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Abbreviations

 T_{s} : Preferred body temperature; T_{b} : Body temperature; $T_{iButton}$: Temperature measured by modified dataloggers.

Introduction

The preferred body temperature (T_s) that a lizard voluntarily selects in a laboratory thermal gradient (indoor enclosure with negligible wind and specific radiant heating regime) provides a reasonable estimate of what a lizard would attain in the wild with a minimum of associate costs in absence of constraints (biotic and abiotic factors) for thermoregulation [1,2].

The recent accessibility of small dataloggers has potential to revolutionize collection of thermal data in small animals [3– 5]. The DS1921 Thermochron iButton (Dallas Semiconductor, TX USA) is an ideal device to quantify temperatures [6]. An iButton is a small lightweight datalogger (~14 mm diameter, 3 mm wide, and 1.5 g) that can store an enormous amount of measurements and their dates (2048 consecutive temperature readings) facilitating long term studies. In addition, iButtons

Research Article Selected body temperature in Mexican lizard species

Abstract

Lizards are vertebrate ectotherms, which like other animals maintain their body temperature (T_{i}) within a relatively narrow range in order to carry out crucial physiological processes during their life cycle. The preferred body temperature (T_s) that a lizard voluntarily selects in a laboratory thermal gradient provides a reasonable estimate of what a lizard would attain in the wild with a minimum of associate costs in absence of constraints for thermoregulation. In this study we evaluated accuracy of modified iButtons to estimate $T_{\rm b}$ and $T_{\rm c}$ of three lizard species (Sceloporus poinsettii and Sceloporus jarrovii in northeastern Durango, and Ctenosaura oaxacana in southern Oaxaca, Mexico). We used linear regression models to obtain equations for predicting T_{b} and T_{s} of these species from iButtons. Results from regression models showed that $T_{iButton}$ is a good indicator of T_{b} for *S. jarrovii* and *S. poinsettii* during calibration process. In the same way, T_{iButton} is a good indicator of T_s for S. poinsettii, S. jarrovii and C. oaxacana through experimental gradient. Thus, external measurements using modified iButtons provided an accurate measurement of $T_{\rm b}$ for S. jarrovii and S. poinsettii and T_s for three species of lizards in this study. In laboratory, body temperature $(T_{\rm b})$, and preferred temperature $(T_{\rm c})$ obtained from *S. jarrovii* and *S. poinsettii* fell within the range of $T_{\rm b}$ s of other lizards in the family Phrynosomatidae. T_s measured for S. jarrovii, S. poinsettii, and C. oaxacana are within the range observed for lizards. Therefore, thermal preferences appear more phylogenetic that certain environmental factors present in each population of lizards of this group.

> are easily programmed and have a simple interface. Robert and Thompson [6], describe a technique for modifying an iButton, which involves removing the stainless-steel housing exposing the electronic board and apply a coating of plastic (Plasti Dip Spray, Performix, USA) to reduce the size and weight of iButtons. The modified iButton can then be glued to the skin of small reptiles. Given the relatively low thermal capacity of iButtons, it is possible to obtain a good estimate of T_b . Although, Robert and Thompson [6], did not verify the accuracy of iButtons to predict T_b of animals.

> In this study we evaluated accuracy of modified iButtons to estimate $T_{\rm b}$ and $T_{\rm s}$ of three lizard species (*Sceloporus poinsettii*, *Sceloporus jarrovii*, and *Ctenosaura oaxacana*). We used linear regression models to obtain equations for predicting $T_{\rm b}$ and $T_{\rm s}$ of these species.

Materials and Methods

Study Sites and Lizard Species

The study of sympatric species *S. jarrovii* and *S. poinsettii* was conducted at Las Piedras Encimadas canyon (25°38'47"N, 103°38'40"W), 25 km northwest of Gomez Palacio, Durango, Mexico (elevation 1,425 m). The climate at the site is seasonal, with the highest temperature and rainfall occurring in spring

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and summer, respectively. Mean annual precipitation is 239 mm and mean annual temperature is 21°C [7]. Vegetation is within the Lechuguilla Scrub biome (Matorral Xerófilo with *Agave lechuguilla* [8].

Sceloporus jarrovii is a lizard species of montane forests and some isolated mountain habitats in the Chihuahuan and Sonoran Deserts with extensive rock exposure and crevices, at elevations between 1370–3550 m [9,10]. Sceloporus poinsettii is largely saxicolous, inhabits vegetated areas in canyons in arid and semiarid regions within Chihuahuan Desert, as well as forested slopes of Sierra Madre Occidental [11,9].

The lizard *Ctenosaura oaxacana* inhabits at Montecillo Santa Cruz in southern Oaxaca (16°22'05"N, 94°35'15.4"W) within Isthmus of Tehuantepec. The study site has tropical climate with a rainfall of 800 mm, and annual average temperature of 27.6 °C. The vegetation is mainly early successional and composed of shrubs and bushes [12–14]. The habitats used by this species are dry forest, Nanchal (*Byrsonima crassifolia*), grassland, riparian vegetation, and mangrove [15].

Calibration

External data loggers generally provide accurate estimates of internal body temperatures in small ecthoterms that have limited physiological capacity to control rates of heat exchange [16].

In order to observe relationship between T_b and temperature measured by modified dataloggers ($T_{iButton}$) of adult lizards, three *S. poinsettii* and two *S. jarrovii* were collected by noosing in October 2012 and fitted with modified iButtons fixed dorsally on the back of lizards with cyanoacrylate glue [6]. Furthermore, in order to measure T_b , we inserted a K-type thermocouple into the cloaca of each lizard attached to a digital thermometer. We used masking tape to keep the thermocouple within the cloaca.

Thermochron iButtons were programmed to measure $T_{iButton}$ per minute and simultaneously T_b of lizards was measured. Lizards were placed within a chamber of approximately 40 x 25 x 30 cm, equipped with incandescent light bulbs controlled by a thermostat for adjusting temperature. The temperature of each of five lizards was measured during one hour by increments of about 5 °C (within chamber) for 20-minute intervals. The experiment began with 25 °C, after 20 min of measurement increased to 30 °C, and finally at 35 °C.

Calibration temperature analysis

Analysis of covariance (ANCOVA) was performed to observe relationship between a change in T_{b} and $T_{iButton}$, and to determine whether there is a difference between those lizard species. Within analysis, T_{b} and $T_{iButton}$ were assigned as covariates and the fixed factor was the species. Then, we developed a regression model for each species. The models met assumptions of normality and homoscedasticity of residuals. All data analysis and figure were performed using statistical package R [17].

Selected body temperature

Adults of S. poinsettii and S. jarrovii were collected by

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noosing in October 2012 and separated into two categories based on individuals with presence or absence of iButtons attached on the back of both lizard species. Preferred body temperatures (T_s) and iButton temperatures $(T_{iButton})$ of captive lizards were measured in a thermal gradient (S. poinsettii: n = 9 lizards without iButtons and n = 8 lizards with iButtons; and *S. jarrovii*: n = 10 lizards without iButtons and n = 6 lizards with iButtons). The thermal gradient consisted of a $1 \times 1.5 \times$ 0.5 m wooden enclosure and divided lengthways into six separate compartments. Heat was provided at one end of each compartment by a 250 W infrared lamp. The laboratory climate was adjusted so that at one end of thermal gradient reached 50 °C and 20 °C at other end, thus forming a continuous gradient [18]. We collected lizards four days before T_s were measured to allow lizards to acclimate for at least 18 h prior to testing. $T_{\rm s}$ of all lizards was measured every hour from 9:00 until 18:00 hours (the normal activity period of each species). We inserted a K-type thermocouple in the cloaca using the same digital thermometer to measure $T_{\rm b}$. Simultaneously; $T_{\rm iButton}$ was measured with iButtons attached on the back of both lizard species. Preferred temperature ranges were generated with upper and lower bounds established by using central 50% of body temperatures per lizard [18]. By using central 50%, the outliers resulting from nonthermoregulatory behavior (e.g., escape or exploratory behavior) were eliminated [18].

Moreover, T_{sel} for *C. oaxacana* (n = 7 lizards) was also measured in a thermal gradient inside a wooden box with similar dimensions to those described above and with same range of temperatures between 20 °C and 50 °C. Individuals were picked by noosing each hour to record simultaneously both temperatures, one automatically registered by modified iButton ($T_{iButton}$), and the other (T_s) by a cloacal thermometer Miller and Weber[®].

Selected body temperature analysis

To test whether there was significant difference between T_s (measured through a digital thermometer) of *S. poinsettii* and T_s of *S. jarrovii*, and between $T_{iButton}$ and T_s in both species, were compared with a Student t test when data met requirements for use of parametric tests (normality and homogeneity of variances, test Kolmogorov–Smirnov and Levene, respectively), and if not we used nonparametric Mann–Whitney test. We also performed a multiple regression analysis to determine relationship of $T_{iButton}$ with T_s and compared slope for each species. Data analysis and graphics processing programs were performed with Systat 13[®]. The tests were performed with $P \leq 0.05$ and means are expressed ± 1 SE, n and total range.

Moreover, for *C. oaxacana* a Wilcoxon two-sample test was performed in order to test whether there was significant difference between T_s measured through a cloacal thermometer and $T_{iButton}$ measured by modified iButtons.

All lizards used in this study were marked with a nontoxic paint to identify lizards that had already been measured so that repeat measurements were not made and then released at the exact site of capture.

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Results

Calibration

The mean T_b and mean $T_{iButton}$ of three individuals of *S.* poinsettii was 29.5 ± 0.31 °C (range, 23.1 – 36.5 °C, n = 118) and 31.1 ± 0.33 °C (range, 23.5 – 38.0 °C, n = 120), respectively. Moreover in two individuals of *S. jarrovii*, mean T_b and mean $T_{iButton}$ was 31.1 ± 0.32 °C (range, 23.7 – 37.0 °C, n = 118) and 31.9 ± 0.33 °C (range, 24.0 – 39.0 °C, n = 120), respectively.

The ANCOVA analysis showed a significant difference between two species ($F_{1, 297} = 70.71$, P < 0.0001), so we proceeded to perform a regression model for each species (Figure 1). The model met assumption of normality of residuals and homoscedasticity. The linear equation obtained for *S. poinsettii* was $T_b = 1.29 + 0.91 \text{ x} T_{\text{iButton}}$ (n = 180, $R^2 = 0.95$, P < 0.0001), and *S. jarrovii* was $T_b = 1.38 + 0.93 \text{ x} T_{\text{iButton}}$ (n = 120, $R^2 = 0.95$, P < 0.0001).

Selected body temperature

The mean T_s and mean $T_{iButton}$ of *S. poinsettii* was 32.3 ± 0.60 °C (n = 76, range, 19.8 - 40.0 °C) and 33.0 ± 0.66 °C (n = 76, range, 19.0 - 45.0 °C), respectively. Moreover for *S. jarrovii*, mean T_s and mean $T_{iButton}$ was 32.7 ± 0.44 °C (n = 57, range, 21.7 - 39.8 °C) and 33.0 ± 0.59 °C (n = 57, range, 20.5 - 41.0 °C), respectively.

A Mann–Whitney *U*-test on T_s between *S. poinsettii* and *S. jarrovii* revealed no significant differences (W = 5,842, P = 0.41). Furthermore, a Student *t*-test revealed no significant differences between T_s and $T_{iButton}$ for *S. poinsettii* (t = 0.67, df = 150, P = 0.501), and a Mann–Whitney *U*-test revealed no significant differences between T_s and $T_{iButton}$ for *S. jarrovii* (W = 5,247.5, P = 0.48).

Both *S. poinsettii* as *S. jarrovii* T_s was positively related with $T_{iButton}$ (Figure 2). The linear equation obtained for *S. poinsettii* was $T_s = 1.08 \text{ x} T_{iButton} - 2.20$ (n = 76, $R^2 = 0.98$, P < 0.0001), and *S. jarrovii* was $T_s = 1.28 \text{ x} T_{iButton} - 9.13$ (n = 57, $R^2 = 0.93$, P < 0.0001).

Furthermore, mean T_s and mean $T_{iButton}$ of *C. oaxacana* was 32.1 ± 0.6 °C (n = 37, range, 13.1 – 39.5 °C) and 32.1 ± 1.0 °C (n = 37, range, 14.1 – 39.1 °C), respectively; which were not significantly different (Mann–Whitney *U*–test, *W* = 720, *P* = 0.7), and we proceeded to perform a regression model between T_s and $T_{iButton}$ (Figure 3) and relationship was positive. The linear equation obtained for *C. oaxacana* was T_s = 0.75 x $T_{iButton}$ + 7.16 (n = 37, R^2 = 0.93, P = 0.02).

Discussion

In laboratory, body temperature (T_b) , and preferred temperature (T_s) obtained from *S. jarrovii* and *S. poinsettii* fell within the range of T_b s of other lizards in the family Phrynosomatidae, with a mean T_b of 35.2 °C [19–21]. However, there is substantial variation in T_b s among species and values range from 26.8 and 41.5 °C. In the present study, T_b (calibration) for *S. jarrovii* and *S. poinsettii* was 31.1 and 29.5 °C, respectively;

whereas $T_{ibutton}$ was 31.9 and 31.1 °C, respectively. Furthermore, T_s for *S. jarrovii* and *S. poinsettii* were 32.7 and 32.3 °C, respectively; whereas $T_{ibutton}$ was 33.0 and 33.0 °C, respectively. Moreover, T_s and $T_{ibutton}$ for *C. oaxacana* were 32.1 and 32.1 °C, respectively. T_s measured for *S. jarrovii*, *S. poinsettii*, and *C. oaxacana* are within the range observed for lizards, which generally approaches 30 °C [22,23]. Therefore, thermal preferences appear more phylogenetic that certain environmental factors present in each population of lizards of this group.







Figure 2: Relationship between iButton temperature $(T_{iButton})$ and selected temperature (T_s) of *Sceloporus jarrovii* and *Sceloporus poinsettii*. The observation of curve for *S. poinsettii* is shown as solid circles with fitted regression as a solid line, and an open triangle with fitted regression as a dashed line for *S. jarrovii*.



temperature (T_s) of *Ctenosaura oaxacana*. Observations of curve are shown as solid diamonds with fitted regression as solid line.

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In this study there was a significant relationship between $T_{\rm iButton}$ and $T_{\rm b}$ for *S. jarrovii* and *S. poinsettii*, as well between $T_{\rm iButton}$ and $T_{\rm s}$ for three species of lizards. Results from regression models showed that $T_{\rm iButton}$ is a good indicator of $T_{\rm b}$ for *S. jarrovii* and *S. poinsettii* during calibration process. In the same way, $T_{\rm iButton}$ is a good indicator of $T_{\rm s}$ for *S. parrovii* and *C. oaxacana* through experiment gradient. External and internal temperature measurements were highly correlated. Thus, external measurement of $T_{\rm b}$ for *S. jarrovii* and *S. poinsettii* and $T_{\rm s}$ for three species of lizards in this study.

Our data confirm that iButtons must be calibrated prior to placing on the lizards. The failure to do so can result in the underestimation of body temperature by as much as 1.4 °C [24]. Slopes and R² data of these study suggest that a linear fit to calibration data is adequate for physiological temperature ranges. The modified iButtons are thus a convenient device for making these preliminary studies and should aid in improving the quality of selected temperature data and other metabolic data obtained in laboratory.

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