	Snout-Vent Length
T_{air}	Air Temperature
T_b	Body Temperature
T_{emerge}	Emerge Body Temperature
T_{opt}	Optimum Temperature
TPC	Thermal Performance Curve
T_{pref}	Preferred Body Temperature
$T_{pref,min,imm}$	Minimum Preferred Body Temperature while Immobile
$T_{pref,min,mob}$	Minimum Preferred Body Temperature while Mobile
$T_{pref,max,imm}$	Maximum Preferred Body Temperature while Immobile
$T_{pref,max,mob}$	Maximum Preferred Body Temperature while Mobile
T_{rad}	Radiation Temperature
$T_{retreat}$	Retreat Body Temperature

T_{sky}	Sky Temperature
T_{soil}	Soil Temperature
VeDBA	Vectorial Dynamic Body Acceleration
Vel	Wind Velocity
Zen	Zenith Angle

1. Introduction

1.1. General introduction and motivation of the study

Biodiversity is being eroded globally by human-related factors, such as climate change and habitat degradation (Butchart et al., 2010). Empirical data have already confirmed the negative effects of climate change on the biology, distribution and interactions of many species (reviewed in Parmesan, 2006), and the projected rates of climate change might prevent many of them from adapting to the new conditions (Sinervo et al., 2010). To quantify the scale of biodiversity loss and to respond with effective management strategies when the risks are greater, it is important to understand how vulnerable organisms are to environmental changes and to predict how these changes might affect them (Lee & Jetz, 2008).

When an animal is exposed to a change in conditions, not only the degree of environmental exposure itself but also the animal's sensitivity to the change is going to determine its vulnerability and its eventual persistence. The sensitivity of a species or an individual is determined by its physiological and ecological traits (thermal limits, behaviour etc.), which in turn are based on its genetics (Williams et al., 2008). The degree to which an organism's performance is affected by its body temperature defines its thermal sensitivity and it is a crucial trait determining its vulnerability to climate change (Angilletta, 2009; Buckley et al., 2022). For example, changes in environmental temperature can alter the body temperature of ectotherms, which in turn affects their physiological performance (Huey et al., 2012).

A species' vulnerability to environmental change is also affected by its potential to adapt to new conditions. In many cases, adaptation by natural selection requires many generations to occur, while global climatic change is progressing at rates that sometimes might not allow the timely evolution of adaptive responses in many animals (Seebacher et al., 2015). Thus, it is expected that mechanisms working at the individual level, like phenotypic and ecological plasticity, may be necessary to minimize the effects of climate change in the short term, rather than evolutionary processes working at a population level (Seebacher et al., 2015; Williams et al., 2008). To better understand species' vulnerability, there is a growing need to describe not only their sensitivity but also the mechanisms that might allow animals to buffer the effects of rising temperatures through behavioural, physiological and morphological variation and plasticity (Helmuth et al., 2005).

Reptiles, as ectotherms, depend greatly on local environmental conditions, such as ambient temperature and humidity, and on the characteristics of their habitat, to maintain

their body temperatures within a range in which daily activity and performance are optimal (Pough, 1980). This environmental dependence makes them especially vulnerable to climate-driven changes in their surroundings, which might bring some reptile populations to extinction (Kearney, 2013). At the same time, these same qualities of reptiles render them good candidates for studies assessing the impacts of climate change on animal thermal biology (Taylor et al., 2021). In addition to being foundational to their ecosystems due to their efficiency in secondary biomass production (Pough, 1980), reptiles are often good indicators of thermal environmental quality precisely because of their dependence on external conditions. For this, conclusions based on ectotherms as model organisms can be relevant for almost all species within their ecosystem (Sinclair et al., 2016; Taylor et al., 2021).

The importance of reptiles for thermal biology research does not end there. Being ectotherms, reptiles buffer fluctuations in their environment through behavioural thermoregulation and moving to more suitable thermal habitats (Kearney et al., 2009). Behavioural thermoregulation, as an aspect of phenotypic variation, can be a critical mechanism for species survival under the thermal stress of climate change (Enriquez-Urzelai et al., 2020), through the control of activity times, body posture and microhabitat selection (Huey et al., 2012). Hence, reptile physiology, behaviour and their interaction with the environment are relevant study topics to understand how animals use their available space and under which conditions this is possible (Huey, 1991).

Finally, apart from their biological attributes, reptiles are important as model organisms for understanding fundamental aspects of animal physiology and thermal ecology due to their ease of study. There are many reptile species that are highly suitable for field and laboratory experiments because of their local abundance, amenity to sampling and handling, as well as resilience to laboratory captivity conditions (Taylor et al., 2021). Reptiles have been long used for studies in evolutionary ecomorphology, behavioural plasticity and functional performance, which provides solid background knowledge (Ariano-Sánchez et al., 2022; Bernich et al., 2022; DeSantis et al., 2020; Kaliontzopoulou et al., 2013, 2018; Muñoz et al., 2016; Zena et al., 2020).

As reptiles are at high risk of extinction due to climate change (Kearney, 2013; Sinervo et al., 2010), there is a growing need for intensive monitoring and conservation actions for affected species. Success in conservation strategies requires a deeper understanding of reptiles' thermal ecology, physiology and habitat use, through the implementation and improvement of the most suitable and advanced methodologies and technologies (Taylor et al., 2021). Still, there is a scarcity of studies regarding the behaviour and

physiology of free-ranging animals in natural settings and in relevant spatial and temporal scales, while laboratory studies cannot adequately imitate all the biotic and abiotic interactions found in nature (Cooke, 2008; Logan et al., 2015). However, ongoing advancements in remote monitoring technology and the miniaturization of biologgers are providing novel – and affordable – methodologies for innovative research on animal ecology and allow us to describe the way animals freely use their habitats, as well as the mechanisms that might limit them in their daily lives.

1.2. Activity and habitat use monitoring

1.2.1. Determinants of variation in ectotherm daily activity patterns

The study of why animals occur in certain areas and how they select and exploit the space available to them is paramount in the effort to conserve the Earth's biodiversity. For ectotherms particularly, the ability to seek and select suitable microhabitats can be crucial for their persistence (Huey & Bennett, 1987). Different microhabitats inside the same habitat often provide different biophysical environments that ectotherms can use to thermoregulate. Understanding the physiological response of individuals to the parameters of their environment, in combination with the extent to which they can buffer them with behavioural thermoregulation, can improve predictions of the effects of climate change on organisms.

A way to record and describe animal behaviour in the context of habitat use is through the detailed documentation of organismal movement or the lack of it (Cooke et al., 2004). Movement patterns and their underlying processes reflect the interaction between intrinsic (e.g. physiological state) and extrinsic factors (environmental conditions, predation etc.), which eventually shape the behavioural decisions of animals. The movement of individuals to different locations is linked to many vital processes, from reproduction, foraging and escaping predators to dispersal, habitat selection and responses to disturbances (Nathan et al., 2008). For instance, basking behaviour and moving to cooler microhabitats during fluctuations in ambient temperatures can protect ectotherms from reaching their thermal limits (Huey et al., 2003). Thus, a more comprehensive understanding of the causes, patterns and mechanisms behind animal movement is essential.

External factors, like ambient temperature and humidity, that affect physiological function, are of particular importance for the movement and daily activity of ectotherms, such as lizards (Huey, 1982). Lizards need to maintain their body temperatures at an optimum value to reach the maximum performance needed for vital activities (e.g. foraging, reproduction). This is achieved through the adjustment of their daily activity periods and the

appropriate selection of available microhabitats when environmental temperatures are pushing them away from their thermal optima (Adolph & Porter, 1993; Gunderson & Leal, 2015). These favourable body temperatures can be maintained only within a small window of ambient temperatures and so, the thermal environment of a lizard can greatly restrict its activity times, its thermoregulatory options and, as a result, its successful survival and reproduction (Adolph & Porter, 1993). Apart from the behavioural plasticity in thermoregulatory strategies, a lizard's sensitivity to changes of temperature is influenced by its range of thermal tolerance (Huey et al., 2012). Therefore, to fully comprehend a reptile's potential to resist climate change, we need a better and in-depth understanding of the intraspecific variation in thermal tolerances, behavioural thermoregulation and their potential interplay.

The variation in habitat use and movement patterns inside a population or a species, apart from external conditions, is also driven by individual traits, such as body size, morphology, sex, etc. (Perry & Garland, 2002). Body size and shape variation can determine the habitat availability for a species, but its cause-and-effect role is often difficult to interpret. The effects of climate change on body size have already been observed. Organism size tends to decline as worldwide temperatures rise (Caruso et al., 2014; Sheridan & Bickford, 2011), since developmental rates vary in response to environmental conditions (Kingsolver et al., 2004). A correlation between habitat and body size at a population level has also been observed, as there is a tendency for an increase in body size in colder conditions, with warmer environments reducing survivorship and the probability of reaching maturity (Angilletta et al., 2004). At the same time, in grown animals, body size may restrict the use of different refuges and microhabitats, as well as affect activity patterns, while it is expected that larger animals will have higher energy requirements and thus will spend more time foraging and looking for resources (Perry & Garland, 2002). Larger body size is however associated with greater performance fitness within populations (Kingsolver & Huey, 2008) and it has been shown that larger lizards can reach higher accelerations over longer distances and periods than smaller lizards (Huey & Hertz, 1984a).

Ectotherms often need to expose themselves to very large daily fluctuations of the conditions of their immediate environment and even very short exposures to extreme conditions can have major consequences for an animal's physiological rates and performance (Kearney et al., 2012). Thus, to understand the mechanisms behind processes that might shape population persistence, it is important to choose the appropriate temporal scales. The choice of daily data, recorded at a high temporal resolution, is becoming more and more prominent in this field. Recent technologies and methodologies facilitate the

acquisition of such data and can help to obtain a detailed view of individual movement, activity budgets, and how these are affected by external factors (Nathan et al., 2008).

1.2.2. Biologging to study mechanisms in ecology

Movement ecology aims to integrate the theory of whole-organism movement with the development of a general framework of research that will help in understanding the causes, mechanisms and patterns behind organismal movement, as well as their role in ecological and evolutionary processes (Nathan et al., 2008). The recent advancement in the technology of animal-borne remote-sensing devices, or biologgers, has facilitated this kind of research and has given the possibility to monitor movements and behaviours at resolutions so far only achievable in laboratory conditions (Allan et al., 2018; Cooke et al., 2004; Kays et al., 2015). This remote measurement of behavioural and physiological data, also known as biotelemetry, provides a powerful tool to answer questions about ecological mechanisms in detail. In this way, it is now possible to observe how animals interact with their natural environments and this, in turn, can be used as a model to understand how animal populations react to anthropogenic environmental stressors (Cooke et al., 2004).

Depending on the questions asked, different types of animal-attached devices may be implemented: some biologgers can determine changes of animal position (GPS; Bernich et al., 2022), animal behaviour (accelerometers; Brown et al., 2013), environmental conditions (temperature, light intensity, relative humidity, wind speed) and/or animal physiology (e.g. heart rate; Cooke et al., 2004). Combining multiple sensors in the same logging device can provide detailed data on the daily behaviour and ecology of animals in the wild, reveal intraspecific patterns and monitor fine-scale movements and environmental conditions, an approach termed ‘daily diary' (Wilson et al., 2008).

Naturally, the implementation of biologgers to wild animals comes with some challenges. One of them is the recovery of the sensors with the logged data after the logging period is over, something that usually requires the recapture of the animal. This process can be difficult for smaller animals or animals that do not tend to repeatedly go back to the same site (Wilson et al., 2008). Another limitation is the size of the study animal. Until very recently, most biotelemetry studies concerned larger-bodied taxa (Brown et al., 2013). Even if recent advancements in biologging technologies have allowed for more and more studies on smaller organisms, like reptiles (Bernich et al., 2022; DeSantis et al., 2020) and amphibians (Halsey & White, 2010), morphological challenges with species of smaller body size remain. Although it would be ideal that logged animals behave in an

identical way as their non-equipped conspecifics, such a scenario is unrealistic, especially for smaller-bodied taxa, and it is safer to assume that these animals' behaviour would be modified in some ways (Wilson & McMahon, 2006).

For this study, it was relevant to obtain measurements of activity and of the conditions experienced by ectotherms daily. Thus, the appropriate biologgers used in this case are accelerometers and sensors recording environmental conditions.

1.2.2.1. Activity monitoring through acceleration logging

Miniature accelerometers are being increasingly used in studies regarding remote monitoring of animal movement and identification of behavioural modes, with the study of Yoda et al. (1999) being the first to apply this kind of technology to free-ranging animals. The great benefit of the accelerometer protocol is, among others, the possibility of obtaining fine-scale and continuous data on animal activity without the limitations of observer presence, weather, time of the day and spatial scale (Brown et al., 2013; Yoda et al., 1999). Combining this data with measurements of environmental parameters (e.g. light, ambient temperature) can give important insights into whether and when animal movements are driven by specific factors (Brown et al., 2013; Wilson et al., 2008).

Tri-axial accelerometers can be used to answer a wide range of ecological questions and to understand in detail the relationships between animal behaviour and their environment (Patterson et al., 2019). They can be implemented to track animals for large periods and with a high temporal resolution, measuring their acceleration in three dimensions of movement (x: forward-backward movement, y: left-right movement, z: upward-downward movement). When the acceleration sensor is deformed (i.e. there is a change in velocity), it produces signals proportional to its acceleration (measured in g; 1 g = 9.8 m/s^2). The sensor can be deformed in all three axes by two components. The first, termed the static component, captures the inertial acceleration caused by the Earth's gravitational field (which, when mounted on an animal, represents the changes in posture). The second, or the dynamic component, corresponds to the actual changes in velocity resulting in the movement of the animal (Shepard et al., 2008).This means that under static conditions (when the animal is immobile) the acceleration signal of an animal-attached accelerometer would not be zero in all axes, but it would capture the gravitational force acting on the sensor (Brown et al., 2013). Based on all the above, calculating the dynamic component of acceleration experienced by the logger already requires further processing of the raw data, first to define the static component and then to subtract it from the overall acceleration of each axis (Wilson et al., 2008).

As valuable as accelerometer data might be, their usage is not yet standardized and can often be challenging. The first issue that a researcher encounters is the ensuing analysis of the produced acceleration data. As seen above, the raw acceleration data provided by the sensors cannot be used as they are but need to be converted into biologically meaningful values, by deriving the dynamic component of acceleration. Moreover, the sheer volume and detailed nature of such tracking device data (usually recording with a frequency of more than 10 Hz) pose a significant computational barrier in the effort of analysing and interpreting them (Collins et al., 2015; Patterson et al., 2019). Such challenges are similar to those encountered in the analysis of 'big data', even when talking about datasets derived from single individuals. Data recorded at such high frequencies are very noisy, something that requires data filtering with smoothing techniques, like the use of running means (Williams et al., 2020).

Even after the acquisition of biologically relevant acceleration values, more challenges arise. For instance, we still lack a computationally simplified method for categorizing and classifying acceleration values into even the coarsest animal behaviours (e.g. movement vs. non-movement) without relying on validation data (e.g. field observations or subjective assessments) (DeSantis et al., 2020), while behavioural classifications from acceleration data might not be transferable between animal species (Collins et al., 2015; DeSantis et al., 2020). There is therefore the need for the development of an unsupervised framework to analyse data for fine-scale activity budgets, which can objectively tackle all the above-mentioned problems and provide a robust method to facilitate the implementation of this technology in ecological research (Patterson et al., 2019).

1.3. Mechanistic niche modelling

1.3.1. Modelling a species niche

Describing and predicting species-environment relationships is a relevant practice in the research regarding species' vulnerability and persistence to climate change. The niche concept is used to describe these relationships. A species niche is defined as the n-hypevolume in the environmental space which represents the set of environmental conditions that allow a species to maintain a viable population (Hutchinson, 1957). In turn, the physical and chemical manifestation of a species' niche in the landscape constitutes the species' habitat (Whittaker et al., 1973). Predictive models have been developed to study species' niches, to understand why species occur in certain areas and to forecast future distributions (Urban et al., 2016).

Ecological Niche Models (ENMs), sometimes also referred to as Species Distribution

Models (SDMs) (Sillero, 2011), are the main tool used to examine the relationship between environmental conditions and the distribution of species. One approach are correlative ENMs, which statistically relate environmental variables with records of species presences and absences (Elith & Leathwick, 2009; Sillero, 2011). The data used for these models are often readily available in databases and they do not require detailed knowledge of the physiology of the studied species (Elith et al., 2006). For this reason, correlative ENMs are widely used to predict species distributions.

However, correlative models do not directly model the mechanisms that might limit species' presence in certain areas, which often depends on the physiological limits of organisms (Kearney & Porter, 2009). Ignoring the physiological responses of individual organisms or populations to past and present environmental conditions can be misleading, as physiology mediates the way that abiotic conditions (e.g. temperature, water availability) affect reproduction, movement, growth and eventually survival (Urban et al., 2016). Physiological parameters, such as preferred temperatures and critical thermal minima and maxima, are key to population range shifts and persistence (Enriquez-Urzelai et al., 2019). For example, knowing the portion of time an organism can spend active during the day under specific conditions provides important information regarding its potential persistence under different climatic scenarios (Sinervo et al., 2010).

Moreover, the environmental variables used in correlative ENMs very often come from macroclimatic environmental layers from weather stations, which are measured 1-2 m above the ground, and they do not normally represent the exact microclimatic conditions which animals experience in their daily lives (Potter et al., 2013). These conditions include air temperature, radiation, humidity, wind speed, soil temperature and soil moisture at the location of the animal and they are shaped by the interaction of macroclimatic conditions and specific habitat features, such as terrain, vegetation and shade (Kearney & Porter, 2017). It is thus important to study physiological performance within the context of the organism's local microclimate, a challenge that a different type of models, i.e. mechanistic and biophysical models, try to address.

1.3.2. Biophysical modelling

Mechanistic models are mathematical models that predict a phenomenon based on its explicit underlying mechanisms, while, more specifically, mechanistic niche models (MNMs) consider additional processes, i.e. metabolic and evolutionary theory, to predict the niche of species (Briscoe et al., 2023). A form of MNMs are biophysical models, which explore the processes of mass and energy exchange between organisms and their microclimatic environment. Specifically, the processes of radiation, conduction, convection and evaporation are taken into account to understand the physical constraints that might limit organismal performance (Kearney & Porter, 2009). In biophysical models, an organism is considered as an open thermodynamic system, with the system boundary being the organism's outer surface. Like any open thermodynamic system, both heat and mass are exchanged between the organism and its environment, which, in equilibrium should sum up to zero or else result in heat storage or loss (Porter, 2016). To estimate how the environment affects the body temperature of the organism, biophysical models solve an energy balance equation based on the thermodynamic law of the conservation of energy (Figure 1). Solving this equation for an ectotherm of specific traits can provide estimates of its core body temperatures under any given environmental conditions (Kearney & Porter, 2009). Thus, the goal of biophysical models is to identify the set of environmental conditions that allow an organism of specific physiological, behavioural and morphological traits to maintain its homeostasis in order to conclude a full life cycle.

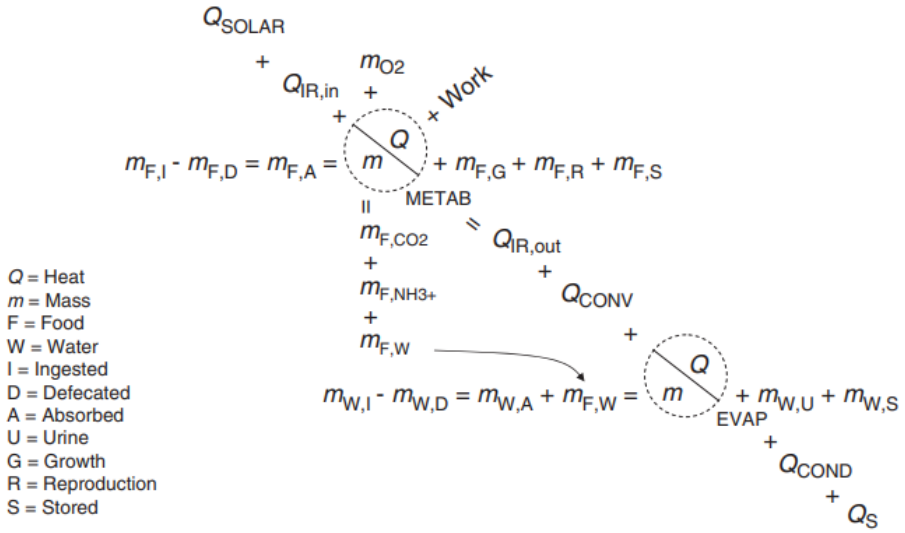


Figure 1. Energy (*Q*) and mass (*m*) balance equation that can be developed for ectotherms and their habitats to estimate the core body temperature under a set of environmental conditions. The energy is exchanged as heat (diagonal heat balance equation) and is intersected by three mass balance equations, two at the metabolism term, representing the mass balance for dry food (horizontal) and for respiratory gases (vertical) and one at the evaporation term, representing the mass balance for water (Kearney & Porter, 2009).

These conditions are found in environments where the interaction between an organism's functional traits and its habitat's abiotic constraints might allow it to survive and reproduce, and thus fall within the fundamental niche of the species, or 'the conditions which would permit the species to exist indefinitely' (Hutchinson, 1957). More precisely, they constitute its 'thermodynamic niche' (Kearney et al., 2013). These mechanistic models describe the thermodynamic niche of organisms, or their capacity to respond to their current or future environmental conditions depending on their physical traits. Hence, while an organism's physical traits remain the same, MNMs can be very precise and detailed in explaining the processes that limit its distribution (Kearney, 2006; Kearney et al., 2013). Thus, mechanistic models are increasingly implemented to model range-limiting processes, even if they have the disadvantage that they require much more data, resources and time than their correlative counterparts (Kearney & Porter, 2009).

1.3.3. The mechanistic niche modelling framework

The first step to model the thermodynamic niche of an animal involves depicting the environment in the specific way the organism experiences it (Kearney & Porter, 2009). Specifically, the environmental conditions that need to be modelled – and that are relevant to understand the processes of mass and energy exchange – are the microclimatic conditions, as opposed to the macroclimate. One can gain information about the microclimatic conditions of a habitat with the use of environmental datalogging devices. However, the information from these devices is limited in space. Thus, it is necessary to model the microclimatic conditions in entire landscapes. To that end, microclimate models (e.g. the widely used and tested NicheMapR's microclimate model; Kearney et al., 2019) compute microclimatic conditions for different shade levels near and below the ground at any point in time, given habitat and terrain properties and macroclimatic conditions from weather stations (Kearney & Porter, 2017).

The outputs of the microclimate model can subsequently be used as input for animal, heat-transfer models and, more specifically, for the scope of this project, the ectotherm model. These biophysical animal models take species-specific morphological, physiological and behavioural parameters. Based on these parameters, they solve the energy balance equation to determine how microenvironments affect the body temperature (T_b) of an organism and thus its potential performance (movement, foraging), as body temperature is critical for physiological function, especially for ectothermic animals (Kearney et al., 2021).

Most biophysical models solve steady-state energy balance equations, i.e. they assume

that the organism's heat storage is negligible between the modelled intervals. In other words, the organism is modelled to reach the equilibrium body temperature within that interval (Kearney & Porter, 2009). These 'steady-state' models are simpler models that allow integrating behavioural data, but they have some constraints. Animals, especially those of bigger sizes, have a higher heat capacity and display a degree of thermal inertia, meaning that their body temperature equilibrates gradually to that of the environment (Stevenson, 1985). To account also for this effect of body size on ectotherms, 'transient-state' mechanistic models can be used. These models also incorporate a term for stored energy (Kearney et al., 2021), which is the component of the model that accounts for thermal inertia.

1.4. Predicting the thermal sensitivity of ectotherm locomotion

Ectotherm body temperatures are directly connected to ambient temperatures and they have a significant impact on their performance (e.g. ability to run, forage, reproduce etc.) by influencing physiological and biochemical reaction rates (Angilletta, 2009). For reptiles specifically, extreme body temperatures are harmful and potentially lethal, while intermediate to moderately high temperatures are preferred and they maximize performance (Huey & Kingsolver, 1989). The degree to which an organism's performance depends on its body temperature is known as its thermal sensitivity. There are animals that can properly function only in a very narrow set of temperatures and, hence, their performance depends strongly on environmental conditions (thermal specialists), while other animals are able to perform adequately in a wide range of temperatures (thermal generalists) (Huey & Slatkin, 1976). These strategies determine the trade-offs that are imposed on each individual organism when experiencing fluctuations in its environment and individual variation in these strategies can determine a population's persistence (Angilletta, 2009).

Moreover, the thermal sensitivity of organisms can also interact with or vary according to their physical traits. For instance, large organisms might be more effective in regulating the thermal variability of their environment due to increased thermal inertia that buffers the effect of the environment on their core body temperatures. On the contrary, smaller organisms might have a reduced capacity to buffer their environments and thus be more affected by rapid fluctuations of ambient temperatures (Slein et al., 2023). Addressing such issues is important to estimate the potential ecological consequences of climate change on organisms.

A standard way of evaluating the consequences of thermal variability on ectotherm

performance is by estimating the actual body temperatures of ectotherms in natural conditions and, subsequently, predicting how these body temperatures affect whole-organism performance and fitness (Huey & Slatkin, 1976). Thermal Performance Curves (TPCs) provide quantitative descriptions of the effect of body temperature on performance (Huey & Stevenson, 1979). Mapping physiological rates (performance) across different body temperatures provides a usually asymmetric curve which gradually rises until it reaches a maximum level, where performance is also maximized, and then drops rapidly (Figure 2). Through these TPCs, one can objectively estimate useful metrics of animal thermal sensitivity, like optimum body temperature (T_{opt}), maximum performance (r_{max}), critical thermal minima and maxima (CT_{min} and CT_{max}) and thermal performance breadth, i.e. the range of temperatures that organisms seek out within a thermally heterogeneous environment and in which they perform above some arbitrary performance level (Huey & Kingsolver, 1989).

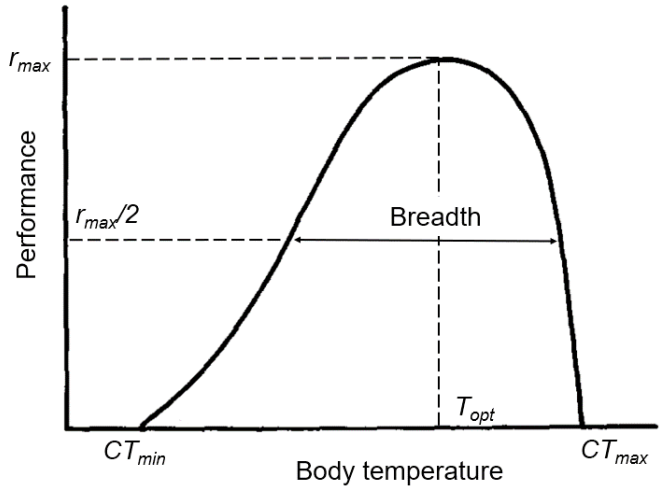


Figure 2. Hypothetical performance curve of an ectotherm as a function of body temperature (T_b), commonly referred to as thermal performance curve (TPC). Depicted are the thermal parameters of the curve: maximum performance (r_{max}); body temperature where performance is maximized, or optimum temperature (T_{opt}); the breadth (width) of the curve where performance is above some level (here half of r_{max}); and critical minimum (CT_{min}) and maximum (CT_{max}) body temperatures, below and above which performance is not possible.

A smaller performance breadth or a lower optimum temperature in a given environment entails a higher risk of overheating as the climate becomes warmer. Furthermore, because of the asymmetrical shape of the curve, the effects of body temperature change on performance can be minimal, insignificant or major, depending on which part of the TPC these changes occur. The integration of body temperatures with TPCs and studying the shape of curves and their derived parameters provides a useful tool for estimating organismal responses to environmental change (Sinclair et al., 2016).

Performance can be defined as the capacity of an organism to function and is usually expressed as a biological rate. TPCs usually measure whole-organism performance and thus, measures of locomotion or growth are typically used, which are higher-level traits that are immediately connected to ecological performance and respond rapidly to changes in temperature (Angilletta, 2009). Locomotion, quantified as the speed or the acceleration of an animal at a given moment, is an ecologically relevant measure of performance for most reptiles and the thermal sensitivity of sprinting has been found to match the thermal sensitivity of other traits in various reptile species (Huey, 1982; Huey & Bennett, 1987). The ability of an animal to accelerate is vital for its daily survival (e.g. to escape predators or catch prey) and it has been shown to be paramount in the ability of animals to gain maximum speeds (Huey & Hertz, 1984a). Acceleration is therefore an excellent performance trait for TPCs.

Understanding the intraspecific variation of TPCs and comparing their parameters between individuals and populations can help to answer fundamental questions regarding a species' thermal sensitivity. Two of these questions are summed up by two evolutionary trade-off hypotheses: the first, known as the 'hotter-is-better' hypothesis, argues that the maximum performance of an organism with a higher optimum body temperature should be greater than that of an organism with a lower optimum temperature, a hypothesis based on the fact that biochemical reactions slowdown in colder temperatures (Angilletta et al., 2010). The second hypothesis, known as the 'jack-of-all-temperatures is a master of none' or 'specialist-generalist' hypothesis, makes the distinction between thermal specialists and generalists and is based on the principle of energy allocation. According to this hypothesis, trade-offs are imposed between maximum performance and breadth of performance: the ability to perform over a broad range of temperatures comes at the expense of low maximum performance (Huey & Hertz, 1984b). Answers to these questions can provide vital information regarding the patterns of species' thermal sensitivity.

1.5. Objectives

The overall aim of this study was to better understand the mechanisms behind lizards' thermal microhabitat and microclimate use. For this purpose, I equipped 26 adult males of ranging body sizes of the ocellated lizard (*Timon lepidus*) with external logging devices which recorded animal locomotion and the environmental conditions in the immediate surroundings of the animals. I then released the lizards into individual mesocosms, where they were left to exploit their available space freely. With the obtained data, I quantified movement patterns and locomotor performance to elucidate how variation in thermal tolerance, behaviour and body size affects organisms' interaction with their bio-physical environment.

First, with my data collection, processing and data analysis pipeline, I aimed to provide a general framework for activity monitoring of small animals and an automated way of interpreting fine-scale biologger data and classifying coarse behaviours (movement vs. immobile states) from raw accelerometer data. Subsequently, I used these data to describe and evaluate fine-scale activity and heat-budgets for individual lizards and to explore how – and if – ambient conditions and individual variation in body size affect their movement patterns.

The second objective of this project was to assess how an ectotherm's body temperature and body size, and the environmental conditions of its surroundings can affect whole-organism performance (acceleration). For this, I first developed mechanistic niche models to predict the exact body temperatures of each lizard at any given moment. Then, I used thermal performance curves (TPCs) to map animal performance onto the predicted body temperatures of each animal, which provided a model of the impacts that temperature variability has on an organism's physiology. Based on the parameters provided by TPCs, I tested several hypotheses. Specifically, I expected that: (i) body size affects performance, with larger individuals reaching higher acceleration values than smaller individuals, (ii) individuals with higher optimum body temperatures would also display higher maximum performance ('hotter is better' hypothesis) and, (iii) an intra-population 'specialist-generalist' trade-off would be present, with animals that are able to perform over a broad range of temperatures reaching lower maximum performances.

Altogether, I aimed to showcase how cutting-edge tools such as biologging and mechanistic modelling can be merged to get insights into fine-scale behavioural and physiological aspects of ectotherms. This in turn is essential for accurate forecasting of the impacts of climate change and, as such, I believe that this work will serve as inspiration for other researchers in the field to adopt these methodologies.

2. Materials and methods

2.1. Study species and sampling process

The ocellated lizard, *Timon lepidus* (Daudin, 1802), is one of the largest green lizard species belonging to the Lacertidae family and one of the biggest European lizards, with snout-to-vent length up to 24 cm and total length up to 70 cm (Arnold et al., 2007). It can be found in most of the Iberian Peninsula, on the southern coasts of France and in the extreme northwest of Italy. Males are larger than females with a massive head. They are active between March and October, or even for longer periods in the warmest parts of their range, while mating takes place during April and May (Speybroeck et al., 2016).

The species can be found in open, dry areas, often close to cultivated lands, from sea level and up to 2.500 m ASL. They are territorial and they prefer areas which offer refuges, like large rocks with holes and crevices (Pleguezuelos et al., 2009; Speybroeck et al., 2016). They have very specific habitat requirements and since they do not dig their own holes, they depend on the pre-existence of appropriate microhabitats that provide shelter from predators, but also from the thermal and hydric fluctuations of their environment (Grillet et al., 2010). Moreover, their large body size requires equally large refuges to accommodate them. Other aspects of the ocellated lizard's biology restrict even more its suitable habitats. Their prey consists mostly of insects usually found in open grasslands, areas which typically do not have enough rocks to act as shelters for such big animals (Castilla et al., 1991). *Timon lepidus* individuals can usually be found basking for considerable amounts of time in the area around their shelter using vegetation as a cover, while they are known to flee from a great distance when approached during their activity times (Arnold et al., 2007; Speybroeck et al., 2016).

Timon lepidus is classified as a Near-Threatened species (Pleguezuelos et al., 2009), with its populations declining across its distribution range. Its decline is caused mainly by human-related factors, like habitat loss, pesticides responsible for decreased prey availability and poisoning. Although many local populations are strongly declining, there are records of populations with high densities of up to 50 individuals per hectare (Pleguezuelos et al., 2009). One such population can be found close to the beach of Castro de São Paio (Labruge, Vila do Conde), where the sampling of *T. lepidus* individuals for the needs of the present study took place. This area has been extensively monitored in the past years and constitutes a convenient location for the sampling required for this project.

Castro de São Paio is a coastal area of approximately 5 ha, located in Northern Portugal

(41° 16’ 54” N; 08° 43’ 50” W). The available habitat types are mainly agricultural fields, grasslands and shrublands (consisting mainly of *Ulex*, *Erica* and *Carpobrotus* species). Trees and higher vegetation are scarce. The landscape is completed by low stone walls, used to separate the farming properties and create walking paths. The structure of these walls, made of sparsely placed stones, creates spacious gaps between them, which are ideal for the large lizards to use as shelter. Animal collection occurred in May and June, a period when ocellated lizards are mating and thus spend more time active (Grillet et al., 2010; Hódar et al., 1996).

This master thesis project belongs to a more extensive project, funded by an FCT project grant and encompassing the PhD thesis of Carolina Reyes-Puig (CRP), which aims to analyse how different phenotypic traits affect the potential of green lizards to cope with past and future environmental change. For the purposes of this general project, CRP conducted the sampling of the animals used also for this master thesis. During the months of May and June, CRP sampled 26 *T. lepidus* individuals of ranging body sizes in groups of four, using nooses attached to fishing rods. Only adult males were sampled to exclude any biases due to ontogeny, sexual dimorphism and gravidity. Every week, the four captured males were taken to the laboratory of CIBIO/InBIO, a research centre of the University of Porto, Portugal, where they were subjected to different ecophysiological experiments (thermal gradients to quantify selected temperature, experiments to measure water loss, and simulated escape runs to quantify locomotion). During this time, all the animals were also weighed and their morphological attributes measured. These experiments were done within the frame of the wider project and some of the data acquired were later used for the analyses of this project.

Every week, the four males spent a week in the lab and, subsequently, I was transporting them to the mesocosm enclosures in CICGE of the Faculty of Sciences of the University of Porto, where the experiment of this thesis took place. Every week, new animals were sampled by CRP and, while the previous animals were in the mesocosms, they were subjected to laboratory experiments before entering the mesocosms as well. This cycle was repeated for seven weeks, for all 26 animals to spend one week in the lab and, afterwards, one week in mesocosms. After these two weeks, animals were released to the field.

All the processes of collection, management, and experimentation with green lizards were carried out with the collection and research permits LICENÇA Nº 346 / 2022 / CAPT and LICENÇA Nº 346 / 2022 / CAPT obtained from the Instituto da Conservação da Natureza e Florestas. In addition, the Órgão Responsável pelo Bem-Estar dos Animais

(ORBEA) was informed about the practices and details of the project.

2.2. Mesocosm setting

The space where the experiment took place is found in the facilities of CICGE - Research Centre on Geo-Spatial Science in the Astronomic Observatory Professor Manuel de Barros of the Faculty of Science of the University of Porto (Vila Nova de Gaia, Portugal). There, a 20×20m open area can be found, reserved for this kind of experiment, and originally designated by my supervisor NS, which, when enclosed, is suitable for creating the necessary mesocosms. To prepare the mesocosms, the area had to be divided into four individual enclosures of 10×10m each, with barriers that would stop any attempts of escaping from the lizards (Figure 3*a*).

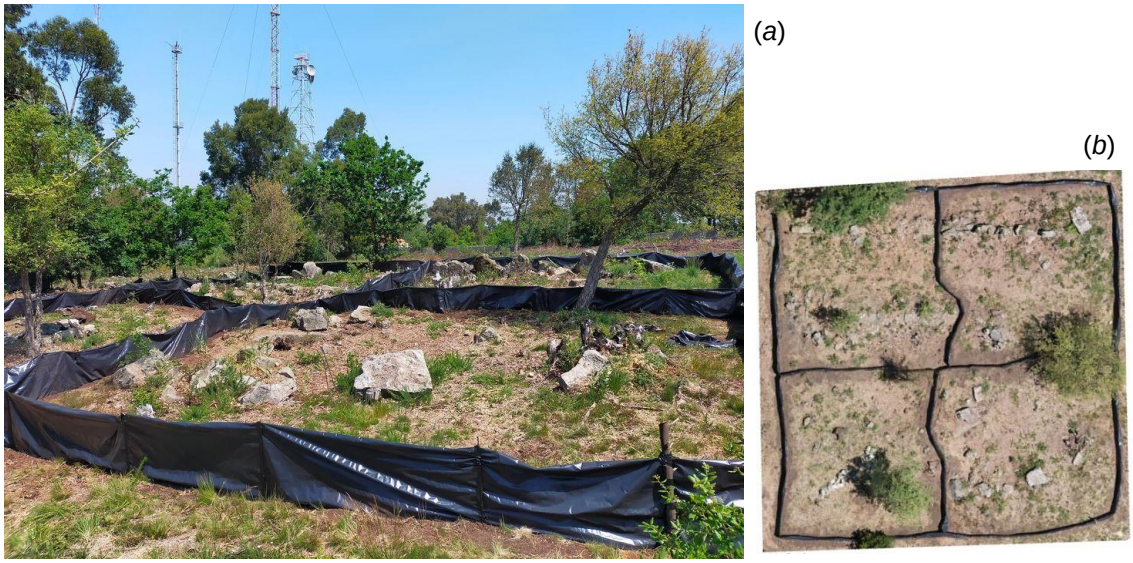


Figure 3. The four mesocosms; (*a*) after their construction, in the beginning of May and (*b*) their aspect from above, through the orthophoto map created by the drone.

I started the process by cleaning the mesocosm space of any dry and dead matter to create an environment as friendly to the lizards as possible and imitating their natural habitat. The natural vegetation of the mesocosm (mostly shrubs and very few trees) was left as it was. Subsequently, I moved some big rocks and stones inside the mesocosms from the area around it, trying to create similar microhabitats and shelters in all four mesocosms. Although it was impossible to create four identical mesocosms, I attempted to construct a similar composition of all the different microhabitats (rock, bushes, open ground) in each of them.

The next step was the creation of the mesocosms themselves, meaning the creation of

the barriers separating them. For this, I used black plastic for greenhouses to create a wall around the whole area and between the four replicate mesocosms. First, I dug a small ditch (of around 10 cm) around the entire area and in a cross, divided the 20x20m space into four 10x10m squares. Inside this ditch, I buried metal sticks (1 m high) in the ground, enough so that they were stable. In total, 80 metal sticks were used for the entire area, placed at 1 m from each other. I then attached the plastic (2 m high) to the sticks with duct tape and folded it in two, so it is more resistant, I inserted the part of the plastic that was in the inside part of the mesocosm in the trench and buried it with soil. This way, it was ensured that there would be no way for the animals to escape under the plastic barrier.

Before the start of the main experiment, one day before the first group of lizards entered the mesocosms, I placed six temperature and relative humidity dataloggers (iButton® DS1923) in or around the mesocosm area, to register the microclimatic conditions available in the mesocosm landscape throughout the course of the experiment. The dataloggers were placed on the surface of different microhabitats (soil and rock) and under different conditions (sun and shadow), while there was also a completely exposed datalogger, recording the conditions of the air, and another one buried, recording the conditions in the soil.

Finally, my supervisor NS produced a high-scale orthophoto map of the mesocosm, which was captured by a drone (DJI Mavic Air 2) and generated with the Agisoft Metashape software (Figure 3*b*). This fine-scale map also contains information about the relative height of the mesocosm microhabitats (digital surface model).

2.3. Data collection

The main mesocosm experiment started with the release of the first group of four lizards coming from the lab. Before their release, I colour-coded the animals with non-toxic dye to distinguish them in case they would trespass across mesocosms.

Before releasing them in the mesocosms, I also equipped each lizard with an external logging device (Axy-Trek; https://www.technosmart.eu/axy-trek-mini/) attached to the base of their tail. The loggers of each animal were activated before being attached to them and contained sensors that record acceleration, temperature, relative humidity and light intensity in the immediate environment of each lizard. The accelerometer of the device was set to record tri-axial acceleration (8-bit resolution, recording range -4 g to +4 g) at a frequency of 10 Hz (s^{-1} in SI units), while the rest of the sensors were recording

at 1 Hz, providing very fine-scale temporal data for each lizard.

Every new week of the experiment, I took the loggers from the previous four animals, downloaded the collected data, recharged their batteries and mounted the loggers to the next four lizards. I attached the logger as a backpack around the hind legs of the animals (Figure 4). The material of the string I used was very soft and stretchy and thus it did not cause trauma to the animals if not attached too tightly. In this manner, the datalogging devices were firmly attached to the back of the animal in a position where they were not seriously restricting locomotion and limb movement, nor were they affected by it. Furthermore, by placing the dataloggers around the hindlimbs of the animals, where the back height is lower than around the forelimbs, the movement of the animals inside burrows or holes was less limited. The loggers, with a weight of around 4 gr, comprised 4-8% of each individual's body mass, which is considered low enough to avoid greatly affecting the normal behaviour of animals (Hawkins, 2004). After the release of the lizards, I placed a fifth logger in an open area next to the mesocosm, which recorded the light conditions of open sky. This was necessary as a reference for the light recordings of the loggers that were attached to the animals in order to get the relative light (percentage) that each animal received at each moment.



Figure 4. The attachment style of the animal logger on the lizards. Two thin elastic silicon strings were wrapped around the front and hind part of the logger. Subsequently, the strings were tied around the front and the back part of the animal's hind legs, stabilizing the logger on the back of the animal. Apart from the string, double-faced stickers were also used to further stabilize the dataloggers on the back of the animals. The stickers usually remained securely attached throughout the entire experiment (1 week) and it was easy to remove them without causing any harm to the animal's skin.

After I released the lizards in their respective mesocosms (Thursday or Friday), I left them to acclimatize to the new conditions for 2-3 days. The logger recordings from these days of acclimation were not considered in the analyses. Then, from Monday until Thursday of each week, I performed sporadic (3-5 times per day) observations of their location

inside the mesocosm, with binoculars and from a distance, so that the animals were left undisturbed to continue with their normal behaviour. I recorded the position of each lizard, when I successfully spotted them, directly over the orthophoto of the mesocosm using QField application in a Getac Z710 field tablet. Eventually, I obtained three and a half days of data recording and observations for each animal. I downloaded the data from the animal loggers onto a PC using the software X-Manager 1.8.3 (Axy, Technosmart, Italy).

This process was repeated for seven weeks and it provided data for 26 animals in total. In this way, it was ensured that in the end, I would have data from enough lizards ranging in body size to proceed with data analyses, while also avoiding extending the sampling period for too long, which would cause major fluctuations in the climatic conditions.

The sunrise and sunset times did not differ substantially from the beginning until the end of the experiment (May 23: sunrise at 06:13 and sunset at 20:48; June 30: sunrise at 06:15 and sunset at 21:05).

2.4. Data analyses

By the end of the mesocosm experiment (seven weeks and ~3.5 days of recording for each individual), the datasets that were considered fit for further analyses corresponded only to 15 animals (Table 1); the rest were subject to unforeseen events during the experimental period and were not further used for the results of this project to avoid any bias (e.g. animals losing their sensors or trespassing into different mesocosms because of initial insufficiencies in the mesocosm structure etc.). Each one of the retrieved datasets, corresponding to one animal, contained data for ambient temperature (°C), relative humidity (%), light intensity (relative value) and acceleration (g) in all three axes (x, y and z). From these raw sensor data, the accelerometer and light sensor data required further processing and transformation (see below), in order to be converted into biologically meaningful values.

Table 1. Body size [Snout-Vent length (SVL), body mass] and logging period (not considering the acclimation period) for the 15 male *Timon lepidus* lizards included in the analyses.

Lizard ID	Logger ID	SVL (mm)	Body mass (gr)	Logging period
TLCRP005	Unit5	131	61.7	23/05 00:00 – 26/05 11:00
TLCRP006	Unit2	147	98.5	23/05 00:00 – 24/05 14:00
TLCRP007	Unit1	148	81.1	23/05 00:00 – 26/05 11:00
TLCRP008	Unit3	144	78.4	23/05 00:00 – 26/05 11:00
TLCRP009	Unit3	150	98.4	30/05 00:00 – 02/06 12:00
TLCRP012	Unit1	131	102.2	30/05 00:00 – 02/06 12:00
TLCRP013	Unit1	147	108.3	06/06 00:00 – 09/06 11:00
TLCRP014	Unit5	148	51.1	06/06 00:00 – 09/06 11:00
TLCRP015	Unit2	144	53.6	06/06 00:00 – 09/06 11:00
TLCRP016	Unit3	150	77.4	06/06 00:00 – 09/06 11:00
TLCRP021	Unit1	158	100.9	20/06 09:00 – 23/06 12:00
TLCRP022	Unit2	158	104.4	20/06 09:00 – 23/06 12:00
TLCRP023	Unit3	144	77.1	20/06 09:00 – 23/06 12:00
TLCRP024	Unit5	122.5	55.1	20/06 09:00 – 23/06 12:00
TLCRP025	Unit5	137	56.4	27/06 00:00 – 30/06 10:00

2.4.1. Data transformation

2.4.1.1. Treatment of acceleration data

To calculate the changes in the velocity of the animals (movement) from the accelerometer-derived data, I calculated the dynamic acceleration by subtracting the static component from the total recorded acceleration of each axis. The static acceleration can be calculated by appropriate smoothing of the acceleration data. The data smoothing method that is generally practised with acceleration data is the calculation of running or rolling means, also known as moving averages. A running mean of a specific length is taken from the raw data of each axis, averaging the data at either side of a mid-point, and represents the static acceleration (Shepard et al., 2008). Usually, the length of the running mean is arbitrarily set to 1 second (Wilson et al., 2006), but in many cases a longer rolling window is opted for, based on the species in question and the type of behaviour to be captured (DeSantis et al., 2020). The length over which the data are smoothed defines the threshold over which the acceleration is attributed to the dynamic component. A too short running mean length can lead to overestimation of the static component, while a too long running mean can have the opposite effect. For animals with bursts of activity that change their posture, the length of the running mean chosen

should not exceed the duration of these behaviours (Shepard et al., 2008). For my data, I decided that the standard 1 second running mean is the most appropriate, since *T. lepidus* is an animal with sudden bursts of movement that happen within very small intervals. After the calculation of the static component, I derived the dynamic body acceleration (DBA) of each axis by subtracting the static acceleration from the raw accelerometer data (Shepard et al., 2008; Wilson et al., 2006).

Subsequently, the dynamic body acceleration of all three axes of movement can be combined to estimate overall body acceleration metrics. Such metrics are the overall dynamic body acceleration (ODBA) (Wilson et al., 2006), which corresponds to the absolute sum of the dynamic body acceleration along the x, y, and z axes:

$$ODBA = |DBA_x| + |DBA_y| + |DBA_z|$$

and the vectorial dynamic body acceleration (VeDBA) (McGregor et al., 2009), the vector of DBA across the three axes:

$$VeDBA = \sqrt{DBA_x^2 + DBA_y^2 + DBA_z^2}\,.$$

From now on, I will use the VeDBA as a measure for overall acceleration. ODBA and VeDBA are strongly correlated to each other and can be used interchangeably as acceleration metrics (Qasem et al., 2012). However, ODBA is more sensitive to the position and the alignment of the datalogger on the animal, something that should not affect a vectorial solution (Gleiss et al., 2011). Apart from the overall acceleration metrics, I also calculated additional 'rolling' summary acceleration statistics using the same running window of 1 s, including metrics of data dispersion (standard deviation), maximum values of acceleration and means (averages).

Having metrics such as VeDBA provides a continuous measure of the lizards' acceleration every tenth of a second. However, it is not enough to classify the animals as mobile or immobile, since VeDBA values are rarely exactly equal to zero. Values close to, but different than zero can still be attributed to immobile phases of the animals, due to slight errors in the calculation of the dynamic component which cannot be avoided. Thus, a classification process is needed to distinguish between 'mobile' and 'immobile' acceleration values. The simplest method to do this would be by establishing movement thresholds that separate different behavioural categories based on observed behaviours. However, in this case, a set of observations is needed, which act as a validation method, taken either by video or by a researcher on the spot (Brown et al., 2013). There are various other classification methods available in the literature, like histogram segregation analysis (Collins et al., 2015), supervised machine learning algorithms that, again,

require a training dataset for the validation of the algorithm (DeSantis et al., 2020), as well as unsupervised machine learning methods (e.g. hidden Markov models; Leos-Barajas et al., 2017), which however are very often needlessly complex (Collins et al., 2015; Patterson et al., 2019).

In the case of the present study, due to the lack of a detailed training dataset of observations, an unsupervised classification method was opted for. These methods usually implement machine learning algorithms that cluster acceleration values with similar characteristics. These clusters can then be assigned to distinct behavioural groups (Brown et al., 2013). Patterson et al. (2019) compared the accuracy of various (supervised and unsupervised) behavioural classification methods and concluded that different approaches had minor effects on classification accuracy, especially for a small number of different behaviours ('mobile' and 'immobile' in the case of this project). Among the unsupervised methods, k-means clustering – the simplest algorithm – has a very small difference in accuracy from the most complex method (hidden Markov models) (Patterson et al., 2019). However, k-means is not fit for large datasets, as is the case for the data of this project. An algorithm that solves this problem is the CLARA (Clustering Large Applications) algorithm. It works by taking random subsamples of the data and clustering the objects of each sample into k subsets. The centre of every subset – or cluster – is its medoid (as opposed to the mean, which is used by k-means). CLARA uses the PAM (Partitioning Around Medoids) algorithm to cluster the data of each sample and the best clustering obtained from the different subsamples is selected by the algorithm (Kaufman & Rousseeuw, 1990).

For a correct clustering of movement modes, the appropriate acceleration metrics need to be chosen, based on how well they appear to distinguish the target behaviours. I ran the CLARA algorithm multiple times for each individual lizard dataset, clustering various acceleration metrics. Mainly four different strategies were followed and compared to decide which gives the best clustering result, or the most appropriate acceleration threshold to distinguish between movement and immobility of the animals. For every attempt, the data of each animal were visualized in a time-series format, which was used to evaluate the result of the clustering.

First, I used various acceleration metrics (VeDBA, ODBA, $DBA_{x,y,x}$, $SD_{DBAx,y,z}$, $max_{DBAx,y,z}$) and the full animal datasets for the clustering. Second, I decided to use three metrics related to the movement of the x-axis (forward movement) which can be better suited to distinguish between the mobile and immobile states: DBA_x, SD_{DBAx} and max_{DBAx}. On a third attempt, I used again all the calculated acceleration metrics, but only the data from

night-time recordings, since during the night *T. lepidus* are mostly immobile and there are fewer behavioural modes to be recognized by the algorithm. On the contrary, during the day, the acceleration values from the animal loggers can reflect a variety of different behaviours and the number of the possible clusters to be defined can be equally variable. Finally, I used only the three metrics related to the movement on the x-axis for the night-time recordings of the animals and this was the strategy eventually used. The CLARA algorithm was run for each animal dataset using the 'cluster' package in R. The clustering was performed with Manhattan distances and for k = 2 clusters (meant to represent the 'mobile' and 'immobile' categories).

After the clustering, every observation (tenth of a second) inside each dataset was classified as a moment of movement or immobility. For each dataset, the maximum acceleration value (VeDBA) of the first cluster (representing the 'immobile' moments) was calculated as the activity threshold for this animal, below which the acceleration values signify an immobile state, and over which they imply movement. To be able to compare results between different individuals and to limit errors which could be attributed to the clustering process for each individual dataset, the same movement threshold was used for the datasets of all the studied animals. The best universal threshold was considered to be the median of the thresholds calculated for each dataset.

2.4.1.2. Light data transformation

The analogue sensor recording light intensity contained in the datalogging device of each animal provides an output that is not the real value of light (in lux), but rather the result of a comparison. Therefore, some calculation was needed to extract the real light intensity. The formula that was used for the transformation and was applied to the light data from all the datasets was provided by the sensors' manufacturer in Technosmart, C. Catoni, and is the following:

$$light = \sqrt{10^{9.8-2\cdot\left(\frac{51000\cdot RawSensor}{1023\cdot RawSensor}\right)}},$$

where *light* is the final value of light intensity (relative value, no unit) and *RawSensor* is the value recorded by the light sensor of the logger. From now on, as a measure of light intensity, I will use the percentage of light that each animal's sensor was receiving from the total light of the sky at any given moment, calibrated with the data from the sensor I had placed in the open sky (individual animal light sensor/open sky reference light sensor x 100).

2.4.2. Mechanistic (biophysical) modelling

I used the data collected by the animal loggers in the mesocosms to develop mechanistic niche models for each individual animal. The mechanistic models were developed with the package 'NicheMapR', an R implementation of the Niche Mapper biophysical models (Porter et al., 1973), using two of its components: the microclimate and the ectotherm model (Kearney & Porter, 2020).

2.4.2.1. Microclimate model

To model the microclimatic conditions in the mesocosm landscape, I used the microclimate model of NicheMapR, as implemented in the micro_ncep function (Kearney et al., 2019). The model uses interpolated macroclimatic data from weather stations, including solar radiation, air temperature, relative humidity, wind speed, air pressure and rainfall, which, in the case of micro_ncep, are available from the National Centers for Environmental Prediction (NCEP) reanalysis dataset (Kemp et al., 2012). To account for local topographic and vegetation effects, the 'microclima' package was used in a complementary manner to the NicheMapR microclimate model. The integration of this package in the estimation of the microclimates serves to correct for terrain-specific effects that drive microclimatic variation (Kearney et al., 2019). The model was run for the specific geographical coordinates where the mesocosms were located and generated an output of the above and below-ground microclimatic conditions in the mesocosm area for the period in which the experiment took place (20/05/2022 – 30/06/2022). These include hourly changes in surface and soil temperature, humidity, radiation and wind speed estimations for two extremes of shade.

Subsequently, I validated the output of the microclimate model using the logged temperature and relative humidity data from the six dataloggers that had been placed in the mesocosm area before the start of the experiment, as explained above. To compute the deviation between the observed conditions of the mesocosm and those predicted by the model I used the root mean square error (RMSE). The parameters of the microclimate model were changed until the results of the model better approached the observed data and the parameter combination for which the prediction had the lowest RMSE was selected. More details on the microclimate modelling and model validation can be found in the accompanying github repository (https://github.com/katsioump/timon_project).

2.4.2.2. Ectotherm model

The outputs of the microclimate model were then used as inputs for the ectotherm model. The hourly estimates of the microclimate model were first interpolated for every second, which is the frequency of the environmental recordings of the animal loggers. Thus, I could obtain models for each second, for every animal.

Regarding the ectotherm (or animal) model, I used the transient-state heat-budget model of NicheMapR. This model uses the output of the microclimate model as forcing variables and a set of animal parameters to solve a heat balance equation (details can be found in Kearney et al. (2021)). By solving this equation, the model returns the animal's transient-state body temperatures (T_b) for every time step (for every second in the case of this project). I ran the model assuming no metabolic heat generation and the 'one lump' scenario was chosen, which assumes that the whole body of the animal is at a uniform temperature at any moment (Kearney et al., 2021). This assumption is reasonable for small ectotherms (Porter et al., 1973). The one-lump algorithm was implemented (Kearney & Porter, 2020), which works by obtaining a solution for the heat-budget model using an ordinary differential equation solver. For this numerical solution, the R package 'DeSolve' (Soetaert et al., 2010) is used by the onelump_var function of NicheMapR. In short, the one-lump algorithm models the rate of change of body temperature of a hypothetical object with convective and radiative heat exchange, considering the defined parameters and forcing variables (Kearney et al., 2021).

Before running each individual model, I defined the animal parameters, such as animal weight, specific heat capacity, animal density etc. (see Table 2). Then, the required forcing data for the function were provided (Table 3). These data came mostly from the microclimate model, including wind speed, zenith angle, radiative temperature and solar radiation. The last two were corrected for the shade that the animal was experiencing at any given moment, using the relative light recordings from the animal's sensor. Lastly, the forcing variable accounting for air temperature was not taken from the microclimate model output but rather from the temperature recordings of the animal's logger. More details can be found on github (https://github.com/katsioump/timon_project/blob/main/analyses/3_onelump_var.R).

Table 2. Animal parameters used as input for the heat-budget mechanistic model, describing the modelled lizards as open thermodynamic systems. These parameters were implemented by the onelump_var function of the NicheMapR package to predict lizard body temperatures.
* The values of body mass were changed according to the lizard modelled.

Parameter	Value	Unit
Body mass	*	*gr*
Specific heat of flesh	3762	*J/kg*
Animal density	1000	kg/m^3
Metabolic rate	0	W/m^3
Thermal conductivity of flesh	0.5	*W/(m·K)*
Emissivity of skin	0.95	-
Animal solar absorptivity	0.9	-
Solar configuration factor	0.4	-

Table 3. Forcing variables (functions of time) used as input for the heat-budget mechanistic model through the one-lump_var function of NicheMapR, together with the animal specific parameters, to solve the differential equation that predicts animal body temperatures.
* The noted variables are not directly used by the differential equation, but are used to estimate radiation temperature, which is a forcing variable for the function.

Term	Variable Definition	Origin
Q_{sol}	Solar radiation	Microclimate model + animal's light sensor (calibration for shadow %)
T_{sky}*	Sky temperature	Microclimate model + animal's light sensor (calibration for shadow %)
T_{soil}*	Soil temperature	Microclimate model + animal's light sensor (calibration for shadow %)
T_{rad}	Radiation temperature	(T_{sky} + T_{soil}) / 2
Vel	Wind speed	Microclimate model
T_{air}	Air temperature	Animal's temperature sensor
Zen	Zenith angle	Microclimate model

2.4.3. Integration of acceleration metrics and MNM output to fit thermal performance curves (TPCs)

To understand the ecological consequences of body temperature on whole-organism performance and to study the thermal sensitivity of *T. lepidus* locomotion, I used thermal performance curves (TPCs). To fit the TPCs, acceleration values (VeDBA) were plotted

against body temperatures (as calculated with mechanistic models) and a thermal performance curve was fitted for each animal. I used a TPC pipeline that consists of the 'rTPC' and 'nls.multstart' R packages, which gives the possibility to fit the data to a number of TPC models using nonlinear least-squares regression (NLLS) (Padfield et al., 2021).

Initially, the data needed to be stored in a long format, where each row contains one measurement of T_b and VeDBA per second, per animal. The body temperatures were then split into bins of 0.2°C and, to reduce the noise and fit the curves with the values most relevant for animal movement, I only kept the rows with the five maximum VeDBA values from each bin. The rTPC package contains 24 mathematical TPC models and gives the possibility to fit multiple model candidates at once and to select the best fit model afterwards. Seven different models (see Table S1) were considered, based on their ecological applicability to data of this kind (see Angilletta, 2006) and their implementation in similar studies (Kontopoulos et al., 2018; Niehaus et al., 2012; Rezende & Bozinovic, 2019). For every model, the corrected Akaike's information criterion (AICc) was calculated and the best-fit model for every animal was determined based on the lowest AICc score. The most common best-fit model across the different animal datasets was chosen to fit the TPCs of all the animals.

After fitting the model to the data of each individual, key TPC parameters (thermal traits) were extracted, like optimum temperature (T_{opt}), maximum performance (r_{max}) and thermal breadth. The thermal breadth was calculated as the range where performance is at least 50% of r_{max} and it was considered to represent the range of body temperatures the animal would try to maintain in the field, since they maximize movement potential (e.g. for foraging, socializing etc.).

2.4.4. Hypotheses testing

Apart from the thermal traits calculated through the TPCs, I also estimated lizard's preferred body temperatures (T_{pref}) while mobile and immobile ($T_{pref,mob}$ and $T_{pref,imm}$ respectively), as the body temperatures where the lizards spent the majority of their time in either state. A range of most preferred body temperatures for mobility and immobility was also calculated for each animal, based on an arbitrary density threshold (minimum preferred T_b while immobile, $T_{pref,min,imm}$; maximum preferred T_b while immobile, $T_{pref,max,imm}$; minimum preferred T_b while mobile, $T_{pref,min,mob}$; maximum preferred T_b while mobile, $T_{pref,max,mob}$). Finally, instead of using critical thermal minima and maxima to describe the thermal limits of the lizards, for every animal I used the lowest T_b where movement was

recorded as its voluntary thermal minimum (T_b where the animal emerges from its shelter, T_{emerge}), and the highest T_b where movement was recorded as its voluntary thermal maximum (T_b at retreat, $T_{retreat}$). Contrary to critical thermal limits, which can have deleterious effects on organisms, sub-critical or voluntary thermal limits describe the behavioural thermal tolerance of animals and can provide a more subtle understanding of the animal's interaction with its environment (Braschler et al., 2021; Camacho et al., 2018).

To study the relationship between body size and lizard thermal ecology, all estimated preferred thermal parameters were regressed on SVL and body mass. Additionally, I performed pairwise Pearson correlation tests between all TPC thermal traits (T_{opt}, r_{max}, breadth) and both SVL and body mass. To understand whether body size affects the ability to reach maximum performance without overheating, or how close an animal's optimum temperature is to its voluntary thermal limit, I performed a correlation test between body size (SVL and body mass) and the difference between an animal's retreat temperature and its optimum temperature ($T_{retreat} - T_{opt}$).

Finally, to infer potential evolutionary trade-offs, I performed pairwise Pearson correlation tests between the lizards' thermal traits: between T_{opt} and r_{max} ('hotter-is-better' hypothesis) and between r_{max} and thermal breadth ('specialist-generalist' hypothesis).

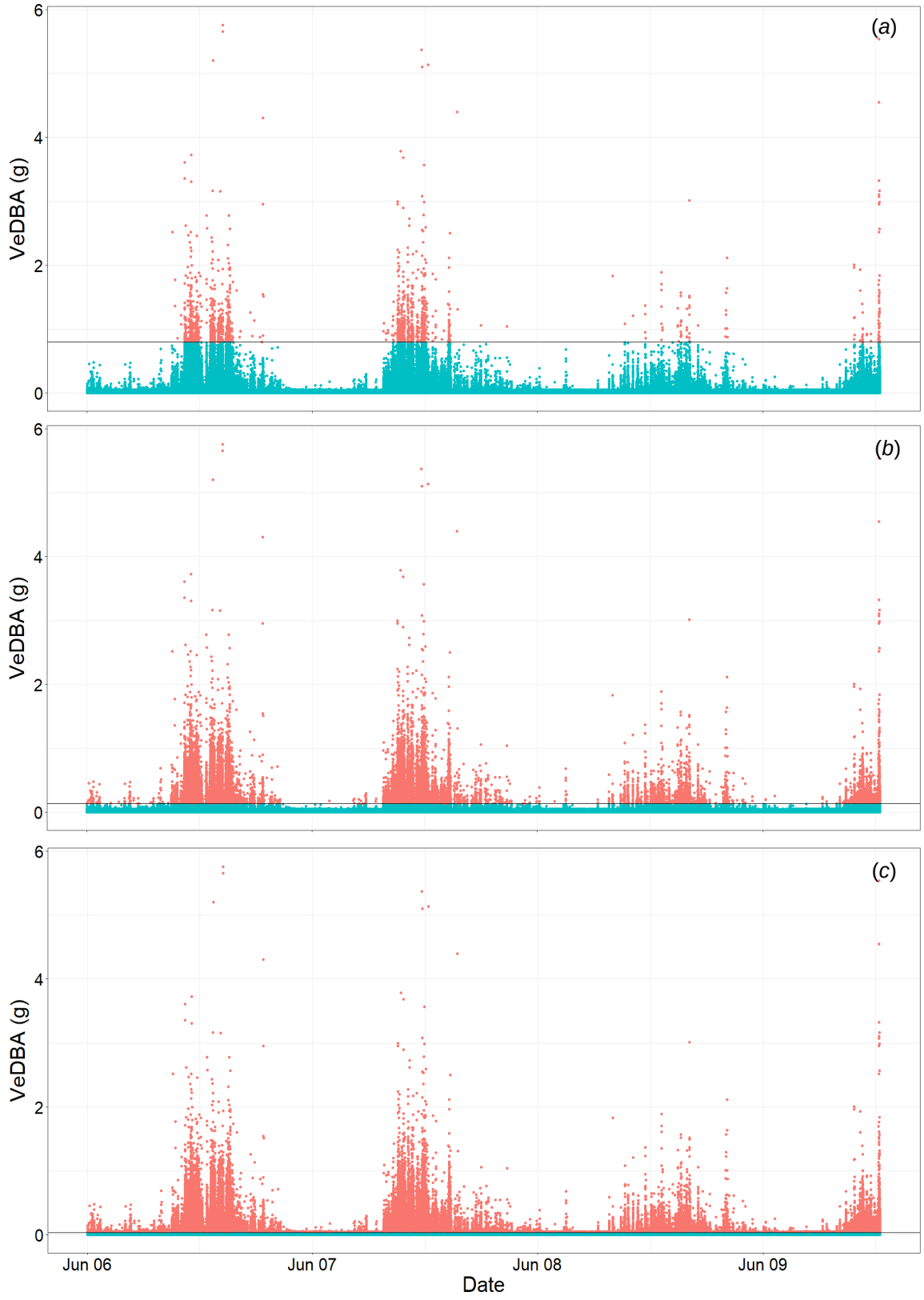
3. Results

3.1. Analysis of animal acceleration data

3.1.1. Movement classification framework

In search of the best metrics and data for the clustering process, I ran the CLARA algorithm using four different strategies. The first and most general strategy, using all calculated acceleration metrics and the full animal datasets to create the appropriate partitioning of the data into two clusters, gave VeDBA threshold values that varied between individuals (*SD* = 0.3; see Table S2) and in some cases the value of the movement threshold was clearly too high to correspond to immobile animals (Figure 5*a*). The second strategy, this time using only the acceleration metrics regarding the x-axis of acceleration, provided clusters with less variable threshold values (*SD* = 0.02), but still generally too high to be considered close to the actual movement threshold (Figure 5*b*). The third clustering strategy, using only nighttime recordings, but again all the acceleration metrics together, gave threshold values that were closer to those expected from observing the nighttime recordings in Figure 5*c*, which are known with certainty to represent immobile animals. The threshold values from this clustering were not very variable between individuals (*SD* = 0.02), but now some of these values seemed to be even too low, classifying clearly immobile phases as movement (Figure 5*c*). So, finally, I tried a fourth strategy, using only acceleration metrics of the x-axis for nighttime recordings (Figure 5*d*). The variability of the produced threshold values based on this clustering of the data was the smallest (*SD* = 0.019) and most of these values seemed to perfectly fit the acceleration values during the mostly immobile night periods. Thus, this method was chosen as the most appropriate for the clustering process.

The resulting clusters produced were separated by a different threshold value of acceleration for each animal dataset, ranging from VeDBA = 0.04 to 1 g (Table 4). The threshold that was subsequently used to standardize the procedure across the different animals was their median, VeDBA = 0.051 g (Figure 5*e*), considering that the distribution of the threshold values was skewed to the right.


(a)
(b)
(c)
VeDBA (g)
0
2
4
6
Jun 06
Jun 07
Jun 08
Jun 09
Date

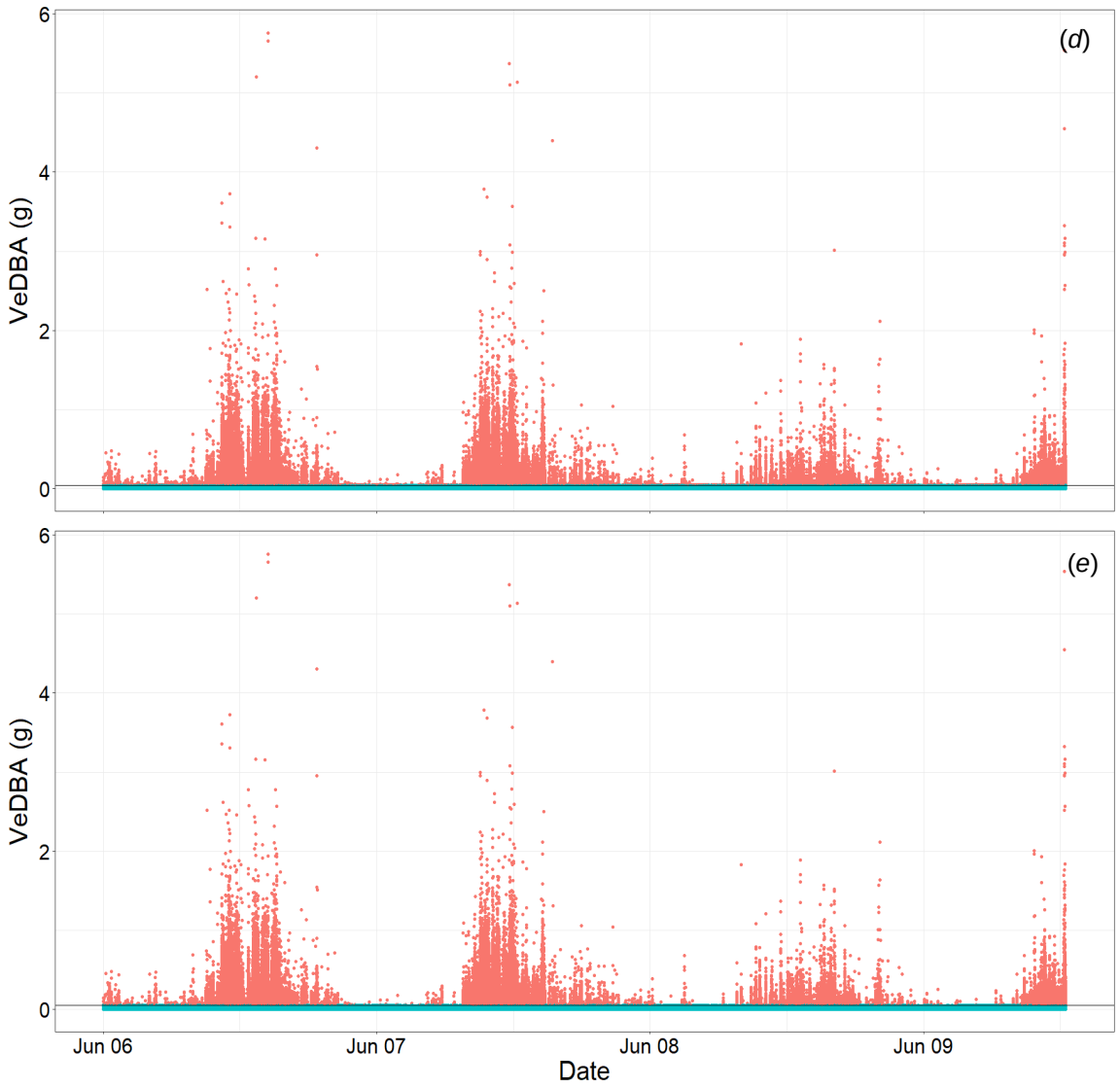


Figure 5. Data from one animal dataset (lizard ID: TLCRP014) showcasing the four different strategies followed for the CLARA clustering analysis, towards the selection of the most appropriate method to establish a threshold acceleration value for movement vs. immobility. The time-series plots correspond to the full duration of the lizard's stay in the mesocosm and depict the animal's movement pattern, as quantified by its acceleration (VeDBA, Vectorial Dynamic Body Acceleration) throughout these 3.5 days. The horizontal line represents the acceleration threshold that was calculated from the clustering procedure for each strategy; (*a*) the clustering algorithm used all the calculated acceleration metrics for the full animal dataset, (*b*) only the x-axis acceleration metrics were used for the full animal dataset, (*c*) all the acceleration metrics were used, but only for the data of the night periods (20:30 – 06:30), when the animal was mostly immobile, (*d*) only the x-axis acceleration metrics of the night periods were clustered, and (*e*) the final generalized threshold, calculated as the median of all threshold values that were estimated through the fourth clustering strategy.

3.1.2. Description of habitat preferences and fine-scale movement patterns

In total, the 15 individuals that were considered in the analyses were followed for ~3.5 days (except for individual TLCRP006, which lost its logger during the second day of observations). During this time, each animal's logger recorded ~3 million observations for acceleration, as well as ~300.000 observations for temperature, humidity and light intensity.

A first exploration and visualization of the raw data illustrated the tendencies of lizard movement across different environmental conditions. The general pattern for all the animals was an increase of activity as ambient temperatures rose until movement reached a peak, usually between 35 to 40°C, after which very few movements were recorded (see example in Figure 6). The opposite tendency was noticed for humidity, with movement reaching a maximum in low percentages of environmental humidity (20-40%).

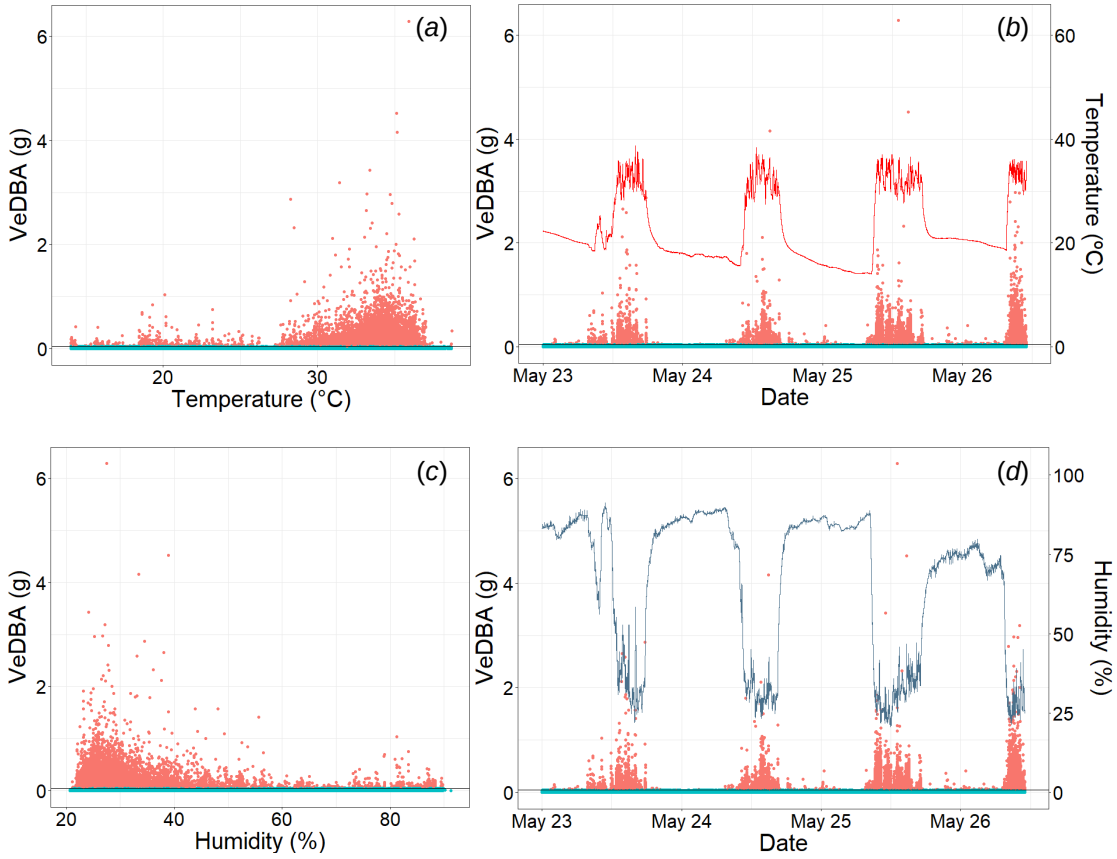


Figure 6. Occurrence of movement, quantified as the vectorial dynamic body acceleration (VeDBA), of a specific lizard (TLCRP005), as associated with different environmental conditions; (*a*) movement intensity along environmental temperatures, (*b*) time series plot depicting the acceleration of the animal on the left y-axis and the environmental temperatures it was experiencing (red line) during its stay in the mesocosm on the right y-axis, (*c*) movement intensity at different percentages of humidity, and (*d*) time series plot depicting the acceleration of the animal on the left y-axis and the humidity of its environment (blue line) on the right y-axis. The pink points of the VeDBA values represent observations of movement, while the blue points indicate immobility. The horizontal line indicates the chosen movement threshold.

The tendency for humidity data was less prominent than that observed for ambient temperature, while for most animals the humidity sensor data were saturated in very high percentages of humidity and the sensor's data were considered unreliable. For this, humidity was not considered in further analyses other than its visual association with the movement patterns of the animals (for the data of all animals see Figure S1). A more thorough and quantitative description of the influence of ambient temperature on lizard body temperature and locomotor performance is presented further on.

Table 4. Movement thresholds (vectorial dynamic body acceleration, VeDBA) as estimated by the CLARA clustering algorithm for the dataset of each animal, below which thresholds acceleration values signify immobility and over which they signify movement. The median of the threshold values is highlighted in bold, and it was used as a generalized movement threshold for all the animals. Based on this last threshold, the total proportion of time spent moving during their 3.5 days in the mesocosm was calculated for all the animals, as well as the total recorded movement in minutes.
* The minutes of recorded movement for the lizard TLCRP006 were not included in the summary statistics because this animal spent only 2 days in the mesocosm.

Lizard ID	Movement threshold (g)	Proportion of time moving (%)	Recorded movement (minutes)
TLCRP005	0.094	2.54	124
TLCRP006	0.050	1.75	40*
TLCRP007	0.043	2.28	112
TLCRP008	0.050	2.31	116
TLCRP009	0.049	1.43	71
TLCRP012	0.074	1.46	73
TLCRP013	0.051	2.08	104
TLCRP014	0.045	2.44	120
TLCRP015	0.051	1.86	90
TLCRP016	0.049	3.04	153
TLCRP021	0.066	2.50	126
TLCRP022	0.085	2.03	98
TLCRP023	0.095	1.99	105
TLCRP024	0.102	0.99	45
TLCRP025	0.071	2.31	113
Mean; median	0.065; **0.051**	2.06; 2.08	103; 108

VeDBA values of acceleration were classified as conveying movement or immobility based on the decided movement threshold. The overall proportion of time classified as movement per individual ranged from 0.99 to 3% (mean ± *SD*: 2 ± 0.5%), which, translated into minutes, ranged from 45 to 153 minutes of movement (mean ± *SD*: 103 ± 26 minutes) for the whole 3.5 days period of each animal in the mesocosm (Table 4). No significant correlation was found between the percentage of time the lizards spent moving and their body size (body mass: r = -0.11, P = 0.67; SVL: r = 0.44, P = 0.09).

Total average movement per hour (in minutes) for all the individuals showed that *T. lepidus* is mostly diurnal, with very few movements recorded during night hours (20:00 – 06:00) (Figure 7). In some animals, the movement pattern displayed a tendency towards a bimodal distribution, with average movement decreasing during the warmest hours in the middle of the day (see for example in Figure 7; TLCRP007, TLCRP014, TLCRP015). In these animals, the highest number of movements during the morning started occurring at around 10:00 and after midday a secondary peak of activity seemed to happen after 14:00. However, there was a great inter-individual variability in the bimodality of movement, with some animals even displaying a peak of activity during the warmer midday hours (Figure 7; TLCRP013, TLCRP025).

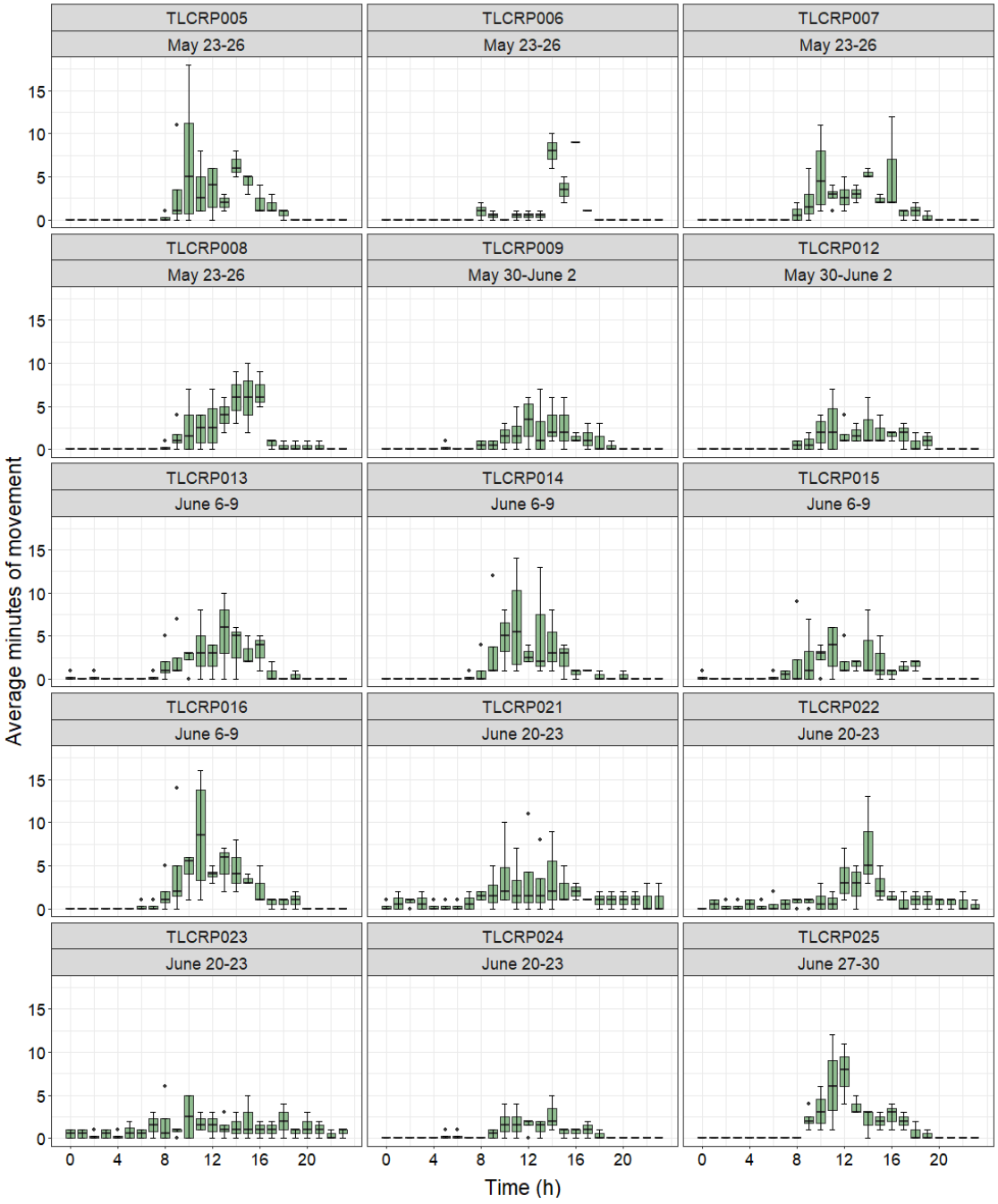


Figure 7. Boxplots showing the average (± s.e.) minutes of movement per hour of the day for the 15 *Timon lepidus* lizards during their stay in the mesocosms. The black dots represent outlier values and the black line of each box the median of the minutes moving.

3.2. Mechanistic modelling

3.2.1. Microclimate model validation

The difference between the microclimatic conditions as predicted by the microclimate model and as observed by the logged data from the mesocosm dataloggers was measured by their root-mean-square error (RMSE), which served as a metric for the evaluation of the microclimate model. After improved parameterization, the parameter combination for which the prediction had the lowest RMSE was selected. For example, with the improved parameters, the RMSE of the predicted rock temperature (temperature at the animal's location) in full sunlight was 6.3°C, while with the standard parameters it was 12.5°C (see Figure 8*a*). For the whole results of the microclimate model validation, see Figures S2-S5.

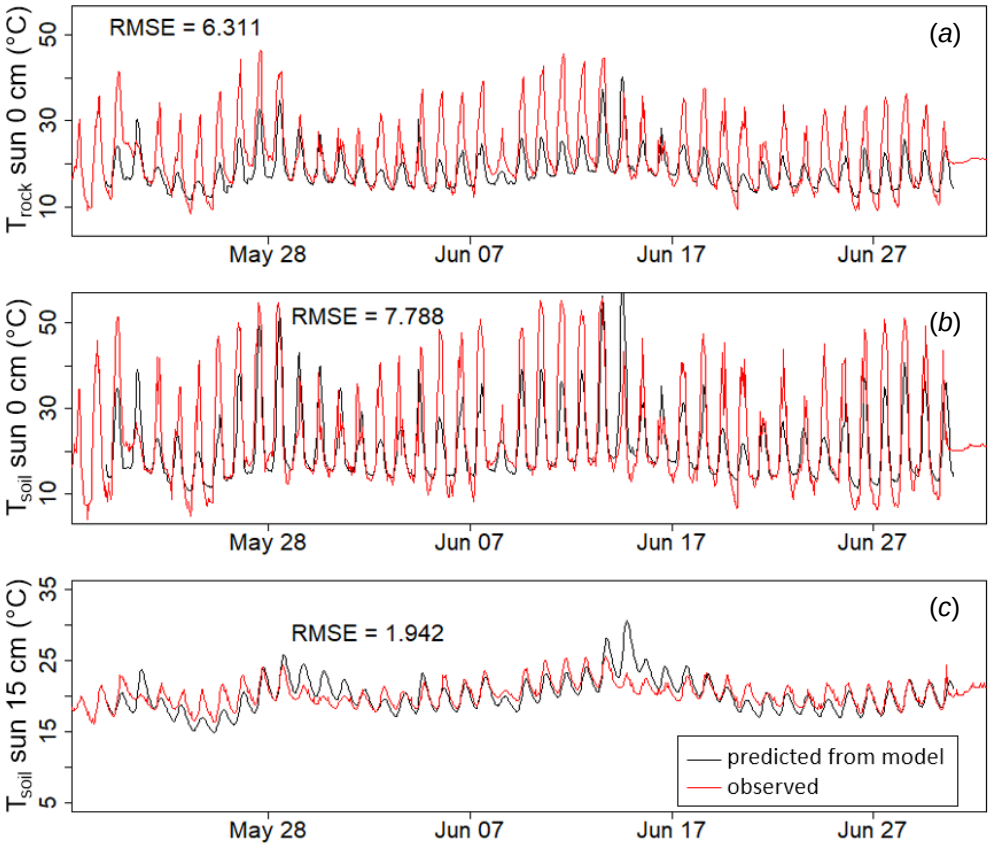


Figure 8. Comparison between the temperatures at full sun in different microhabitats of the mesocosm landscape, as predicted by the microclimate model after improved parametrization, and as observed by the dataloggers recording temperature and humidity in the same microhabitats; (*a*) surface rock temperatures, (*b*) surface soil temperatures, and (*c*) soil temperatures at 15 cm of depth. The root-mean-square error (RMSE) of each prediction shows the average distance between the prediction of the model and the recorded values.

3.2.2. Body temperature

Predicted body temperatures for every animal via the transient-state model showed that, regardless of the sudden fluctuations of environmental temperatures (as recorded by the logger on their back), T_b changed gradually. T_b as predicted by the model also seemed to depend on the exposure of the animal to solar radiation (i.e. sunlight), with mismatches between air temperature and T_b of up to 15ºC (Figure 9*a*).

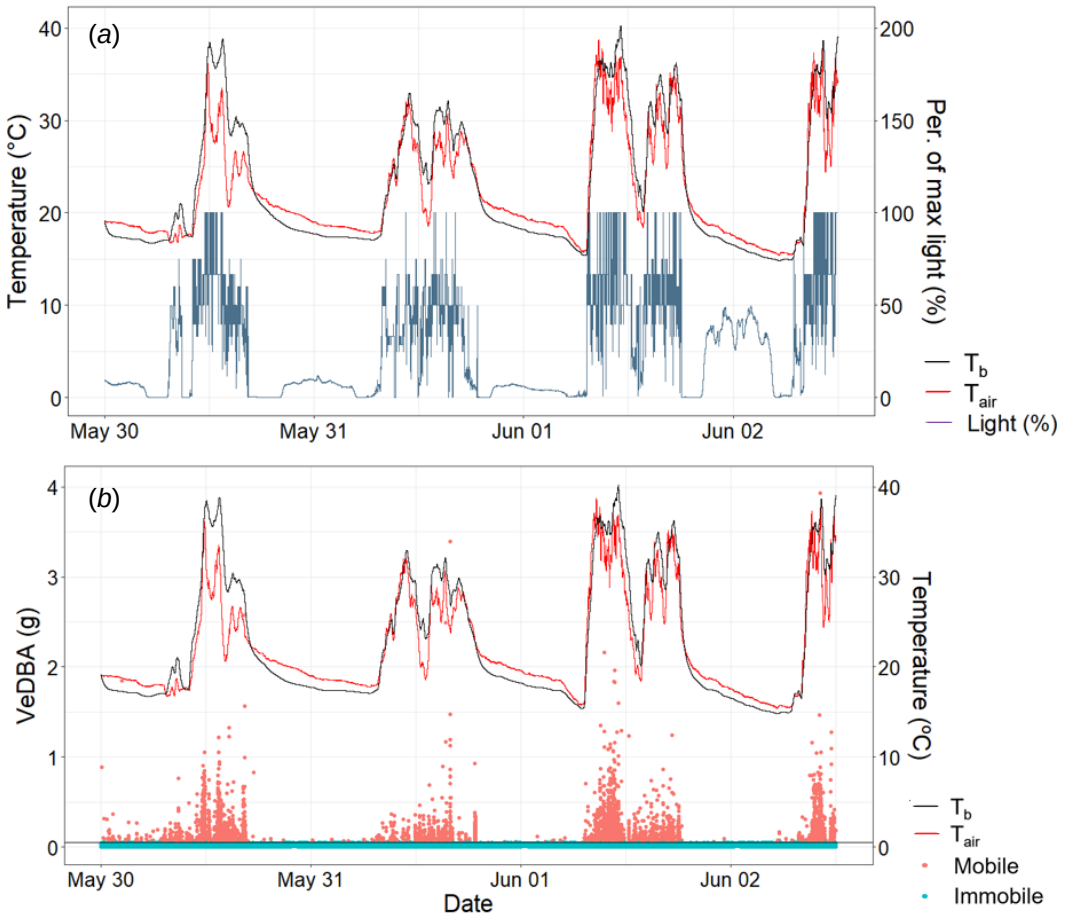


Figure 9. Plots showing the body temperatures (T_b) of a single animal (TLCRP012), as predicted by the ectotherm model of NicheMapR, during its stay in the mesocosm, together with the temperatures of its immediate environment (T_{air}) as recorded by the logger on its back; (*a*) plotted together with light intensity (percentage of light that the animal was receiving at each moment) and (*b*) plotted together with its acceleration (VeDBA, vectorial dynamic body acceleration), showing intensity of movement. The horizontal line indicates the movement threshold, below which the animal was immobile.

Specifically, the predicted T_b of animals in full sunlight exceeded at times the temperature of their surroundings, with the maximum difference being the T_b exceeding air temperature by 11ºC (mean ± *SD*: 6.6 ± 2.4ºC). On the contrary, the T_b of animals in the shadow (possibly in refuges), would often never reach their ambient temperatures, with the biggest observed difference being a T_b of 15ºC below T_{air} (mean ± *SD*: 8.6 ± 3.2ºC). There was also an evident association between higher body temperatures and higher movement rates (Figure 9*b*; see below for more detailed analyses).

The predicted body temperatures of the lizards were distributed differently during moments of movement and during moments of immobility. The most common (or most 'preferred') T_b for immobile lizards ranged from 12 to 19°C, while in moving animals, the highest densities of T_b ranged from 31 to 37°C, apart from one animal (TLCRP023), whose T_b density distribution while moving was bimodal and the first T_b peak of 14°C had been observed slightly more often (Figure 10; TLCRP023). These values represent the peaks of the density plots for each animal, when immobile and when moving (see Figure 10).

The T_b range in which each animal was spending the majority of its time, while moving versus while immobile, was here arbitrarily defined as the range of T_b with density over 0.05. Based on this, the minimum and maximum body temperatures of this preferred range of each animal were calculated (see Table S3). Linear regression analysis suggested that the highest T_b in the preferred T_b range of immobile animals (hereby mentioned as $T_{pref,max,imm}$ – maximum preferred T_b of immobile animals) is affected by their SVL (R^2 = 0.37, F(13) = 7.7, P = 0.01). On one side of the spectrum, a small lizard (TLCRP024) of SVL = 122 mm was not spending a substantial amount of time in body temperatures over 14°C when immobile. On the other side, a longer lizard (TLCRP022) of SVL = 158 mm, was able to spend a longer time inactive while maintaining a high body temperature ($T_{pref,max,imm}$ = 32°C). A similar relationship was not found between $T_{pref,max,imm}$ and body mass (R^2 = 0.01, F(13) = 0.2, P = 0.6).

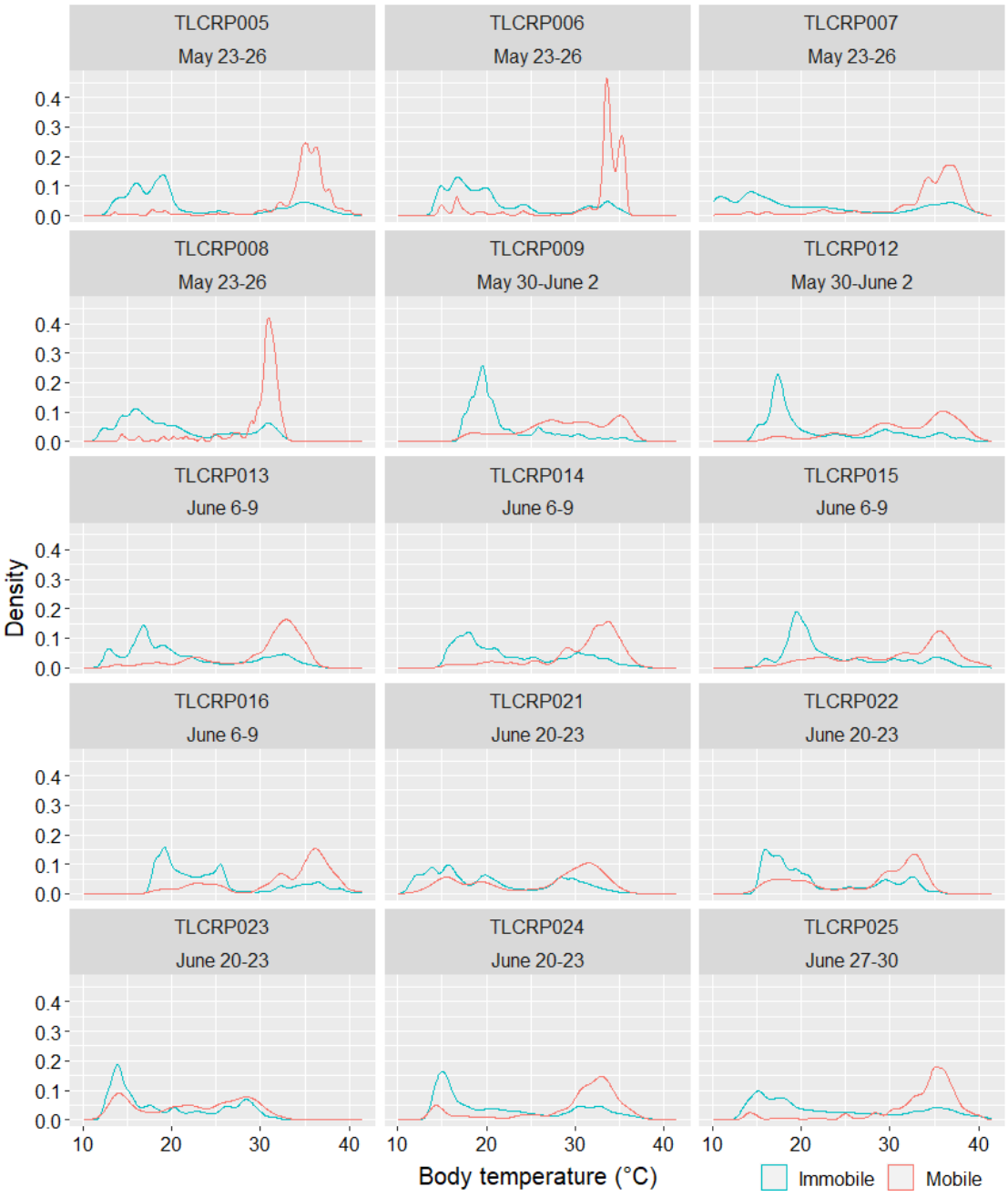


Figure 10. Density plots of predicted body temperatures (T_b) for the 15 *Timon lepidus* when they were immobile (blue line) versus moving (red line). The code of the animal, as well as the period of its stay in the mesocosm when the recording took place, are mentioned on top of each density plot.

3.3. Thermal sensitivity of locomotion

A total of seven thermal performance curve models were fitted to the acceleration data of each lizard. The models that gave the lowest AICc scores for more than one animal were the Sharpe-Schoolfield and the Rezende models. The Sharpe-Schoolfield model was the best-fit model for nine animal curves (60% of the analysed animals), while in many of the other animals the best-fit model had an AICc score of fewer than 2 degrees higher than the Sharpe-Schoolfield model (e.g. for TLCRP008, Rezende model: AICc = 562.3 and Sharpe-Schoolfield model: AICc = 564.1). For the AICc scores of all fitted models for the different animals, see Table S4. Thus, the Sharpe-Schoolfield model was selected to fit the thermal performance curves of all animals.

The thermal performance curves (TPCs) of the different lizards varied in shapes (see Figure S6 for the curves of all animals). The distribution of body temperatures generally followed the classic TPC shape, with T_b gradually increasing with performance in the beginning and, after reaching the maximum performance, rapidly dropping. However, the skewness (asymmetry) of the curves and their width (breadth) varied between lizards (Figure 11). The parameters extracted from each individual TPC are summarized in Table 5. Calculated optimum temperatures (T_{opt}) for the 15 lizards ranged from 29.5 to 37.6°C (mean ± *SD*: 34.7 ± 2.3°C) and maximum locomotor performance (r_{max}) ranged from 0.45 to 1.67 g (mean ± *SD*: 1 ± 0.3 g). The preferred temperature range for locomotion, here estimated through the TPCs, or the body temperatures where performance is at least 50% of the maximum (based on the thermal breadth of the TPC), ranged from 32.6 to 35.6°C for the animal with the narrowest breadth (TLCRP006; breadth = 2.98°C) and from 15 to 32°C for the animal with the widest breadth (TLCRP023; breadth = 17°C).

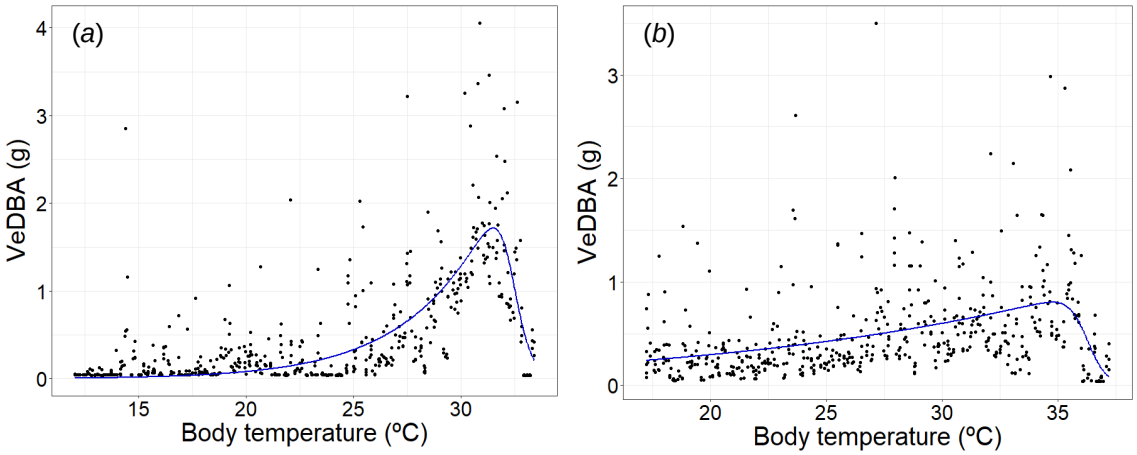


Figure 11. Thermal performance curves (TPCs) for locomotor performance (vectorial dynamic body acceleration, VeDBA) of two lizards, based on their predicted body temperatures during the duration of their stay in the mesocosms; (*a*) TLCRP008 and (*b*) TLCRP009.

No significant correlation was found between animal body size (body mass, SVL) and their thermal traits (T_{opt}, r_{max}, thermal breadth) (see Table S5). The 'hotter-is-better' hypothesis was also rejected, since there was no correlation between optimum temperature and maximum performance (Pearson's r = 0.27, P = 0.3) (Figure 12*a*). However, maximum performance and thermal breadth showed a significant negative correlation (Pearson's r = -0.7, P = 0.001), supporting the 'specialist-generalist' hypothesis (Figure 12*b*).

Finally, a significant negative correlation was found between lizard body size and the difference between their maximum T_b for movement, or their retreat temperature, and their optimum T_b ($T_{retreat}$ - T_{opt}). Both animal body mass and SVL were negatively correlated with this difference, which signifies how close an animal's optimum temperature is to its voluntary thermal limit (body mass: r = -0.72, P = 0.002; SVL: r = -0.59, P = 0.02).

Table 5. Thermal performance curve (TPC) derived parameters and important body temperature thresholds for the 15 *Timon lepidus* lizards included in the analyses: maximum performance – acceleration – rate (r_{max}); thermal breadth (body temperatures where performance is at least 50% of the maximum); emerge temperature (T_{emerge}); minimum body temperature in the 50% performance breadth ($T_{B50,min}$); optimum temperature (T_{opt}); maximum body temperature in the 50% performance breadth ($T_{B50,max}$); and retreat temperature ($T_{retreat}$).

Animal ID	r_{max} (g)	Breadth (°C)	T_{emerge} (°C)	$T_{B50,min}$ (°C)	T_{opt} (°C)	$T_{B50,max}$ (°C)	$T_{retreat}$ (°C)
TLCRP005	1.47	6.40	13.58	32.56	36.39	38.96	41.08
TLCRP006	1.18	2.98	14.52	32.60	35.10	35.57	35.73
TLCRP007	1.26	6.89	10.30	32.01	37.44	38.90	40.33
TLCRP008	1.67	4.92	12.03	27.94	31.64	32.86	33.43
TLCRP009	0.79	13.04	17.20	23.50	34.94	36.54	37.21
TLCRP012	0.83	9.48	14.87	29.62	37.61	39.10	40.25
TLCRP013	1.25	8.06	13.29	27.83	34.20	35.90	36.61
TLCRP014	1.25	7.41	15.32	28.84	34.44	36.25	37.79
TLCRP015	1.11	10.35	15.53	29.70	37.27	40.05	40.80
TLCRP016	1.11	11.42	17.97	28.82	37.40	40.24	41.44
TLCRP021	0.96	11.33	12.58	23.63	33.35	34.96	36.12
TLCRP022	0.91	9.28	15.45	25.56	33.38	34.83	35.63
TLCRP023	0.45	17.00	12.89	15.18	29.55	32.18	33.04
TLCRP024	0.75	7.91	14.04	27.91	33.02	35.82	38.62
TLCRP025	1.02	8.75	13.72	30.52	35.61	39.27	40.45

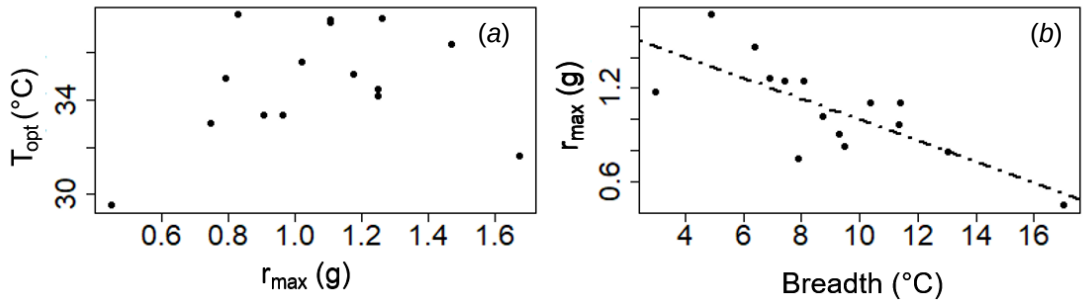


Figure 12. Plots showing the relationship between the thermal performance curve (TPC) derived thermal traits of the 15 *Timon lepidus* lizards regarding two major evolutionary trade-off hypotheses; (*a*) the non-significant relationship between optimum temperature (T_{opt}) and maximum performance rate (r_{max}), failing to support the 'hotter-is-better' hypothesis, and (*b*) the negative relationship between r_{max} and thermal breadth, supporting the 'specialist-generalist' hypothesis.

4. Discussion

4.1. Description of activity patterns

4.1.1. Framework for activity monitoring of small animals

For the first part of this study, I developed and explored a comprehensive methodology to be used for interdisciplinary studies of fine-scale activity budgets and thermal habitat use on small ectotherms. Through this protocol, two important goals were achieved: first, I introduced and tested a reproducible experimental procedure for fine-scale recording of acceleration and environmental variables using low-mass, animal-mounted loggers in semi-natural enclosures. Second, I developed a new method for classification of coarse-scale animal behaviours (movement vs. immobile) from tri-axial accelerometer data, using an unsupervised machine learning technique.

Trying out different combinations of datasets and accelerometer-derived metrics gave important insights as to how cluster analysis can be used for behavioural assignment of accelerometer data. Even if, initially, the clustering procedure was attempted using many acceleration metrics, the variability in the results of different lizard datasets coming from such an unfiltered clustering showed that using as many predictor variables as possible is not necessarily useful (Patterson et al., 2019). On the contrary, too many metrics might confuse the classification algorithm by adding random noise and make the result too complex in its interpretation. It is easier to interpret clusters that are obtained through fewer and biologically meaningful acceleration metrics, which have an intuitive relationship to known behaviours (e.g. changes in the x-axis can correspond to sprinting behaviour in lizards). Furthermore, it has been shown that to distinguish between coarse behaviours, even a small amount of acceleration metrics can give a high classification accuracy, while also being more computationally and time efficient (Patterson et al., 2019). This was evident also from the strategies followed in my study, where the use of only three acceleration metrics concerning the x-axis improved the consistency of the classification among the different individuals.

However, the classification results improved substantially with the use of only the nighttime recordings from each animal dataset. During the night, diurnal lizards, as *Timon lepidus*, are known to be generally inactive. Using only nighttime recordings for the clustering, which uniformly corresponded to immobile states, cleared the noise of other behaviours, irrelevant for the clustering, and, thus, reduced the complexity of information that was given to the algorithm. However, here it should be noted that such a strategy is possible only when the researcher can be relatively confident that the animals are

inactive during the night and should be appropriately adjusted otherwise, considering the ecology of the species in question.

Clustering only the nighttime data but all available metrics, resulted in clusters that, in some instances, classified even clearly inactive moments of the animals as ‘movement’ (see Figure 5*c*). This can be attributed to different and more subtle behaviours, such as changes in posture, that are related to changes in the acceleration values in the y and z-axes and they do not account for actual forward movement. Using only the x-axis acceleration metrics of the night period resulted in a cleaner clustering image (Figure 5*d*). DeSantis et al. (2020) had similar results when they assessed the relevance of various metrics in the distinction between the ‘mobile’ and ‘immobile’ phases of a tagged pitviper, based on a training dataset. They concluded that summary statistics and data dispersion metrics of the x and y-axes are more useful in distinguishing between coarse behavioural categories like movement and non-movement in animals that do not generally display upward-downward movement (z-axis). They also noted that overall body acceleration metrics (ODBA and VeDBA) do not improve the classification of simple and few behaviours, even if they can be more appropriate when the clustering aims to identify differences in activity intensity (DeSantis et al., 2020).

It is important to understand that CLARA, as well as other unsupervised clustering methods, tries to find a suitable partition in the data that maximizes the similarity inside the resulting clusters without any prior knowledge (Shraddha & Emmanuel, 2014). All mathematical algorithms make assumptions that do not always correspond to biological reality. The prior knowledge in this case must come from the researcher, who must consider the appropriate acceleration metrics and the type of data to be used, so that the algorithm distinguishes among the desired behaviours in a biologically meaningful way (Collins et al., 2015; S. Halle & Weinert, 2000). In the case of this study, the visual examination of a graphic representation of the acceleration data proved to be of extreme importance for the selection of the appropriate classification pipeline and to shape prior decisions based on the correct biological understanding of the data.

The methods chosen in this work fill a very important gap in the biologging research for small animals. So far, comprehensive protocols for behavioural assignment of accelerometer data have mainly been tested in large animals like birds (e.g. seabirds and vultures; Collins et al., 2015; Nathan et al., 2012; Patterson et al., 2019) and mammals (e.g. cows and pinnipeds; Shuert et al., 2018; Vázquez Diosdado et al., 2015) due to weight limitations of smaller-bodied species (Brown et al., 2013). Even today, with the advancements in the miniaturization of animal-borne logger devices, there are few

methodological studies of this kind for small animals, let alone for reptiles, while even these have their limitations: for instance, too complex methods, such as hidden Markov Models (Hammond et al., 2016) or the need of a training dataset through direct observations (DeSantis et al., 2020). There was thus the need for a simple protocol for small animals that does not require direct observations for behavioural validation. This is especially important for smaller-bodied species that are cryptic and sensitive to observer effects. Training datasets for supervised machine learning methods can be challenging to obtain for secretive species, while the normal behaviour of the animal may be affected, introducing further bias into the data (Kays et al., 2015).

The above-mentioned framework can be used to construct daily activity budgets of small animals in very fine temporal scales and to provide detailed descriptions of the timing and duration of movement patterns depending on the microclimate conditions of the animals at each moment. These methods can be further improved and extended to other efforts to study lizards and, more generally, small-sized animals.

4.1.2. Detailed movement patterns of *Timon lepidus*

The fine-scale activity budget estimates that were produced by this work allowed for detailed visualization and examination of the timing and duration of *T. lepidus* individual movement patterns (see Figures 5, 6, 9*b*). The low proportion of total time spent moving (> 97% of the time was classified as immobility in all animals) is expected for these ectotherms, that are known to show high fidelity to the site of their refuge, where they spend extensive amounts of time thermoregulating motionless (Grillet et al., 2010). Furthermore, the limited space of the mesocosm landscape, the short period of recording for each animal, as well as possible effects of the dataloggers on the normal animal behaviour, must be taken into account and might contribute to an underestimation of the potential movement capacity of these lizards.

In most animals, morning movement started around 8:00, when the animals possibly first exited their shelters to bask and the last substantial movements during the day were recorded until 20:00. However, the movement patterns during the day varied substantially between the different lizards (see Figure 7). The expected bimodal activity that has been observed in this species during summer months, characterized by reduced movements during midday when temperatures are higher (Busack & Visnaw, 1989; Rouag et al., 2006), was indeed prominent in some individuals (see example in Figure 9) but in many others the modality of activity was not so clear. This can be likely attributed to both inter-individual variation in physical traits like thermal preferences and tolerances (no

relation was observed between movement patterns and body size) and to the environmental conditions that the animals were subjected to. The second scenario is the most likely in most cases, as from the activity patterns of the 15 *T. lepidus* it seems that all animals recorded in the same week were uniformly displaying either a unimodal pattern (see in Figure 7, June 20-23) or a bimodal one (Figure 7, May 30-June 2). From observing the ambient microclimatic temperatures of the two above mentioned weeks (Figure 8*a*), a drop in temperatures seems to have happened during June 20-23, which is in agreement with the fact that the movement patterns of the lizards spending these days in the mesocosms display lower intensity in activity and unimodal activity patterns. A shift from a unimodal activity pattern in spring to a bimodal one in summer has been previously reported in lacertids (Busack & Visnaw, 1989) and this seems to be the case here, with lizard activity modality varying depending on the temperatures even at a daily and not only seasonal scale.

Of particular interest are the sporadic and infrequent, yet existent movements recorded during the night period in some animals. *T. lepidus* is a strictly diurnal species and such behaviours have not been observed before through standard sampling methods since nighttime recordings in natural settings are nearly impossible without the use of animal-mounted sensors (Brown et al., 2013). However, from the detailed data provided by this study it seems that *T. lepidus* can display limited nocturnal activity, possibly in summer when temperatures allow short bursts of movement. This is not the first instance that possible nocturnal activity is suggested for *T. lepidus*, as Hódar et al. (1996) reported similar findings in a population of south-east Spain.

T. lepidus activity intensity was found to be highly associated with increased ambient temperatures (Figure 6*a*,*b*), as usually expected by such thermoregulating lizards (Sannolo et al., 2019). However, this does not necessarily establish a cause-and-effect relationship between the environmental temperature and lizard movement. It is important to note that T_{air} in the microhabitat chosen by a lizard is not a measure of the available ambient temperatures and does not necessarily reflect the heterogeneity of the available microhabitats. Rather, the microclimate conditions (temperature, humidity, solar radiation) as recorded by the animal-attached dataloggers describe the conditions preferred by the animals based on their thermal traits (Hertz et al., 1993). Thus, the picture of the lizards' activity patterns as given by the animal dataloggers already illustrates the complex interplay between the animal's thermoregulatory behaviour and its physiological capacities (influenced by its body size and thermal tolerance) (Huey, 1982).

Observing the time-series visualization of each individual's movement intensity and

temperature data (Figure 6*b*) gives us an exact picture of the environment the animal needs to operate but also sheds light on the timing and causation of movements, i.e. seeking particular conditions, like shade when the temperature is too high, or going to bask after spending too much time in a shelter where temperatures are lower. This exemplifies the importance of combining the collection of environmental data at the local scale of the animal with the collection of data regarding movement intensity, when one wants to reconstruct in detail how animals move given the conditions they face (Williams et al., 2020).

Here it is important to acknowledge the relatively small sample size of different individuals that was used in the analyses (N = 15), as well as the short recording period for each lizard (3.5 days), which does not allow for conclusions on seasonal patterns or robust tendencies in daily movement variation. However, with further study, these methods can provide important insights into the inter-individual variation in daily activity rates. These in turn have been shown to be potentially related to life-history traits and can account for the heterogeneity in fitness inside a population, when activity patterns are correlated with more successful foraging or thermoregulation (Biro & Stamps, 2008).

4.2. Physiological effects of body temperature on *Timon lepidus* locomotor performance and thermal habitat selection

Body temperatures of active *Timon lepidus* during the time of the mesocosm experiment, as predicted by the animal model, generally agreed with the available published data on this species, obtained through other methods, such as infrared technology (preferred temperatures: 32.16 ± 3.11°C; Sannolo et al., 2019), thermal gradients (preferred temperatures ~32°C; Barroso et al., 2016) and cloacal readings (range of observed body temperatures: 21.2-34.5°C; Busack & Visnaw, 1989). This similarity supports the results of the model and is especially clear when compared with the predicted body temperatures preferred by active lizards in my study (32.8 ± 5°C; Figure 10).

The body temperatures that the lizards selected within the thermally heterogeneous environment of the mesocosm (T_{pref}) through behavioural thermoregulation, were generally lower than their optimum temperatures, or the temperature where locomotor performance is maximized (T_{opt}), as calculated through the TPCs. Thus, the preferred temperature range was centred around ‘suboptimal’ temperatures. This result agrees with the theory of Martin & Huey (2008), which predicts that ectotherm-preferred temperatures should be shifted to temperatures lower than those that are optimal for performance. This

tendency is related to the typical asymmetrical shape of the TPC. Because of this asymmetry, if ectotherms were to choose temperatures close to their optimum, potential increases of ambient temperatures would push body temperatures towards values higher than T_{opt}, causing locomotor performance to drop rapidly, together with the animal's physiological function and survival rates (Huey & Stevenson, 1979). Because of the costs in energy and time associated with behavioural thermoregulation and locomotion, lizards are not perfect thermoregulators (Gunderson & Leal, 2015; Huey & Slatkin, 1976), thus experiencing a range of body temperatures seasonally and daily (Hertz et al., 1993). Therefore, the tendency of *T. lepidus* individuals to prefer microhabitats that help them maintain suboptimal body temperatures might serve to increase fitness in the long term by protecting them from sudden thermal increases, even if this comes at the cost of not reaching maximum short-term performance (Martin & Huey, 2008).

4.2.1. Does body size affect lizard thermal habitat use?

Variation in body size did not have any significant effect on TPC parameters (T_{opt}, r_{max}, thermal breadth) of *T. lepidus* individuals of this study. This is possibly due to the low sample variability (body mass: 51-108 gr, SVL: 122-158 mm), since the lizards used in the analyses were only adult males of the same population. Rubalcaba et al. (2019) found that body size variation influences the thermoregulatory accuracy of terrestrial ectotherms across different regions when modelling lizards of various body sizes. Other studies also found a correlation between intra-specific body size variation and physiological thermal parameters across altitudinal or latitudinal gradients (Aguado & Clusella-Trullas, 2021) or even between lizards of different species (Clusella-Trullas & Chown, 2014; Wang et al., 2013). Yet, studies investigating more subtle inter-individual, intra-populational differences in lizards of not too variable body sizes, as this one, did not find any relationship between physiological thermal parameters and body size variation (Cecchetto & Naretto, 2015; Gilbert & Miles, 2017; Sannolo et al., 2019). This suggests that body size variation of this scale might not relate to thermal habitat variation inside the same species or population of lizards.

However, when looking into more subtle details of each animal's thermal preferences and tolerance, some patterns were revealed. Individual snout-vent length (*SVL*) and the maximum body temperature of the preferred range during immobility ($T_{pref,max,imm}$) displayed a significant positive relationship. This relationship implies a potential for larger lizards to maintain their body temperature in high values for a longer period while immobile. Larger body sizes have greater thermal inertia and, thus, this relationship may

indicate a more gradual change of body temperature in larger animals, which would allow them to retain their T_b for a longer time while immobile (Stevenson, 1985). However, a similar relationship was not found between $T_{b,max,imm}$ and the body mass of the lizards. Thus, the previous result is likely related to a more complicated mechanism.

The heat balance of ectotherms is determined both by their body size and their morphology, which affect their heat and water fluxes (Kearney et al., 2021; Stevenson, 1985) and by their behaviour, which determines the use of different thermal microhabitats (Malishev et al., 2018). From the perspective of the heat balance mechanism, and taking into account the nonlinear relationship between an animal's skin surface area and body mass (Porter & Kearney, 2009), the surface-mass ratio of longer, but not necessarily larger, lizards could influence their ability to maintain high body temperatures while immobile. Longer lizards should have a greater skin surface area, thus facilitating convective heat exchange with their environment, similar to the pattern observed in small ectotherms with low thermal inertia (Huey & Slatkin, 1976; Stevenson, 1985). Therefore, if the ability of bigger lizards to maintain higher T_b while immobile does not correspond to the effects of thermal inertia, it could be due to their surface-mass ratio facilitating faster heating rates while basking during the hot hours of the day.

Lizard body size was also found to be negatively correlated with the difference between the animal's optimum temperature (T_{opt}) and its retreat temperature ($T_{retreat}$), which may also reflect its voluntary thermal maximum (Braschler et al., 2021). According to this relationship, larger lizards perform better in body temperatures closer to their thermal limits, regardless of their maximum performance or optimum temperature being higher or lower (since no relationship was found between body size and other TPC thermal parameters). $T_{retreat} - T_{opt}$ could also describe the rate and severity of performance decline at temperatures above the T_{opt} (Huey & Kingsolver, 1989). In other words, when the difference between T_{opt} and a $T_{retreat}$ is smaller, performance starts to decline at a higher T_b within the animal's T_b range. Such a relationship might suggest that bigger lizards cannot maximize their performance without risking getting closer to their thermal limits (Buckley et al., 2022). Thus, based on the individuals included in this study, bigger *T. lepidus* seem to be more sensitive to increases in body temperatures above their T_{opt}.

Since body size affects so many aspects of an organism's biology and ecology and it is generally accepted that bigger body size is related to higher fitness (Kingsolver et al., 2004), it is expected that selection should act on traits related to morphology and body size. Yet, until now, research regarding the relationship between body size and thermal traits in ectotherms has been inconclusive (Buckley, 2021). Various works have

concluded that larger ectotherm species are at greater risk of overheating as the temperatures of their habitats rise (Meiri, 2008; Rubalcaba et al., 2019; Sunday et al., 2012). Leiva et al. (2019) noted a relationship between body size and variability in the critical thermal maxima (CT_{max}) of ectotherm species. However, in many cases the role of body size in lizard habitat use and thermal preferences has remained unclear and the complex patterns that are observed can be a result of the interplay between developmental, physiological and behavioural mechanisms (Meiri, 2008; Olalla-Tárraga et al., 2006). Studying the relationship between body size and thermal traits in fine-scales and from the perspective of different mechanisms, as demonstrated in this project, might help in revealing new patterns on the influence of body size on animal physiology.

4.2.2. Thermal sensitivity of locomotor performance in *T. lepidus* is constrained by specialist-generalist trade-off

There was high variation in maximum performance, optimum temperature and performance breadth between individuals. This is a significant observation, since it shows that the shape of performance curves varies within the same population and there are constraints within individuals that limit the way they respond to various environments (Seebacher et al., 2015). The first tested hypothesis that explains TPC variability, the 'hotter-is-better' trade-off hypothesis, was rejected, showing that lizards with higher optimum temperatures did not exhibit a concomitant increase in maximum performance (Angilletta et al., 2010). This suggests that this thermodynamic constraint in locomotor performance at least does not affect lizards inside the same population, in agreement with other studies on ectotherms that have found only a minor effect, even when studying variation among populations (Enriquez-Urzelai et al., 2018).

The second tested hypothesis regarding TPC variability, the 'specialist-generalist' trade-off hypothesis, was corroborated for the individuals of this study. Based on this, a phenotypic variation of physiological traits was present within the *T. lepidus* population of this study, consisting, on one end of the spectrum, of animals that tend to perform better but only over a relatively restricted range of body temperatures (thermal specialists) and, on the opposite end, of animals able to perform over a wide range of body temperatures, but at the cost of having low maximum performance (thermal generalists) (Huey & Hertz, 1984b).

The implications for the fitness and microhabitat use of the individuals of this population might be various. 'Specialist' individuals that have a very strong preference for a specific range of body temperatures (which implies low plasticity of T_{pref}) might not be able to

function in body temperatures far below or above their preferred range, thus reducing their activity times and available microhabitats. Thus, a potential increase in T_b caused by climate warming would have a larger impact on a specialist than on a generalist (increased thermal sensitivity) (Huey et al., 2012). However, this temporal reduction of activity should come with an increase in performance capacity while active (e.g. more successful reproduction, prey capture, predator escape), thus possibly increasing the fitness of these animals when the environmental conditions allow it (Gilbert & Miles, 2017). On the contrary, 'generalist' individuals that are more flexible in their selection of body temperatures might be able to increase their activity time and exploit a larger part of their surroundings (Huey et al., 2009). By having a wider range of preferred temperatures for locomotion, lizards on the generalist side of the spectrum are not so dependent on shaded microhabitats and thus they can prolong the time period for foraging and other activities before they have to retreat into refuges (Gunderson & Leal, 2015). A longer activity period increases the probability of successful reproduction and foraging, as well as the thermoregulatory opportunities to reach T_{opt} or T_{pref}. Despite this, due to the confirmed 'specialist-generalist' trade-off, these lizards might have a performance disadvantage during their time of activity.

However, despite the environmental variability *T. lepidus* experiences daily and seasonally being a temperate species, the coexistence of both thermal specialist and generalist phenotypes in the same population likely indicates that none of the two opposing performance phenotypes enhances fitness, at least for the current environmental and habitat conditions this population faces (Seebacher et al., 2015). Nonetheless, knowing of the existence of such phenotypes inside the population gives the possibility to study further how lizards on the opposite ends of the spectrum might use their available microhabitats and eventually reach a better understanding of how each phenotype would respond in the face of future environmental change.

4.3. Concluding remarks and future perspectives

In this study I implemented a combination of innovative technologies and methods, including biologging techniques, mechanistic niche modelling and a pipeline to fit thermal performance curves, aiming to disentangle the mechanisms that define the relationship between thermal physiology, performance and habitat use in lizards. From a methodological perspective, the results of the study illustrate the importance of obtaining time-matched behavioural along with microclimatic data for creating a detailed reconstruction of animal activity patterns and inferring environmental preferences. Moving forward, the

methodologies used here can be improved and extended for other species. Some suggestions would be the incorporation of magnetometers in the animal dataloggers, which can be used to infer orientation, thus giving the possibility to reconstruct detailed animal movements in 2D and 3D using the 'dead-reckoning' technique (Williams et al., 2020), or improved datasets containing more animals and perhaps from different populations, to study intra-specific variation and the potential for thermal adaptation. Nevertheless, the methods presented here should improve the potential for remote observation of the mechanisms behind the movement and habitat selection of small animals.

Understanding how animals avoid extreme temperatures and the consequences of failing to do so is paramount to comprehend the impact of anthropogenic climate warming. My results demonstrate the significance of physiological information and especially of thermal ecology traits, for understanding and conserving reptile species. Even at a within-population level, I found evidence that *T. lepidus* thermal preferences might correlate with body size. Furthermore, there was a clear phenotypic pattern inside the population matching the 'specialist-generalist' trade-off, which suggests a potential limit for adaptive plasticity under future climate change scenarios (Thompson, 1991). Even if there are disputes over the degree that phenotypic plasticity in thermal physiology within a population is sufficient to buffer species from warmer temperatures in the long term (Gunderson & Stillman, 2015), through the data collected in this project it is possible to explore further the implications of variation in thermal traits and habitat use to forecast population persistence.

Planning conservation strategies for species and populations requires taking into account variation in thermal traits and opportunities within the landscape that the animals experience (Sgrò et al., 2011). Through this project, I quantified the physiological effects of body temperature on whole-organism performance. Combining this data with a detailed map of the microclimates available in the habitat used by the lizards – as is already possible with the data obtained from the mesocosm landscape – the physiological suitability of different microhabitats for lizards of different phenotypes can be obtained (Huey, 1991). In this way, the implementation of additional functions of biophysical modelling can help us predict how climate change would affect the microhabitat use of a generalist versus a specialist *T. lepidus* of various body sizes and study the mechanistic limitations of phenotypic plasticity on ectotherm performance (Briscoe et al., 2023). This is a promising venue for future research, which was not further explored in the duration of this MSc project due to time constraints alone.

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Supplementary material

Table S1. Equations and parameters of the 7 thermal performance curve models of the rTPC package used in this project to determine the best-fit model for the *Timon lepidus* lizards' data.

Model	Equation	Parameters
Sharpe-Schoolfield - high temperature (Schoolfield et al., 1981)	$rate = \frac{r_{tref} \cdot \exp^{\frac{-e}{k}\left(\frac{1}{temp + 273.15} - \frac{1}{temp + 273.15}\right)}}{1 + \exp^{\frac{e_h}{k}\left(\frac{1}{t_h} - \frac{1}{temp + 273.15}\right)}}$	*temp*: temperature (°C); r_{tref}: rate at t_{ref}, i.e. the standard temperature at which rates are not deactivated by high temperatures; *e*: activation energy (eV); *eh*: high temperature deactivation energy (eV); t_h: temperature (°C) at which enzyme is 1/2 active and 1/2 suppressed due to high temperatures
Weibull (Angilletta, 2006)	$rate = a \cdot \left(\frac{c - 1}{c}\right)^{\frac{1 - c}{c}} \left(\frac{temp - t_{opt}}{b} + \left(\frac{c - 1}{c}\right)^{\frac{1}{c}}\right)^{c - 1} \exp - \left(\frac{temp - t_{opt}}{b} + \left(\frac{c - 1}{c}\right)^{\frac{1}{c}}\right)^{c} + \frac{c + 1}{c}$	*temp*: temperature (°C); *a*: metric regarding height of the curve; t_{opt}: optimum temperature; b: defines breadth of the curve; *c*: defines curve shape
Beta (Niehaus et al., 2012)	$rate = \frac{a\left(\frac{temp - b + \frac{c(d - 1)}{d + e - 2}}{c}\right)^{d-1} \cdot \left(1 - \frac{temp - b + \frac{c(d - 1)}{d + e - 2}}{c}\right)^{e - 1}}{\left(\frac{d - 1}{d + e - 2}\right)^{d - 1} \cdot \left(\frac{e - 1}{d + e - 2}\right)^{e - 1}}$	*temp*: temperature (°C); *a*, *b*, *c*, *d* & *e*: model parameters
Rezende (Rezende & Bozinovic, 2019)	if temp < b : $rate = a \cdot 10^{\frac{\log_{10}(q_{10})}{\left(\frac{10}{temp}\right)}}$ if temp > b : $rate = a \cdot 10^{\frac{\log_{10}(q_{10})}{\left(\frac{10}{temp}\right)}} \cdot \left(1 - c \cdot (b - temp)^2\right)$	*temp*: temperature (°C); *q10*: the factor by which the reaction rate increases when the temperature is raised by ten degrees by 10°C; *a*: parameter describing shifts in rate; *b*: threshold temperature (°C) beyond which the curve starts declining; *c*: parameter controlling the rate of decline beyond the threshold temperature, *b*
Spain (Spain, 1982)	$rate = a \cdot \exp^{b \cdot temp}\left(1 - \left(\frac{temp - t_{opt}}{c}\right)^2\right)$	*temp*: temperature (°C); *a*: constant determining the steepness of the rising part of the curve; *b*: constant determining the position of t_{opt}; *c*: constant determining the steepness of the declining part of the curve; *r0*: the rate at 0°C
Gaussian (Lynch & Gabriel, 1987)	$rate = r_{max} \cdot \exp^{\left(-0.5\left(\frac{\lvert temp - t_{opt} \rvert}{a}\right)^2\right)}$	*temp*: temperature (°C); r_{max}: maximum rate at optimum temperature; t_{opt}: optimum temperature (°C); *a*: related to curve width
Quadratic (Montagnes et al., 2008)	$rate = a + b \cdot temp + c \cdot temp^2$	*temp*: temperature (°C); *a*: defines the rate at 0°C; *b* & *c*: model parameters

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Table S2. The movement thresholds (vectorial dynamic body acceleration (VeDBA) values) as estimated by the CLARA clustering algorithm for the dataset of each animal, below which thresholds acceleration values signify immobility and over which they signify movement, and for each of the four clustering strategies attempted.

Animal ID	Threshold (g) Str. 1	Threshold (g) Str. 2	Threshold (g) Str. 3	Threshold (g) Str. 4
TLCRP005	0.7	0.14	0.09	0.094
TLCRP006	0.1	0.1	0.1	0.050
TLCRP007	0.07	0.46	0.05	0.043
TLCRP008	1	0.09	0.03	0.050
TLCRP009	0.14	0.14	0.03	0.049
TLCRP012	0.17	0.17	0.04	0.074
TLCRP013	0.11	0.11	0.04	0.051
TLCRP014	0.79	0.13	0.03	0.045
TLCRP015	0.13	0.13	0.05	0.051
TLCRP016	0.6	0.1	0.04	0.049
TLCRP021	0.13	0.13	0.09	0.066
TLCRP022	0.05	0.12	0.06	0.085
TLCRP023	0.08	0.09	0.06	0.095
TLCRP024	0.11	0.13	0.05	0.102
TLCRP025	0.45	0.1	0.07	0.071
SD	0.3	0.022	0.021	0.019

Table S3. The preferred body temperature (T_b) range of each lizard, while moving versus while immobile, defined as the range of T_b-*s* with density over 0.05. Based on this, the following were calculated: the minimum and maximum preferred T_b values of this range, as well as the most preferred T_b of immobile animals ($T_{pref,min,imm}$, $T_{pref,max,imm}$, $T_{pref,imm}$ respectively) and the same values during movement ($T_{pref,min,mob}$, $T_{pref,max,mob}$, $T_{pref,mob}$).

Animal ID	$T_{pref,min,imm}$ (°C)	$T_{pref,imm}$ (°C)	$T_{pref,max,imm}$ (°C)	$T_{pref,min,mob}$ (°C)	$T_{pref,mob}$ (°C)	$T_{pref,max,mob}$ (°C)
TLCRP005	33.44	35.09	38.16	13.52	19.62	20.25
TLCRP006	16.51	33.57	35.93	14.23	17.18	21.03
TLCRP007	33.09	36.87	38.66	10.28	14.97	16.80
TLCRP008	28.72	30.93	32.70	13.68	16.38	31.53
TLCRP009	25.20	35.07	36.26	17.14	18.35	21.69
TLCRP012	28.10	35.95	38.59	15.03	16.32	19.59
TLCRP013	29.98	32.90	35.70	12.54	16.82	20.36
TLCRP014	28.44	33.73	35.90	15.38	17.81	30.43
TLCRP015	31.56	35.59	37.72	17.93	18.49	22.40
TLCRP016	31.50	36.16	38.89	17.66	18.64	26.13
TLCRP021	14.78	31.53	33.88	11.72	14.78	29.91
TLCRP022	28.74	32.74	34.48	15.15	14.62	32.91
TLCRP023	12.88	14.06	30.13	12.38	12.72	29.23
TLCRP024	29.49	32.97	35.49	13.73	14.71	17.44
TLCRP025	31.76	35.19	37.97	13.70	15.45	19.06

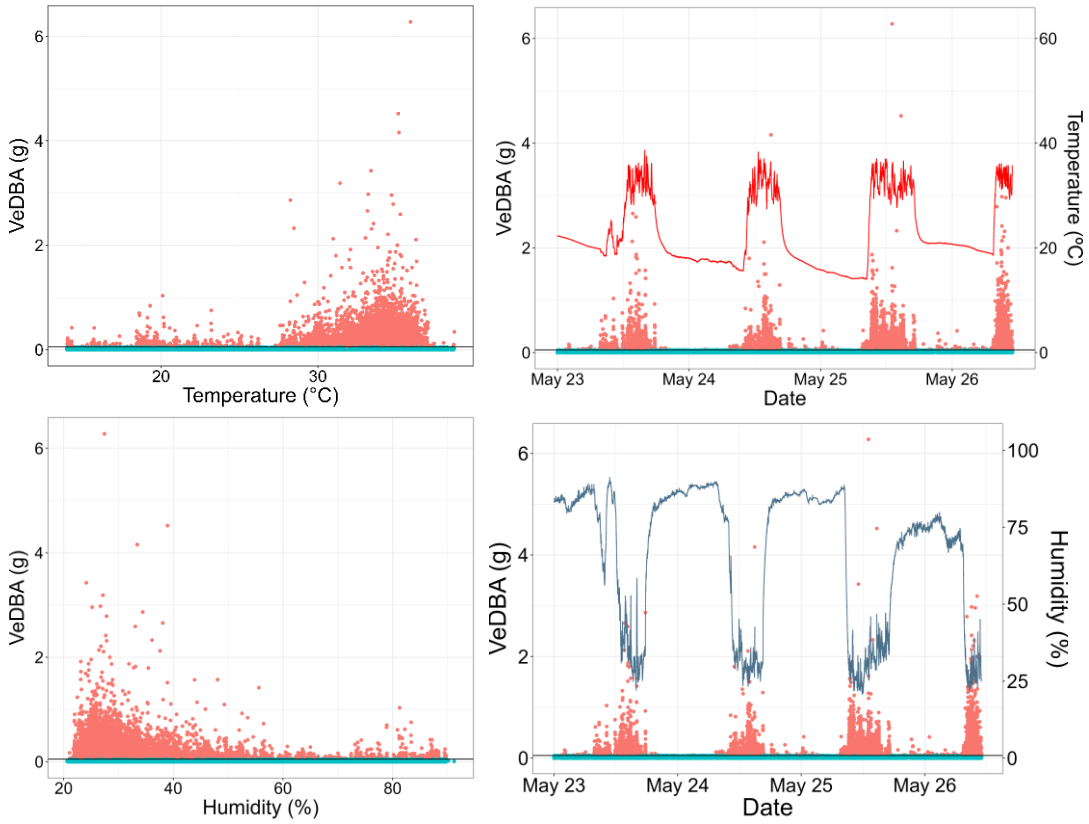
Table S4. The AICc scores for each one the 7 thermal performance curve models of the rTPC package used in this project to determine the best-fit model for the *Timon lepidus* lizards' data. The lowest AICc scores that defined the best-fit model for each animal are highlighted in bold.

	Model (*AICc*)						
Animal ID	**Sharpe-Schoolfield**	**Weibull**	**Beta**	**Gaussian**	**Rezende**	**Spain**	**Quadratic**
TLCRP005	**764.5**	770.6	777.6	781.3	807.6	820.4	942.1
TLCRP006	346.1	347.4	339.6	347.6	647.2	**315.3**	333.1
TLCRP007	**526.6**	599.2	587.4	680.8	551.1	569.6	692.9
TLCRP008	564.1	600.5	588.9	639.1	**562.4**	572.1	632.5
TLCRP009	**512.2**	530.2	523.3	531.2	513.2	516.7	535.2
TLCRP012	**449.3**	469.1	465.4	472.1	452.6	458.0	473.5
TLCRP013	477.3	533.4	499.4	560.4	**475.1**	479.9	573.6
TLCRP014	**419.6**	467.3	463.0	522.9	435.8	445.6	619.2
TLCRP015	1124.8	1143.8	1137.2	1148.8	**1122.6**	1128.7	1147.4
TLCRP016	**761.0**	797.6	782.5	817.7	762.1	771.3	828.5
TLCRP021	**729.7**	785.2	762.9	789.5	731.0	737.6	786.7
TLCRP022	666.7	698.9	685.4	705.9	**664.8**	669.5	667.6
TLCRP023	-44.9	-23.1	-33.0	-21.8	**-46.3**	-43.2	-20.2
TLCRP024	**485.6**	494.9	512.6	508.4	496.2	503.8	553.9
TLCRP025	**477.7**	484.0	490.4	493.5	489.3	499.4	587.1

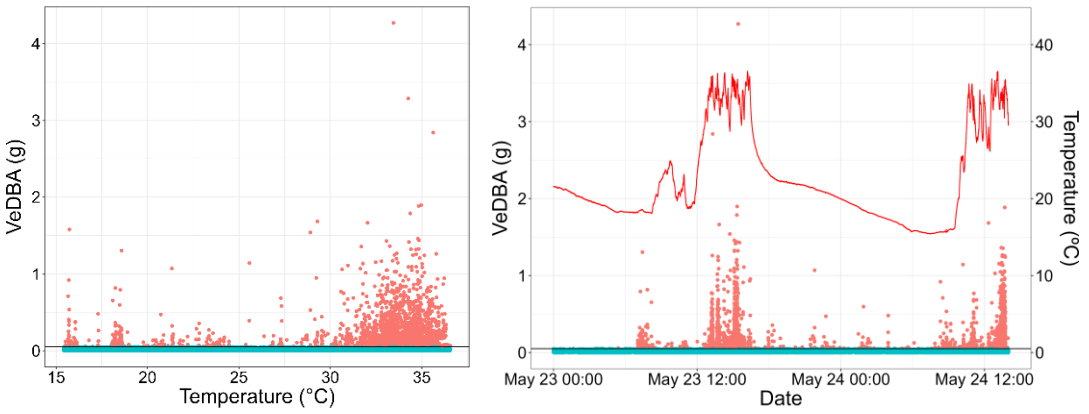
Table S5. Results of correlation between animal body size [body mass, snout-vent length (SVL)] and their thermal traits [optimum temperature (T_{opt}), maximum performance (r_{max}), thermal breadth].

	Body mass		SVL	
	r	*P*	*r*	*P*
T_{opt}	-0.07	0.77	-0.12	0.65
R_{max}	-0.14	-0.6	0.05	0.8
Breadth	0.06	0.8	0.19	0.4

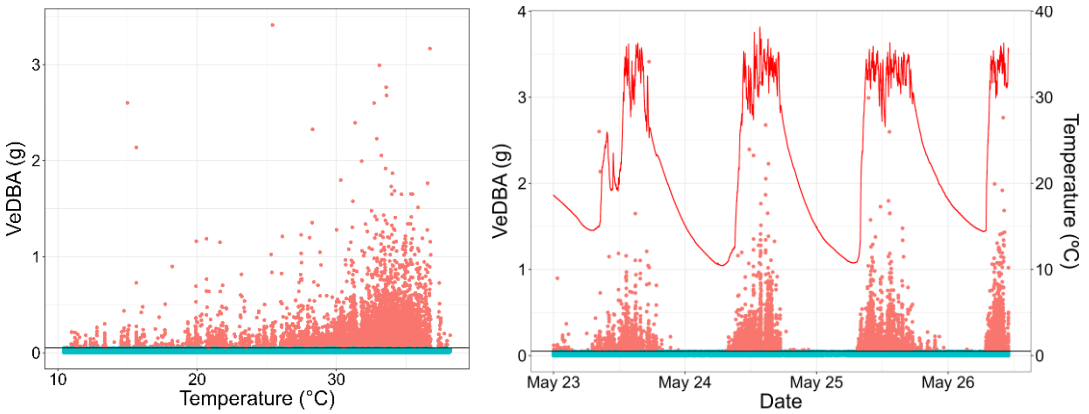
TLCRP005



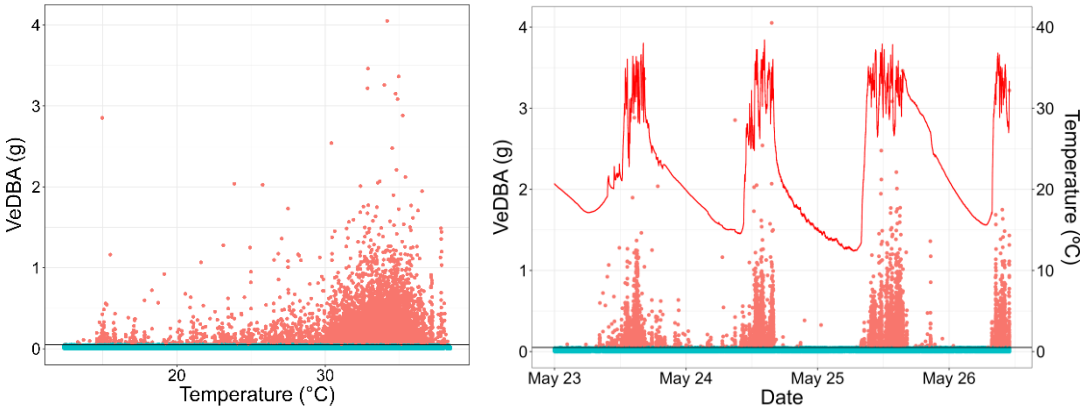
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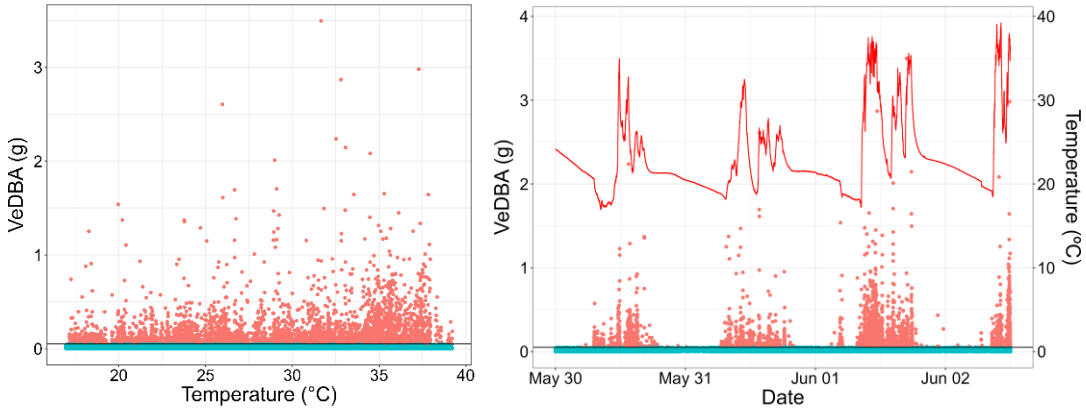
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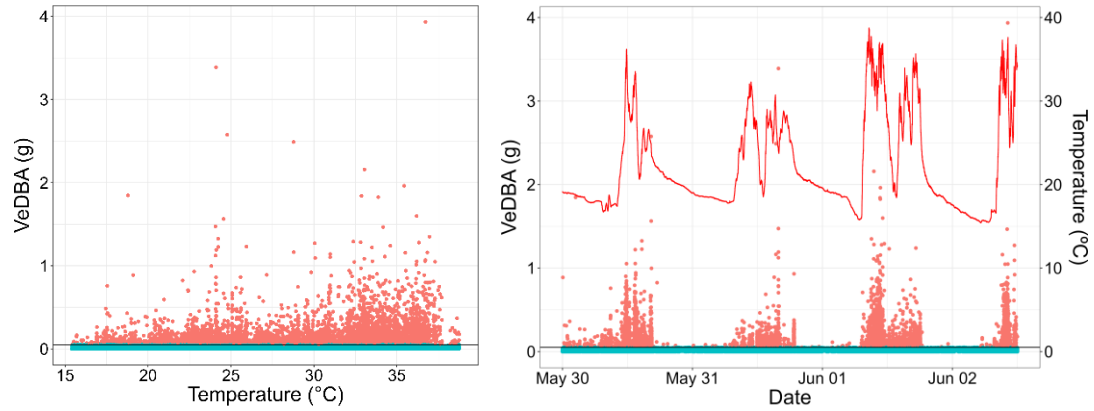
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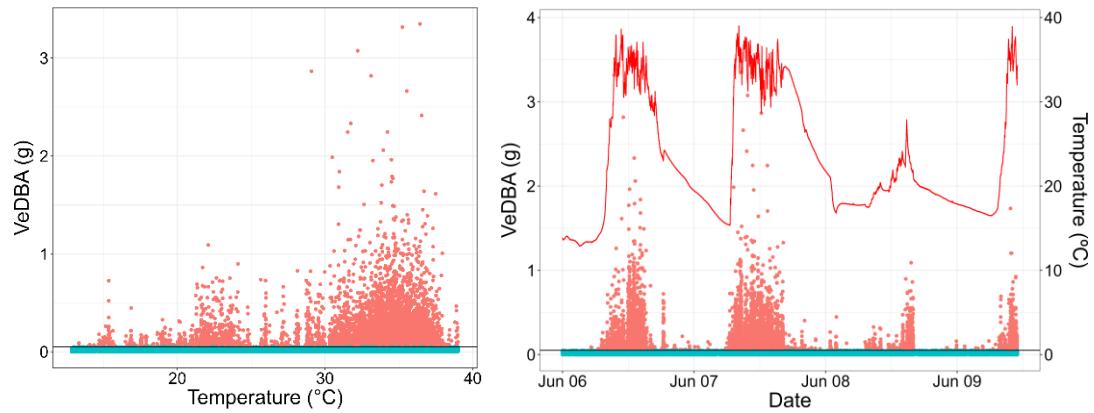
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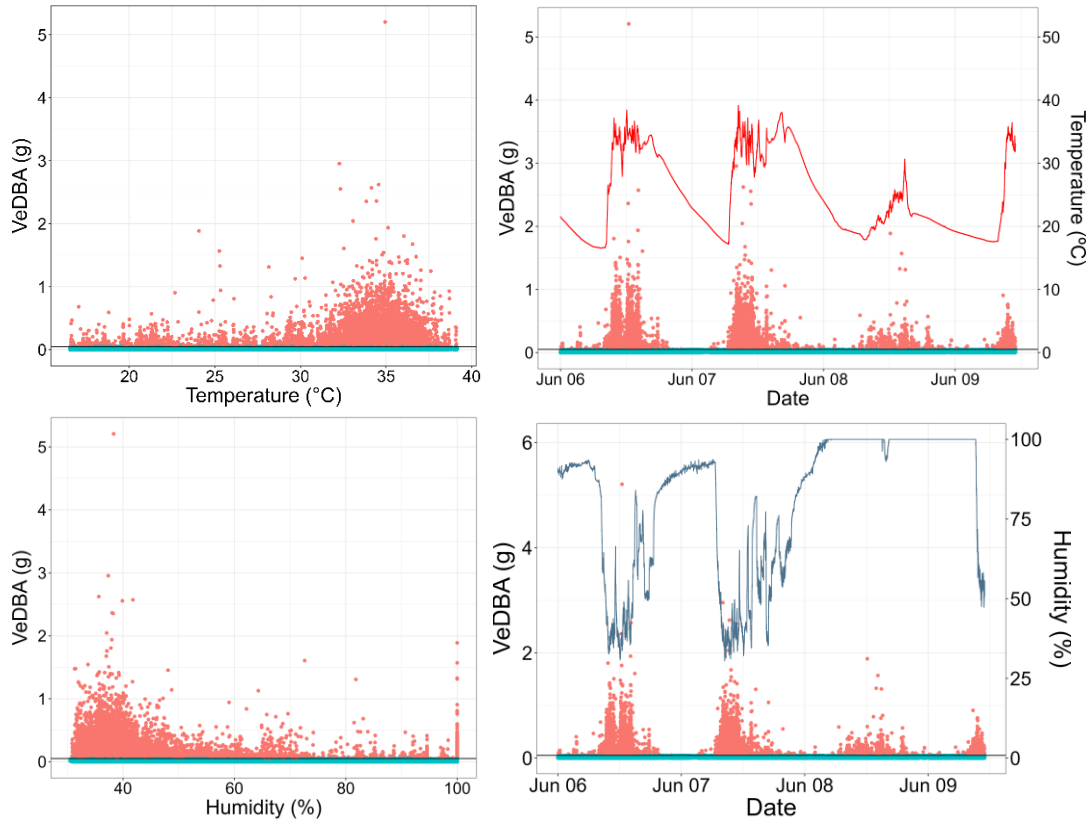
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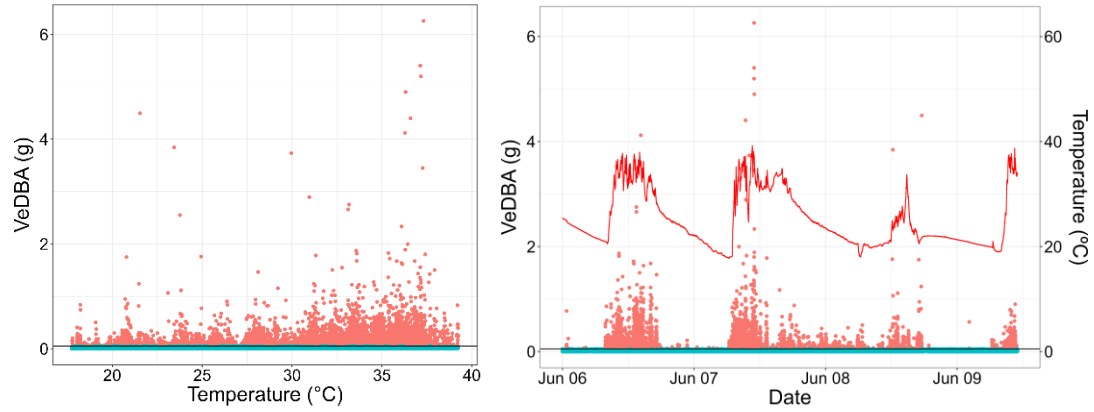
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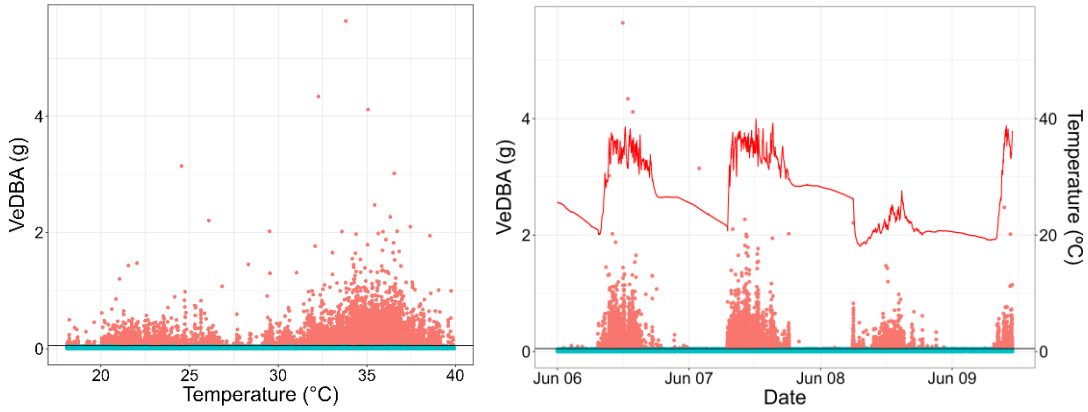
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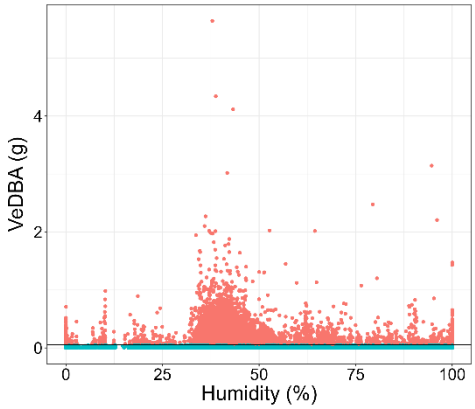
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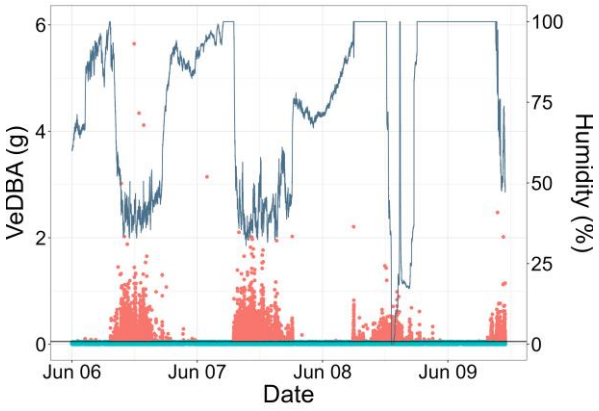


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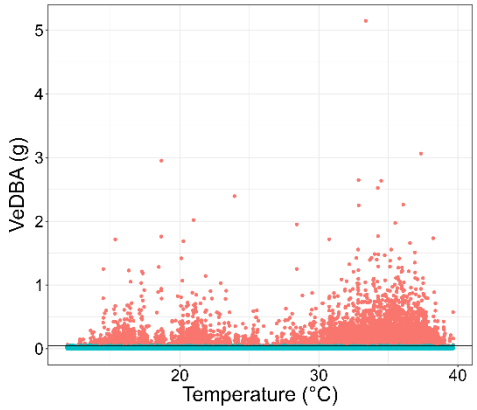


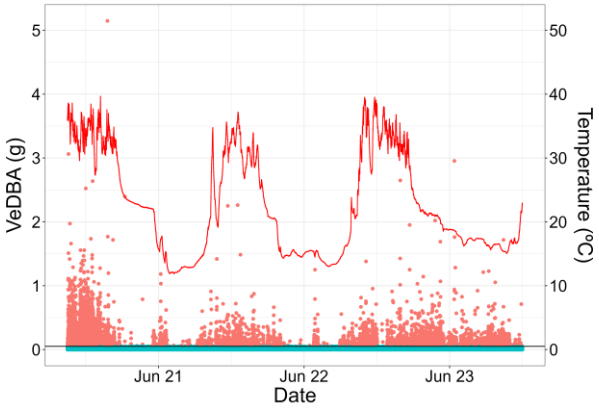
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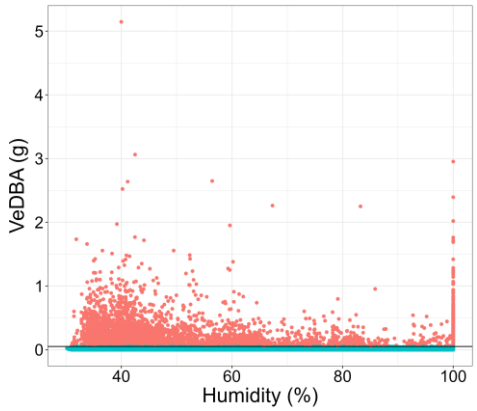


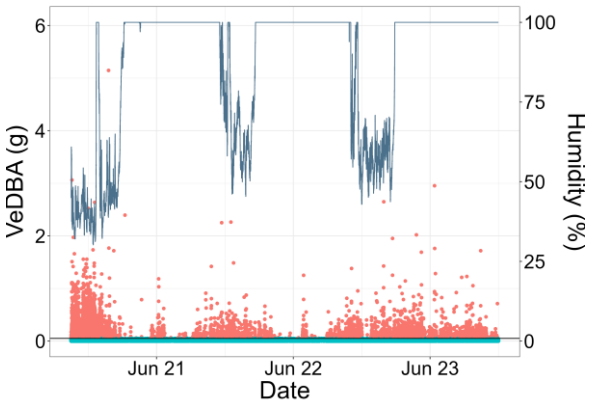


TLCRP021

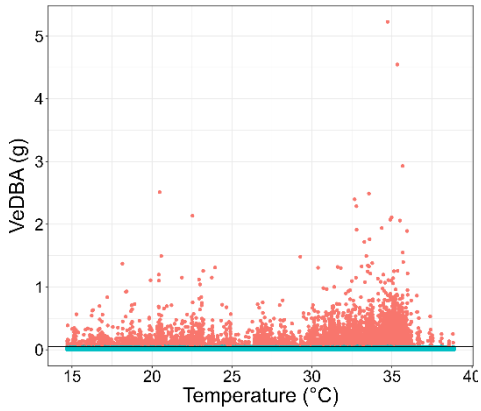


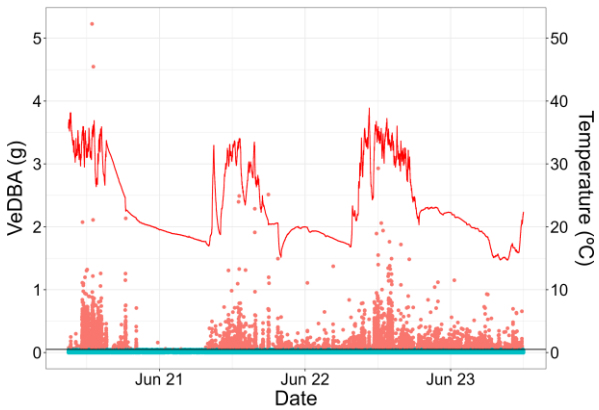






TLCRP022





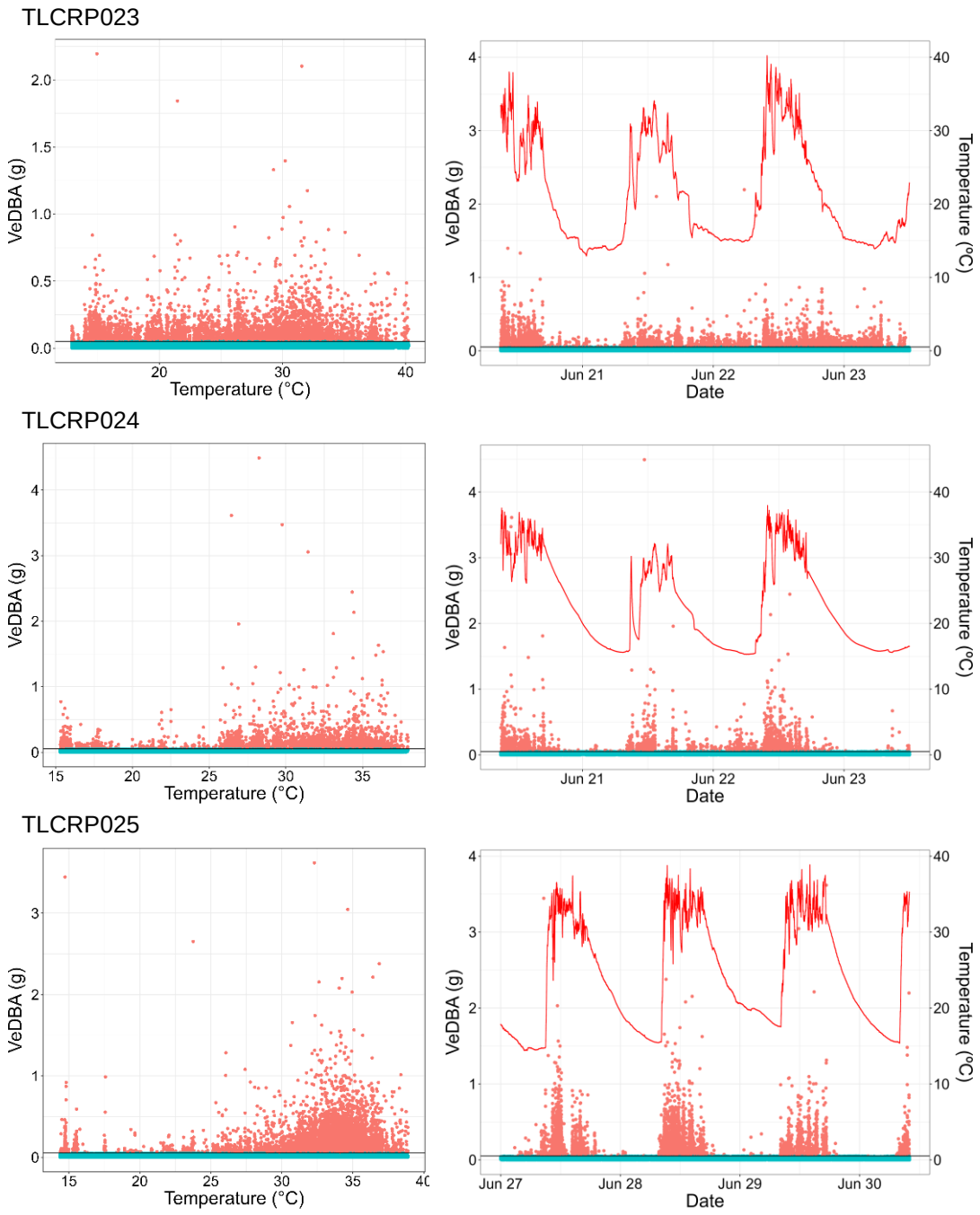


Figure S1. Occurrence of movement, quantified as the vectorial dynamic body acceleration (VeDBA), of all lizards that were included in the analyses of this study, as associated with different environmental conditions: movement intensity along environmental temperatures and/or humidity (left-side plots); time series plots depicting the acceleration of the animal on the left y-axis and the environmental temperatures and/or humidity it was experiencing (red/blue line) during its stay in the mesocosm on the right y-axis (right-side plots). The pink points of the VeDBA values represent observations of movement, while the blue points indicate immobility. The horizontal line indicates the chosen movement threshold. Most animal humidity sensors (except for lizards TLCRP005, TLCRP014, TLCRP16 and TLCRP21) were saturated at very high values and were considered unreliable, thus displaying here their results was not needed.

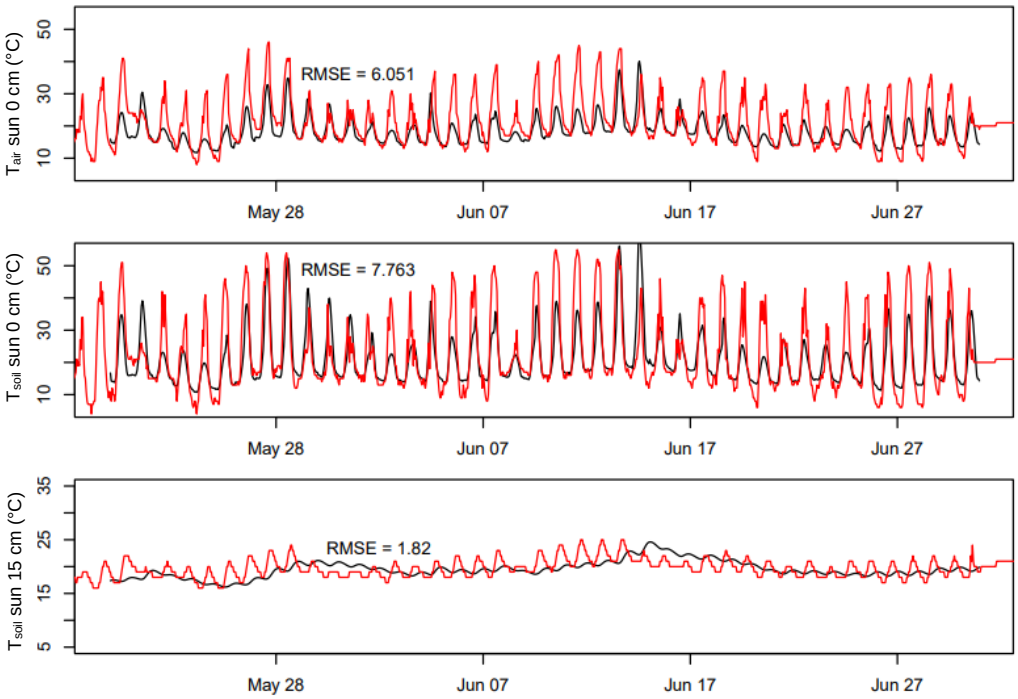


Figure S2. A comparison between the temperatures (T) at full sun in different microhabitats of the mesocosm landscape, as predicted by the microclimate model after improved parametrization and observed by the dataloggers recording temperature and humidity in the same microhabitats; (a) surface rock temperatures, (b) surface soil temperatures, and (c) soil temperatures at 15 cm of depth. The root-mean-square error (RMSE) of each prediction shows the average distance between the prediction of the model and the recorded values. The red line represents the temperature values recorded by the mesocosm dataloggers and the black line represents the predicted temperatures from the microclimate model.

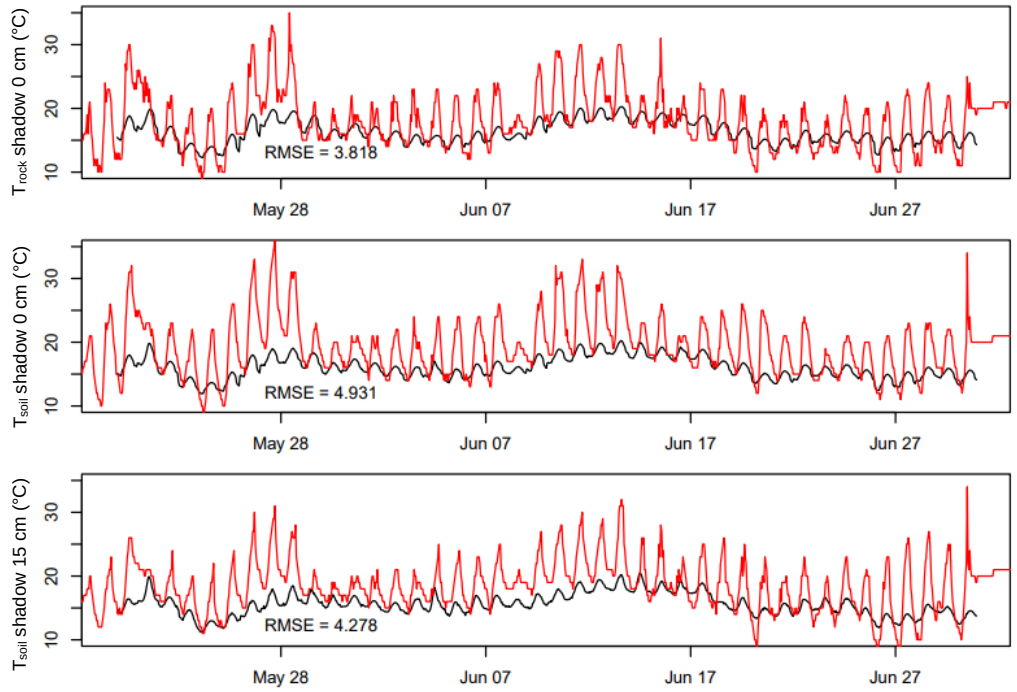


Figure S3. A comparison between the temperatures (T) at 90% of shade in different microhabitats of the mesocosm landscape, as predicted by the microclimate model after improved parametrization and observed by the dataloggers recording temperature and humidity in the same microhabitats; (a) surface rock temperatures, (b) surface soil temperatures, and (c) soil temperatures at 15 cm of depth. The root-mean-square error (RMSE) of each prediction shows the average distance between the prediction of the model and the recorded values. The red line represents the temperature values recorded by the mesocosm dataloggers and the black line represents the predicted temperatures from the microclimate model.

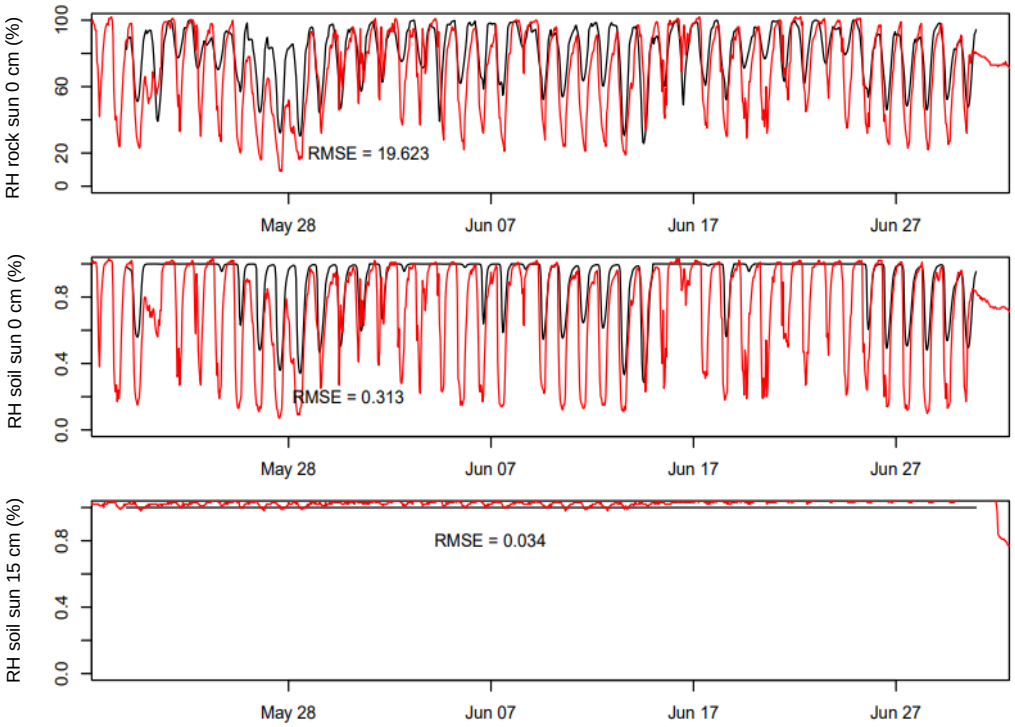


Figure S4. A comparison between the relative humidity (RH) at full sun in different microhabitats of the mesocosm landscape, as predicted by the microclimate model after improved parametrization and observed by the dataloggers recording temperature and humidity in the same microhabitats; (a) humidity at rock surface, (b) humidity on soil surface, and (c) soil humidity at 15 cm of depth. The root-mean-square error (RMSE) of each prediction shows the average distance between the prediction of the model and the recorded values. The red line represents the humidity values recorded by the mesocosm dataloggers and the black line represents the predicted humidity from the microclimate model.

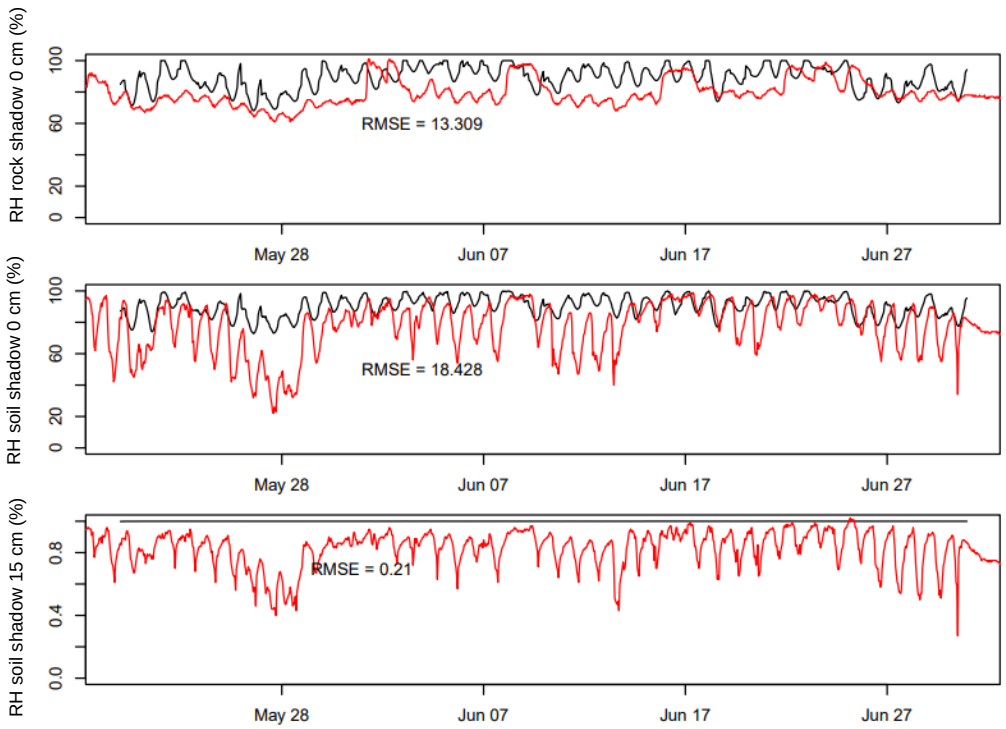
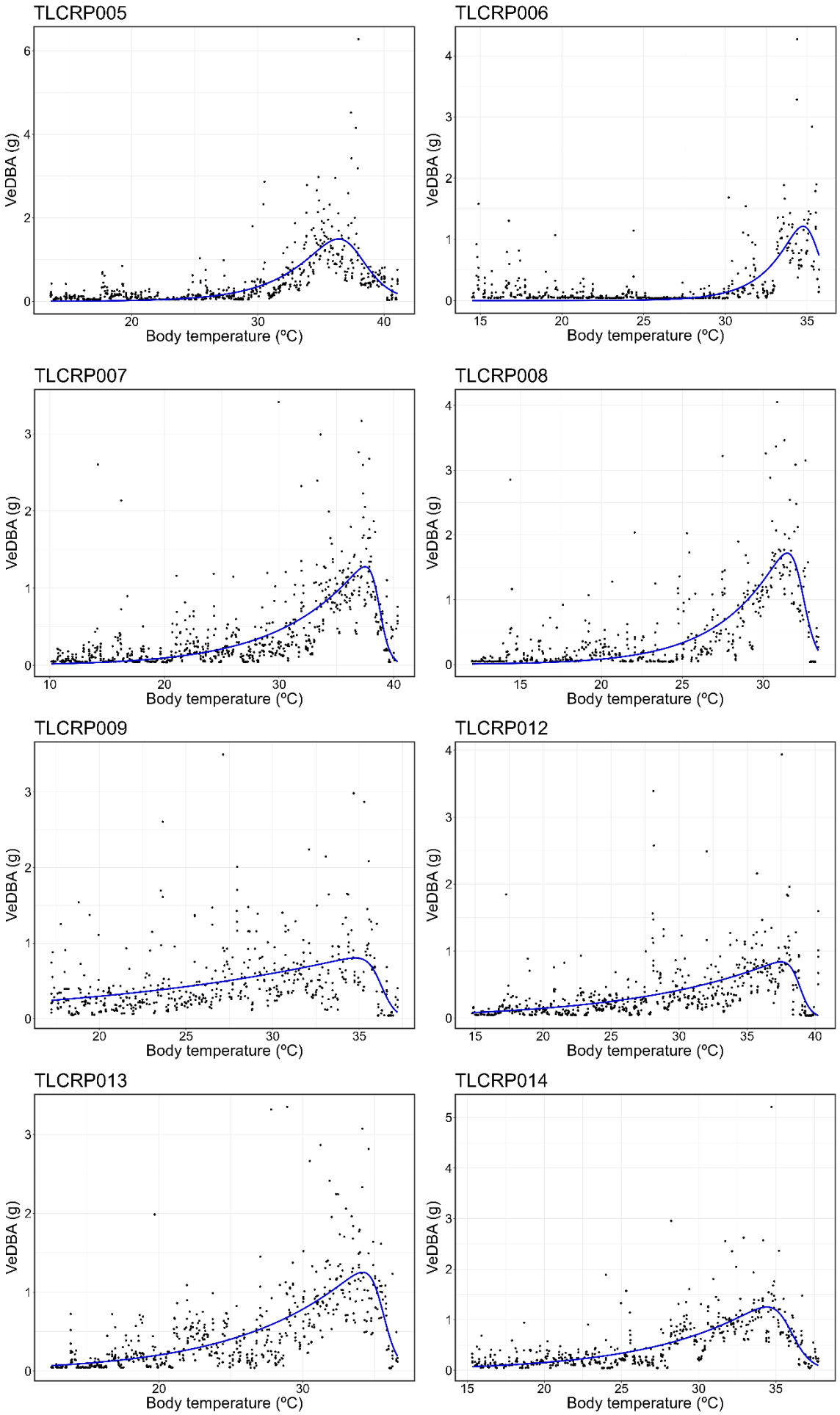


Figure S5. A comparison between the relative humidity (RH) at 90% of shade in different microhabitats of the mesocosm landscape, as predicted by the microclimate model after improved parametrization and observed by the dataloggers recording temperature and humidity in the same microhabitats; (a) humidity at rock surface, (b) humidity on soil surface, and (c) soil humidity at 15 cm of depth. The root-mean-square error (RMSE) of each prediction shows the average distance between the prediction of the model and the recorded values. The red line represents the humidity values recorded by the mesocosm dataloggers and the black line represents the predicted humidity from the microclimate model.


TLCRP005
TLCRP006
TLCRP007
TLCRP008
TLCRP009
TLCRP012
TLCRP013
TLCRP014
VeDBA (g)
Body temperature (°C)

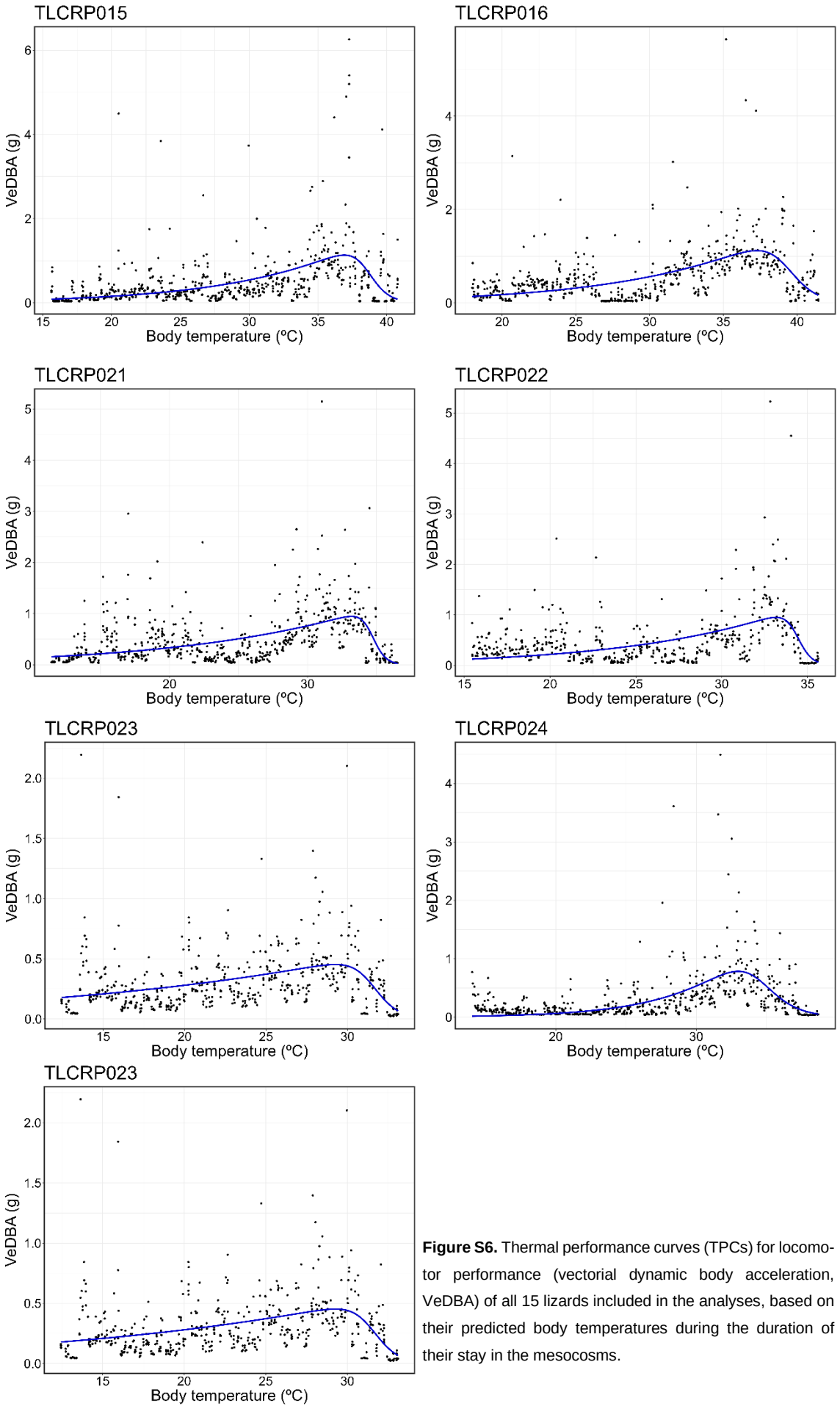


Figure S6. Thermal performance curves (TPCs) for locomotor performance (vectorial dynamic body acceleration, VeDBA) of all 15 lizards included in the analyses, based on their predicted body temperatures during the duration of their stay in the mesocosms.