



ARTÍCULO ORIGINAL

## Thermal biology of adults *Peltophryne peltcephala* (Amphibia: Anura: Bufonidae) from one population of Central Cuba: Natural and laboratory assays

*Thermal biology of adults Peltophryne peltcephala (Amphibia: Anura: Bufonidae) from one population of Central Cuba: Natural and laboratory assays*

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### RESUMEN

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Algunos modelos de calentamiento global sugieren efectos negativos sobre el rango de distribución geográfica de los anfibios cubanos, no existen estudios que exploren las posibles respuestas frente a estos cambios basados en la biología térmica de las especies. El Sapo Gigante Oriental, *Peltophryne peltcephala* es un sapo grande, endémico, ampliamente distribuido a lo largo del archipiélago cubano. Analizamos la relación entre la temperatura corporal de adultos de *P. peltcephala* activos durante la noche, y la temperatura del aire y del sustrato durante tres meses diferentes de muestreo en una localidad del centro de Cuba. Además, examinamos la respuesta de individuos adultos en un experimento térmico con condiciones estáticas (temperatura del agua 12 -36°C). La temperatura corporal individual fue función de la temperatura del sustrato y del aire, mayores coeficientes de correlación fueron detectados entre la temperatura corporal y la temperatura del sustrato para ambos sexos de *Peltophryne peltcephala* en la naturaleza. Las mayores diferencias entre las temperaturas máximas del aire y del cuerpo, y las máximas del sustrato y del cuerpo, se registraron durante el mes más cálido. Aunque la temperatura corporal se mantiene dentro de un rango térmico estrecho tanto en condiciones de campo y como de laboratorio, valores de temperaturas ambientales extremas, particularmente por encima de los 32°C podrían representar un riesgo para la supervivencia. Las hembras son menos eficientes durante la termoaclimatación.

**Palabras clave:** biología térmica, bufónido cubano, temperatura corporal, temperatura del sustrato, termoaclimatación

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## ABSTRACT

Some models suggest negative effects of global warming on the geographic distribution range of Cuban amphibians. However there are no studies that explore the possible responses to these changes based on the thermal biology of the species. The Eastern Giant Toad, *Peltophryne peltocephala*, is an endemic large toad widely distributed along the Cuban archipelago. We analyzed the relationship between the body temperature of nocturnally active adults of *P. peltocephala* and air and substrate temperatures during three different months of survey in one locality of Central Cuba. Additionally, we examined the response of adult individuals in a thermal experiment (water temperature 12-36°C) with static conditions. Individual body temperature was a function of substrate and air temperature; highest coefficients of correlations were detected between body temperature and substrate temperature for both sexes of *Peltophryne peltocephala* in nature. The higher differences between maximum air and body temperatures and maximum substrate and body temperatures were registered during the warmest month. Although body temperature is maintained within a narrow thermal range under both field and laboratory conditions, extreme ambient temperature values, particularly above 32°C, could represent a risk to survival. Females are less efficient during thermoacclimation.

**Keywords:** body temperature, Cuban bufonid, substrate temperature, static trial, thermal biology, thermoacclimation

## INTRODUCCIÓN

As a consequence of cumulative increases of concentrations of anthropogenic greenhouse gas emissions, each of the last three decades has been successively warmer at the Earth's surface than any preceding decade since 1850. The period from 1983 to 2012 was likely the warmest of the last 1400 years in the Northern Hemisphere (IPCC, 2022). Several models have been developed to predict the impact of climate change on biodiversity. Such changes can alter the biology of organisms, its physiological performance and vulnerability, particularly in terrestrial ectotherms (Deutsch et al., 2008; Huey et al., 2012; Hoffman et al., 2013; Moritz and Agudo, 2013)

Amphibians are ectotherm vertebrates with complex life cycles with aquatic and terrestrial life stages, shell-less eggs, permeable and exposed skin; therefore they are very sensitive to changes of environmental quality (Hillman et al., 2009). Several behavioral and physiological processes are dependent of temperature environment (e.g. feeding, communication, locomotion, development, metamorphosis, growth, etc.), that makes them very susceptible to the progressive global warming (Blaustein et al., 2010; Calosi et al., 2010; Duarte et al., 2012). Body temperature is perhaps the most important ecophysiological variable affecting the performance of ectotherms (Angilletta et al., 2002). Body temperature

of an amphibian in its natural environment results from complex interactions of numerous environmental factors, internal physical changes and physiological adjustments (Navas et al., 2016). Amphibians can escape from the environmental challenges by selection of appropriated microhabitats, employment of behavioral mechanisms or secondary physiological regulation (Hutchison and Dupré, 1992). Behavioral thermoregulation, therefore buffers body temperature from variation in ambient thermal conditions, whereas acclimation buffers biochemical and physiological rate functions from changes in body temperature (Little and Seebacher, 2016).

Although some studies have evaluated the possible impacts of the climate change on amphibian populations in Cuba, most of them are based on the modelling of thermal niche and estimations of possible changes on species distribution ranges under different future climatic scenarios (Suarez et al., 2014; Cobos and Alonso Bosch, 2016; 2018; Mancina et al., 2022). However, thermal biology of Cuban amphibians is barely known. The effects of environmental temperature variation on the ecological and physiological species performances have received little attention. Only a few papers documented the relationship between environmental temperature and acoustic parameters variation of the advertisement calls of some species (Hernández et al., 2010; Rodríguez et al., 2010; Alonso Bosch et al., 2017). It is

necessary to integrate current field information and laboratory assays, in order to be able to understand the sensitivity, vulnerability and possible responses of the species to the adverse effects of the climatic change.

The Eastern Giant Toad (*Peltophryne peltocéphala*, Tschudi 1838) is one of the terrestrial large-bodied toads from Cuba (Schwartz, 1960). It is widely distributed along the Cuban archipelago, with the exception of western Cuba. It occurs from the central region to the eastern edge of Cuba, including the Zapata's Peninsula, Isla de la Juventud, some keys in the Sabana-Camaguey archipelago and keys in the northern coast of Holguin province (Alonso Bosch, 2011). This species shows an extraordinary ecological plasticity, it occupies very dissimilar habitats, from sea level to approximately 800 m a.s.l., in xeric or wet conditions. It inhabits broadleaf forests, along stream banks, and coastal deserts. This species is also common in cultivated fields and in backyards, and around houses in rural areas (Henderson and Powell, 2009). Taking into account the life history traits and the ability of *P. peltocéphala* to colonize diverse microhabitats and climates, this species could be a good model to quantify the relationship between body temperature and air and substrate temperatures, paying attention to possible sex-specific differences. In this study, we analyzed the relationship between the body temperature of nocturnally active adults of *P. peltocéphala* and air and substrate temperatures during three different months of survey in one locality of Central Cuba. Additionally, we analyzed their behavioral and thermoregulatory responses to different water temperatures under laboratory conditions.

## MATERIALS AND METHODS

### DETERMINATION OF FIELD BODY TEMPERATURES AND ENVIRONMENTAL VARIABLES

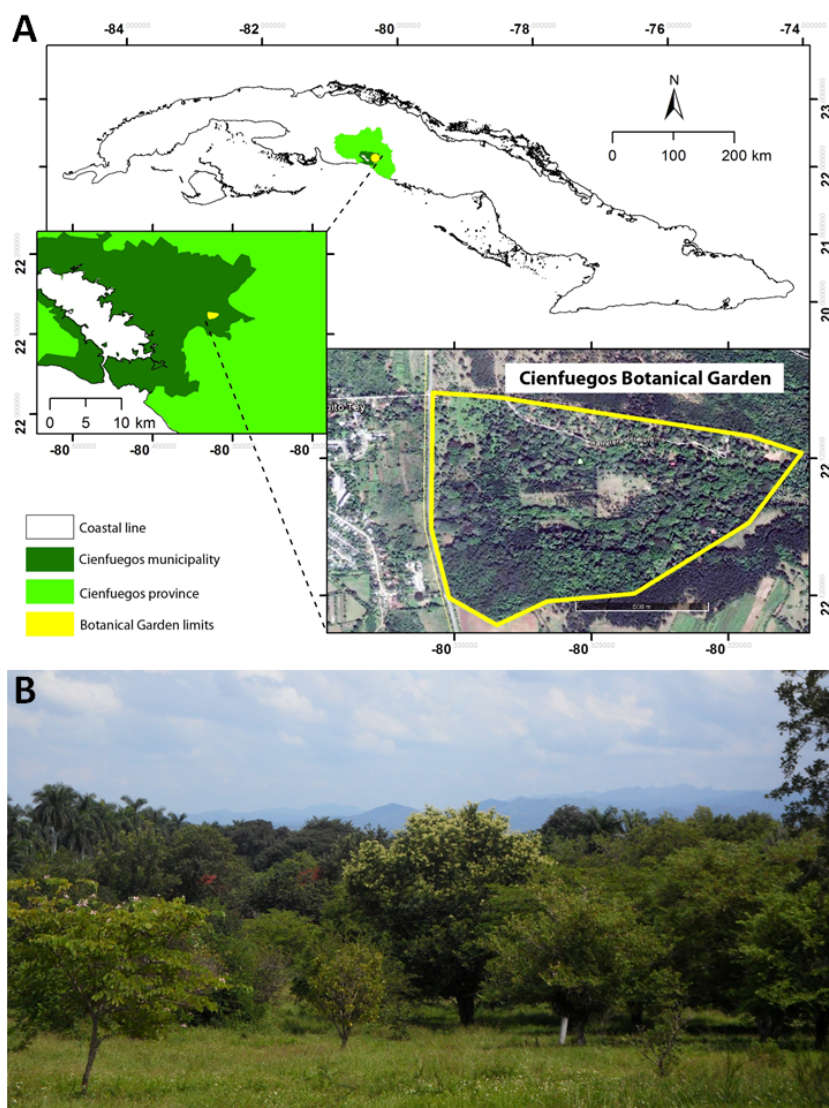
Adult individuals of Eastern Giant Toad (*Peltophryne peltocéphala*) were studied at a semi natural environment at the Cienfuegos Botanical Garden (22.12722°N, 80.33056°W, datum WGS 84), Cienfuegos province, in Central Cuba (Fig. 1a). Seven hectares correspond to a relatively well-preserved natural forest (Fig. 1b). The garden is characterized by

tropical climate with a mean annual temperature of 24.5°C, a mean maximum temperature of 30.4°C, a mean minimum temperature of 20.0 °C, and a mean annual rainfall of 1400 mm (most of which falls during the wet season in summer months). The study was conducted during two consecutive nights in September (summer and wet season), December (winter and beginning of dry season) 2015, and March (middle dry season) 2016. The animals were captured during the peaks of activity of this species (Sampedro *et al.*, 1982), along a closed (semicircular) transect of 500 m, covering diverse types of vegetation with different levels of anthropogenic perturbation inside the garden.

The animals were located with the use of headlights, and captured manually. With a fast reading thermometer (error  $\pm 0.1$  °C, Miller and Webber instruments) the body temperature ( $T_b$ ) of each animal was measured a few seconds after capture, avoiding over-manipulation. Afterwards, snout-vent length (SVL) was measured with a Vernier Caliper (error  $\pm 0.05$  mm) and body mass ( $W$ ), using Pesola dynamometers (error  $\pm 2$  g). The sex was determined based on sexually dimorphic characters (size, nuptial pads, vocal sac, etc.). We measured substrate temperature ( $T_s$ ) at the site of capture using a Fisher Scientific sensor (error  $\pm 0.1$  °C). Finally, we measured the relative humidity (HR, error  $\pm 5$  %) and air temperature ( $T_a$ , error  $\pm 0.5$  °C) at 20 cm above the soil with a thermohygrometer (HANNA Instruments). All animals were released on the site of collection after taking measurements.

## EXPERIMENT AND LABORATORY DATA COLLECTION

In order to explore the behavior and immediate responses of both sexes of this species in a changing thermal environment, we collected 19 adult individuals (seven males and twelve females) in the summer 2015. Individuals were transported to the laboratory and maintained for two days at 25 °C, with a photo-period of 12x12. We used a static trail placing each individual for five minutes in a thermostatic bath (Nüve laboratories, error  $\pm 1$  °C) with a capacity of 7 liters and programmed to achieve the desired constant temperature in the water. After this procedure, each experimental subject was removed from the bath and



**Figure 1.** Geographic location of the Botanical Garden of Cienfuegos. The transect plotted for the data collection is within the delimited area (A.). Panoramic view of the Botanical Garden of Cienfuegos (B.). Photograph by RAB.

its body temperature ( $T_b$ ) was measured immediately using the fast reading thermometer. This procedure was repeated for each individual in an increased gradient of seven water temperatures at 4 °C intervals (12-16-20-24-28-32-36 °C). We observed the animal behavior (movements, postures and ability to escape) during each treatment. The loss of the righting response, defined as the moment when a frog cannot right itself from being placed venter-up for a period longer than 5 sec (Brooks and Sassman, 1965;

McManus and Nellis, 1975; Navas, 1997, Catenazzi *et al.*, 2014; von May *et al.*, 2019) was checked just at the end of each temperature treated. Temperatures were considered to be critical if toads were no longer able to return to their original position. Each individual was exposed to a constant temperature, and it was not stimulated again after, at least three hours later. The bath water was replaced for each animal. The analysis of the thermo- acclimation and behavioral response of adult individuals of *Peltophryne peltoccephala* to the

abrupt temperature variation served to identify its comfort temperature range in laboratory conditions. At the end of the laboratory experiment, all toads were released during the night in the original capture site.

### STATISTICAL ANALYSES

We calculated the mean, standard deviation, maximum and minimum for all the thermal parameters. We tested for data normality with the Shapiro–Wilk test. All statistical analyses were performed with Statistica 8.0 software (StatSoft Inc. 2007). We analyzed the relationship between air and substrate temperatures with body temperature. Body mass influences the physiological characteristics of individuals such as behavior, reproduction, foraging and / or growth (Angilletta *et al.*, 2002). Here we explored the relationship between snout-vent length and body mass of the animals. Using a regression model as a body condition indices (BCI), based on allometric relationships between snout-vent length and body mass, a Residual index was calculated using log<sub>10</sub>-transformed data that improved the linearity of the relationship between these variables (Bancila *et al.*, 2010). Body condition is considered as a good indicator of environmental stress, prey availability and/or habitat quality (Sztatecsny and Schabetsberger, 2005). Then, we examined the relationship between

the body condition (residual index) and body temperature for each sexes.

### RESULTS

A total of 121 adults *Peltophryne peltoccephala* were collected in the field during the study. Table 1 presents a summary of the thermal ecology of these animals with information about snout-vent length and mass for both sexes. The number of males sighted inside the transect was similar among the studied months, while the number of females found was variable between the months, but always higher than the number of males (Table 2). A significant relationship ( $R=0.873$   $p<0.01$ ) was observed between body mass and snout-vent length (Fig. 2). Females were significantly larger than the males ( $t = 9,304$ ,  $p<0.05$ ). The residual index showed no correlation with SVL ( $r = 0.031$ ,  $P = 0.973$ ).

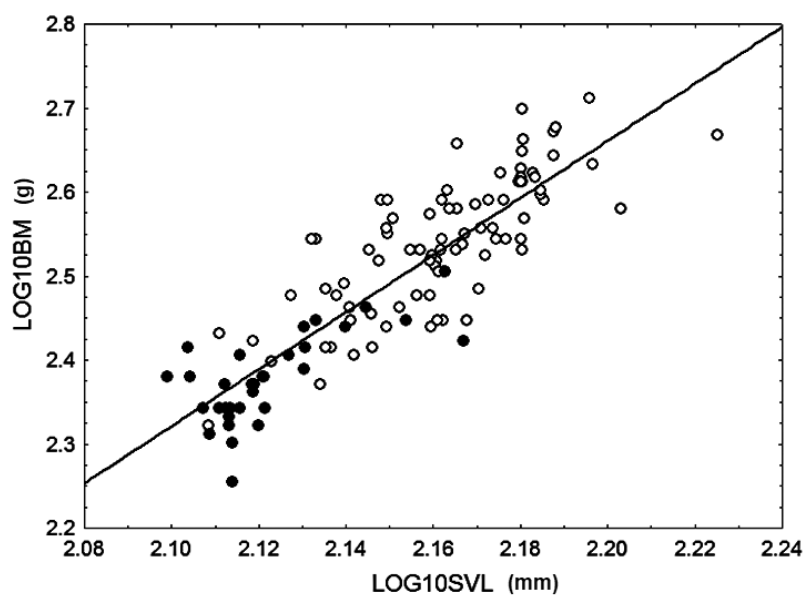
Body temperature varied in a relatively reduced range, from 18.6 - 24.4 °C in males, and from 18.5 - 25.3 °C in females (Table 1). In contrast with the distribution of frequencies observed for the variable air temperature, most of the toads varied its temperatures closer to the range of variation of substrate temperature during the study, mainly between 19-25°C. (Fig. 3). Body temperatures were significantly higher on September in both sexes ( $F=96.25$ ,  $P < 0.001$ ), but no significant differences were

**Table 1.** Mean, DS and range (min-max) of snout-vent length (SVL), Body Mass (BM), and thermal parameters [Air temperature (Ta), Substrate temperature (Ts) and Body temperature (Tb)] measured in adults *Peltophryne peltoccephala* from Botanical Garden of Cienfuegos, Cuba. Number of individuals (N).

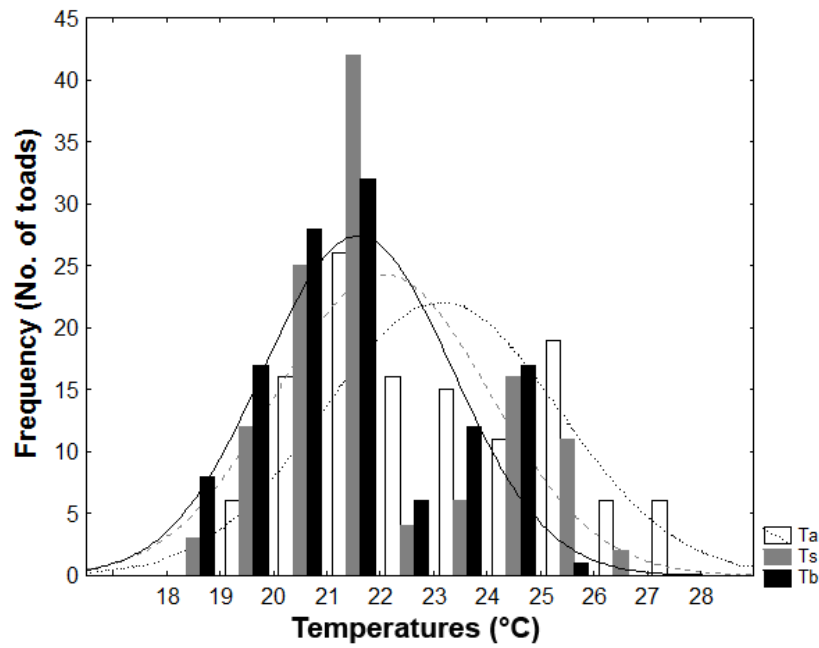
Parameter	All sample	
	Males (N=31)	Females (N=90)
SVL (mm)	132.7±5.2 (125.6-146.8)	145.5±7.07 (128.3-168.0)
BM (g)	240.6±30.5 (180.0-320)	353.1±64.8 (210.0-515.0)
Ta (°C)	22.9±2.5 (19.6-27.5)	23.2±2.06 (19.6-28.0)
Ts (°C)	22.0±2.2 (18.9-26.0)	22.0±1.9 (18.3-26.8)
Tb (°C)	21.5±1.9 (18.6-24.4)	21.6±1.7 (18.5-25.3)

**Table 2.** Field collected data from the two nights of survey during three months of the study. For each thermal parameters measured in adults of both sexes of *Peltophryne peltoccephala* we show Means  $\pm$  Standard Deviation and Minimum-Maximum in parenthesis. Air temperature (Ta), Substrate temperature (Ts), and Body temperature (Tb).  $\Delta T_{max1}$ = Tamax-Tbmax,  $\Delta T_{max2}$ = Tsmax-Tbmax.

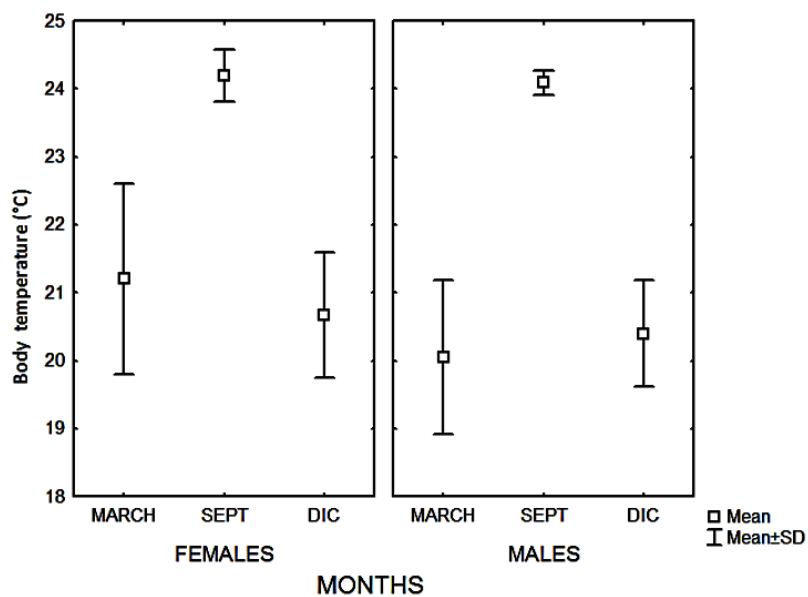
Thermal parameter	September		December		March	
	Males (N=10)	Females (N=16)	Males (N=12)	Females (N=25)	Males (N=9)	Females (N=49)
Ta (°C)	26.2 $\pm$ 1.2 (23.8-27.5)	26.0 $\pm$ 0.7 (25.1-28.0)	21.5 $\pm$ 0.6 (20.5-22.5)	21.4 $\pm$ 0.7 (20.1-22.4)	21.2 $\pm$ 1.5 (19.6-23.1)	23.1 $\pm$ 1.8 (19.6-26.3)
Ts (°C)	24.9 $\pm$ 0.5 (24.3-26.0)	25.0 $\pm$ 0.8 (23.8-26.8)	20.9 $\pm$ 0.6 (20.2-21.9)	21.0 $\pm$ 1.1 (18.3-23.4)	20.3 $\pm$ 1.0 (18.9-21.8)	21.5 $\pm$ 1.6 (18.4-26.0)
Tb (°C)	24.0 $\pm$ 0.2 (23.9-24.4)	24.2 $\pm$ 0.4 (23.4-25.0)	20.4 $\pm$ 0.8 (19.1-21.4)	20.7 $\pm$ 0.9 (18.7-22.0)	20.0 $\pm$ 1.1 (18.6-21.9)	21.2 $\pm$ 1.4 (18.5-25.3)
$\Delta T_{max1}$	3.1	3	1.1	0.4	1.2	1
$\Delta T_{max2}$	1.6	1.8	0.4	1.4	0.1	0.7



**Figure 2.** Relationship between log<sub>10</sub> transformed body mass (g) and snout-vent length (mm) (A.) in adult Eastern Giant Toads, *Peltophryne peltoccephala* (n = 121). Influence of sex on residual index as a measure of the body condition. Graphics represent mean residual index scores ( $\pm$  SD). Males represented with black-filled circles, open circles for females



**Figure 3.** Frequency of adult toads in the field according to its body temperatures (solid bars and continuous line), substrate temperature (gray bars and discontinuous line) and air temperature (white bars and dotted line) in the Botanical Garden of Cienfuegos, Cuba.



**Figure 4.** Comparisons of body temperatures between females and males *Peltophryne peltoccephala* from Botanical Garden of Cienfuegos, Cuba, during three months of study of its thermal biology. See results for details of statistical analysis.

found between December and March (Fig. 4, Table 2). When we analyzed the differences inside month, we

found intersexual differences only during March ( $t=2.342$ ,  $p<0.05$ ). The higher differences between maximum air and body temperatures and maximum substrate and body temperatures were registered during the warmest month, September, these differences were always more lowest in males (Table 2). September was the wettest month with 91.4 % of maximum value of relative humidity, this parameter was most similar between March (82.1 %) and December (80.5 %).

The residual index showed no significant correlation with  $T_b$  ( $r = 0.0646$ ,  $P = 0.4817$ ). Individual body temperature was function of substrate and air temperature (Fig. 5, Table 3), but a small dispersion is observed when we analyzed the relationship between body and substrate temperatures, particularly in males at the coldest range of this curve (Fig. 5, Table 3). During our nocturnal surveys we recorded seven substrate categories in the microhabitat (leaf litter, mud of the bare ground, grass, straw, puddle, branch and asphalted pavement) for *P. peltocephala* ( $N = 121$  individuals) from the seminatural environment of the Cienfuegos Botanical Garden (Fig. 6). In open areas, the mud of the bare ground (33.8%) and grass (21.9%) were the microhabitats where most toads were first sighted, while in the leaf litter of the forest ground the animals were also common (18.2%). Asphalted areas were also frequently used in the most perturbed zones (12.4%). These preferences were maintained during the three months of sampling (Fig. 6). Females were more abundant and more widely dispersed by microhabitats in the study area.

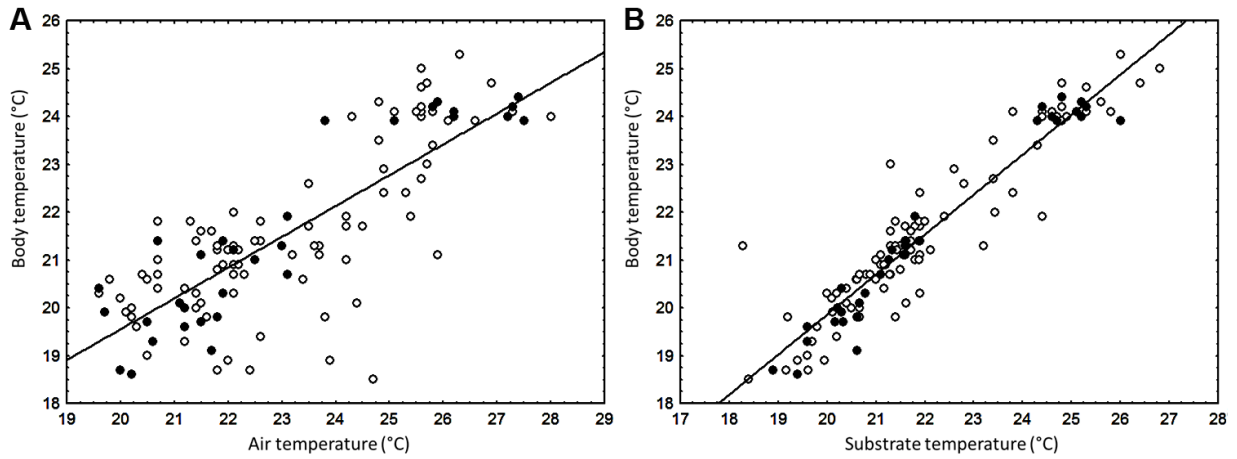
In the extreme temperatures of the experiment (12 and 36° C, respectively), the individuals showed stress behaviors. These were initially characterized by series of vigorous swimming and upward escape movements. After these behaviors, the locomotor activity becomes disorganized and the animals lost its ability to escape from these thermal conditions, up to a sudden and total inactivity. However, all animals returned to their original positions when we tested the loss of the righting response. All stress behaviors were less evident when the temperature of the water moved away from 20-28°C. When the temperature was set at 24 °C the individuals remained practically immobile, keeping the lungs inflated and the hind limbs stretched, thus increasing the contact surface resulting in the buoyancy of its bodies. In this context, body temperature ( $T_b$ ) in both sexes practically equaled water temperature (Males:  $T_b = 24.2 \pm 0.3$ , Females:  $T_b = 24.0 \pm 0.5$ ). With an increasing or decreasing,  $T_b$  varied with a tendency toward acclimation (Fig. 7). Both sexes exhibited better acclimation ( $T_b \sim T_s$ ) towards low temperatures, while at the high and extreme temperatures of the gradient, the relationship  $T_b$ - $T_s$  showed greater differences (Fig. 7).

## DISCUSSION

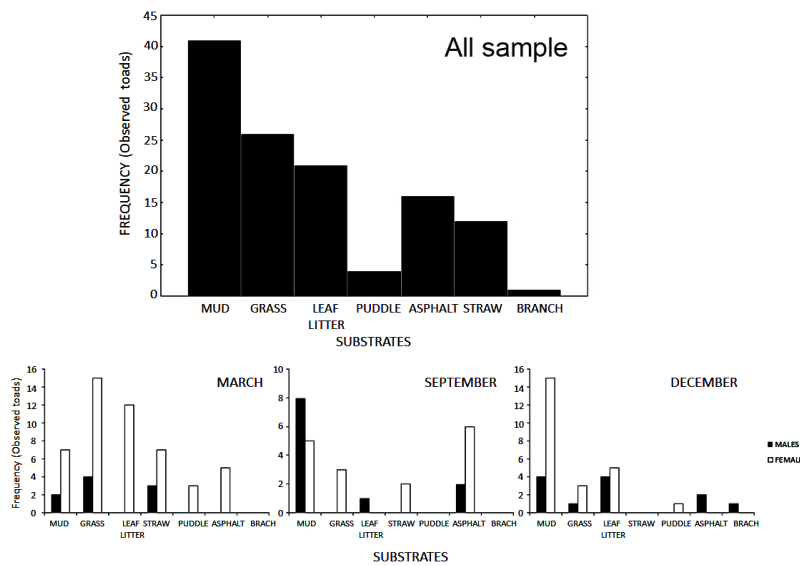
The maintenance of optimal  $T_b$ , through the selection of suitable microclimate sites, determines how ectotherms interact with their environment. During behavioral thermoregulation, ectotherms subsample the environment, thereby reducing the gap between environmental temperature and optimal body temperature (Little and Seebacher, 2016). *Peltophryne peltocephala* used all the temperatures available in its

**Table 3.** Relationship between thermal parameters obtained of adults *Peltophryne peltocephala* from Botanical Garden of Cienfuegos, Cuba. Air temperature ( $T_a$ ), Substrate temperature ( $T_s$ ) and Body temperature ( $T_b$ ). Pearson correlation coefficients ( $r$ ) and values of significance ( $p$ ) are shown in each case.

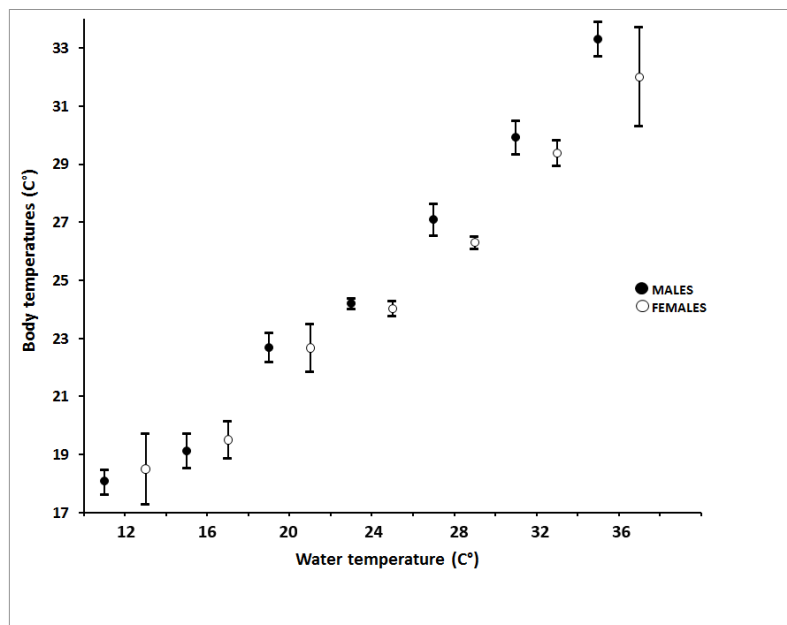
	All sample (N=121)	Females (N=90)	Males (N=31)
Ta vs Tb	$r = 0.8002$ , $p = 0.0000$	$r = 0.7429$ , $p = 0.0000$	$r = 0.9179$ , $p = 0.0000$
Ta vs Ts	$r = 0.7707$ , $p = 0.0000$	$r = 0.7023$ , $p = 0.0000$	$r = 0.9210$ , $p = 0.0000$
Ts vs Tb	$r = 0.9424$ , $p = 0.0000$	$r = 0.9269$ , $p = 0.0000$	$r = 0.9786$ , $p = 0.0000$



**Figure 5.** Scatterplots visualizing the individual body temperature as a function of air (A.) and substrate (B.) temperatures in adults *Peltophryne peltoccephala* from Botanical Garden of Cienfuegos, Cuba. Open circles (females), filled circles (males).



**Figure 6.** Microhabitats (substrates) used by adult males and females *Peltophryne peltoccephala* during the night of three different months in Botanical Garden of Cienfuegos, Cuba.



**Figure 7.** Variation in body temperature ( $T_b$ ) in relationship to water temperature ( $T_s$ ) for males ( $N = 7$ ) and females ( $N = 12$ ) of *Peltophryne peltoccephala* under laboratory experimental conditions.

environment, shuttling between microhabitats, regulating activity time and adjusting posture. The

diversity of used substrates probably is related to the abundance and distribution of thermal resources in space (Angilleta, 2009). Like other terrestrial toads, formerly included in genus *Bufo*, the body temperature of *Peltophryne peltoccephala* showed a strong correlation with environment temperature, apparently by selection of substrate temperatures in available microhabitats (Sinsch, 1989; O'Connor and Tracy, 1992; Raush *et al.*, 2008; Oromi *et al.*, 2010; Noronha-de-Souza *et al.*, 2015; Sanabria *et al.*, 2015; Mokhatla *et al.*, 2019).

The apparent reduction in activity during the warmest conditions could be related to the control of water loss, rather than the avoidance of extreme temperatures. Anderson and Andrade (2017) suggested that as dehydration becomes more severe when thermal tolerance range is narrowed. Our observations in *P. peltoccephala* revealed that the higher differences detected between maximum air and body temperatures and maximum substrate and body temperatures were registered during the warmest month. Body temperature elevation might be

compromising on dehydrated toads. Further studies are needed to understand the physiological and biochemical mechanisms behind the acclimatory behavior of *P. peltoccephala* as a promissory model for the studies of thermal biology of Cuban amphibians.

Eastern Giant toad (*Peltophryne peltoccephala*) appear to regulate its body temperature during the night by conduction from ground and/or radiation from ground and vegetation (Brattstrom, 1979), by pressing its ventral body surface on selected moisturized substrates. Hydric balance may impose limits on thermoregulation, restricting individuals to moist microhabitats, which in turn may limit ranges of opportunity for body temperature regulation (Tracy, 1976; Anderson *et al.*, 2018; Bovo *et al.*, 2018). The intrinsic association between body temperature regulation and water balance should be explored in this species along its wide distribution, paying attention to the variability of the combined effects of both parameters on feeding, locomotion, and metabolism (Preest and Pough, 2003)

In nature, this species appears to be active in conditions close to their thermal tolerances. *P. peltoccephala* adults show a relatively narrow thermal

range in congruence with its tropical ectotherm condition. They were not able to acclimate properly and they did not achieve to match their body temperatures with the extreme temperatures environment, particularly in higher temperatures. Thermal stress can directly reduce survival of individuals in habitats where maximum daily temperatures regularly approach their CTmax (Nowakowski *et al.*, 2015). Minimum critical temperature (CTmin), has been described as the Tb below which performance is minimum, while a maximum critical temperature (CTmax), which represents Tb above which performance is also minimum, with an optimum temperature (Topt), which represents Tb at which performance is maximum. In our conditions, 24°C appear to be Topt in both sexes, and this represents 100% of the efficiency of the activity of the species ("Relative performance"). The body temperatures measured in the intermediate zone of the gradient could represent the 70% of the efficiency of the activity according to the observed behavior. The minimum body temperature, as well as the maximum reached by the individuals used in the experiment could coincide with 50% of the efficiency of the activity ("Relative performance") taking into account initial stress of the animal that finish in quiet postures. CTmin and CTmax could be near to 12 and 36° C, respectively.

The mechanisms involved in thermal biology *Peltophryne peltoccephala* may change across its wide distribution along Cuban archipelago (Rivalta *et al.*, 2014), in response to the temporal and spatial scales over which micro-climatic conditions vary. This species could display higher thermal tolerance in other sites of its wide distribution range, exploit different bioclimatic microhabitats. *P. peltoccephala* has been recorded in very contrasting thermal habitats, for example in the semi-desert climate of the southern coast of the province of Guantánamo, and the tropical montane climate of the Sierra Maestra (Díaz and Cádiz, 2008; Rivalta *et al.*, 2014). Further studies are needed to understand the plasticity in thermoregulatory behavior in nature and the susceptibility of this species to environmental extremes, that will provide an excellent opportunity to address behavioral constraints induced by thermal and water availability.

The future climate of Cuba could be characterized by a mean air temperature rising up to 4°C, a decrease in

annual rainfall that, depending on the scenario, would range between 15- 63%, accompanied by an increase in the potential of evapotranspiration and real evaporation (Planos *et al.*, 2013). These climatic predictions joined to the ectothermic condition of *P. peltoccephala* and its apparently narrowed thermal tolerance could represent true threats to adaptability, dispersal, reproduction and survival of this endemic Cuban amphibian. Some studies indicate that, in the future, areas of habitats suitable for other Cuban toads will be reduced (Cobos and Alonso Bosch, 2016; 2018, Mancina *et al.*, 2022).

During our experimental conditions, females were unable to acclimate at high temperatures, suggesting that they could be more vulnerable than males to extreme events, such as a sudden temperature change (Li *et al.*, 2013). Females need to maintain their body temperature at optimal intervals that guarantee the survival of their gametes. Thermal constraints can exist in aquatic and terrestrial environments, but the cost of thermoregulation depends very much on mean and variance of operative temperatures (Sears *et al.*, 2011). Considering that amphibian eggs are deposited in thermal environments that optimize growth and development, an additional challenge is therefore, to explore how adults and tadpoles from other populations of *P. peltoccephala* would acclimatize in their respective breeding conditions.

## CONCLUSIONS

Adults *Peltophryne peltoccephala* used all the temperatures available in its environment. Body temperature (Tb) showed a strong correlation with environment temperature, apparently by selection of substrate temperatures in available microhabitats. Individual body temperature was function of substrate (Ts) and air temperature (Ta), but the highest coefficients were detected between body temperature (Tb) and substrate temperature (Ts) for both sexes of *Peltophryne peltoccephala* in nature. We observed significant difference in mean residual index scores (as a measurement of body condition) between males and females, but the residual index showed no significant correlation with body temperature. In experimental conditions, both sexes exhibited better acclimation (Tb~Ts) towards low temperatures, while at the high and extreme temperatures of the gradient the Tb-Ts relationship showed greater differences. Females were

unable to acclimate at high temperatures, suggesting that they are less efficient during thermoacclimation could be more vulnerable than males to extreme events, such as a sudden change in temperature.

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