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DO COGNITIVE ABILITIES VARY UNDER DIFFERENT THERMAL REGIMENS IN WALL LIZARDS (*PODARCIS BOCAGEI*)?

VOLUME 1

Dissertação no âmbito do Mestrado em Ecologia, orientada pelo Professor Doutor Miguel Ángel Carretero e pelo Professor Doutor Paulo Jorge Gama Mota e apresentada ao Departamento de Ciências da Vida da Universidade de Coimbra.

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Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção de grau de Mestre em Ecologia, realizada sob a orientação do Professor Doutor Miguel Ángel Carretero e do Professor Doutor Paulo Gama Mota.

Alexandre Domingos Correia

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A captura de 39 machos da espécie *Podarcis bocagei* e subsequente manutenção para a realização das experiências deste ano foi realizada ao abrigo das licenças no. 873 a 876/2021/CAPT concedidas pelo Instituto da Natureza e das Florestas à equipa de investigação

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Abstract

Temperature deeply shapes the biological landscape since all biological functions depend on temperature and are possible only within a certain thermal interval. However, ectotherms such as reptiles, find themselves in a special bond with the external temperatures. Most reptiles are effective thermoregulators, attaining optimal temperatures at daringly substantial periods, by means of behavioural strategies. In this context, behaviour of ectotherms is expected to be thermally optimised to enhance fitness. This should include cognition, that is, the organism's ability to acquire and process information to interact with the environment, which in ectotherms should include the thermal context. In our study we aimed to establish a link between temperature and cognition in a reptile model by investigating whether different cognitive programs exist to accommodate different body temperatures. To do so, we turned to the species of wall lizards *Podarcis bocagei*, a small and polymorphic lacertid. We devised two experiments in which the animals would be tested on cognition under two distinct thermal regimens, Cold and Hot (basking and full activity temperatures, respectively). First, in a Learned Association Test, we looked into the time lizards spent near the focus coloured Petri dish and the number of primary interactions (in previously trained lizards). Secondly, in a Prey Quantity Preference Test, two distinct pairs of prey item quantities were exposed for the lizard to choose, recording the time interacting with each choice. Our results indicate some influence of body size on time spent near the coloured focus Petri dish, but showed no differences in the thermal regimens. This suggests that a single cognitive program of male *Podarcis bocagei* is able to deal with different thermal contexts. These findings are interpreted considering the functional ecology and life history of the model organism. Perspectives for future cognition research in ectotherms are discussed.

Key words: Reptile, Cognition, Thermal Ecology, Quantity Preference, Learned Association

Resumo

Temperatura modifica severamente o panorama biológico, uma vez que todas as funções biológicas são invariavelmente dependentes da temperatura, sendo apenas possíveis dentro de um determinado intervalo térmico. Contudo, animais ectotérmicos, como os répteis, encontram-se num vínculo característico com as temperaturas do ambiente circundante. A grande maioria dos répteis são termorreguladores exímios, atingindo temperaturas ótimas com substancial rapidez através de estratégias comportamentais. Ainda neste contexto, é esperado que o comportamento de animais ectotérmicos seja termicamente otimizado para aumentar a aptidão dos mesmos. Como tal, cognição deverá estar incluída nesta otimização, isto é, a habilidade de um organismo em adquirir e processar opinião, de forma a interagir apropriadamente com o meio ambiente, deverá abranger o contexto térmico em animais ectotérmicos. No nosso estudo, procurámos determinar a relação entre a temperatura e cognição em répteis como organismo modelo, investigando assim a possibilidade de existirem diferentes programas cognitivos que acomodem diferentes cenários de temperatura corporal. Para tal, utilizámos a espécie de lagartixa *Podarcis bocagei*, que é um pequeno lagarto polimórfico. Desenhámos então duas experiências, nas quais os animais seriam testados para cognição sob a influência de dois regimes térmicos distintos, Frio e Quente (temperaturas de “basking” e completa atividade respetivamente). Primeiramente, num Teste Associação Aprendida, avaliámos o tempo passado perto da caixa de Petri colorida focal em conjunto com o número de interações primárias com a mesma, após treino prévio dos lagartos. Em seguida, num Teste de Preferência de Quantidades, dois pares distintos de quantidades de presas foram dadas aos lagartos para escolher, sendo registado o tempo em cada escolha. Os nossos resultados mostram alguma influência do tamanho corporal no tempo passado perto da caixa de Petri colorida focal, mas não se verificaram diferenças em relação ao regime térmico. Estes resultados sugerem que existe apenas um programa cognitivo machos de *Podarcis bocagei* que consegue colmatar as necessidades de diferentes contextos térmicos. Estas descobertas são interpretadas tendo em consideração Ecologia Funcional e “life history” do organismo modelo. São também discutidas perspetivas para futuros estudos de cognição em animais ectotérmicos.

Palavras-chave: Répteis, Cognição, Ecologia Térmica, Preferências de Quantidades, Aprendizagem Associativa

CHAPTER 1

Introduction

1. Introduction

1.1. Behavioural ecology and Cognition

The discipline of behavioural ecology was founded in 1986 (Gross, 1994), in order to understand why organisms behave accordingly to biotic and abiotic context. To behavioural ecologists, the term "behaviour" means much more than a simple "response to a stimulus"; behaviour refers to everything an organism does. Since then, until now, behaviour has been seen as one of the key functional components of organisms' fitness. As such, it is under selection and can evolve. But may also respond plastically to environmental challenges (e.g., relations with conspecifics, reproduction, avoidance of predators, foraging, and, in the case of ectotherms, thermal and hydric environment) (see Davies et al., 2012).

One key subject in behavioural ecology is the study of cognition. In her book, *Cognition, Evolution, and Behaviour*, (Shettleworth, 2010) defines cognition as the mechanisms of processing, storing, acquiring and acting on information given by the environment; including concepts as perception, learning, decision-making and memory. (Shettleworth, 2010) also states that studying cognition is inferring on how information is processed by analysing input-output relations with disregard for the kind or extent of the study subject awareness - consciousness and private experiences -, even though the study of consciousness (in humans, animals and similarities between both), became an active and respectable area of research (e.g., Hampton, 2005; Heyes, 2008; Staddon, 2000). A distinction in behaviour can be made between first order and higher-order processes. First-order processes comprise perceptual input, i.e., when a stimulus creates a trace in memory or an immediate response. Second order (higher-order) processes operate on top of the first-order processes in assessing the memory of an event in a given individual (Heyes, 2008; Penn et al., 2008). Only the latter of any interest when talking of cognition.

Cognition, through a combination of all its mechanisms, can be revealed as a key for survival success of a variety of species in a given habitat: taking advantage of sensory input (such as vision) to locate food and take better profit of foraging trips; memorising where food is stored during abundance seasons to use in seasons with harsher conditions (Sonnenberg et al., 2019); learning and retaining information from previous experiences with predators gaining an edge in later encounters with them (e.g., Vila Pouca et al., 2021), or learning to distinguish higher quality food by its characteristic colours; and all the decisions made during foraging, mate choice, rival recognition or during predator encounters, that in turn lead to better survival rates, reproductive success, and improved health (Dukas, 2004). The success in a specific habitat due to cognitive traits can come at a cost to said habitat when talking about invasive species; through a combination of behaviours like exploratory behaviour, boldness/shyness and flexibility in foraging behaviour (e.g., Damas-Moreira et al., 2019; Madelaire et al., 2020), a species becomes much more prone to successfully invade and outcompete native species (Chapple et al., 2012). In order to understand how such species can establish and spread should not overlook the behavioural traits of the invasive species, since these can provide a greater ability to predict possible invasive species and spread one patterns (Carere & Gherardi, 2013). Furthermore, the study of cognition can also aid in the protection of different species and environments by providing insight regarding species interactions with human-modified environments, since these introduces new threats like ecological traps and challenges (Burunat-Pérez et al., 2018; Griffin et al., 2017; Patten & Kelly, 2010; Ward-Fear et al., 2016). Therefore, understanding the cognitive processes of a species, and how these processes might be impaired by human activity or anthropogenic environments, as well as how they can facilitate the integration and spread of a certain species in these novel environments gains great relevance (Griffin et al., 2017).

1.2. Reptiles and Cognition

When looking to understand the evolution of cognition, it is of the utmost necessity to evaluate and examine cognitive abilities across a broad range of vertebrates. Non-avian reptiles are a heterogeneous assemblage of groups paraphyletic to birds and mammals, taking advantage of thermoregulation to maintain body temperature (Vitt & Caldwell, 2014).Both ectotherms and endotherms are dependent on thermal environment. However, ectotherms must behave under more variable conditions, being able to produce phenotypic variation in the population dependent on temperature fluctuations, making them really interesting to study in this context (Matsubara et al., 2017). Even though ectotherms have to behave under changing thermal regimens in contrast with endotherms, this does not mean that the latter are independent from thermal environment. In those cases, effects mostly act throughout associated costs, for instance thermal stress (Soravia et al., 2021).

Since reptiles show such a substantial difference in their development patterns from mammals and birds, they can be used to investigate factors that shape cognition that cannot be investigated in endotherms (Matsubara et al., 2017). The study of similarities and differences between vertebrate groups in the performance of the same tasks, as well as the study of brain structure and function, pose a great possibility for better understanding of the evolution of cognitive abilities(Matsubara et al., 2017). Reptiles can play a very important role in the study of homologies and analogies of cognitive mechanisms between all amniotes (Fitch et al., 2010).

Even though an increase in the diversity of species being studied has been seen, the bias towards the study of mammals and birds remains (Shettleworth, 2009), as well as a lack of investigation of reptiles (Roth et al., 2019). However, when tested, reptiles show an impressive repertoire of cognitive abilities (e.g., Leal & Powell, 2012; Mueller-Paul et al., 2014; Wilkinson & Huber, 2012) (Matsubara et al., 2017). Nonetheless, it is worth mentioning that studies with lizards have risen and doubled their number since 2012 (Szabo et al., 2019). The growing literature has been raising questions underlying cognitive performance to be asked through the lenses of reptile models. Consequently, bringing forth the potential of reptiles for addressing questions fundamental for cognitive ecology (Szabo et al., 2020). Furthermore, reptiles were regarded in their majority as being precocial, having at birth a considerably more developed brain in comparison to altricial species, resulting in impacts to their learning abilities at an early life stage (Szabo, Noble, et al., 2019). A distinct way in which reptiles can help answer fundamental questions central to cognition ecology, is the study of social behaviour, being grossly viewed as solitary, rather "asocial" animals (Szabo et al., 2020). However, beyond the scope of cognition, social behavior is well studied in many reptiles, but focusing on reproductive and territorial interactions (e.g., Brattstrom, 1974; Mason & Parker, 2010; Mc Coy, 2003). Since a large body of studies is focused on social vertebrates (Galef & Laland, 2005), further research should be made with reptiles that in have been found to learn socially (e.g., Damas-Moreira et al., 2018; Gavriilidi et al., 2022; Szabo et al., 2020).

Reptiles are known for inhabiting rapidly changing and extreme environments (e.g. Díaz de la Vega-Pérez et al., 2019; Dunlap & Wingfield, 1995; Espinoza et al., 2004), which has been shown to have a link with selection for complex cognition (Rodrigues & Kohlsdorf, 2019; Roth et al., 2010). Besides, changing environments may end up modifying cognitive abilities via developmental plasticity, although these shifts do not always come as adaptative shifts. The fastest responses to environmental changes are based on phenotypic plasticity, while selection and adaptation will act long-term. However, mechanisms for plasticity also depend on genes. On the other hand, many reptiles, including *Podarcis bocagei*, are good thermoregulators. That means they can maintain their body temperature relatively stable when active, if the environment is not too restrictive (Chevin et al., 2010; West-Eberhard, 2003). Taking further into account the environment in which reptiles live, specially under the climate change threat, studying reptiles can provide further understanding of developmental plasticity of cognitive abilities (Refsnider & Janzen, 2012). Since rapid changes in temperatures might become too fast for genetic plasticity to account for, behavioural plasticity may account for acclimatization to new environment in shorter time accompanying those changes, by for example females choosing adequately where to lay their eggs in order to diminish the effect of temperature in sex ratios due to temperatures (Refsnider & Janzen, 2012). However, is worth mentioning that reptiles in itself is a paraphyletic group, and that some representatives of different reptile groups display thermal sex determinations, but many others present chromosomic sex. What is common throughout the different reptile groups, is that oviparous females carefully select the nesting places. On the other hand, viviparous females are extremely careful when thermoregulating accordingly to the requirements of the embryos (Carretero et al., 2005).

1.3. Reptiles/Temperature Relationship

Temperature has a profound and ubiquitous effect in developmental processes and biological functions (Angilletta Jr & Angilletta, 2009; Feiner et al., 2018). Since temperature can function as a catalyst, when it decreases so does the velocity of biochemical reactions and the rate of growth and differentiation of growth and differentiation (Angilletta Jr & Angilletta, 2009; Gillooly et al., 2002). Focussing on ectotherms , the dependence of an external source of heat makes them uniquely sensitive to different temperatures required for different life processes (Forsman, 2000). Although many aspects of the life history of reptiles is deeply affected by temperatures (due to physiology needs like energy usage), many species display fine-tuned thermoregulation, and as many ectotherms rely on behavioural thermal regulation (especially terrestrial ectotherms/reptiles) so that they can achieve and maintain physiologically optimal temperature for long periods, thus having behavioural thermal regulation as a buffer to temperature changes, even in the face of global warming, given a heterogenic thermal environment is present (Huey et al., 2012; Shine, 2005).

During gestation, reptiles are able to develop under a floating range of temperatures; this fluctuation affects the phenotype of the hatchlings including sex, physiology, growth rate, morphology, locomotion, pigmentation, and behaviour (Abayarathna & Webb, 2019; Booth, 2006; Deeming, 2004). When referring to thermal traits specifically (like basking time behaviour or temperature tolerance), development plasticity seems to be at the core, since temperatures experienced during embryonic stages can predict temperatures that the individual will experience during its lifetime. Studies have shown that different effects in early life-stages might impact fitness, however the effects of the temperature experienced during embryonic development on later life-stages is still understudied (Refsnider et al., 2019).

Temperature may also come into play in influencing individual cognitive abilities (Amiel et al., 2017; Clark et al., 2014), which may lead to later implications in the fitness of the individuals (Matsubara et al., 2017). Different species of reptiles will perform differently in cognitive tasks, after being hatched in opposite temperatures deemed “cold” and “hot” (Amiel et al., 2014; Clark et al., 2014). Later studies show that incubation temperature seems to only have a short-term effect; with some time passed, incubation temperature seems to not have any long-term effect on cognitive abilities (Siviter et al., 2017). Other important aspect is that normal brain functionality depends on temperature and ceases at low temperatures (reaching 0ºC) in ectothermic vertebrates (Lillywhite et al., 2016). One important aspect to keep in mind is that there’s still an inconsistent terminology to what is considered “hot” to reptiles (Abayarathna & Webb, 2019). Nevertheless, a good approach the inconsistency in terminology of “hot” or “cold” temperatures is through thermal reaction norm concept (Angilletta, 2009). A temperature should be deemed too “hot” / “cold” when a labile trait (e.g., running speed, growth rate) becomes negatively impacted by the external factor temperature, always taking into account the thermal plasticity of the study species (Angilletta, 2009).

1.4. Study Species

This study was preformed using Bocage’s wall lizard, *Podarcis bocagei* (Seoane, 1884), belonging to the Animalia Kingdom, Chordata Phylum, Reptilia Class, Squamata Order, Lacertidae Family and *Podarcis* Genus. *P. bocagei* is a small sized lizard, nonetheless slightly bigger than other sympatric and parapatric species of the same Genus, reaching in average the adult Snout-Ventral Length of 57 mm, for the males, and 55 mm for the females (Galán & Milla, 2015). Being sexually dimorphic, mature males have larger heads and shorter trunks than females and brown flanks and a- green dorsum. Their bellies are usually yellowish, although they can also present orange/red, yellow or white (Galán & Milla, 2015). Mature females and juveniles are mostly brown on the dorsal region and have the same ventral colours as the males. Both sexes have light coloured continuous lines along the dorsolateral regions (Galán & Milla, 2015). Endemic to the north-western part of the Iberian Peninsula; especially abundant in Galicia and

Minho and Douro Litoral regions in Spain and Portugal respectively (Galán & Milla, 2015), this species is considered an Atlantic species, and occupies a large variety of habitats: dunes, grasslands, walls (in urban or rural areas), and rocky terrains (Ferreira et al., 2016; Galán & Milla, 2015). The International Union for the Conservation of Nature (IUCN) attributes the status of Least Concern (LC) to *P. bocagei* (IUCN, 2008). *Podarcis bocagei* is mostly an active hunter, stalking prey during foraging, however, it uses sit-and-wait foraging, when basking. Consequently, its diet is composed of a wide spectrum of various Arthropods (preferring arachnids); although it does not consume too small and/or too mobile prey (Galán & Milla, 2015).

Both adults and immatures of this species may remain active all year, but in the winter, restrict to midday, Nevertheless, many individuals may seek refuge and ~undergo brumation (ectotherm inactivity). Especially deeper into continental lands they become lethargic in the colder months, usually between March and October (Galán & Milla, 2015). In the areas with mild climate, such as coastal Portugal, males and females of *P. bocagei* has its sexually active season generally from late March to the beginning of late July (Ribeiro et al., 2006), resulting in each female (depending on body size) laying three clutches yearly, from April to July (with decreasingly numbers of eggs per clutch) (Galán & Milla, 2015). In the reproductive season, males will form a hierarchical chain with bigger individuals on top (Galán & Milla, 2015). *Podarcis* spp. males maintain their testosterone levels stable, during copulating season, so their behaviour is not influenced by drastic fluctuations. In females, it is hard to assess if they are pregnant or if they are post parturient (gravidity can be assessed by palpation and recent egg-laying can be assessed by observing flank folds), and their hormones fluctuate in each reproductive stage, therefore there is the risk that their behaviour can be greatly influenced by these fluctuations.(Damas-Moreira et al., 2019).

When possible, *Podarcis bocagei* will maintain their body temperature around ca. 33 ºC, if there is water available, but they will select ca. 29 ºC if water is not available, if females are pregnant or if they are immatures, to restrict dehydration (Ferreira et al., 2016; Sannolo & Carretero, 2019). It is expected that individuals from this species experience colder temperatures in their more active seasons (spring or early autumn) as well as the mornings of summer, while doing their daily foraging routine.

1.5. Objectives

By further exploration of the unique link between temperature and reptile behaviour, this study aims to better understand the cognition capabilities of reptiles within a thermal ecology framework, specifically in Bocage's wall lizards (*P. bocagei*); this will be evaluated by experiments of quantity assortment and colour association memory.

The working hypothesis is that individuals of the species *P. bocagei* will display more complex cognitive repertoire under increasing thermal regimes, closer to their physiological optima. In particular, it is expected that under colder thermal regimens they I) will have low discrimination of prey quantities (because they will be less likely to repeat predation acts when cold) ; II) will display learned abilities (because cold lizards will be less able to perform complex capturing strategies); and in both regimens you would expect that individuals with small size and low body condition will show less effect of temperature regimen (because weak animals will be more likely to display a simplified cognitive behaviour regardless of the temperature).

CHAPTER 2

Materials and Methods

2. Materials and Methods

2.1. Sampling Procedures

Due to the reasons stated in the previous chapter, regarding stronger fluctuations in female's body condition and hormones (Carretero, 2006), only males of *P. bocagei* where collected. To do this, a noose technique was employed, allowing the capture of multiple animals in one field trip, this technique is executed by using a small adjustable thread noose at the tip of a rod (see Sillero & García-Muñoz, 2010), thus, allowing for fast captures and minimizing the risk of autotomy of the tail due to stress. On the 25th and 26th of May of 2021, thirty males of *P. bocagei* with different belly colour morphotypes were caught: ten orange, ten yellow, and ten white. We deemed colour morph a relevant factor to take into consideration because it has been demonstrated to influence the behaviour of *Podarcis* sp. (Abalos et al., 2016; Coladonato et al., 2020; Perez i de Lanuza et al., 2017; Pérez i de Lanuza et al., 2013, 2016; Sreelatha et al., 2021). All males were caught in Porto region, 14 of these males were caught in Gião (40° 58' 50.259'' N 8° 28' 41.834'' W), along agricultural walls; Sixteen were caught in Vairão on the agricultural walls around and within the Entre Douro and Minho Agricultural Museum (41° 19' 32.774'' N 8° 40' 30.506'' W). These 30 males were the ones used in the main tests. For the temperature pilot test, on the 4th and 5th of July of 2021, eight more males were caught with the same noosing technique on the agricultural walls around and within the Entre Douro and Minho Agricultural Museum: three orange belly, four yellow belly, and two white bellies. In total, we used 39 male individuals for this study.

2.2. Morphometry and Husbandry

Once in the laboratory, all males had the Snout-Ventral Length (SVL) measured using a digital calliper (precision 0.01 mm) and were weighted on a precision scale (precision 0.0001 g). All animals were assigned individual codes.

All males were put in an individual plastic terrarium (30 x 19.5 x 20.5 cm). Each terrarium had one side covered with a paper sheet in such a way that the animal inside could not see the neighbouring animals, reducing stress. Besides, in every terrarium existed three distinct spaces: the basking spot with a brick piece; the refuge spot, that consisted of two stacked tiles with three edges, covered with sponge, making a thin opening (Abalos et al., 2016); a space in between the basking spot and the refuge, where there was a bottle cap with water so that the animals could drink, that was the same spot where we dropped the mealworms before the start of the colour association test (see Figure 1). All the terrariums were exposed to a diurnal natural light and the artificial lights (heating lamps) were turned off after 17:00 h, so that the animals experienced a natural light cycle. Each terrarium was also exposed on the basking side, to a 150 W infrared lamp for heating. However, each morning, before the trials, animals were only exposed to daylight. If the animals had no trials on that day, the lamps would automatically turn on at 10:00 h and off at 17:00h. If the animals had trials, the heating lamps would only be turned on at end of the trials (which was between 13:00 h and 15:00 h). The laboratory was kept at a room temperature of 19 ºC/20 ºC daily, via air conditioning.

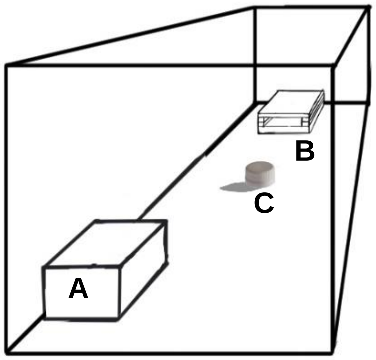

Figure 1. Disposition of the terrarium during acclimatization to the enclosure period, temperature control pilot-test~~, ~~and Prey quantity preference test. The A letter marks the brick piece used by the males for basking; B refers to the refuge tiles; C is the water bottle cap that contained the drinking water.

The 30 males had a period of one week of acclimatization before trials and got accustomed to eating mealworms (*Tenebrio molitor*). The extra nine males used on the pilot test had a smaller period of acclimatization of two days, being also fed mealworms since the behaviour was not evaluated.

All animals were tested in eight identical arenas (50 cm (length) x 30 cm (width) x 40 cm (height). Made with Perspex® acrylic sheets, these arenas had a removable separator (opaque PVC sheet) in the middle, that was lifted when the trial started, after the thermoregulation period. Furthermore, each arena was covered in white opaque plastic so that the animal inside could not see the male in the arenas next to it.

All trials had a duration of 15 minutes: 10 minutes for thermoregulation, and five minutes to perform the test. This time window was chosen because it is expected that given more time, the animal inside the arena will thermoregulate approaching the preferred temperature. Furthermore, at the end of each trial, the same person took a thermal image of the animal, to minimize inter-observer variances. The image for the temperature control test was taken at four separate times (0 min, 5min, 10 min, 15 min). To do these, a thermal camera (model FLIR T335; lenses FOL 18mm) was used. Every image was then processed using the software FLIR Tools 6.4 (2022 © Teledyne FLIR LLC All rights reserved.; http://www.flir.com), where the temperature taken from the dorsal zone and one of the eyes was measured.

2.3. Tests Performed

2.3.1. Temperature control pilot-test

Since there is no consensus on the broad definition of what “cold” temperatures are for reptiles (Abayarathna & Webb, 2019), we ran a pilot test to ensure different, but realistic, temperature of the lizards during the experiments. Firstly, the temperatures were considered Cold when significantly below the preferred temperature, but still possible to be recorded for animals observed in the field. Since *P. bocagei* males preferred body temperatures around 32 °C, in thermal gradients (Ferreira et al., 2016; Sannolo & Carretero, 2019) and minimal temperature recorded in the field was 22 °C, we selected a range of temperatures between 22 ºC and 25 ºC as "Cold" and a range of 29 ºC - 33 ºC, as "Hot". Remarkably, running speeds of the lizards differ by 20 %, between both thermal regimens (Sannolo, 2019). No lizards were tested under a regimen hotter than the preferred temperature, because, even if they survived, they could approach Critical Thermal Maximum and suffer a high increase in dehydration. (Sannolo & Carretero, 2019),

The temperature pilot test was therefore designed to decide on which heating lamps and at how high they should be placed at, above the arena in such manner that it generated a clear difference in the temperatures deemed "Cold" and "Hot" in the temperature brackets referred previously (ca. 22 ºC/25 ºC and ca. 29 ºC/33 ºC).

To assess what the most adequate lamp wattage should be used, four different kinds of lamps were used with four different wattages: 100 W, 70 W, 60 W, 46 W; also, each lamp was tested at two different heights (from the ground of the arena): 65cm and 45cm. The tests were conducted on the 7th, 9th, 11th, 14th, and 17th of June of 2021.

Despite the small sample, after making a boxplot graph using package ggplot2 on RStudio (RStudio, 2022; Wickham, 2016), and analysing the means and standard deviation, we were able to ascertain the best height and wattage to do the other tests. For the "Hot" temperatures the chosen lamp was the one with 100 W, at a height of 45 cm (Eye temperature at 10 min: mean = 29.7 ºC, s. d. = 1.87; Eye temperature at the 15 min: mean = 31.2 ºC, s. d. = 1.97; Dorsal temperature at 10 min: mean = 32.5 ºC, s. d. = 2.17; Dorsal temperature at the 15 min: mean = 33.8 ºC, s. d. = 2.7). For the "Cold" temperature the lamp chosen was the one with 60 W at a height of 65 cm (Eye temperature at 10min: mean = 22.5 ºC, s. d. = 0.60; Eye temperature at 15 min: mean = 22.5 ºC, s. d. = 0.97; Dorsal temperature at 10min: mean = 23.8, s.d. = 1.0; Dorsal temperature at 15 min: mean = 24.2 ºC, s. d. = 1.3). This test began at room temperature, as would happen in the main tests.

In these trials the animals were recorded using a CCTV system as would happen during the other tests. Videos were scored blindly, after experiments were completed. In each trial, animals were put in the arena separated by a white opaque PVC sheet, that was later used to separate the animals from the test, during trials of the other tests (Figure 2).

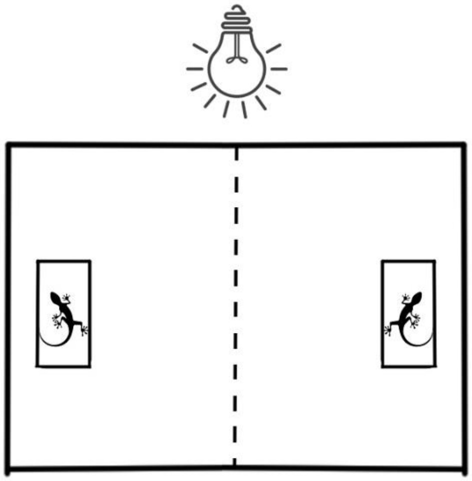

Figure 2. Disposition of the animals in the arena during the Temperature control pilot-test

2.3.2. Prey Quantity Preference Test

The prey quantity test was performed in order to ascertain if: 1) *P. bocagei* males to discern different quantity of prey; 2) there was difference in preference of prey quantity under different thermal regimens, exposing the plasticity of hunting behaviour to accommodate the thermal demands of the individual (being the answer to the first question true).

The basis of this test was the experiment done with *P. sicula* by Petrazzini *et al.* (2017, 2018). Similarly, to what was done in that experiment, the animals were exposed to different quantities of prey inside petri dishes, equidistant to the starting point where the animal was placed in the arena. In our experiment, we placed two quantity pairs: the first pair had one mealworm inside one petri dish and five mealworms inside the other ("1vs5"); the second pair had two mealworms inside one of the petri dishes and three mealworms inside the other ("2vs3"). All mealworms were small and had similar sizes. Since *Podarcis sicula* is able to discriminate prey sizes, but showed an abnormal difficulty in discriminating quantities compared to other vertebrates (Petrazzini et al., 2017); we expected the same abilities and difficulties to appear in other *Podarcis* spp. We must also consider that *P. sicula* has demonstrated to be more proactive than other *Podarcis* sp. which make it better invader and competitor even compared with P. bocagei (Damas-Moreira et al., 2018). We also expect that in the case of a preference in prey quantities, such difference would be more pronounced in the pair "1vs5" than in the pair "2vs3", considering that the ratio of quantities is bigger in that pair, since the ability to discriminate is enhanced by bigger disparity between prey quantities presented (Petrazzini et al., 2018).

The test started on 18th of June of 2021 and ended on the 26th of June of 2021. During the experiment run time, the animals were separated into equal groups, one group was experimented with one pair of quantities and the second group was experimented with the other pair. Both groups were experimented on the same day, however only one temperature regimen was tested per day, either Cold (60 W lamp, 65 cm heigh) or Hot (100 W lamp, 45 cm high), having one day in between which the animals were not fed for motivational purposes, since it is a well-known fact that reptiles can go without food for sizeable periods of time (e.g., Bustard, 1967; Lueth, 1941; Manrod et al., 2008). All animals performed only two trials randomly selected by individual, one for each temperature, to make sure that the animals did not learn that they could not get to the prey items.

The trials lasted for 15minutes (as in the temperature control pilot-test) and started by placing a male in the arena and one of the halves of the refuge (8 males and arenas simultaneously), that acted as starting point and a familiar place to the animals to minimize the effect of neophobia of the arena. At this point a white PVC opaque sheet would block the passage to the interaction zone, where two small plastic petri dishes (38.95 mm wide and 9.69 mm high) would contain the mealworms with the respective pair amounts (either "1vs5" or "2vs3"), the placement of the quantities was randomized between A or B, thus minimizing side tendencies (Figure 3). Each Petri dish was sealed with transparent tape and the males could not assess the mealworms, so that the animals didn't only attack and eat he first amount that they entered in contact with.

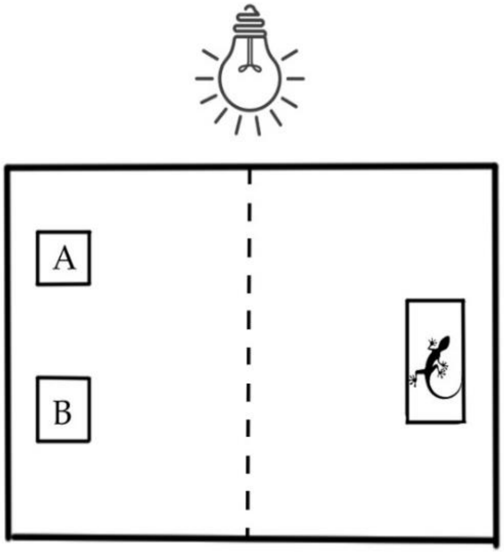

Figure 3. Prey quantity preference test set-up. “A” and “B” refer to the placement of small petri dishes with the mealworms; the dotted line in the middle shows the placement of the PVC sheet barrier; the lamp was directly above the barrier heating both sides of the arena at the same time.

For the first ten minutes, the barrier was kept in place as in the temperature control pilot test, according to the Cold (60 W lamp, 65 cm high) or Hot (100 W lamp, 45 cm high) set up, allowing the animal to thermoregulate and reduce neophobia of the arena itself. After the ten-minute mark, the PVC sheet was removed and the trial lasted five more minutes, where the animals could interact with the petri dishes. At the end of each trial, a thermal image was taken and everything (arena and petri dishes) was cleaned with ethanol (95 %), so that the acute sense of smell of these animals (Galán & Milla, 2015) did not detect the presence of another male, which could alter their behaviour in between trials. When all trials were finished, the animals were fed two mealworms in their individual terrarium.

All trials were recorded using a CCTV system. These recordings were edited using both GIMP (The GIMP Development Team, 2021) and Kdenlive (Mardelle et al., 2022) software, in which an area of 0.5 cm extra was added to the petri dishes. This area was used to record the time spent interacting with the respective petri dish when any part of the head of the lizard was inside said area, therefore being a proxy for interest; to record this time the software BORIS (Friard & Gamba, 2016) was used.

2.3.3. Learned Association Test

This test was performed in order to assess the effects of colder temperatures on the ability of male *P. bocagei* to perform a learned behaviour, where food was associated to a coloured small petri dish. Lower temperatures having an effect on such ability as learned information, colder temperatures may show itself detrimental to fitness as well as show an underlying less advanced behavioural “program” for these lizards.

For this test, a similar design to that in Damas-Moreira *et al.* (2018) was made, not only because they also used *P. bocagei* as representative of native species, but also, they attributed the ability to acting on learned information as beneficial to fitness and even a likely explanation to the success of the invasive *P. sicula*. tested.

Before starting this test, the basking brick piece in each individual terrarium was replaced by a little platform made with white PVC sheets, and the different parts were made by cutting the PVC sheets in pieces and then gluing everything together. The PVC platforms were ≈ 1.5 cm tall, ≈ 6.8 cm wide and ≈ 14.8 cm in length. On top of these platforms, using transparent tape, three small plastic petri dishes (38.95 mm wide and 9.69 mm high) were glued. Each of these petri dishes was wrapped around with a plasticized coloured cardboard ring, being the chosen colours blue, red and yellow. Each ring was large enough so that the animal could not see the contents inside unless peeking through the top (Figure 4). The blue colour was chosen as the one where the mealworms were placed, the other colours acted as decoy. Furthermore, blue is a rare colour in nature and these animals do not express this colouration (Galán & Milla, 2015). This new terrarium set up was put in place in order to minimize the neophobia towards the identical set up that was placed in the trial arenas.

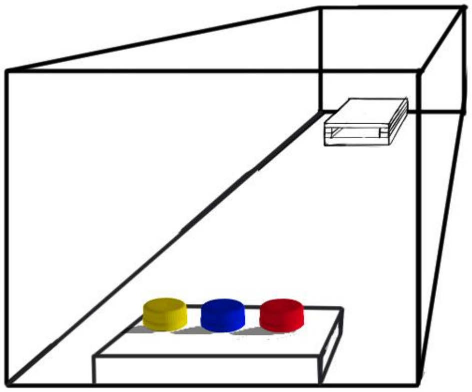

Figure 4. Disposition of the terrarium during the Learning association test.

The first part of this trial was training the animals to associate the blue coloured petri dish with food. The aforementioned training was executed by placing each animal in the arena and running a mock test. It started by putting each animal (that were at room temperature) in the arenas with the 100 W lamp at 45 cm, in the Hot set up as tested in the temperature pilot test, being the lamp already turned on when they were put inside; furthermore, the animals were simultaneously put in the arenas in rounds of eight (one by arena) and the refuge tiles were also put inside to act as a safe and starting point. The first ten minutes of the test were dedicated to thermoregulation and acclimatization. During this time the barrier of white PVC sheet obstructed the passage and view to the platform with the coloured petri dishes (Figure 5).

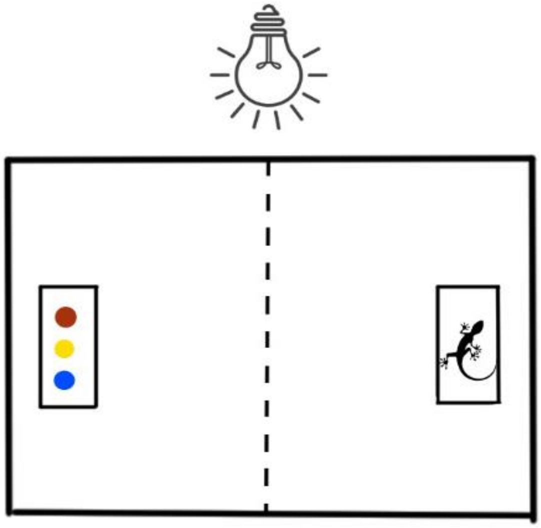

Figure 5. Learned association test setup. The coloured petri dishes and the PVC platform in opposition to the male and refuge half; dotted line in the middle shows the placement of the PVC sheet barrier; the lamp was directly above the barrier heating both sides of the arena at the same time. This would be the set-p for the learning trials and the actual trials

During training, in the blue coloured Petri dish was placed a small mealworm; this size was chosen to prevent animals to be full, hence, unmotivated in the next days (normally after eating a big mealworm they would not eat in the next day). At the ten-minute mark, all barriers were removed, giving time for the animal to interact with the novel set up. Five minutes after the training, trial stopped and thermal image was taken. If the animal ate the mealworm inside the blue petri dish, it would not get more food for the day. If that was not the case the animal would receive, in the blue Petri dish inside the individual terrarium, the mealworm that it had not eaten. In addition, in each trial, the position of the blue Petri dish on the acrylic table was exchanged with other colours to eliminate the possibility of the learned behaviour being the spatial positioning of the blue petri dish. At the end of each trial, the arena was cleaned thoroughly with ethanol (95 %) before the start of the trials for the next set of animals. This was needed for the acurate chemosensory ability of these animals (Barbosa et al., 2005, 2006; Cooper, 1991; Cooper & Perez-Mellado, 2002; Galán & Milla, 2015; Van Damme & Quick, 2001), since the next male could detect the existence of the previous male and alter their behaviour. These mock trials lasted for nine consecutive days, from the 28th day of June to the 6th of July of 2021. At the end of the nine-day period, we deemed the animals trained, since the animals seemed to be losing interest in the novel apparatus and the number of successes, which was having the first interaction with the blue petri dish in the arena, seemed to be decreasing after the day seven. During the training one of the animals (PBW12) died overnight (5th of July of 2021), which later we found that it had an abnormal number of blood parasites (haemogregarines). Stress rather than the parasites themselves is the likely cause of death, since haemogregarines may respond opportunistically to immunosuppression due to environmental or social factors (Arakelyan et al., 2019), being this infection a common one *P. bocagei* (Roca & Galdón, 2010).

The start of the actual trials was on the 7th day of July and lasted until the 12th of July. In these trials there was no food in the blue petri dish during the trial, thus allowing us to only evaluate the effect of the learned behaviour (blue petri dish = food), without possible interferences of prey smell and sound. Half the animals passed the trial under the Cold set up (60 W lamp, 65 cm heigh) and the other half under the Hot set up (100 W lamp, 45 cm high). Always beginning the trials with the Cold set up in order to minimize the risk of the influence of the Hot set up on the overall temperature of the arenas. Like in the mock trial, the placing of the blue petri dishes on the acrylic table was also exchanged with the other colours throughout the days. At the end of each trial, each arena was cleaned in the same manner as in mock trials, before the next set of trials, and a thermal image was taken, to measure the body temperature of the animal. In addition, there was a 30-minute pause, with the lights turned off, between the Cold trials and the Hot trials, so that the beginning conditions of each set of trials was similar, in terms of arena temperatures. At the end of each experiment, the animals were fed on the blue petri dish inside the individual terrarium a small mealworm.

At the end of these test, CCTV footage was edited using software Kdenlive (Mardelle et al., 2022) and GIMP (The GIMP Development Team, 2021) making an “area of interaction” around the petri dishes of 0.5cm away from the edge of the petri dish)where the time would be recorded when any part of the head of the animal entered the delimited area; this measure would be synonymous with interest by animal with respective coloured petri dish. The recording of time inside the area of interaction and the success behaviour was made using the BORIS software (Friard & Gamba, 2016).

2.4. Statistical Analysis

2.4.1. Learned Association Test

All statistical analyses were done using SPSS (IBM Corp., 2017) and RStudio (RStudio, 2022) with the packages: IPSUR Plugin for the R Commander (Kerns, 2019); lme4 (Bates et al., 2015, p. 4); Envstats (Millard, 2013); pastecs (Grosjean & Ibanez, 2018), BSDA (Arnholt & Evans, 2021). For the graphs the packages ggplot2 (Wickham, 2016) and ggpubr (Kassambara, 2020).

The response variables assessed in this test were time spent in the previously mentioned area of interaction of the blue petri dish and if the blue petri dish was the first interaction that they had with the novel set up.

Body size (SVL in this case) a good predictor of foraging, regardless other factors (Amaral et al., 2012). Analysis was based on three models of Generalized Linear Mixed Models (GLMM). Thermal regimen and morph as fixed factors, individual as random factor, and SVL as continuous predictor, because body size is a good predictor of foraging, regardless other factors (Amaral et al., 2012).

The first dependent variable was the time spent in the area of the blue petri dish, for this we did a small transformation, dependent variable was added 1 to deal with zeros form the Gamma distribution. The second dependent variable is the number of “primary interactions with the novel apparatus being with the coloured Blue petri dish”, on which we did a Poisson GLMM (Logarithmic Link Function) The first model was GLMM model for Poisson probability distribution with logarithmic link function and had as the baseline factors for comparison the morphotype Yellow and the temperature Hot. The second model was a GLMM for Gamma probability distribution with identity link function. Like the previous model, this model had as the baseline factors for comparison the morphotype Yellow and the temperature Hot. The last model is also a GLMM for Gamma probability distribution with identity link function. This last model for this experiment looks into the relationship between time spent in the area of interaction of the blue Petri dish considering temperature and SVL. As in the previous models, the baseline temperature for comparison is the Hot temperature.

The time variable was tested for normality with Shapiro-Wilk normality test, which showed that it deviated significantly from a normal distribution (W = 0.78708; p = 0.000368).

2.4.2. Prey Quantity Preference Test

All statistical analysis was done using SPSS (IBM Corp., 2017) RStudio (RStudio, 2022) with the packages: IPSUR Plugin for the R Commander (Kerns, 2019); lme4 (Bates et al., 2015, p. 4); Envstats (Millard, 2013); pastecs (Grosjean & Ibanez, 2018), BSDA (Arnholt & Evans, 2021). For the graphs the packages ggplot2 (Wickham, 2016),rstatix (Kassambara, 2021) and ggpubr (Kassambara, 2020).

The response variable in this test was time spent in the mentioned area of interaction around and including each of the Petri dishes. This variable that we called Time Difference is a transformation of data. This time variable is the result in the difference between total time spent on the Petri dish containing the higher number of mealworms minus the total time spent on the Petri dish containing the lower number of mealworms (for both pairs of quantities). Therefore, a negative value means that the male *P. bocagei* spent more time in the area of the Petri dish containing the lower number of mealworms, and vice-versa. This transformation allowed us to compare the time spent in either quantity independently of the tested pair. Only time of interaction was used since were trying to extract a cognitive response with minimal effect of movement, which would be directly influenced by temperature via metabolism and muscle contraction. We performed a Shapiro-Wilk normality test that showed that this variable did not deviate from a normal distribution (W = 0.94721; p-value = 0.09299)

The last step in the statistical analysis was to perform a Generalized Linear Mixed Model (GLMM), using an Identity Link Function, for all the variables. The personal codes (ID) of the animals were put in the model as a randomized factor, the time difference variable was the response variable. Morphotype of the animal, SVL variable and quantity pair variable were introduced in the model as fixed factors. This GLMM model had as the baseline factors for comparison the morphotype Yellow, the temperature Hot, and the quantity pairing of 2vs3.

CHAPTER 3

Results

3. Results

First, we should consider that the experimental design was effective to induce stable, separate temperatures separated creating to distinct thermal regimens for all the animals in both tests (Figure 6, Figure 7 and Figure 8). This was the result of the Temperature control pilot-test.

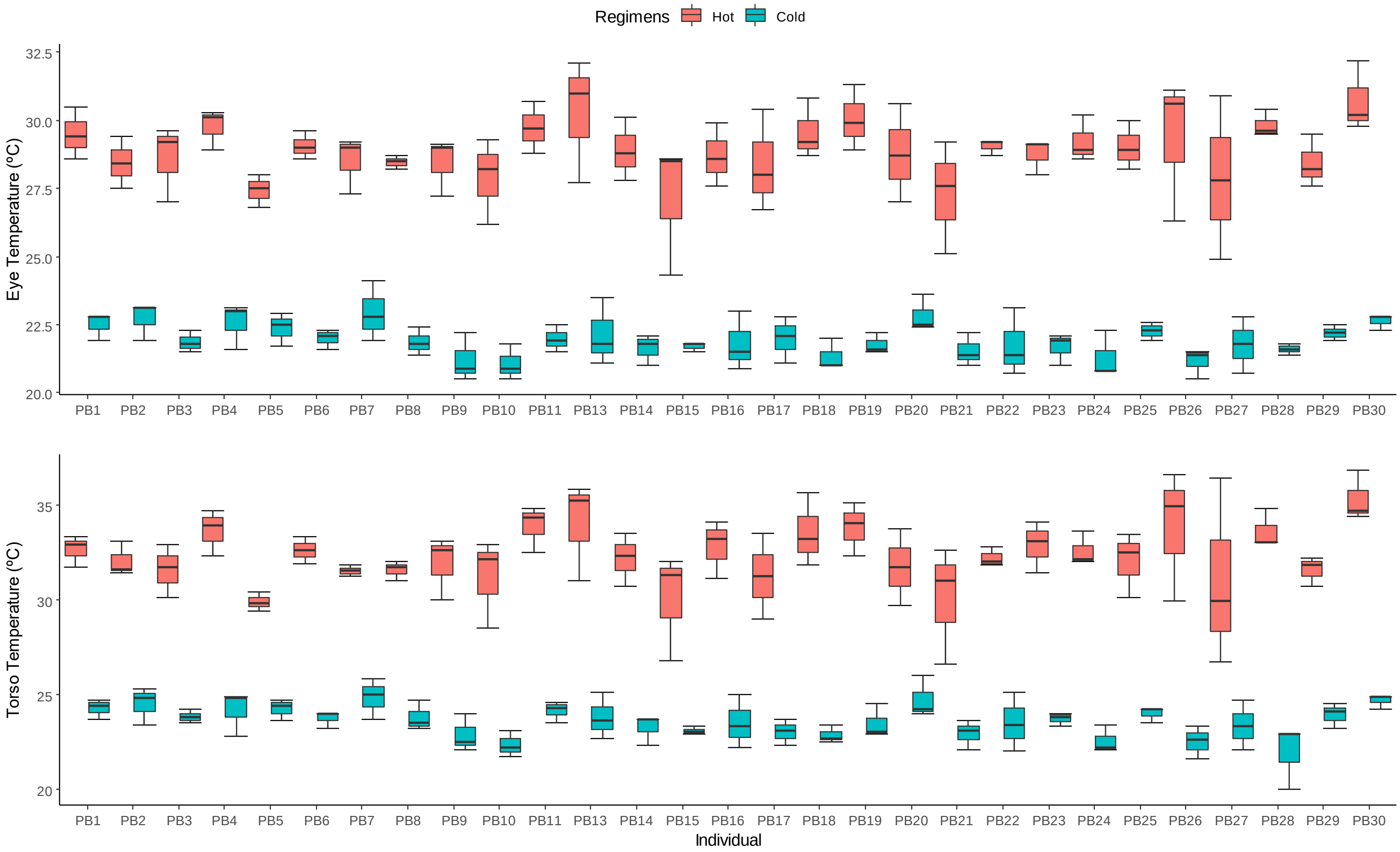

Figure 6. Boxplot for the temperatures pe individual during the trials of the Learned Association Test

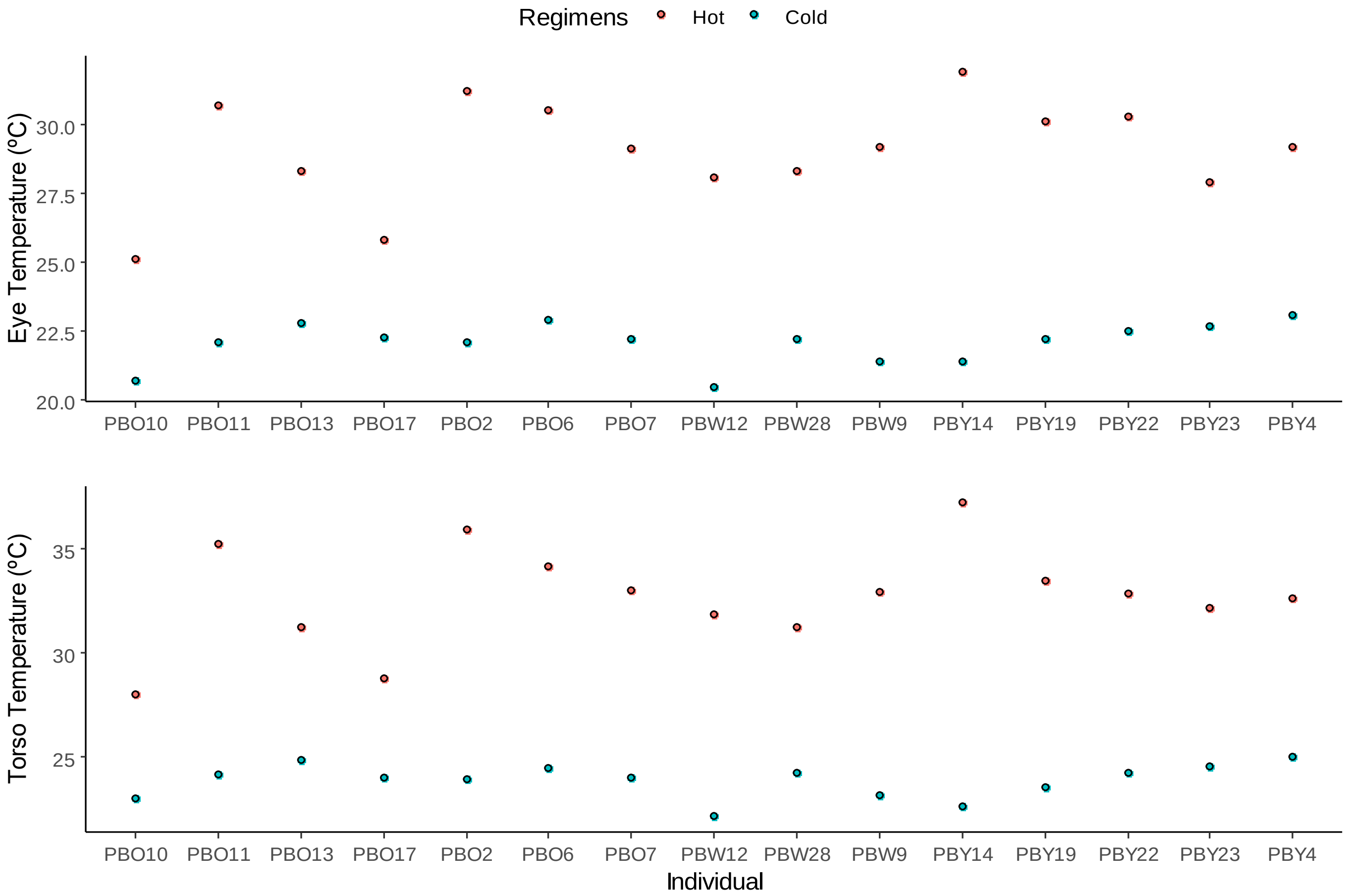

Figure 7. Boxplot for the temperatures per individual during the trials Prey Quantity Preference Test, group that did 1vs5.

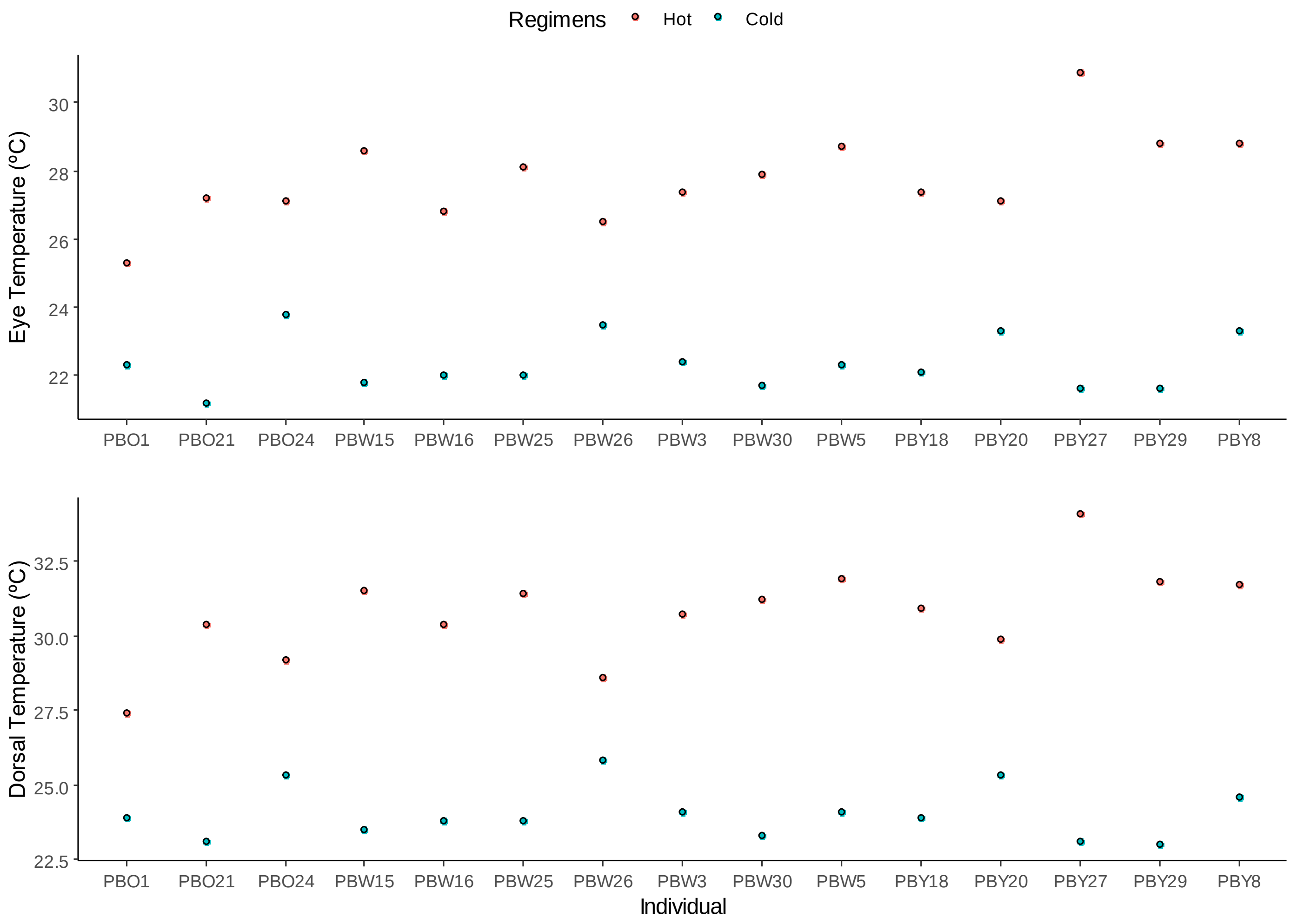

Figure 8. Boxplot for the temperatures per individual during the trials Prey Quantity Preference Test, group that did 2vs3.

We proceeded to analyse if SVL was dependent on the Morph, for this we did a One-way ANOVA. This ANOVA (F = 3.461; p = 0.045; degrees of freedom = 2) showed that the morphotype only explains 20.4 % of the variance in body size (SVL), within morphotype. However, doing a Multiple Comparison of Means (Tuckey Contrasts), we did not recover any significant difference between morphotype pairs (Table 1). However, in the biological context we could say that the difference in size between the Yellow morphotype and White morphotype could be relevant within our dataset.

Table 1. Result of the Multiple Comparison of Means (Tuckey Contrasts) test. Estimates (pairwise comparison), SE, t-values and probabilities are presented.

Deviation from zero	Estimate	Std. Error	t value	Pr(>|t|)
White - Orange	-3.569	1.620	-2.203	0.0889
Yellow - Orange	0.232	1.620	0.143	0.9888
Yellow - White	3.801	1.620	2.347	0.0662

3.1. Learned Association Test

The first model applied to this experiment (GLMM model for Poisson probability distribution), showed that there was no significant difference in the number of primary interactions being with the blue coloured petri dish by the animals, considering temperature and morphotype (Table 2).

Table 2. Test of the effect of temperature and morphotype on the number of primary interactions with the blue petri dish, using a GLMM (Poisson Probability Distribution; Logarithmic Link Function). Coefficients (difference to zero assumed for Hot in temperature and for Yellow in morphotype), SE, t-values and probabilities are presented. AIC = 105.971; BIC = 116.689

Model Terms	Coefficient	Std. Error	t - values	Pr(>|t|)
Intercept	-0.405	0.398	-1.019	0.314
Temp (Cold)	-0.182	0.590	-0.309	0.759
Orange	-0.981	0.796	-1.232	0.224
White	-0.065	0.590	-0.109	0.913
Orange * Cold	0.875	1.030	0.850	0.400
White * Cold	-0329	0.925	-0.355	0.724
SVL	0.013	0.062	0.216	0.830

On the second model applied to this experiment (GLMM, Gamma Probability Distribution; Identity Link Function), all variables showed no significant differences in the time spent inside the established area of interaction of the blue petri dish, considering temperature, morphotype, and SVL (Table 3.). However, the value obtained in this model for SVL was so close to significance (p-value = 0.061) that prompted the necessity to construct a third model, taking only into consideration temperature and body size.

Table 3. Test of the effect of temperature, SVL and morphotype on the time spent in the area of interaction of the blue Petri dish, using a GLMM (Gamma Probability Distribution; Identity Link Function). Coefficients (difference to zero assumed for Hot in temperature and for Yellow in morphotype), SE, t-values and probabilities are presented. AIC = 405.400; BIC = 417.184

Model Terms	Coefficient	Std. Error	t - values	Pr(>|t|)
Intercept	-63.857	44.066	-1.449	0.155
Temp (Cold)	0.121	11.236	0.011	0.991
Orange	-3.461	9.997	-0.346	0.731
White	0.473	10.094	0.047	0.963
SVL	1.484	0.770	1.928	0.061
Orange * Cold	5.680	15.606	0.364	0.718
White * Cold	-8.277	12.968	-0.638	0.527

In the third model applied to his experiment (GLMM, Gamma Probability Distribution; Identity Link Function), body size showed to be significant in the model, having a positive impact in time spent in the area of the blue petri dish (Table 4). Bigger animals spent more time in the previously mentioned area of interaction at Cold temperatures and Hot temperature (Figure 9).

Table 4. Test of the effect of temperature and SVL on the time spent in the area of interaction of the blue Petri dish, using a GLMM (Gamma Probability Distribution; Identity Link Function). Coefficients (difference to zero assumed for Hot in temperature and for Yellow in morphotype), SE, t-values and probabilities are presented. AIC = 396.801; BIC = 403.560

Model Terms	Coefficient	Std. Error	t - values	Pr(>|t|)
Intercept	-94.009	38.607	-2.435	0.019
Temp (Cold)	-4.035	4.647	-0.869	0.390
SVL	2.034	0.723	2.813	0.007

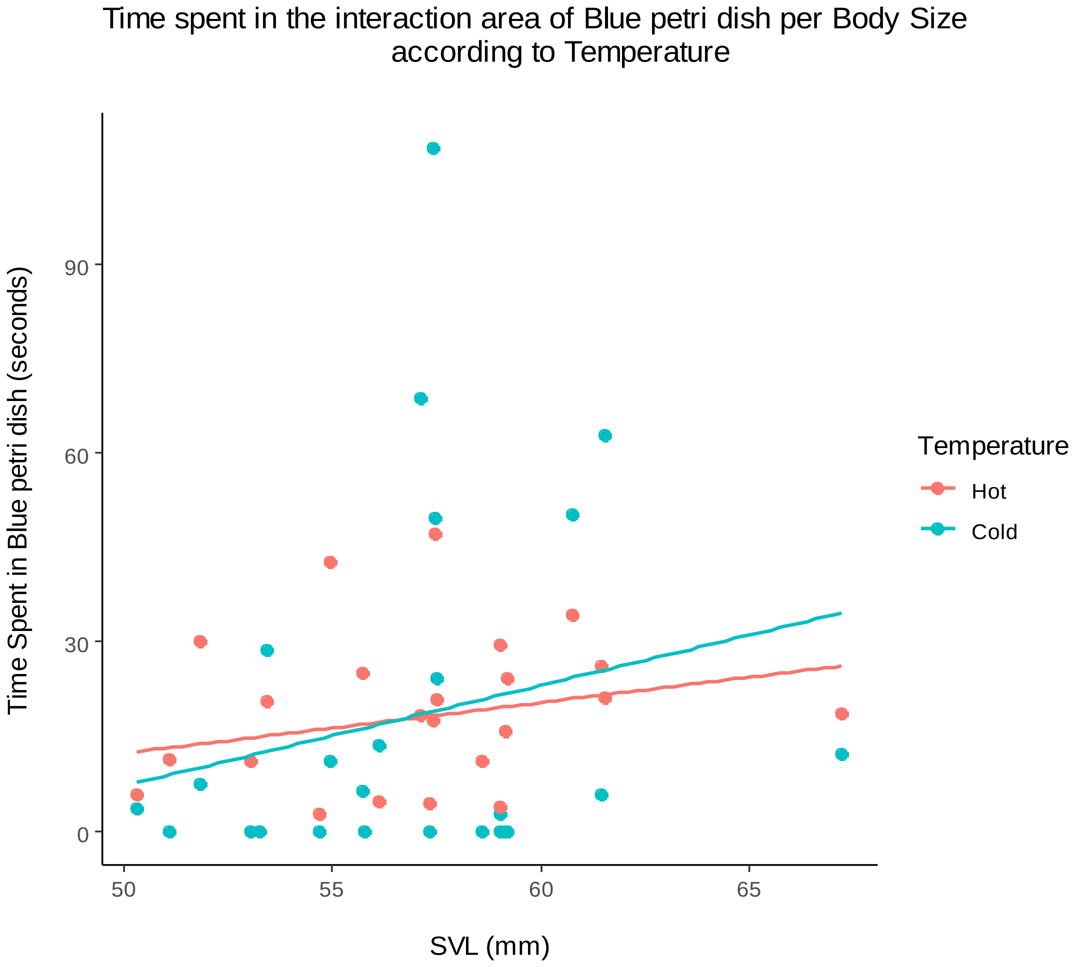

Figure 9. Distribution of time spent in blue petri dish in relation to SVL, according to temperature regimen. The lines refer to the Linear Regression of these two variables according to Temperature.

3.2. Prey Quantity Preference Test

The GLMM executed for this test showed that there was no significant difference between time spent in the area of interaction by the animals, considering temperature, morphotype, SVL and the test performed (1vs5 or 2vs3) (Table 5).

Table 5. Test of the effect of temperature, quantity pairing and SVL on the time spent in the area of interaction of either quantity Petri dish within tested pair, using a GLMM (Gamma Probability Distribution; Identity Link Function). Coefficients (difference to zero assumed for Hot in temperature and for Yellow in morphotype), SE, t-values and probabilities are presented. AIC = 233.627; BIC = 234.923.

Model Terms	Coefficient	Std. Error	t-value	Pr(>|t|)
Intercept	-7.249	31.659	-0.229	0.821
Cold	1.926	4.779	0.403	0.690
1vs5	2.211	4.268	0.518	0.640
SVL	0.594	4.707	-0.470	0.642
Orange	-1.973	4.198	-0.470	0.642
White	0.594	4.707	0.126	0.900
Cold * 1vs5	-3.069	6.482	-0.473	0.640

CHAPTER 4

Discussion

4. Discussion

In both experiments, our results show that neither temperature regimen nor morphotype influence the cognitive abilities male *P. bocagei*. This is true for the number of primary interactions and time spent near the blue Petri dish associated with food (through training). Similarly, morphotype and temperature did not significantly alter the time spent near Petri dishes with lower or higher quantities of prey items. However, body size (SVL) showed to significantly alter the time spent near the blue Petri dish (associated with food), larger individuals spent more time in the focus Petri dish, independently of the temperature regimen they were under during the trials.

Before further discussion of the results, it is important to mention that *Podarcis bocagei* is a species that is more neophobic and slow to interact with novel objects compared to other *Podarcis* spp. (Damas-Moreira et al., 2018). Furthermore, we did not have a complete context on the age and incubation temperatures of the caught animals, and this piece of information has been shown to be significant in cognition tests done in other species, although more so in neonates (e.g., Amiel et al., 2014; Clark et al., 2014; Deeming, 2004; Siviter et al., 2017).

4.1. Learned Association Test

In this experiment we wanted to assess if males *P. bocagei* performed worse or better at finding food in a learned marked spot, under the influence of two separate thermal regimens, Hot and Cold, using only visual cues. Regarding the behaviour of going directly to the colour associated with food, no difference was observed between the number of times this behavior under different thermal regimens or according to morphotype. This absence of significance leads to two possible hypotheses. The first hypothesis is that there is in fact only one cognitive program for both thermal regimens. This hypothesis can be justified by high thermoregulatory efficiency in *Podarcis* wall lizards, rendering a simplified program for colder temperatures unnecessary. Besides, *Podarcis bocagei* occupies a temperate and coastal climate (Galán & Milla, 2015), meaning that they may rarely be in a situation of suboptimal thermal conditions as other species of lizards or populations from mountainous or islands habitats. The second hypothesis assumes that the training in our experiments insufficient and ineffective. Associative training was obtained by Damas-Moreira et al. (2018) in *Podarcis bocagei* and *Podarcis sicula*, but a complete different method was employed here. In the aforementioned study, demonstrators from both species were used, and the requirement for considering the behaviour as learned was consecutively choose seven to eight times the colour associated with food, across 40 trials (Damas-Moreira et al., 2018). In our experiments the animals could not see where the food was placed before the start of the trial, resulting in a "blind hunt" during the trial. Additionally, in our experiment the lizards only got nine days to learn the behaviour (one trial each day), since interest in the novel apparatus seemed to be dwindling. However, we tried to counter this loss of interest tendency by setting up a replica of the novel apparatus inside the personal terrarium, where a mealworm was placed inside the blue petri dish after each trial. Then again, this strategy might have incentivized the lizards to wait for food inside the terrarium and not in the open arena. Nevertheless, pairing these results with the ones of Damas-Moreira team (2018), we can hypothesize that *Podarcis bocagei* is not capable of rapid associative learning like other lizards can (e.g., Batabyal & Thaker, 2019; Leal & Powell, 2012; Manrod et al., 2008).

The only factor that impacted time spent in the blue Petri dish area was body size (SVL). Morphotype and temperature did not show any effect. In lizards, there is a partial relationship between body size and age and smaller animals are often younger, but larger animals of similar size can have a difference of one to two years from each other, resulting in different behavioural histories indistinguishable from size (Caetano et al., 1986; Castanet, 1981; Damas-Moreira et al., 2019; Eroğlu et al., 2017, 2018). Although the lizards in our experiment did not show a tendency to pick the colour blue as the first exploration checkpoint, it is possible to hypothesize that larger and more experienced individuals learned that the colour blue potentially had food, and chose to visit it more often, and/or spend more time in its area. Moreover, it was shown with other *Podarcis* spp. that larger animals are more bold (Damas-Moreira et al., 2019). This relationship between size and boldness can explain the results obtained in this experiment regarding SVL, given that larger animals would approach and spend more time near the novel objects. Regarding morphotypes, specific morphotypes showed different behaviours in other studies regarding, boldness, aggression, escape tactics and mobility (Brock & Madden, 2021; Coladonato et al., 2020; Scali et al., 2013; Sreelatha et al., 2021). However, our results indicate cognitive abilities (specifically learned association) does not vary within different morphotypes.

As mentioned before, this is species may not have the need for two distinct cognitive programs due to the climate they inhabit, where periods of suboptimal temperatures are short along the entirety of activity cycles (Diego-Rasilla & Pérez-Mellado, 2000; Grbac & Bauwens, 2001; Pérez-Mellado, 1983; Rugiero, 1995). Our results seem to support the hypothesis of an unnecessary cognitive program for suboptimal, yet they do not allow us to discard the possibility of variations between morphotypes. Additionally, is possible that the one cognitive program they have can be all encompassing, considering the variability in the prey they eat, such prey being different in behaviour, size, and colour. Moreover, while costly, learned behaviours can heavily favour the fitness of the individual (Davies et al., 2012; Shettleworth, 2010). Thus, losing the capacity to access learned information or switch between the most suitable cognitive program as a direct result of external factors, would come at a great cost for the fitness of the individual.

4.2. Prey Quantity Preference Test

We predicted a difference between the choosing of prey quantities by male *P. bocagei* under the influence of distinct thermal regimens, Hot and Cold. However, such difference was not observed. Although *Podarcis bocagei* display two distinct hunting strategies, sit and wait at colder temperatures or when basking and active foraging at hotter temperatures, our experimental design could only detect the behaviour of lizards that moved, that is, active foragers. Since we had the animals and the Petri dishes at opposite ends of the arena, this might result in only subjects that thermoregulated enough to start comfortably exploring the environment were accounted for. Consequently, we might have inadvertently excluded the sit and wait foraging strategy from our experiment. It is not a farfetched reasoning that if the Petri dishes with prey were closer to the opaque PVC barrier, more and meaningful interactions would happen during the five-minute interaction time. As such a test based on the direction of the first movement (by placing prey in opposite lateral sides of the arena) would have been more representative. Since body temperature plays a big role in locomotion (Angilletta, 2009; Booth, 2006; Lailvaux, 2007), animals that did not move might have shown more interest or even less neophobia if the prey were closer, breaching the thermoregulation threshold for prolonged movement and better exposing a possible preference under the sit and wait behaviour.

We were expecting that the morphotype groups might show some differences in prey quantities, since *Podarcis* spp. have shown to have different personalities (Sreelatha et al., 2021) and more aggressive behavior (Coladonato et al., 2020) according to morphotype. However, there were no significant differences between the morphotypes and the time spent in either quantity. As such, a possible interpretation for this result is that independently of the morphotype, the hunting strategies are equal between morphotypes within the same population of *P. bocagei*. In *Podarcis muralis* there is partial divergence in microhabitats use by different morphs (Pérez i de Lanuza & Carretero, 2018); however, *Podarcis bocagei* morphotypes have a fairly homogeneous distribution along the territory occupied by the whole species (Galán & Milla, 2015). Therefore, the lack of significance in prey quantity preference by morph may be true for different populations of this species. This hypothesis is in line with the paucity in evidence of different hunting strategies in polymorphic species, although some small variance in diet was found in *Podarcis muralis* (Scali et al., 2016).

Body size did not show any significance effect in the model. Although we were expecting such results, since larger animals can take down larger prey, unlike smaller conspecifics, but the number of prey per foraging trip is similar (Carretero, 2004). Additionally, in our sample, representatives of the three colour morphs were not significantly different in size, even accounting for morphotype (average SVL by morphotype: Orange = 58.447; Yellow = 58.679; White = 54.878), as the One-way ANOVA showed. If we had captured a more heterogeneous group, the SVL might have shown as a more relevant variable, although it is arguable that if SVL samples were more tailored to be purposefully distinct, we might incur sampling bias error. All the males were caught in rural agricultural walls, and SVL has been shown as an important variable in *Podarcis bocagei* individuals affected by agrochemicals (Amaral et al., 2012). These individuals showed more difficulty in subduing prey when they had higher concentrations of chlorpyrifos in the blood (Amaral et al., 2012). We did not take into consideration pollution, although the possibility of the animals we collected being contaminated is still a present. Unlike the animals caught in corn fields by Amaral et al. (2012), we collected animals in university campus fields (other than copper sulphate, chemical pollution is negligeable), allowing us to not take pollution into consideration. However, future studies should take chemical pollution into consideration as well as body size if collecting animals from less chemically regulated habitats.

The pair of prey quantity did not have an effect on time spent in the area of interaction of either quantity pair. When tested *P. sicula* showed a limitation in optimizing food intake, when posed with a choice between one prey item and four prey items (Petrazzini et al., 2017). Our results also fall in line with the results obtained in that study. In the aforementioned study motivation was a factor taken into account; however, the animals in the study were trained allowing to set aside the relevance of concomitant factors like motivation (Petrazzini et al., 2018). In our study we also took motivation into consideration, having the lizards being fed only on the day of the trials, with a gap day between trials. However, in our experiment the animals were not trained, therefore motivation might still be an impacting factor. We also explored the possible link between body temperature and the ability to optimize food intake, but such link appears to be non-existent. Since the *P. sicula* showed the ability to discriminate prey size, a future study should look into the relationship between temperature and the ability of *Podarcis* spp. to discriminate between body sizes.

An important consideration for this quantity prey preference test is that we only did one trial per temperature. This was done out of fear that the animals would lose interest in the novel situation by remembering that they could not access the food. A solution to this problem could be repeat the test several times; but accounting for the order of trials, to control for habituation

4.3. Conclusions

Temperature to which the animals can be subjected under different seasons and times (simulated by the thermal regimens Hot and Cold in this study) did not result in different cognitive abilities (e.g., simplified vs. complex cognitive program) of male *Podarcis bocagei*. Despite the evidence for alternative different behavioural personalities associated with colour morphotypes, no cognitive differences between them were found.

Lizards did not vary their performance regarding learned association towards a food reward at different thermal regimens or between colour morphs. Only body size showed as significant effect in time spent on the trained stimulus of food reward, this is likely due to greater general activeness of large individuals rather than related with cognitive capabilities.

Lizards did not express a difference in ability to distinguish between different prey quantities at different temperatures or between colour morphotypes. In this case, body size showed to have no effect on such ability.

Overall, the results of these experiments strongly suggest that there is only one cognitive program in male *Podarcis bocagei*, acting at both optimal and suboptimal temperatures. Seemingly, cognition abilities are flexible enough to operate under the spectrum of the thermal conditions this species face, likely associated to its high thermoregulatory performance.

4.4. Future Perspectives

The analysis of the relationship between cognitive abilities and temperature is still open. This is because lizards could develop other cognitive abilities not related with foraging, but space use or social relations. As such, other experimental designs and even with other target species (e.g., less efficient thermoregulators), can be tested.

In the future, it is also advisable to repeat these experiments with a larger sample of individuals and by having lengthier training periods. Furthermore, a special care should be taken regarding the age (e.g., experience) of the animals in order to further research the impacts of temperature in fully adult males.

Despite our negative results, the area of cognition in reptiles remains promising, and there are many aspects of the relationship between cognition and temperature that deserve exploration.

5. References

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