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Assessing the reliability of thermography to infer internal body temperatures of lizards

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ABSTRACT

For many years lizard thermal ecology studies have relied on the use of contact thermometry to obtain internal body temperature (T_b) of the animals. However, with progressing technology, an interest grew in using new, less invasive methods, such as InfraRed (IR) pyrometry and thermography, to infer T_b of reptiles. Nonetheless few studies have tested the reliability of these new tools. The present study tested the use of IR cameras as a non-invasive tool to infer T_b of lizards, using three differently body-sized lacertid species (*Podarcis virescens, Lacerta schreiberi* and *Timon lepidus*). Given the occurrence of regional heterothermy, we pairwise compared thermography readings of six body parts (snout, eye, head, dorsal, hind limb, tail base) to cloacal temperature (measured by a thermometer-associated thermocouple probe) commonly employed to measure T_b in field and lab studies. The results showed moderate to strong correlations (R^2=0.84–0.99) between all body parts and cloacal temperature. However, despite the readings on the tail base showed the strongest correlation in all three species, it was the eye where the absolute values and pattern of temperature change most consistently followed the cloacal measurements. Hence, we concluded that the eye would be the body location whose IR camera readings more closely approximate that of the animal's internal environment. Alternatively, other body parts can be used, provided that a careful calibration is carried out. We provide guidelines for future research using thermography to infer Tb of lizards.

1. Introduction

Body temperature is a fundamental aspect in the ecology and physiology of ectotherms due to its effects on individual growth, survival, reproduction (Angilletta et al., 2002; Huey and Stevenson, 1979; Savage et al., 2004), as well as on species density and diversity (Angilletta et al., 2004; Brown et al., 2004; Wiens et al., 2006). Reptiles represent a particularly well studied group in which most species utilise external heat sources and behavioural and physiological adaptations to thermoregulate (Seebacher and Franklin, 2005; Tattersall and Cadena, 2010). By doing this, many reptiles maintain their body temperatures within a preferred range, often referred to as "set-point range" (Gans and Pough, 1982; Hertz et al., 1993) which optimises a variety of metabolic functions (e.g. digestion, locomotion, growth, incubation – Huey and Stevenson, 1979; Van Damme et al., 1991). The temperatures and precision to which reptiles thermoregulate is dependent on many factors such as species, sex, age, season, reproductive, nutritional and health state (Beal et al., 2014; Gans and Pough, 1982; Gunderson and Leal, 2015). Yet, most species can be thought of being somewhere amid a continuum between the perfect thermoregulator and the perfect thermoconformer (Huey and Slatkin, 1976), but the position in this scale is neither static nor absolute, but rather a dynamic range affected by many ecophysiological traits (Hertz et al., 1993; Angilletta, 2010).

Among reptiles, lizards have commonly been used as model-organisms in thermal ecology and ecophysiology studies (Castilla et al., 1999). For example, lizards have been widely used to study thermoregulation patterns (Gunderson and Leal, 2015) and adaptation of thermal niche (Aguado and Braña, 2014; Ma et al., 2014), effects of current climate change on geographic distribution (Bestion et al., 2015; Woods et al., 2015), and preferred body temperature and thermal heterogeneity (Allen and Powell, 2014; Goller et al., 2014).

The previous studies resulted in well-established protocols for collecting data, such as the use of cloacal probes to measure body temperatures. Such wide adoption of this procedure is the results of the

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ease of applying this tool in both field and lab conditions. However, the increasing perception of the complexity behind reptile thermal ecology led to a growing interest in developing new tools and techniques to investigate this aspect of reptilian ecology.

For example, some studies have demonstrated the utility of the use of infrared (IR) technology as an alternative to contact thermometers to measure body temperature. The use of IR technology, such as IR thermometers (pyrometry) and IR thermal imaging cameras (thermography), allows to collect temperature data without the need to capture the animal, and often with great speed, short lag and in high-resolution (Hare et al., 2007; Sannolo et al., 2014). This opens the possibility of recording large amounts of data while potentially minimising the effect of the observer on the studied system (Langkilde and Shine, 2006), possibly resulting in more representative data (Tattersall and Cadena, 2010). Additionally, thermography allows gathering high-resolution temperature data (Tattersall et al., 2009), with comparably lower background noise relative to other IR tools (Kastberger and Stachl, 2003). Finally, thermography permits more freedom to analyse complex data using available dedicated software, increasing the potential applications of the technology (Sannolo et al., 2014).

The high-resolution temperature data collection capabilities of thermography can also be integrated with the knowledge of the occurrence of regional heterothermy in many reptiles. Since Heath (1964) first described this phenomenon in *Phrynosoma coronatum*, many other groups of reptiles have been shown to demonstrate such capabilities (Sannolo et al., 2014). However, some authors have denied the occurrence of this phenomenon in very small reptiles (Stevenson, 1985).

Nonetheless, the onset of thermography has facilitated the further study of this phenomenon in reptile (Bosch, 1983; Burns et al., 2015; Sannolo et al., 2014; Tattersall and Cadena, 2010) and non-reptile groups (McCafferty et al., 2015; Tattersall and Cadena, 2010, Cadena and Tattersall, 2009). Thus, IR tools unlock the possibility of exploring new methods of obtaining comparable temperature data using modern, less intrusive procedures.

This study aims to explore the effects of thermal inertia and regional heterothermy in order to compare the temperature of different body parts of heating and cooling lizards, measured using IR thermal imaging, to their cloacal temperature. Therefore, the aim of this study was ultimately to determine whether it is possible to infer internal body temperature using thermography.

2. Methods

2.1. Study species

This study tested 46 adult male lizards belonging to three different lacertid species, representing three distinct body size-classes observed in European lizards: small (26 *Podarcis virescens*, *sensu* Geniez et al., 2014), medium (10 *Lacerta schreiberi*) and large (10 *Timon lepidus*). Lizards where noosed from the field in Évora (38.57°N, 7.91°W; *P. virescens*) and Vila do Conde (41.33°N, 8.67°W; *L. schreiberi* and *T. lepidus*) municipalities (Portugal) during spring. Only individuals with intact tails were used for this experiment. The animals were brought to the lab where their snout-vent length (SVL) was recorded to the nearest 0.01 mm using a digital calliper and their weight measured to the nearest 0.0001 g using a precision balance (Sartorius M-Pact AX224, Sartorius AG, Goettingen, Germany).

2.2. Experimental setting

The animals were kept in individual cages with food (*Tenebrio molitor* larvae) and water supplied *ad libitum* and exposed to a natural light cycle regime. During the day air temperature was set at 28 °C, while during the night was set at 20 °C. Within less than seven days, all animals where returned to their respective sites of capture.

All lizards were individually subjected to a thermal gradient (± 20–50 °C) in an acrylic terrarium (100×30×40 cm) covered with a $<$ 0.5 cm layer of vermiculite acting as a substrate. A 150 W infrared reflector bulb, fixed 25 cm above the substrate at one end of the terrarium was used as the main heat source (Carretero, 2012). Ambient air temperature was maintained around 20 °C by an air conditioning system.

To obtain readings from heating and cooling animals, lizards were subjected to 4 h of testing in the gradients with the heat lamp turned on followed by 4 h with the lamp turned off. Every hour, a FLIR T335 thermal camera (sensitivity: < 0.05 °C; accuracy: ± 2%; IR image resolution: 320×240 pixels; Flir Systems Inc., Wilsonville, Oregon, USA) was used to simultaneously take an IR and a regular photo of each lizard's entire body (skin emissivity=0.96). IR camera was hand-held and photos were shot at 30–40 cm depending on the subject size. This approach allowed us to maintain always the same resolution in every IR image, irrespective of species and body size. Immediately ($<$ 20 s) after photographing each animal, the subject was captured and its cloacal temperature measured with a contact thermometer (Hibok 18, precision: 0.1 °C, accuracy: ± 0.2%) fitted with a k-type thermocouple probe. The reading was obtained by inserting the probe few millimetres into the cloaca of the animal.

2.3. OLS and RMA regressions

Upon completion of all tests, IR photos were analysed using the software FLIR Tools 2.1 (Copyright 2014 FLIR Systems, Inc; http://www.flir.com). The *Spotmeter* function of this software was used to measure the temperature at six body locations, as shown in Fig. 1: snout, eye, head (at the base of the parietal scales), dorsal (centrally), base of tail (dorsally, above the location of the cloaca) and left hind limb (at the knee articulation). When these were not easily identifiable in IR photo, the corresponding normal photograph was used to determine the location of where to obtain the reading from.

Given the non-normality of the data (Shapiro-Wilk tests < 0.05 for all species individually), a Kruskal-Wallis test and Dunn post-hoc tests were used to test the statistical significance of the difference of both the SVL and the weight, between the three species groups.

Due to the lack of normality of the residuals and given the repeated measures design of the experiment, a Method II regression with resampling was deployed to perform an Ordinary Least Square (OLS) to test for a relationship between cloacal temperature and the temperature of each body part measured using IR imaging (Legendre and Legendre, 2012). OLS was also deemed a more appropriate method than Reduced Major Axis (RMA) regression. Cloacal temperature, even though it is measured with a certain degree of error, is being used as a proxy for inferring internal body temperature. In this case, the variables assigned to the X and Y axes are not arbitrary and, hence, the presence or absence of symmetry in the regressions of Y on X and X on Y becomes irrelevant (Smith, 2009). Nonetheless, with large correlation coefficients, the slopes of OLS and RMA, should not differ to a large extent anyway (Smith, 2009).

2.4. Linear mixed-effects models

We further investigated the relationship between body temperature (from both cloacal and IR readings) and variables that could possibly affect it. Given the unbalance structure of the data and the possible subject-specific effects, we fitted a Linear Mixed-Effects Model. Body temperature was set as the dependent variable and three variables and their interactions as predictors. The set of variables was treatment*body position*species (where treatment is either heating or cooling) and individuals were treated as random effects. The starting model were reduced following Zuur et al. (2009) and normality of the model's residuals were checked graphically (Pinheiro and Bates, 2000).

All the statistical analysis was performed in R (R Development Core