

Thermal biology of the toad *Rhinella schneideri* in a seminatural environment in southeastern Brazil

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Abbreviations: d_b , accuracy of thermoregulation; d_e , thermal quality of habitat; E , effectiveness of thermoregulation; T_b , body temperature; T_e , operative environmental temperature; T_{set} , preferred body temperature selected in a thermal gradient

The toad, *Rhinella schneideri*, is a large-bodied anuran amphibian with a broad distribution over South America. *R. schneideri* is known to be active at night during the warm/rainy months and goes into estivation during the dry/cold months; however, there is no data on the range of body temperatures (T_b) experienced by this toad in the field, and how environmental factors, thermoregulatory behaviors or activity influence them. By using implantable temperature dataloggers, we provide an examination of T_b variation during an entire year under a seminatural setting (emulating its natural habitat) monitored with thermosensors. We also used data on preferred T_b , allowing us to express the effectiveness of thermoregulation quantitatively. Paralleling its cycle of activity, *R. schneideri* exhibited differences in its daily and seasonal profile of T_b variation. During the active season, toads spent daytime hours in shelters and, therefore, did not explore microhabitats with higher thermal quality, such as open areas in the sun. At nighttime, the thermal suitability of microhabitats shifted as exposed microhabitats experienced greater temperature drops than the more insulated shelter. As toads became active at night, they were driven to the more exposed areas and, as a result, thermoregulatory effectiveness decreased. Our results, therefore, indicate that, during the active season, a compromise between thermoregulation and nocturnal activity may be at play. During the estivation period, *R. schneideri* spent the entire day cycle inside the shelter. As toads did not engage in nocturnal activity in those areas with low thermal quality, the overall effectiveness of thermoregulation was, indeed, elevated. In conclusion, we showed that daily and seasonal variation in T_b of an anuran species is highly associated with their respective pattern of activity and may involve important physiological and ecological compromises.

Introduction

Body temperature (T_b) affects behavioral and physiological functions of animals, such as locomotion, foraging and growth.¹ The maintenance of T_b within narrow and controlled ranges can optimize these functions and, ultimately, affect fitness. In ectotherms, behavioral thermoregulation is a common strategy for buffering temporal and spatial variations in environmental temperature. Thermoregulatory behaviors include shuttling between microhabitats, basking, regulating activity time and adjusting posture.^{2–4}

Anuran amphibians have been shown to thermoregulate by behavioral means in natural environments.³ Behavioral thermoregulation is also observed in laboratory conditions, where anurans show less variation in preferred T_b in thermal gradients^{5–8} than displayed in the field.^{8,9} Most anurans, however, offer little

or no resistance to evaporative water loss.^{9–12} It is known that thermoregulatory adjustments need to accommodate the potentially conflicting compromises related to water balance¹³ and the prominent tendency of amphibians to lose water is thought to prevent a more efficient regulation of T_b . As a consequence, amphibians, and particularly terrestrial anurans, may have their activities limited, daily and seasonally, by the availability of adequate thermal microhabitats that allow for thermoregulation without serious consequences to water balance.^{14–16}

The toad, *Rhinella schneideri*, is a terrestrial large-bodied amphibian (Anura, Bufonidae) with a broad distribution over South America (Argentina, Bolivia, Brazil, Paraguay, and Uruguay), inhabiting open areas at Chaco, Cerrado (a savannah-like habitat), and Atlantic Forest biomes.^{17,18} This toad is also easily found in open and urban areas.¹⁸ During the dry and cold season in Southeastern Brazil, *R. schneideri* estivates buried in shallow

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burrows excavated in the soil or under rocks, or fallen logs. In the laboratory, during estivation season, this species shows reductions in metabolic and heart rates,^{5,19} independent of T_b ; furthermore, it selects lower preferred T_b in a thermal gradient during winter.⁵

Due to its large body size and abundance, *R. schneideri* has been subjected to a number of physiological studies, including some devoted to aspects of its thermal biology.^{5,6,19-24} However, we still lack some basic and important information about the thermal biology of this species. In fact, we still do not know the range of T_b 's experienced by this toad in the field and how environmental factors and thermoregulatory behaviors determine it, or even how T_b might be related to changes in activity. Herein, we aimed to fill this gap by providing an examination of T_b , recorded over a year, in a group of toads maintained under semi-natural conditions within their original area of occurrence. In doing so, we adopted the protocol described by Hertz et al.²⁵ to examine daily and seasonal variations in T_b and environmental temperatures, which, combined with preferred T_b of this species in a thermal gradient, allowed us to calculate indexes that evaluate the accuracy and effectiveness of thermoregulation.

Materials and methods

Animals

*Rhinella schneideri*²⁶ toads (formerly known as *Bufo paracnemis* and *Chaunus schneideri*^{27,28}) of both sexes, weighing 335–590 g, were captured under the permit issued by the “Instituto Brasileiro do Meio Ambiente e dos Recursos Renováveis” – IBAMA (Animal License n° 025/2005). Toads were fed beef liver twice a week up to 2 d before surgery for implantation of temperature dataloggers (see below). Throughout the entire duration of the experiment (~1 year), toads were frequently inspected for their general appearance, presence of ectoparasites, position inside the arena and any other noticeable occurrence.

Location and experimental arena

The experiment was carried out in the municipality of Rio Claro, São Paulo state, southeastern Brazil (22°24'53.648"S, 47°33'54.501"W at 626 m a.s.l.). This location is included within the geoclimatic domain of the Tropical Atlantic.²⁹ The climate is mesothermic, with dry winters and rainy hot summers, according to the Köppen-Geiger's system.³⁰ During the experiments, rainfall and relative humidity (RH) data were collected daily by the local meteorological station (CEAPLA, UNESP). Autumn months (March-June) were characterized by 186.8 mm of rainfall (mean 2.00 mm/day), whereas winter months (June-September) had 120.3 mm of rainfall (mean 1.3 mm/day), spring months (September-December) had 389.4 mm of rainfall (mean 4.3 mm/day) and summer months (December-March) had 361.0 mm of rainfall (mean 4.2 mm/day). Maximum, minimum and mean RH (mean ± SEM) were, respectively, 99 ± 0.1, 46 ± 1.4 and 73 ± 0.8 % during autumn, 96 ± 0.4, 39 ± 1.3 and 66 ± 1% during winter, 97 ± 0.3, 49 ± 1.9 and 69 ± 1.4% during spring and 97 ± 0.1, 45 ± 1.3 and 67 ± 1.2% during summer. Minimum ($p <$

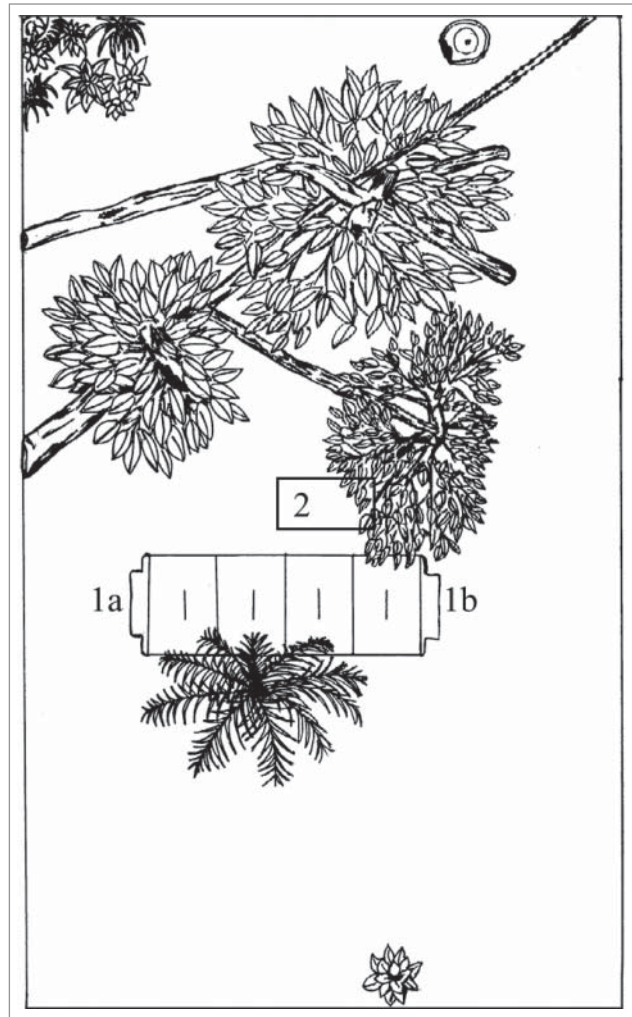


Figure 1. Schema of the superior view of the experimental arena where toads expended a year implanted with temperature dataloggers. Numbers indicate the shelter (1a and 1b are the 2 entrances to the den) and the water access (2). Shade (under trees and other plants) and open areas can be seen here. See text for details.

0.001; $F_{(3,314)} = 7.7$) and mean ($p < 0.001$; $F_{(3,314)} = 8.33$) RH were lower during winter and maximal RH was greater during autumn ($p < 0.001$; $F_{(3,314)} = 18.3$).

The experimental arena (Fig. 1) consisted of an outdoor 5 × 10 m enclosure located next to the Laboratory of Herpetology and Animal Physiology at São Paulo State University. This arena was fenced in by a 0.9 m high cement wall, topped by a metal net fence dome about 3 m high that covered the enclosure entirely. Inside the enclosure, half buried in the soil, there were 2 concrete dens (2.7 × 1.0 × 0.37 m) to serve as shelters (number 1, Fig. 1). The substrate was planted with grass (*Paspalum notatum*) and the pen was enriched with logs, trees (*Dyopsis lutescens*, *Eugenia uniflora*, *Schefflera arboricola*) and other plants (*Philodendron bipinnatifidum*). The animals had permanent access to water (number 2, Fig. 1).

Intra-abdominal implantation of dataloggers

Animals were anesthetized by submergence in an aqueous 0.3% solution of 3-aminobenzoic acid ethyl ester (MS-222, Sigma, St. Louis, USA) and a temperature datalogger (TidbiT, Onset Computer Corporation, Bourne, MA) was implanted in the abdominal cavity. To this end, each animal was positioned in lateral decubitus, and an incision was made in the skin and muscles of the left side of the abdomen just large enough to insert the datalogger into the coelomic cavity. After suturing the incision, animals were treated with enrofloxacin (Baytril®, Bayer, 3 mg/kg s.c.), a broad-spectrum fluoroquinolone, and an analgesic (Flunixin Meglumina, Banamine®, Schering-Plough, 1.0 mg/kg s.c.), and allowed to recover from anesthesia. These procedures lasted approximately 20 minutes. Animals were allowed to recover for at least one week from surgery before being placed in the experimental arena.

Temperature recordings in the experimental arena

Body temperature of 7 toads was automatically measured by the temperature dataloggers implanted in the abdominal cavity. Dataloggers were calibrated, before and after the experiment, against a mercury thermometer (Labortherm-N, 75 mm) at 5 different temperatures. Before being implanted, the dataloggers were programmed (BoxCar® Pro 4.0 for Windows, Onset Computer Corporation) to collect and store the temperature data every 16 min for a period of 12 months, starting 30 d after implantation.

The same dataloggers were used to sample the temperature of representative thermal microhabitats within the experimental arena: i) in a permanently shaded spot; ii) inside the shelter; iii) in the water bowl provided (25 × 54 × 88.5 cm); and iv) in an exposed open area. The dataloggers registered temperatures every 16 min between February of 2005 and January of 2006. Data from all 4 seasons were grouped by daytime (0600–1800) and nighttime (1800–0600) and means were calculated every 32 min.

In order to validate the environmental temperature readings collected by the dataloggers as representative of operative temperatures (T_e) during the experimental period, we simultaneously recorded temperature using a “naked” datalogger, along with a second datalogger that was implanted inside the body cavity of freshly dead toads.³¹ Both the “naked” dataloggers and the dead toads with implanted dataloggers were placed in each of the 4 microhabitats (2 toads of similar size to the experimental animals were used in each: shaded, open, shelter and water) under the same conditions prevalent during the experiment. Recordings lasted for 2 to 3 d each and were repeated under variable climatic conditions. Dead toads were replaced after 48 h, at most, to avoid any thermal effect of putrefaction. We found no significant differences in the heat exchange rates between the 2 conditions (naked datalogger vs within-carcass) for the temperatures recorded inside the shelter and water. Therefore, the raw data, as recorded by the naked dataloggers, were accepted as representative of T_e 's for these microhabitats. For the permanently shaded and open area microhabitats, however, there was a significant

difference between the heat exchange rates for the naked datalogger and the toad carcass ($p < 0.001$). In these cases, we found that T_e could be confidently predicted from a linear combination between time of day and ambient temperature (taken from the naked datalogger) (Multiple Linear Regression: $R_{sq} = 0.904$, $p < 0.001$); thus, allowing us to convert all our ambient temperature readings (from the naked datalogger) for the entire duration of the experiment to T_e .

Thermal preference in a thermal gradient chamber

The range of preferred T_b 's (T_{set}), was measured in a thermal gradient, where thermoregulatory costs and constraints are null.²⁵ The experiments in the thermal gradient during summer and winter presented here were performed by our research group for other purposes (unpublished data) and studies.^{5,6} Briefly, the thermal gradient consisted of a chamber (1.00 m long, 0.15 m high and 0.20 m wide) with an aluminum floor. One end of the floor was cooled to 10–11°C by a copper pad connected to a refrigerated water bath, and the other end was heated to 38–40°C by another bath or an electrical resistor. Petri dishes filled with tap water (from an artesian well), placed throughout the chamber, provided access to water in all temperature ranges. Each animal, equipped with a temperature probe secured 3 cm into the colon through the cloaca with skin sutures, was placed in the center of the thermal gradient. The thermistor output was continuously displayed on a chart recorder. After an exploratory period of about 5–12 h, the preferred T_b was recorded continuously and computed each h for 5 (winter) or 10 h (summer). Measurements were taken between 9:00 a.m. and 5:00 pm, and we assumed no difference in T_{set} between night and day. This assumption was based on the fact that no significant change in preferred T_b was observed in a 24 h period in the same species¹⁹ by us or by others in a closely-related toad, *Rhinella marina*.^{32,33}

Individual T_{set} was estimated from the central 50% of recorded temperatures selected in the thermal gradient (25% quartile representing the lower bound and 75% quartile representing the upper bound of T_{set}). The range of T_{set} for the species was calculated by the average of individual lower and upper bounds of T_{set} , according to Hertz et al.²⁵

Thermoregulatory indexes

Once T_b , T_e and T_{set} were obtained, the protocol described by Hertz et al.²⁵ was used to estimate the accuracy of thermoregulation (db), thermal quality of the habitat (de), and effectiveness of thermoregulation (E). Indexes were calculated for day and night for all 4 seasons. Summer and winter T_{set} were assumed for calculating indexes during spring and summer and during autumn and winter, respectively.

The accuracy of thermoregulation is indexed by \overline{db} , which represents the extent to which T_b 's overlap T_{set} .²⁴ The db from individual readings were interpreted using the following criteria: if the T_b reading fell within T_{set} , db was zero; if T_b fell below the lower bound of T_{set} , db was the difference between the lower bound of T_{set} and T_b ; if T_b fell above the upper bound of T_{set} ,

Table 1. Body temperature (T_b), environmental temperature (T_e), deviation of T_b (\overline{db}) and of T_e (\overline{de}) from T_{set} and E , calculated for daytime (0600–1800) and nighttime (1800–0600), during the 4 seasons

	autumn		winter		spring		summer	
	day	night	day	night	day	night	day	night
T_b	20.5 ± 0.3	20.6 ± 0.2	18.1 ± 0.2	18.8 ± 0.2	21.6 ± 0.1	21.0 ± 0.2	23.2 ± 0.2	22.5 ± 0.2
T_e	Shelter							
	21.1 ± 0.1	21.5 ± 0.1	18.2 ± 0.04	18.7 ± 0.04	21.8 ± 0.03	22.1 ± 0.03	24.3 ± 0.03	23.9 ± 0.3
	Shaded area							
	20.7 ± 0.1	21.1 ± 0.1	18.2 ± 0.1	19.0 ± 0.1	22.0 ± 0.1	22.1 ± 0.1	23.7 ± 0.04	22.8 ± 0.1
	Water							
	19.9 ± 0.1	20.5 ± 0.1	16.8 ± 0.1	17.9 ± 0.1	20.7 ± 0.1	21.3 ± 0.1	23.3 ± 0.1	22.6 ± 0.1
	Open area							
	20.7 ± 0.1	15.1 ± 0.1	19.8 ± 0.1	13.9 ± 0.1	21.9 ± 0.1	16.3 ± 0.1	17.2 ± 0.1	22.6 ± 0.1
\overline{db}	3.5 ± 0.02	3.4 ± 0.02	5.9 ± 0.02	5.2 ± 0.02	4.5 ± 0.02	5.0 ± 0.02	3.1 ± 0.02	3.5 ± 0.02
\overline{de}	3.9 ± 0.03	4.6 ± 0.04	6.0 ± 0.04	6.6 ± 0.03	4.6 ± 0.03	5.6 ± 0.03	3.3 ± 0.03	4.0 ± 0.04
95% confidence interval of E	0.09 – 0.12	0.26 – 0.28	0.00 – 0.03	0.20 – 0.22	0.01 – 0.05	0.09 – 0.11	0.05 – 0.09	0.11 – 0.15

db was the difference between T_b and the upper bound of T_{set} . db 's were averaged for day and night, during autumn, winter, spring and summer. High db indicates low accuracy of thermoregulation, since individuals often experience T_b 's out of the range of T_{set} . \overline{db} that approaches zero represents a species that experiences T_b 's within the ranges of T_{set} , and has, therefore, a high accuracy of thermoregulation.

The thermal quality of the habitat, from the organism's perspective, is represented by \overline{de} , and is calculated analogously to \overline{db} .²⁵ Environments in which \overline{de} is zero offer T_e 's that are exclusively within the range of T_{set} and, therefore, have a high thermal quality; the higher the \overline{de} , the lower the thermal quality of the environment.

\overline{db} and \overline{de} were combined to calculate the index E , which represents the effectiveness of thermoregulation of the species. The index E is calculated as $1 - (\overline{db}/\overline{de})$. An E value that approaches 1 indicates that animals thermoregulate carefully, and T_b deviations from preferred range (T_{set}) are much smaller than those of T_e , while a value that approach 0 indicates that animals do not thermoregulate and their deviations from T_{set} are similar to those of T_e . A negative value of E indicates that animals, for some reason, do not use microhabitats that offer temperatures within the range of T_{set} . In order to compare E values from different seasons and time of day, confidence intervals of E were calculated by bootstrap resampling from the distribution of T_e and T_b , as described by Hertz et al.²⁵

Statistical analyses

All the results are reported as mean ± SE. Operative temperature (T_e) data were reduced to means every 32 minutes, which were used in all analyses; the existence of differences in T_e among seasons was tested by one-way ANOVA. Within each season, a 2-way ANOVA was performed to test if T_e differed between time of day and among microhabitats. The T_b data were averaged for daytime and nighttime in each season for each toad ($n = 7$) and a 2-way ANOVA was used to test the possible differences between time of day and among seasons. For each individual tested in the thermal gradient, the lower and upper bounds of

T_{set} (25 and 75% quartiles, respectively) were calculated and compared between summer ($n = 14$) and winter ($n = 11$) by t test. Comparisons of \overline{db} and \overline{de} were performed by 2-way ANOVA (factors: season and time of day). In all the analyses, when a significant effect was found, a Tukey test was performed to compare the means. A significant difference was accepted when $p < 0.05$.

Results

Operative temperatures (T_e)

T_e in the 4 representative microhabitats in the experimental arena (shaded area, shelter, water and open area) differed among seasons, being, as expected, higher during summer and lower during winter (one-way ANOVA, $p < 0.001$; $F_{(3,65036)} = 5015$).

Within each season, T_e was influenced by time of day and microhabitat (Tables 1 and 2). The Tukey test revealed that, in

Table 2. Results of the 2-way ANOVAs testing for the effects of time of day and microhabitat on environmental temperatures (T_e) during summer, autumn, winter and spring

Predictor	F	df	P
autumn			
time of day	425.5	1	<0.001
microhabitat	894.5	3	<0.001
time of day × microhabitat	898.4	3	<0.001
winter			
time of day	297.5	1	<0.001
microhabitat	285.2	3	<0.001
time of day × microhabitat	1086.9	3	<0.001
spring			
time of day	730.0	1	<0.001
microhabitat	1027.1	3	<0.001
time of day × microhabitat	1217.9	3	<0.001
summer			
time of day	388	1	<0.001
microhabitat	1845	3	<0.001
time of day × microhabitat	1258	3	<0.001

general, daytime temperatures within the shelter, shaded area and water were lower than nighttime temperatures ($p < 0.05$). In the open area, on the other hand, daytime temperatures were higher than nighttime temperatures ($p < 0.05$). The temperatures between microhabitats also varied, especially at night, where temperatures in the open area dropped considerably compared to the other microhabitats ($p < 0.05$).

Hourly mean temperatures were calculated by microhabitat for each season (Fig. 2). Year-round, the shelter presented the lowest daily temperature amplitude, and the open area, the highest. The lowest mean temperature reached in the open area was 12.7, 11.2, 14.4 and 13.9°C for autumn, winter, summer and spring, respectively. These temperatures are at least 3.7°C lower than the minimal temperature reached by other microhabitats, and as much as 6°C higher during summer. At the other end, the highest hourly mean temperature was also observed in the open area: 25.4, 25.1, 26.3 and 25.8°C for autumn, winter, summer and spring, respectively. The difference between the maximal temperature in the open area and the maximal temperature in the other microhabitats was lowest during summer. During nighttime, the shelter presented the highest hourly mean temperatures.

Body temperatures (T_b) in the arena

Body temperature changed throughout the year (2-way ANOVA, $p < 0.001$, $F_{(3,48)} = 153.9$; interaction factor: $p = 0.006$; $F_{(3,48)} = 4.71$). Daytime T_b differed among all seasons, and was higher during summer and lower during winter. Nighttime T_b was also higher during summer and lower during winter, but did not differ between spring and autumn (Tukey test; $p < 0.05$).

Hourly mean T_b 's averaged across all individuals are plotted in Figure 2. The difference between the highest and the lowest hourly mean temperatures was greatest during summer (4.1°C) and lowest during winter (2.9°C).

Thermal preference in the thermal gradient (T_{set})

The lower bound of T_{set} did not differ between summer and winter (26.0 ± 0.6 for summer; 24.0 ± 1.1 for winter; $p = 0.1$). In contrast, the upper bound of T_{set} was higher during summer (28.4 ± 0.7) compared to winter (25.8 ± 1.0) ($p = 0.03$).

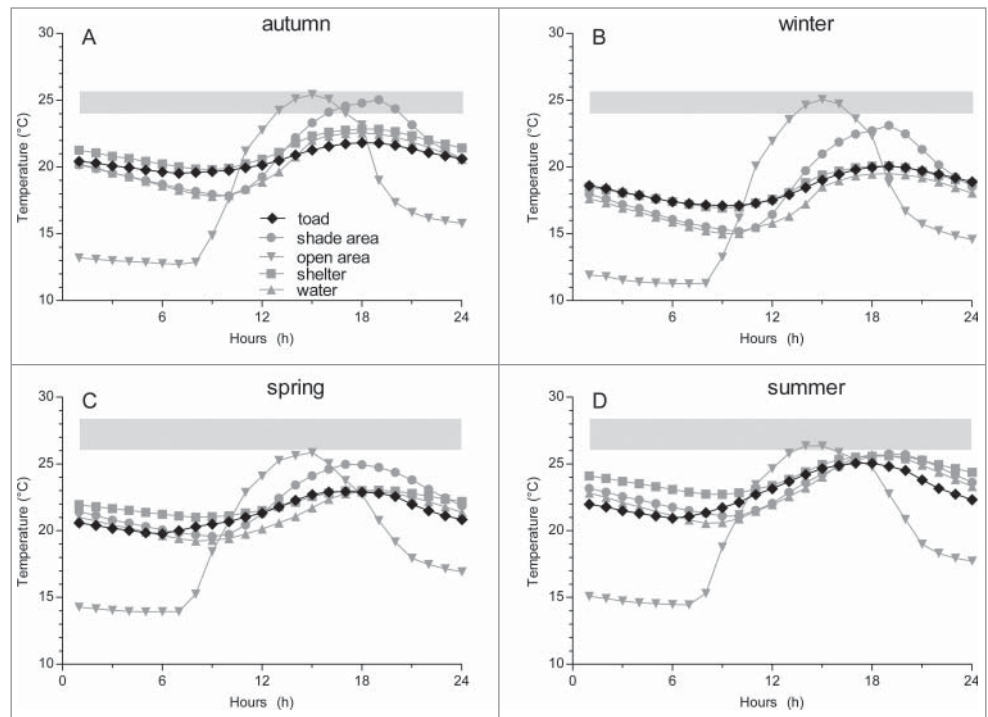


Figure 2. Hourly means of environmental temperatures (T_e) at 4 representative microhabitats and body temperatures (T_b) of toads, *Rhinella schneideri*, during autumn, winter, spring and summer. The shaded area represents the ranges of preferred T_b (T_{set}) calculated in the laboratory during the summer and winter seasons.

Comparisons between T_e , T_b and T_{set}

All hourly means of T_e fell below the upper bounds of T_{set} while all hourly mean T_b 's fell below the lower bound of T_{set} (Fig. 2). Hourly mean T_b 's closely matched the T_e 's from the shelter during winter. During summer, on the other hand, mean T_b 's matched the temperature of the shelter only for some phase of day. During nighttime, mean T_b 's dropped and matched the temperatures of the open area (Fig. 2).

More than 80% of T_e and T_b readings during all seasons fell outside T_{set} (Figs. 3 and 4). The proportions of T_e and T_b readings falling inside and outside T_{set} are shown in Figures 3 and 4. A greater proportion of T_e readings fell within T_{set} during summer (11.4% for daytime and 9.2% for nighttime) and autumn (11.1% for daytime and 8.5% for nighttime) compared to winter (4.9% for daytime and 1.3% for nighttime) and spring (5.9% for daytime and 1.6% for nighttime) (Fig. 3). The proportion of T_b readings that fell within T_{set} was also greater during summer (5.1% for daytime and 3.1% for nighttime) and autumn (8.9% for daytime and 8.8% for nighttime) compared to winter (0.4% for daytime and 0.6% for nighttime) and spring (1.5% for daytime and 0.5% for nighttime) (Fig. 4).

Thermoregulatory indexes

Within each season \overline{de} and \overline{db} were calculated for daytime and nighttime (Table 1). The thermal quality of the environment, represented by de , was influenced by season ($p < 0.001$, $F_{(3,65032)} = 2052$) and time of day ($p < 0.001$, $F_{(1,65032)} = 880$;

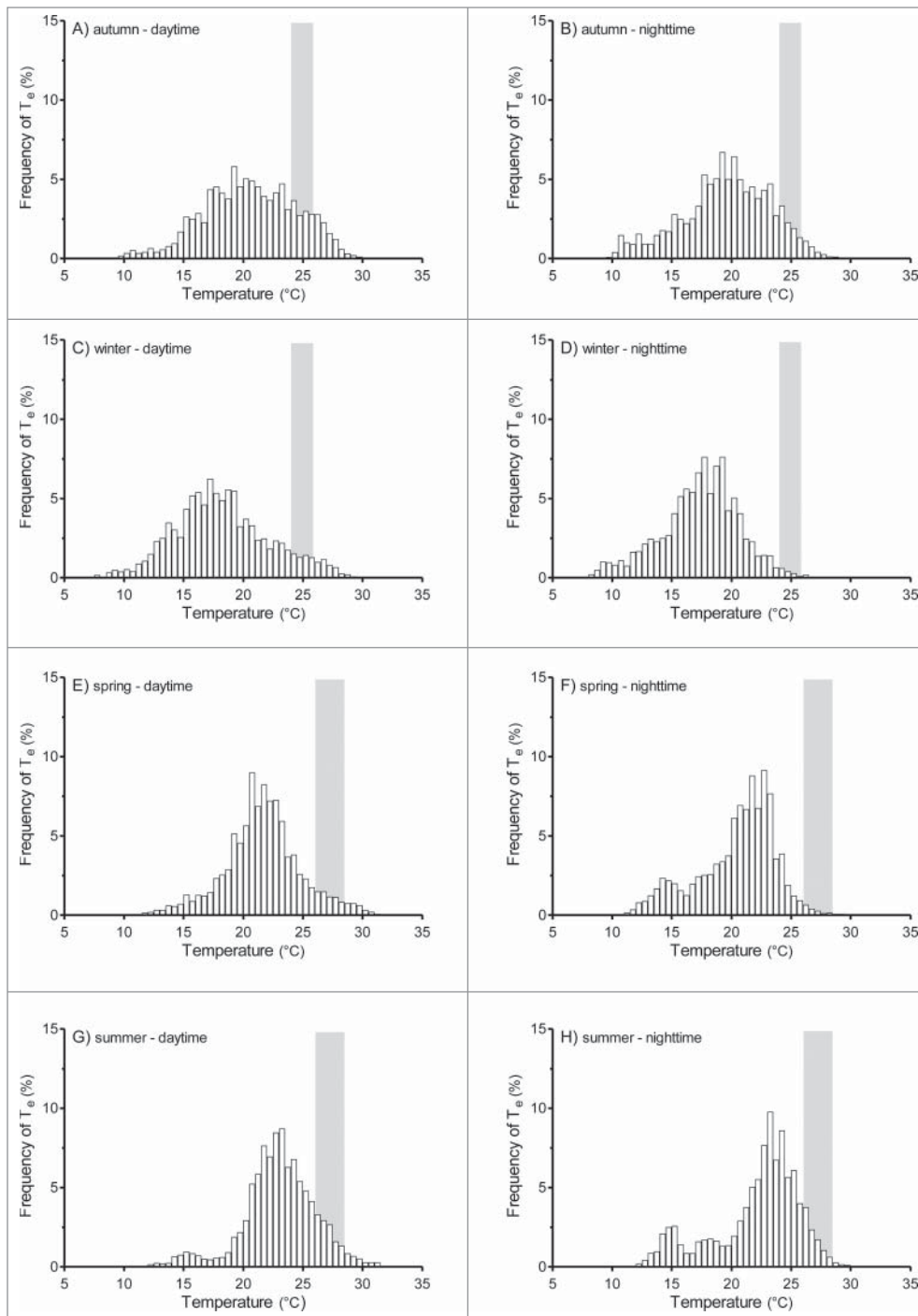


Figure 3. Frequency distribution of environmental temperatures (T_e) registered during the 4 seasons at daytime and nighttime. Daytime: 0600–1800; nighttime: 1800–0600. The shaded area represents the ranges of preferred T_b (T_{set}) calculated in the laboratory during the summer and winter seasons.

interaction factor: $p < 0.001$, $F_{(3,65032)} = 9.1$). Environmental temperatures were closer to T_{set} (higher thermal quality) during summer and, within each season, during daytime. The accuracy of thermoregulation, represented by db , was also influenced by season ($p < 0.001$; $F_{(3,104838)} = 7746$) and time of day

($p < 0.001$; $F_{(1,104838)} = 11$; interaction factor: $p < 0.001$; $F_{(3,104838)} = 27$). Toads kept T_b closer to T_{set} during summer and autumn.

All values of E were positive and did not exceed 0.28, indicating only moderate thermoregulation (Table 1). During all seasons, E was higher during nighttime compared to daytime. Considering nighttime E , since toads are nocturnal, E indicates a higher degree of thermoregulation during winter and autumn compared to summer and spring.

Discussion

Our seminatural experimental conditions tried to emulate, as much as possible, the same conditions that *R. schneideri* is bound to be exposed to in nature. In terms of climatic variables, animals were subjected to same natural weather conditions that free-ranging animals (that do occur in the region) were also exposed. In terms of microhabitat availability, we tried to provide landscape elements that allowed for plenty of thermoregulatory opportunities. No other interventions, other than occasional food supplementation and visual inspection, were introduced during the experiments. Thus, we are confident that if any captivity-related bias was present in our data, its effect is most probably negligible. In agreement with that, we did not observe unnatural behaviors of toads in terms of daily and seasonal activity. Animals were active during the early hours after sundown during the summer and spring and were inactive during day and night during the winter when they spend their time in the provided shelter or,

more rarely, under shallow burrows in the soil covered with plant debris.

Our data show seasonal and daily variation in environmental temperatures, as well as variation between microhabitats tested. In general, the shelter presented stable temperatures throughout

the entire daily cycle (i.e., day and night) and, as a result, the nighttime temperatures of the shelter were closer to T_{set} compared to other microhabitats. The seasonal cycle of activity of the toads^{5,34} was clearly reflected in our body temperature data. For example, during winter when the toads became inactive and retreated into the shelter, their T_b was practically indistinguishable from the T_e 's registered for this microhabitat. Thus, the thermal inertia of the shelter is likely to have provided the toads with a stable range of temperatures suitable to endure the estivation period. Indeed, selection of the shelter as the estivation site buffered the T_b of toads from the extreme variation prevalent in the other microhabitats during this season (see Fig. 2). During spring and summer, T_b 's closely matched the temperature of the shelter only during daytime. At night, when toads were active, T_b 's dropped further from T_{set} and matched the temperatures of the open area, indicating that the toads, as they become active, leave the shelters to explore other microhabitats, even though these microhabitats have lower thermal quality compared to the shelter. Thus, at night, E values during summer and spring are closer to zero compared to winter and autumn, reflecting the engagement of the toads in activities in a low thermal quality environment. During winter, on the other hand, despite being inactive, the choice of microhabitat in which to permanently retreat to for the season allows for T_b to be closer to T_{set} compared to summer and spring, resulting in higher E values.

The low thermoregulatory index of the active toads illustrates clearly the potential compromises between body temperature regulation and other ecologically relevant activities.³⁵ Indeed, the lower T_b 's, in relation to the range of T_e 's, were the result of the toads leaving the shaded/sheltered area and exploring other microhabitats where lower air temperatures prevailed. Although it is difficult to ascertain the

exact nature of such movements, foraging and reproduction seem to be the most likely candidates. In fact, the beginning of spring is the reproductive period for this species,^{36,37} and during spring and summer nights, it was possible to observe insects (possible prey) inside the arena. Finally, another possible explanation for the mismatch between activity T_b and T_{set} may be that T_{set} is a

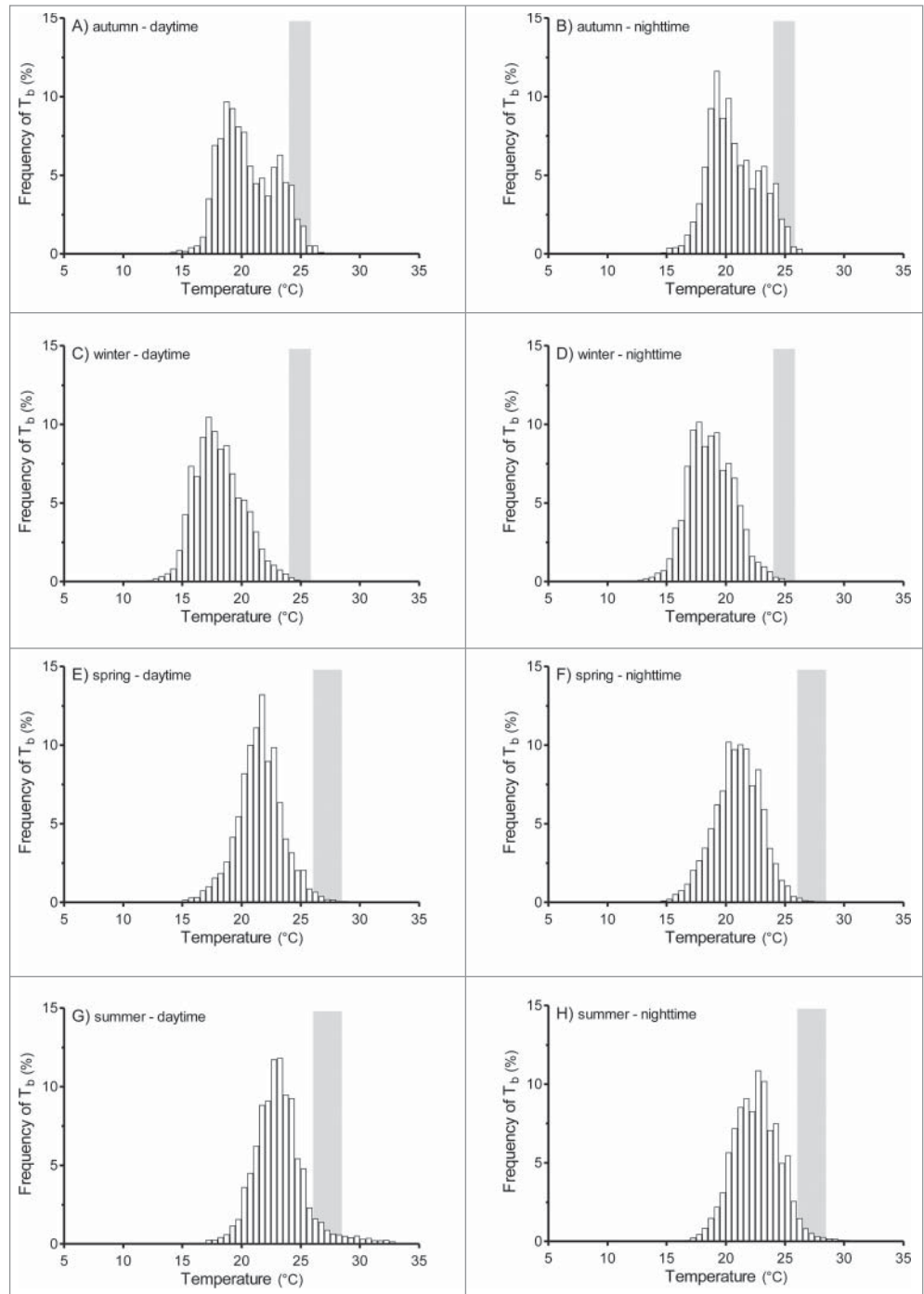


Figure 4. Frequency distribution of body temperatures (T_b) registered during all seasons at daytime and nighttime. Daytime: 0600–1800; nighttime: 1800–0600. The shaded area represents the ranges of preferred T_b (T_{set}) calculated in the laboratory during the summer and winter seasons.

conservative condition, rather than an adaptation to the current thermal environment.¹ Such an idea, in the specific case of the *Rhinella* group, remains to be tested.

The reduction in activity during the cold and dry season by tropical terrestrial toads seems often related to the control of water loss, rather than the avoidance of extreme temperatures.¹⁵ *Rhinella schneideri* estivates during winter without forming a cocoon and, in the laboratory, downregulates its heart rate and metabolic rate,^{5,19} which may help to save energy and reduce respiratory water loss. In the present study, although winter presented slightly lower RH, rainfall was more than 2 times lower than spring and summer, which may reflect the actual reduction of the levels of humidity within the micro-habitats explored by the toads. Thus, spending all the winter inside the shelter may be a strategy to avoid water loss, as microhabitat selection becomes an effective way to minimize evaporation^{14,31,38} in toads due to the high permeability of their skin.^{39,40} In this context, our results discussed above indicate that the seasonal decrease in the thermal quality of habitats may also be an important component influencing the onset of estivation in this species.

Our study provides a detailed account of seasonal and daily variation in T_b of an abundant and widespread anuran of the Neotropics under conditions quite similar to their natural habitats. As far as we know, no other study has provided this kind of information for any other anuran species, including those from all other temperate or tropical areas. This lack of information is unfortunate, both in terms of preventing a more refined ecological contextualization for physiological effects of temperature under experimental conditions, as well as the potential effects of temperature change and its possible consequences to the

conservation of amphibians under a global climate change scenario. In terms of procedure, we demonstrated that it is viable to apply the current procedures for thermoregulatory quantification²⁵ under a more controlled experimental situation. In the specific case of *R. schneideri*, the adoption of such an approach revealed that nocturnal activity may be an important constraint to T_b regulation in anurans, even though such activity is largely limited to seasons with a higher thermal quality. On the other hand, during seasons characterized by an overall lower thermal quality (winter/autumn), the onset of inactivity (estivation), combined with microhabitat selection, may allow for a more accurate (although largely passive) maintenance of T_b .

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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