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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

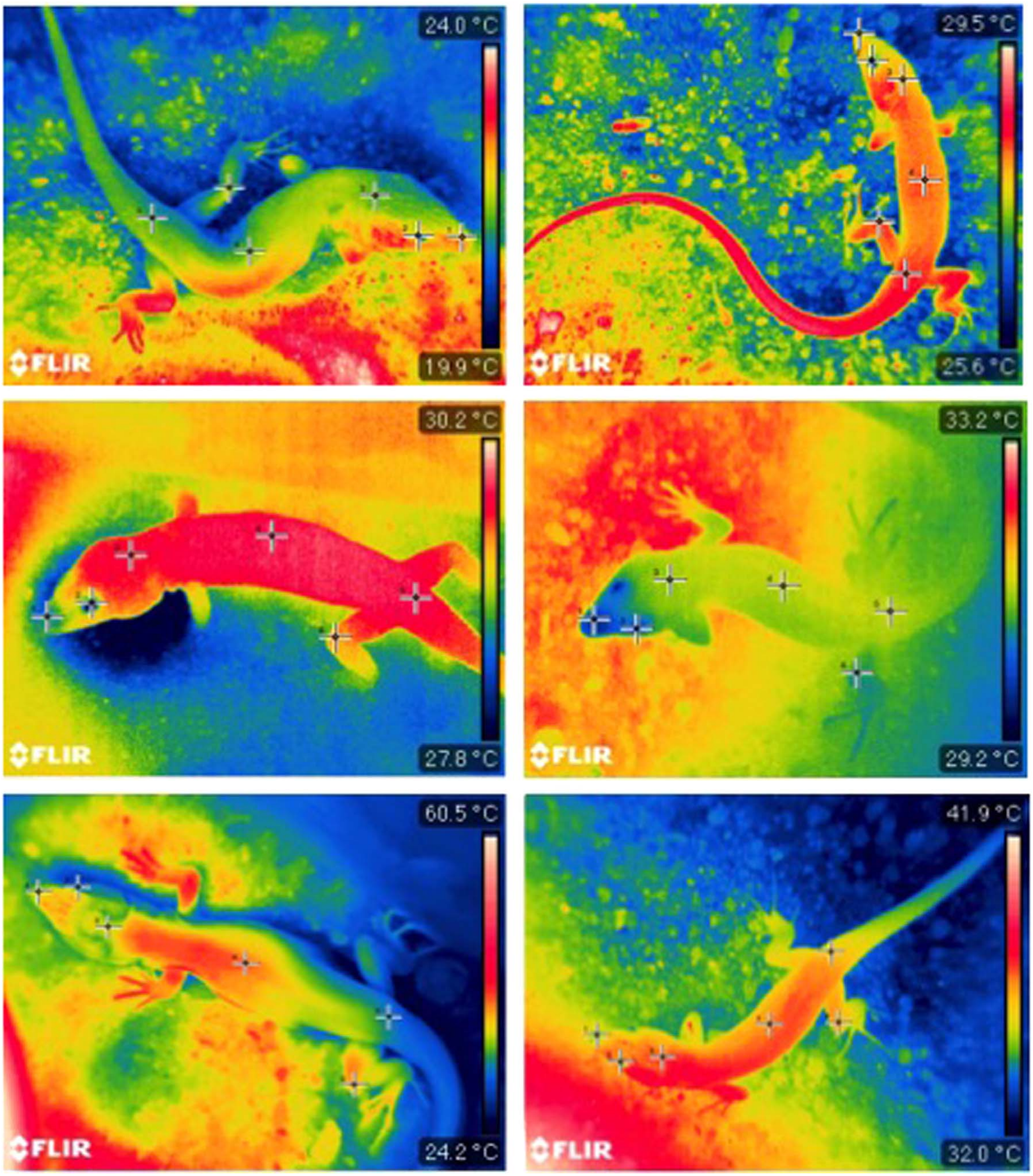

Fig. 1. IR photographs of *Timon lepidus* (left) and *Lacerta schreiberi* (right), showing thermal heterogeneity, while basking in the thermal gradients. The pictures also serve to show from where temperatures were measured on the lizards' bodies.

Team, 2015) using the lmodel2 v1.7-2R package (Legendre, 2012) for the resampling procedure, the lme4 package (Bates et al., 2015) for the linear mixed models and the lmerTest package for the Post-hoc tests on the mixed models.

3. Results

3.1. Size categories

Podarcis virescens was the smallest lizard (mean weight=4.41 ± 0.24 SE; mean SVL=57.97 ± 1.55 SE), followed by *Lacerta schreiberi* (mean weight=25.92 ± 1.47 SE; mean SVL=98.61 ± 2.37 SE) and *Timon lepidus* (mean weight=69.05 ± 3.43 SE; mean SVL=137.42 ± 2.77 SE). Kruskal-Wallis rank sum tests and following pairwise Wilcoxon rank sum tests, corrected with False Discovery Rate (FDR), showed that species were different with respect to weight, SVL and body temperature ($P < 0.0001$ for all pairwise comparisons for each variable,).

3.2. Cloacal vs. IR readings

Podarcis virescens had an overall lower temperature than the two green lizards during both heating and cooling, a larger range and reduced variability in the temperatures measured (Figs. 2b and 3). The green lizards also showed more similar measured temperatures and ranges between them than with *P. virescens* (Supplementary material).

OLS regressions models, summarized in Table 1, showed moderate-to-good fits (R^2 from 0.838 to 0.968). For all the species, the body location that provided the best statistical fit (highest R^2) was the base of the tail. Yet, as shown by the confidence intervals in Table 1, only the

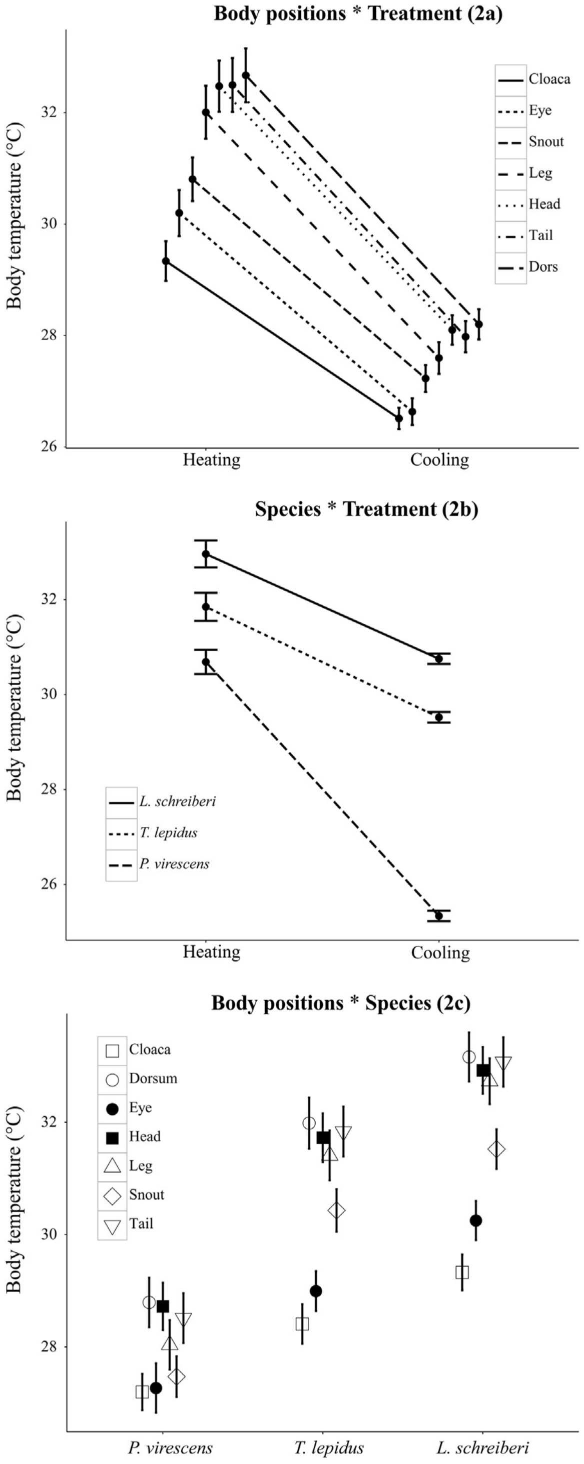

Fig. 2. Graphs describing the three significant interaction terms of the Linear Mixed-Effects models. Top (2a): temperatures for all body positions under heating and cooling conditions. Middle (2b): body temperature of the three species for both treatments. Bottom (2c): temperature of each body position for the three species.

snout did not show an OLS slope different from 1, despite a considerably lower statistical fit.

The eyes of the two green lizards showed the same relationship, also accompanied by a decrease in the strength of the model. On all other body parts, the slopes of the best-fit lines exceeded 1, thus suggesting that the IR readings increased at a faster rate than those taken from the animals' cloaca.

This effect is displayed in Fig. 3 where the pattern of cloacal temperature change over time is less exaggerated than that of most of the other body part temperatures obtained with the IR camera. IR camera tended to inflate the body temperature readings since in all body parts and for both treatments (except for the eye and leg of *P. virescens*) the lines of the body temperatures, measured with the IR camera, are above the line of the cloacal temperature (Figs. 2c, and 3).

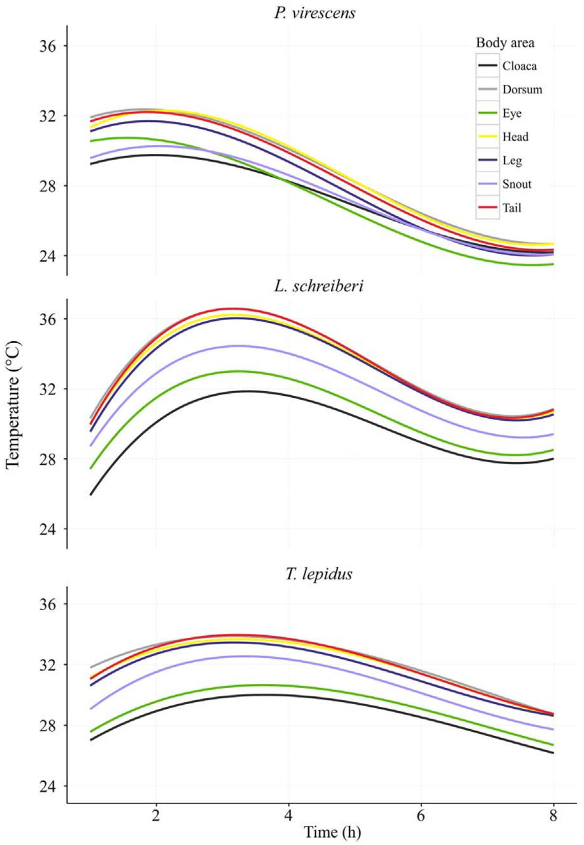

Fig. 3. Graphs showing the patterns of heat gain (hours 1–4) and loss (hours 5–8) in the different body parts of *Podarcis virescens*, *Lacerta schreiberi* and *Timon lepidus* (top, middle and bottom, respectively) over the course of the experiment. Each colour corresponds to one body area. Lines were smoothed using a third order polynomial function.

3.3. Linear mixed-effects models

According to the model, the following variables had a significant effect on body temperature: the random effect ($P < 0.0001$; d.f.=1), and the three interactions treatment*species ($P < 0.0001$; d.f.=2), body position*species (P=0.0011; d.f.=12), and treatment*body position ($P < 0.0137$; d.f.=6). Extensive post-hoc analysis is available as Supplementary materials (S1).

Fig. 2 provides some insight into the pattern of the three significant interactions for the three species. In Fig. 2a can be appreciated that temperature relationship among body parts was consistent in both treatments (regardless of the species). Fig. 2b shows that body temperature was actually higher under heating conditions for all species. *P. virescens* showed an abrupt change in body temperature passing from heating to cooling, possibly because of the reduced body size. Finally, in Fig. 2c it can be observed that the relationship of body temperature with each body position is consistent across species. The dorsum, head, tail and leg reached the highest temperature, followed by the snout and the eye, while cloacal temperatures were consistently the lowest among all body positions.

Table 1
Results of the OLS regressions fitted on the models of *temperature of body part* x (measured with IR camera) ~ *cloacal temperature*. Underlined values indicating the body part that gives the best fit for each species and bold values indicate where the slope was not significantly different from 1. *Italics* indicate significant P-perm values for regression slopes.

	Podarcis virescens					*Lacerta schreiberi*					*Timon lepidus*				
	R^2	Slope	Inter-cept	Slope 95% CI's	P-perm	R^2	Slope	Inter-cept	Slope 95% CI's	P-perm	R^2	Slope	Inter-cept	Slope 95% CI's	P-perm
Snout	0.838	0.996	0.28	**0.934** **1.058**	*0.001*	0.884	0.997	2.27	**0.920** **1.073**	*0.001*	0.900	1.038	0.86	**0.964** **1.112**	*0.001*
Eye	0.879	1.087	−2.22	1.023 1.151	*0.001*	0.934	0.972	1.70	**0.917** **1.028**	*0.001*	0.914	0.972	1.31	**0.909** **1.035**	*0.001*
Head	0.910	1.247	−5.18	1.193 1.300	*0.001*	0.955	1.215	−2.68	1.159 1.271	*0.001*	0.961	1.183	−1.91	1.133 1.234	*0.001*
Dorsal	0.916	1.302	−6.63	1.248 1.356	*0.001*	0.956	1.258	−3.70	1.201 1.315	*0.001*	0.952	1.213	−2.50	1.156 1.272	*0.001*
Tail	0.931	1.316	−7.29	1.267 1.366	*0.001*	0.964	1.272	−4.17	1.219 1.324	*0.001*	0.968	1.206	−2.43	1.160 1.252	*0.001*
Leg	0.924	1.298	−7.26	1.246 1.349	*0.001*	0.959	1.194	−2.25	1.141 1.248	*0.001*	0.925	1.155	−1.39	1.084 1.226	*0.001*

4. Discussion

An increasing number of studies use thermography to obtain temperature data from a large variety species (Bosch, 1983; Heath, 1964; Sannolo et al., 2014; Tattersall and Cadena, 2010). Despite this, it seems to be few attempts to validate the use of IR equipment to infer internal body temperature of reptiles by comparing this technology to more traditional methods, like cloacal thermometer (Luna et al., 2013). Most studies, like Carretero (2012) regarding pyrometry, warn towards the uncertainty of using IR technology to measure surface temperature and infer internal temperatures of small-sized ectotherms, cautioning for larger thermal inertia and bigger effects of regional heterothermy and skin-core temperature gradients with increasing body sizes (Jones and Avery, 1989). Therefore, the aim of the present work was to assess the employability of infrared thermography in lizard thermal ecology studies and determine the potential of using such tool to infer internal body temperatures.

The results from the present study suggest that measurements taken with an IR camera from several body locations correlate strongly ($R^2 > 0.83$) with cloacal temperature obtained through contact thermometry. Contrastingly, the study by Carretero (2012) found much weaker correlations (R^2=0.308–0.668) between cloacal and dorsal temperature, measured with an IR thermometer in four species of the genus *Podarcis*. The strong correlations found in the current study suggest the presence of a definite relationship between skin and internal temperatures of the lizards. Nonetheless such finding is not novel. It has been widely reported that reptiles are able to regulate body temperature distribution (thus creating core-surface thermal gradients) behaviourally and physiologically by adjusting blood flow between the skin and core, through thermal hysteresis, or by eye bulging (Bucklin, 2006; Dzialowski and O'Connor, 2001; Tattersall et al., 2006).

The strength of the reported correlations holds for small to large body sized lacertid lizards, albeit the exact pattern seems to be body-part specific, but consistent in all species (Fig. 2c). This outcome is also supported by the results of the linear mixed-effects model. In fact, we are able to quantify (Figs. 2 and 3, Supplementary material) that the green lizards achieved larger differences between cloacal and skin surface temperatures than the much smaller *P. virescens*. We consider that such effect is most likely attributable to the larger thermal inertia of the green lizards but we cannot discard underlying species-specific physiological and/or behavioural mechanisms being the cause of the observed pattern (Bell, 1980; Zamora-Camacho et al., 2014). However, the relationship among the temperature of the different body positions, as well as the effect of cooling and heating, was consistent irrespective of the body size. We thus believe that, at least for the studied species, body size does not represent an impediment to the use of IR thermography to investigate their thermal ecology.

Different body parts correlate differently with cloacal temperature. For instance, the strongest correlations (highest R^2), like the tail, are not the ones that more closely mimic temperature changes in the cloaca. For instance, the eye followed very closely the absolute values of cloacal temperature, followed by the snout, for all species (Fig. 2c). However, neither the eye nor the snout showed the strongest correlations to cloacal temperature (compare the lines slopes in Fig. 2a). The remaining body parts (dorsal, tail, head, limb) all experienced temperatures larger than the eye and the cloaca, during both heating and cooling (Fig. 2c).

This complex outcome could be due to a multitude of reasons, namely the smaller thermal inertia of the head region (Georges, 1979; Heath, 1964), larger temperature ranges experienced by the snout due to evaporative cooling and variable breathing rates (Bosch, 1983; Tattersall et al., 2006), small size of the measured area that made it more susceptible to the pixel size effect (Faye et al., 2016), and the effect of background radiation.

Several studies suggested that reptiles may regulate their head temperature more precisely than the rest of the body by resorting to evaporative cooling from moist surfaces such as the eyes (King and Green, 1999) or the mouth and the snout (Georges, 1979; Heath, 1964; Hillenius, 1992; Tattersall et al., 2006). This implies that the tissues surrounding these areas should be highly irrigated with blood coming from the rest of the body (Bruner, 1907). These surfaces are therefore very likely to be isothermal with the internal environment of the animal and hence will likely follow the pattern of temperature change in the brain, where the hypothalamus (the temperature regulation centre) is located (Seebacher and Franklin, 2005). These wealth of evidence support our findings that suggest the eye and snout as potentially the best direct proxies for internal body temperatures.

Since the other body points are further away from the hypothalamic environment, they may be expected to experience larger temperature fluctuations (Gordon and Heath, 1983). In fact, our results partly support these expectations as we observed overall higher temperatures on the tail, limbs and dorsal skin surfaces than those measured on the eye or snout. Additionally, these areas also experienced increasing ranges of temperature from the basking to the cooling phases.

Interestingly, the temperatures measured on the head (skin surface temperature) closely followed the pattern of the more peripheral body points, which is quite discrepant from the pattern observed in the eye, snout and the cloaca. Such discrepancy most likely relates to the fact that current technology is sensitive to surface temperatures (Goller et al., 2014; Sannolo et al., 2014; Vollmer and Möllmann, 2010). The skin therefore is more likely to undergo larger fluctuations in temperature than the internal organs since it is in contact to external thermal environment while the temperature of the internal organs is buffered by the whole animals' thermal inertia (Bakken, 1989;

Tattersall and Cadena, 2010).

We acknowledge that obtaining good quality IR images containing the eye (or the snout) of small animals might be challenging, particularly under field conditions. This emphasizes the need of careful consideration in planning studies with IR thermography. Taking together the results concerning the strong but variable relationship between cloacal temperature and other body parts, the effect of body size and the practical difficulties in obtaining eye temperature in the field, we believe that the best approach for using IR thermography could be represented by a species-specific calibration of the tool under use. Alternatively, data analysis should be carried out on IR picture only, without a direct comparison with other tools (Goller et al., 2014). Finally, some authors are already suggesting using skin temperature (as measured with IR tools) as a proxy for body temperature in lizards (Berg et al., 2015). This latter approach, however, poses problems in comparing new data obtained with IR tools with the available literature, which is almost entirely based on internal (cloacal) temperature.

Finally, IR thermography represents a tool that causes little or no stress to the studied animals. Even though calibrating the device against the study species might be time-consuming, we believe that the advantages can largely compensate for the need of proper calibration. This technology allows collecting more data with a better precision and in less time, if compared with cloacal probes. Likewise, the amount of stress induced to the studied animals during repeated manipulations and insertions of the probes is completely eliminated if contactless tools, like IR thermography, are used.

5. Conclusions

From our study, we conclude that thermography can be an invaluable tool in thermal ecology studies, particularly for those researchers wishing to obtain high-resolution temperature data of several body parts simultaneously and/or continuously (Faye et al., 2016; Goller et al., 2014; Tattersall and Cadena, 2010).

We provided evidence to support the use of eye temperature as the best and direct approximation of internal temperature. Otherwise, other body parts can be used, provided that a careful calibration is carried out before inferring internal temperature from IR readings.

Protocols relying on thermography must be meticulously planned with caution not to depart from the assumptions haunting the use of this equipment (i.e. thermal inertia effects, regional heterothermy, thermal hysteresis, skin-core thermal gradients). Finally, future studies should focus on gaining further insight into the potential of this technology applied to thermal ecology studies.

Conflict of interest

There are no conflicts of interest to declare.

Author contributions

FMB, MAC and MS conceived and designed the research; FMB and FS collected the data; FMB and MS performed the analyses; FMB, MAC and MS wrote the manuscript.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jtherbio.2016.10.004.

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