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NYCOLLE AGNYS OLIVEIRA DA SILVA

Variação sazonal na termorregulação, desempenho locomotor e hidratação em campo da perereca-de-banheiro, *Scinax fuscovarius* (Hylidae)

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BANCA EXAMINADORA:

Prof. Dr. Luis Miguel Senzano Castro

Prof. Dra. Thaís Barreto Guedes da Costa

Prof. Dr. Fábio Perin de Sá

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RESUMO

Anfíbios, por serem ectotérmicos e possuírem pele permeável, são especialmente sensíveis às variações de temperatura e disponibilidade de água no ambiente. Assim, em regiões Neotropicais de clima marcadamente sazonal, a atividade de muitos anfíbios pode estar restrita temporalmente aos meses mais quentes e úmidos do ano. Algumas espécies, porém, ajustam sazonalmente tanto seu comportamento termorregulatório quanto a sensibilidade térmica de diferentes processos fisiológicos, permitindo a manutenção de suas atividades ao longo das estações. Esses ajustes podem envolver alterações sazonais nos comportamentos e custos associados à termorregulação e também na sensibilidade térmica do desempenho comportamental. Finalmente, períodos com baixa disponibilidade de água podem expor os anfíbios a uma perda excessiva de água e aumentar o risco potencial de desidratação. Neste contexto, nós investigamos a variação sazonal no comportamento termorregulatório, estado de hidratação em campo e do desempenho locomotor da perereca de banheiro, *Scinax fuscovarius*. Embora essa espécie concentre suas atividades na primavera e verão, ela permanece ativa ao longo do ano, inclusive durante os meses mais frios e secos do ano. Nós determinamos a temperatura corpórea dos animais em campo (T_c) e a distribuição de temperaturas operativas (T_e) em seus microambientes, o que nos permitiu estimar os custos termorregulatórios. Também determinamos o grau de hidratação em campo e a sensibilidade térmica do desempenho locomotor. O estudo foi realizado no município de Rio Claro, SP, sudeste do Brasil, onde o clima pode ser caracterizado pela alternância de uma estação quente-úmida (primavera e verão) e outra fria-seca (outono e inverno). A T_c foi menor na estação fria-seca (21,2 °C) do que na quente-úmida (26 °C). As temperaturas operativas (T_e) diminuíram, em média 6,5°C durante a estação frio-seca, o que reduziu a qualidade térmica (d_e) dos microhabitats. Apesar disso, a espécie manteve uma T_c acima da T_e em ambas as estações, refletindo alta precisão termorregulatória (d_b), ou seja, T_c próxima ao intervalo sazonal de temperaturas preferidas (T_{pref}). Como resultado, a eficácia da termorregulação (*E*) foi alta (> 0.5), indicando termorregulação ativa das pererecas nas duas estações. Os animais mantiveram altos níveis de hidratação (~98,7%) em ambas as estações. O desempenho locomotor variou sazonalmente, com redução na temperatura ótima (T_{opt}), no desempenho máximo (P_{max}) e na faixa ótima de desempenho 80% (B_{80}) durante a estação frio-seca. Além disso, a B_{80} foi mais ampla no inverno. Nossos resultados indicam que *S. fuscovarius* exibe ajustes sazonais importantes em seu comportamento termorregulatório que compensam, ainda que parcialmente, a redução sazonal da qualidade térmica do seu ambiente durante a estação fria e seca do ano. Por outro lado, diferenças sazonais na disponibilidade de água não afetaram o estado de hidratação das pererecas. Finalmente, nós encontramos que o desempenho locomotor diminui significativamente durante o outono-inverno, o que pode estar refletido no menor

nível de atividade exibido por essa espécie durante este período.

Palavras-chave: Anfíbios; Sazonalidade; Qualidade térmica do habitat.

Título em português: Variação sazonal na termorregulação, desempenho locomotor e hidratação em campo da perereca-de-banheiro, *Scinax fuscovarius* (Hylidae).

ABSTRACT

Amphibians, due to being ectothermic and having permeable skin, are especially sensitive to variations in environmental temperature and water availability. Thus, in Neotropical regions with markedly seasonal climates, the activity of many amphibians may be temporally restricted to the warmer and wetter months of the year. Some species, however, seasonally adjust both their thermoregulatory behavior and the thermal sensitivity of various physiological processes, allowing them to remain active throughout the seasons. These adjustments may involve seasonal changes in behaviors and in the costs associated with thermoregulation, as well as in the thermal sensitivity of behavioral performance. Finally, periods of low water availability may expose amphibians to excessive water loss and increase the potential risk of dehydration. In this context, we investigated seasonal variation in thermoregulatory behavior, field hydration state, and locomotor performance of the *Scinax fuscovarius* treefrog. Although this species concentrates its activity in spring and summer, it remains active year-round, including during the coldest and driest months. We measured the frogs' body temperature in the field (T_b) and the distribution of operative temperatures (T_e) in their microhabitats, which allowed us to estimate thermoregulatory costs. We also determined their hydration level in the field and the thermal sensitivity of their locomotor performance. The study was conducted in the municipality of Rio Claro, SP, southeastern Brazil, where the climate is characterized by alternating hot-wet (spring and summer) and cold-dry (autumn and winter) seasons. T_b was lower in the cold-dry season (21.2 °C) than in the hot-wet season (26 °C). Operative temperatures (T_e) decreased by an average of 6.5 °C during the cold-dry season, reducing the thermal quality (d_e) of the microhabitats. Despite this, the species maintained a T_b above T_e in both seasons, reflecting high thermoregulatory accuracy (d_b), i.e., T_b remained close to the seasonal preferred temperature range (T_{set}). As a result, thermoregulatory effectiveness (*E*) was high (> 0.5), indicating active thermoregulation by the frogs in both seasons. The animals maintained high hydration levels (~98.7%) in both seasons. Locomotor performance varied seasonally, with reductions in optimal temperature (T_{opt}), maximum performance (P_{max}), and the 80% optimal performance range (B_{80}) during the cold-dry season. Additionally, B_{80} was broader in winter. Our results indicate that *S.*

fuscovarius exhibits important seasonal adjustments in its thermoregulatory behavior that compensate, at least partially, for the seasonal reduction in the thermal quality of its environment during the cold and dry season. On the other hand, seasonal differences in water availability did not affect the frog's hydration state. Finally, we found that locomotor performance significantly decreases during autumn-winter, which may be reflected in the lower level of activity displayed by this species during this period.

Keywords: Amphibians; Sazonality; Habitat thermal quality.

SUMMARY

1 INTRODUCTION

Amphibians are ectotherms with usually highly permeable skin, making them strongly dependent on environmental conditions to regulate both body temperature and hydration (Angilletta, 2009). Higher environmental temperatures not only elevate body temperature (T_b) but also accelerate evaporative water loss, which increases desiccation risk and tightens thermal and water balance (Anderson & Andrade, 2017; Rozen-Rechels et al. 2019). Hence, marked environmental variation often forces amphibians into trade-offs between maintaining elevated T_b, generally favorable for promoting optimal physiological performance (Kingsolver & Huey, 2008), and conserving water. In Neotropical regions, where hot–wet summers alternate with cold–dry winters (Reboita et al., 2015), the seasonal changes in thermal and hydric conditions create particularly challenging environments. Thermoregulation thus plays a central role, since both physiological performance and field activity time will depend on the ability to maintain suitable body temperatures without compromising body hydration (Huey and Stevenson, 1979; Angilletta, 2009).

Thermal preference (T_{pref}) represents the range of temperatures selected in a cost-free environment and is thought to closely reflect the thermal range at which most physiological processes are optimized (Hertz et al., 1993; Angilletta, 2009). Seasonal declines in environmental temperatures can therefore increase the difficulty of achieving T_{pref}, raising the costs of thermoregulation. For example, during seasonal declines in environmental thermal quality driven by lower operative temperatures (T_e), field body temperature (T_{field}) is more likely to deviate from T_{pref}, raising the costs of attaining preferred ranges and ultimately constraining thermoregulatory accuracy (Anderson et al., 2018; Sanabria et al., 2011). Under such conditions, animals may either thermoconform, allowing T_{field} to passively track the surrounding thermal environment, or actively seek scarce warmer spots to maintain T_{field} as close as possible to T_{pref} (active thermoregulation) (Hertz et al., 1993). The extent to which individuals either thermoconform or actively thermoregulate to attain preferred temperatures will be reflected in the effectiveness of thermoregulation, and ultimately, how successful they are in buffering seasonal constraints (Taylor et al., 2020).

Seasonal regulation of T_b also has direct consequences for locomotor capacity, since organismal performance is strongly determined by the range of T_{field}

that animals can achieve (Kingsolver & Huey, 2008). Thermal performance curves (TPC) describe the sensitivity of performance to changes in T_b, typically increasing from a critical minimum temperature (CT_{min}) until it peaks at an optimal temperature (T_{opt}) and then decreases until it reaches a critical thermal maximum (CT_{max}) (Huey & Stevenson, 1979). The range of T_b below T_{opt} that allows performance to remain near-optimal, though not maximal, is described as the thermal performance breadth (B_{80}) (Angilletta et al., 2002). If operative temperatures deviate from T_{opt}, performance capacity will decline, creating mismatches between physiological optima and achievable T_{field}. Such mismatches may be mitigated if thermal performance breadth (B_{80}) broadens, allowing performance to remain relatively high across a wider range of environmental temperatures, although this could come at the cost of lowering peak performance (Gilchrist, 1995; Boyles et al., 2011).

In addition to thermal constraints, amphibians are profoundly affected by seasonal variation in water availability, largely due to their highly permeable skin, which makes them especially prone to high evaporative water loss (EWL) (Spotila & Berman, 1976; Tracy et al., 2014). While peak activity in many tropical anurans coincides with the warm and wet months, when abundant water bodies facilitate rehydration and high humidity minimizes EWL (Prado et al., 2005; Mitchell & Bergmann, 2015), conditions are markedly different during the cold–dry season. During colder and drier months, the risk of excessive EWL increases while opportunities for rehydration diminish (Duellman, 1995; Huey et al., 2012). Under such conditions, amphibians are faced with the critical trade-off of either prioritizing water balance by enduring suboptimal T_b and reduced performance, or maintain optimal performance by venturing into the few warmer, yet drier microhabitats available, at the expense of accelerated dehydration (Little & Seebacher, 2016; Oromí et al., 2010). While most Neotropical amphibians cope with these unfavorable conditions through aestivation (Carvalho et al., 2009), those that remain active year-round must continuously balance thermal and hydric demands despite the unfavorable seasonal hydric constraints.

In the present study, we examined the seasonal thermoregulatory trade-offs of thermal preference and locomotor performance, jointly with the seasonal field hydration in the snouted treefrog *Scinax fuscovarius* (Hylidae). Distributed across Neotropical regions characterized by pronounced hot-wet and cold-dry seasons, this species remains active year-round, exposing itself to cold-dry periods where ambient

temperatures frequently fall below its thermal preference (Kiss et al., 2009; Reboita et al., 2015; Goldberg et al., 2017). Seasonal shifts in water balance and thermal biology have been documented for this species, including increased dehydration tolerance (EWL_{tol}) and rehydration capacity during the hot-wet season, along with reductions in T_{field} and T_{pref} during colder periods (Maenaka et al., 2023). Specifically, we assessed the seasonal costs of thermoregulation associated with variations in environmental thermal quality between the hot-wet and cold-dry seasons, as well as the thermal sensitivity of locomotor performance parameters, including optimal temperature (T_{opt}), performance breadth (B_{80}), and maximal performance (P_{max}). We expect lower thermal quality during the cold-dry season. Yet, because this species lower its T_{field} and T_{pref} during cold periods, we expect such adjustments to buffer these costs, resulting in similar thermoregulatory accuracy between seasons. To address this, we contrasted T_e and T_{field} distributions between seasons through thermoregulatory indices (Hertz et al., 1993), which offers a complete framework to integrate these components by the quantification of habitat thermal quality, thermoregulation accuracy, and effectiveness of thermoregulation. We also predict lower field hydration during the dry season, when rehydration opportunities are expected to be lower. We also expect a greater mismatch between thermal performance parameters (T_{opt} and B_{80}) and the lowered T_e prevailing during the cold-dry season, along with a reduction in P_{max}.

2 METHODS

2.1 Species and study site

Scinax fuscovarius (A. Lutz, 1925) is a member of the family Hylidae. The species has a broad geographic distribution across South America, occurring in Brazil, Argentina, Paraguay, Uruguay and Bolivia (Frost, 2025). And is primarily nocturnal with arboreal habits, with individuals that can be found on the ground or in low vegetation, indicating behavioural plasticity (Silva et al., 2009). The species shows significant habitat flexibility, it is frequently found in open areas such as fields, marshes, lakes, temporary ponds, and anthropogenic landscapes (Haddad et al., 2008). Also, the distribution and activity of the species is associated with climatic conditions, especially humidity and precipitation, which play a crucial role in the reproductive process (Silva et al., 2009).

This study was conducted with adult individuals collected in the municipality of Rio Claro (22°23'49.7"S, 47°32'36.0"W), São Paulo State, southeastern Brazil. The region is classified as humid subtropical (Cwa) with two well-defined seasons: a hot-wet summer (September to March) and cold-dry winter (April to August) (Köppen, 1936). Summer temperatures range from 19.8°C to 28.2°C, with an average precipitation of 231.5 mm annually, while winter temperatures range from 13.8°C to 25.8°C, with average precipitation of 47 mm (CLIMA, 2025).

2.2 Fieldwork and housing conditions

Fieldworks were carried out between Jun and July of 2024 and December 2024 and February 2025. Animals were located by active nocturnal searches between 18:00 h and 03:00 h in the vicinity of the Laboratory of Experimental and Integrative Biology at the Institute of Biosciences, UNESP – Rio Claro campus. Animals were captured by hand and their T_{field} measured by using a digital thermometer (Incoterm, model 6132, accuracy of 0.1 °C) gently inserted into the cloaca within 10 seconds of capture. Animal handling was conducted using double powder-free nitrile gloves to reduce heat transfer between the researcher's hands and the animal's body (Navas & Araújo, 2000). We recorded the local microenvironmental conditions at the capture site, including air temperature (T_{air}), substrate temperature (T_{sub}), and relative humidity (RH), using a digital

thermo-hygrometer (PCE Instruments, model PCE-320). Capture time was also recorded as it provides a proxy measure for body water loss exposure as a function of activity time, a potential factor capable of influencing field hydration status. T_{field} data were collected from 35 adults (15 in the cold-dry season and 20 in the hot-wet season), while opportunistic searches later increased the total sample to 60 individuals (27 and 33 per season, respectively), although T_{field} measurements were restricted to the original 35 individuals.

Following capture, individuals were transferred to the laboratory, located about a 2-min walk from the collecting site, and randomly assigned to either the immediate assessment of field hydration status or their housing for later locomotor performance tests (detailed below). The next day, all animals were measured for snout-vent length (SVL) using a digital caliper (Mitutoyo, model CD-6"PSX, 0.01 mm accuracy) and dorsally photographed for individual identification (Maenaka et al., 2023). Frogs used in field hydration assessments were released the following night after capture, whereas those assigned to performance trials were kept individually in 29 L plastic containers (45.7 × 32.6 × 28 cm) in an air-conditioned room at 25 °C and under natural photoperiod. Containers were supplied with PVC tubes and dry leaves for shelter, along with two shallow water dishes (3 × 10 cm). Animals were not fed during captivity to avoid post-prandial effects on performance and were kept for a maximum of one week before being released back at the collecting site. Photographic records of individuals were used to prevent repeated measurements in the case of recaptured frogs.

2.2.1 Operative temperatures

Operative environmental temperatures were determined using the agar replica method (Spotila & Berman 1976). Agar replicas were produced by pouring heated 5% agar solution (95% water, 5% agar; Senzano et al., 2022) into rubber molds taken from real *S. fuscovarius* frogs. Agars were colored by adding a small drop of green-yellow food coloring during agar preparation to approximate the coloration of live animals. Once solidified, replicas were deployed under field conditions.

Agars were prepared and field deployed 12 h before scheduled fieldwork to coincide with T_{field} measurements collected from captured animals. Agar monitoring was continuous over 24 h until the completion of fieldwork, with agars replaced three

times per day: morning (07:00 h–08:00 h), afternoon (13:00 h–14:00 h), and evening (19:00 h–20:00 h). Temperature data were recorded by temperature sensors (HOBO Pro v2 External Temperature, accuracy of 0.001 °C) inserted inside each agar and programmed to collect temperature readings at 5-min intervals. We considered two microhabitat types for agar deployment: sunny (without vegetation cover, fully exposed to direct sunlight during daytime hours) and shady (85–90% canopy cover with natural vegetation above ground). Within each microhabitat type, two microhabitat conditions were stablished: wet (agar in direct contact with a wet substrate continuously moistened by a thin water layer supply from a plastic reservoir) and dry (agar in direct contact with dry natural substrate). The four resulting microhabitat combinations (sunny-wet, sunny-dry, shady-wet, and shady-dry) represent the extremes of a continuum in canopy cover and moisture, thus encompassing the broadest range of potential thermal and hydric conditions present at the collecting site (Lertzman-Lepofsky et al. 2020).

2.3 Experimental protocols

Following capture, animals were assigned to one of two experimental groups: 37 individuals for field hydration assessments (n = 17 for cold-dry season; n = 20 for hot-wet season) and 23 individuals for locomotor performance trials (n = 10 for cold-dry season; n = 13 for hot-wet season).

2.3.1 Field hydration

Immediately upon capture, animals were placed in sealed plastic bags and transported to the laboratory, where they were removed from bags, blotted with paper tissue, and had their urinary bladder emptied by gently pressing the abdominal region. Then, they were weighed on a high-precision scale (precision of 0.0001 g, model AR2140, Ohaus Adventurer) and placed individually in plastic chambers (7x14x10 cm) containing dechlorinated tap water and kept for two hours in a temperature-controlled chamber (D.B.O, Eletrolab model EL 101/2RS) at 25 ºC for full hydration. After this period, frogs were blotted, their bladder emptied and weighed again. We assumed this final measurement as the frogs' standard mass (fully hydration state with their bladder emptied; Ruibal et al. 1969). Field hydration was

calculated as the relative difference (%) between the first and second weighing (Preest et al. 1992; Tracy et al. 2014; Vimercati et al. 2018).

2.3.2 Locomotor performance

Locomotor performance tests were conducted 24 h after capture and performed at night (usually between 18:00 and 20:00h) to coincide with the species' nocturnal peak activity. Performance tests were conducted at six temperatures (10, 15, 20, 25, 30, and 35ºC) using the same individuals over six consecutive days. The individuals were tested once per day, with both the daily test temperature and the testing order of individuals completely randomized. All animals were fully hydrated before measurements. To do so, frogs were individually placed in plastic containers (7 x 14 x 10 cm) with ~30 ml of water covering the bottom and kept inside a climatic chamber (D.B.O, Eletrolab model EL 101/2RS) set at the experimental temperature for two hours. Jumping tests were conducted in an arena built with two flat MDF boards (Medium Density Fiberboard; 250 x 100 cm, 1 cm thick) positioned opposite each other, forming a 5-m long jumping track. The entire arena was kept inside an air-conditioned room, with temperature maintained as close as possible to the experimental temperature using a split-system air conditioner (Midea, MSE1-9CR model, 9000 BTU capacity, control range capacity: from 17 to 30°C). At the start of each test, frogs were removed from their hydration container and gently blotted with tissue paper while inducing urination by applying light pressure to the abdomen region. They were then released at one end of the arena and allow to jump five consecutive times voluntary (or by gently tapping their urostyle region when necessary). Jumping tests were brief, typically completed within 1–2 min. The landing point of each jump was manually marked on the arena floor with chalk and later measured with a steel tape measure (Uyustools, precision of 0.1 mm). Immediately after jumps, body temperature was measured by inserting a digital thermometer probe (AKSO, model AK16S, accuracy of 0.5°C) into the cloaca, and individuals were weighed (as described above) before being returned to their maintenance cages. Locomotor performance was evaluated based on the longest jump distance out of the five consecutive jumps for each frog recorded at each temperature.

We fitted thermal performance curves (TPCs) relating the longest jump distance at each temperature and the T_b recorded immediately after the performance

test. Using the nls.multstart and rTPC R packages (Padfield et al., 2021), we identified for each season the best-fitting TPC model from 17 TPC candidates using Akaike Information Criterion corrected for small sample sizes (AICc; Supporting Information S1). We used the seasonally CT_{min} and CT_{max} values empirically determined for this same population of *S. fuscovarius* (Maenaka et al., 2023) to define the lower and upper limits of the TPCs curves. The best-fitting curve for each season were then used to fit TPCs for each frog, capturing individual performance profiles and extracting the associated performance parameters: optimal temperature (T_{opt}), peak performance (P_{max}), and the lower (B_{80} lower) and upper (B_{80} upper) bounds at which performance remains at least 80% of the maximum (the thermal performance breadth, B_{80}).

2.4 Data analysis

Thermoregulatory indices were calculated following the protocol described by Hertz et al. (1993). First, to evaluate the thermal quality (d_e) of microhabitats, we calculated the mean deviation of operative temperatures (T_e) relative to the 50% interquartile range of T_{pref} (the 25^{th}–75^{th} quartiles) reported by Maenaka et al. (2023) for *S. fuscovarius*, which spans 22.9–24.75°C in the cold-dry season, and 26.97–28.82°C in the hot-wet season. Values of T_e within the T_{pref} range have a deviation of 0; thus, lower d_e values indicate higher thermal quality of the microhabitat. Next, we quantified the accuracy of thermoregulation (d_b) as the deviation of T_{field} from the 50% interquartile range of T_{pref} for each season. Likewise, values of T_{field} within the T_{pref} range have 0 deviation; thus, lower d_b values indicate that frogs maintain their T_{field} close to their T_{pref} range. The effectiveness of thermoregulation (*E*) was calculated as: $E = 1 - (d_b/d_e)$. If animals thermoregulate effectively, d_b will be significantly smaller than d_e, and *E* will approach a value of one, indicating that animals thermoregulate effectively (active thermoregulation). Conversely, animals not engaging in thermoregulatory behavior will exhibit T_{field} deviations similar to those observed in agar models ($d_b \approx d_e$), rendering *E* values close to zero (indicative of thermoconformity). *E* can become negative in cases where animals exhibit a T_{field} distanced from their T_{pref} range, despite T_e providing the opportunity for thermoregulation within that T_{pref} range (Hertz et al. 1993).

We performed ANCOVA (Analysis of Covariance) tests prior to all analyses to check for effects of body mass and sex. We also examined parametric assumptions of normality using the Shapiro-Wilk test and homogeneity of variances using Levene's test. Field body hydration of frogs between seasons was compared with one-way ANCOVA, with season as a factor and sex as a covariate. We used multiple linear regressions, separated for season, to evaluate the influence of potential predictors (T_{air}, T_{sub}, RH, body mass, and time of capture) to field hydration. Time of capture was quantified as the number of minutes elapsed from the beginning of frogs' activity (the earliest time recorded for an active frog in each season) to the time of capture. To standardize predictors' effect sizes, we scaled them to a mean = 0 and a standard deviation (s.d.) of 1 (Baguley, 2009). Seasonal differences in T_{field}, T_{opt}, P_{max}, T_e and d_e were evaluated with separate unpaired t-tests, whereas B_{80}, B_{80} lower, B_{80} upper, d_b and E were evaluated with nonparametric Mann–Whitney tests because they violated normality assumptions. All data analysis were made using R software (version 4.2.1, R Core Team). All values are presented as mean ± s.d. Differences were deemed significant at $p < 0.05$.

3 RESULTS

Scinax fuscovarius exhibited an average T_{field} of 21.2 °C during the cold-dry season, significantly lower than that recorded for the hot-wet season (26 °C) ($t_{(35)}$ = -8.64, $p < 0.001$). Operative environmental temperatures also differed between seasons ($t_{(21)}$ = -17.06, $p < 0.001$), being on average 6.41 °C lower during the cold-dry season (T_e = 16.39 °C) than in the hot-wet season (T_e = 22.8 °C) (Table 1). Environmental thermal quality declined markedly in the cold-dry season (d_e = 6.51 °C) compared to the hot-wet season (d_e = 4.17 °C) ($t_{(21)}$ = 6.24, $p < 0.001$). The accuracy of thermoregulation was slightly lower in the cold-dry season (d_b = 1.68 °C) than in the hot-wet season (d_b = 1.24 °C), though this difference was not significant (W = 8, p = 0.22). Frogs thermoregulated effectively in both seasons (E = 0.89 and 0.95, respectively), with no significant differences between them (W = 33, p = 0.055) (Table 1). In the cold-dry season, capture times ranged from 19:27 h to 23:05 h (the earliest and latest recorded active frogs, respectively), whereas in the hot-wet season they extended from 19:26 h to 02:23 h.

Frogs maintained high hydration levels while active in both seasons (~98.7%), with no significant seasonal difference observed ($F_{(1,30)}$ = 0.055, p = 0.82; Figure 3). During the hot-wet season, field body hydration showed a negative correlation with time of capture (Coef. = -1.47, p = 0.04), whereas all other predictors (T_{ar}, T_{sub}, RH and body mass) were nonsignificant in both seasons (Table 2; Fig. 3).

All performance traits varied seasonally. During the cold-dry season, there was a shift in T_{opt} toward colder temperatures ($t_{(21)}$ = -10.12, $p < 0.001$) and a reduction in both the lower (W = 0, $p < 0.001$) and upper (W = 1, $p < 0.001$) bounds of B_{80}. Additionally, B_{80} was broader during the cold-dry season (W = 130, $p < 0.001$), whereas P_{max} reduced during the cold-dry season ($t_{(21)}$ = -5.05, $p < 0.001$) (Table 3; Fig. 4).

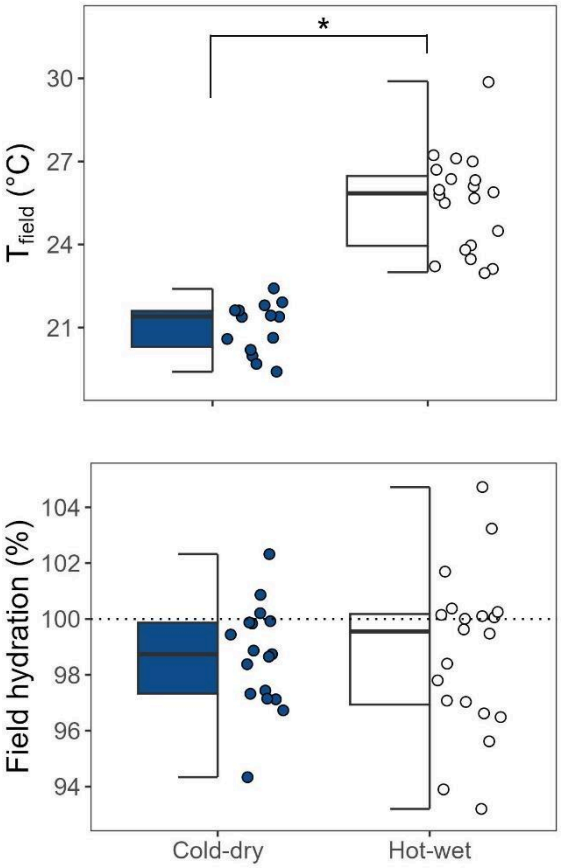
Table 1 – Summary of the physiological traits and thermal indexes for *Scinax fuscovarius* frogs for the cold-dry and hot-wet seasons. Field body temperature (T_{field}), optimal temperature (T_{opt}), maximal performance (P_{max}), locomotor performance breadth (B_{80}), lower and upper bounds of B_{80} (B_{80} lower and B_{80} upper, respectively), field body hydration, accuracy of thermoregulation (d_b), operative temperature (T_e), environmental thermal quality (d_e), and effectiveness of thermoregulation (*E*). Values are presented as mean ± standard deviation.

Trait	Cold-dry	Hot-wet
T_{field} (°C)	21.22 ± 0.86	26.07 ± 1.68
T_{opt} (°C)	25.05 ± 0.53	29.5 ± 1.24
P_{max} (cm)	90.89 ± 14.7	130.7 ± 20
B_{80} (°C)	12.65 ± 0.49	9.27 ± 0.63
B_{80} lower (°C)	18.27 ± 0.71	23.99 ± 1.25
B_{80} upper (°C)	30.92 ± 0.42	33.26 ± 0.9
Field hydration (%)	98.65 ± 1.82	98.79 ± 2.81
d_b (°C)	1.68 ± 0.87	1.24 ± 1.23
T_e (°C)	16.39 ± 0.83	22.8 ± 0.87
d_e (°C)	6.51 ± 0.83	4.16 ± 0.87
E (°C)	0.89 ± 0.05	0.95 ± 0.05

Table 2. Summary of multiple linear regressions examining the seasonal relationship between field body hydration and microclimatic variables, body mass and time of capture. Standardized coefficients (Std. Coef.) are provided for each predictor, with standard error of the mean (s.e.). Predictors with significant p-values are highlighted in bold.

Predictors	Std. Coef.	s.e.	p-value	Adjusted R^2 (%)
Cold-dry season				
intercept	98.66	0.47	**<0.001**	0.25
air temperature	-0.42	0.8	0.605	
substrate temperature	-0.16	0.57	0.778	
relative humidity	-0.64	0.96	0.517	
time of capture	-1.25	0.74	0.117	
body mass	0.3	0.7	0.676	
Hot-wet season				
intercept	98.79	0.49	**<0.001**	0.56
air temperature	0.49	0.62	0.44	
substrate temperature	-0.33	0.65	0.619	
relative humidity	1.06	0.63	0.119	
time of capture	-1.47	0.67	**0.04**	
body mass	0.5	0.51	0.347	

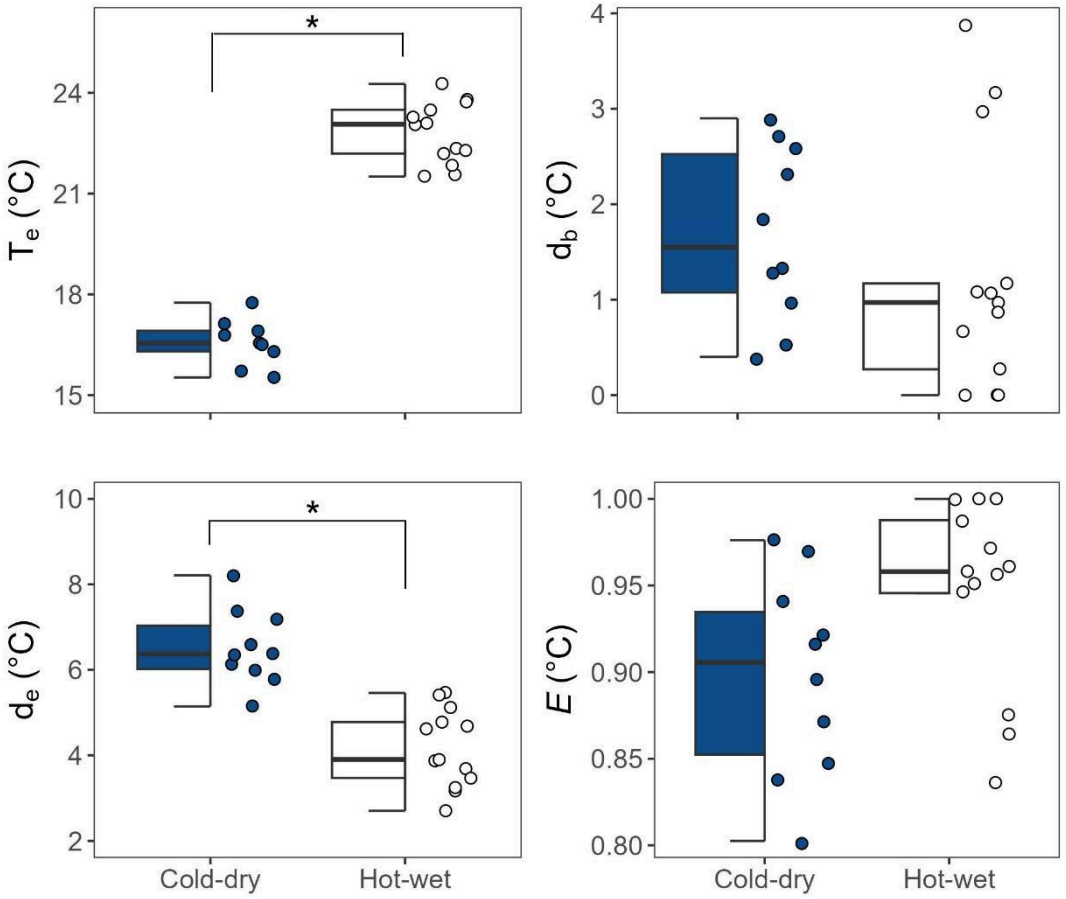
Figure 1 – Field body temperature and body hydration



Source: Prepared by the author, 2025

Figure 1. Seasonal variation in field body temperature (T_{field}) and field body hydration (T_{field}) for *Scinax fuscovarius* frogs. Dots represent individual observations. The lower and upper edges of the boxes correspond to the first (Q1) and third (Q3) quartiles, respectively; the horizontal lines inside the boxes indicate median values, and the vertical whiskers represent the lower and upper data limits. Horizontal lines and asterisks indicate seasonal statistical differences ($p < 0.05$).

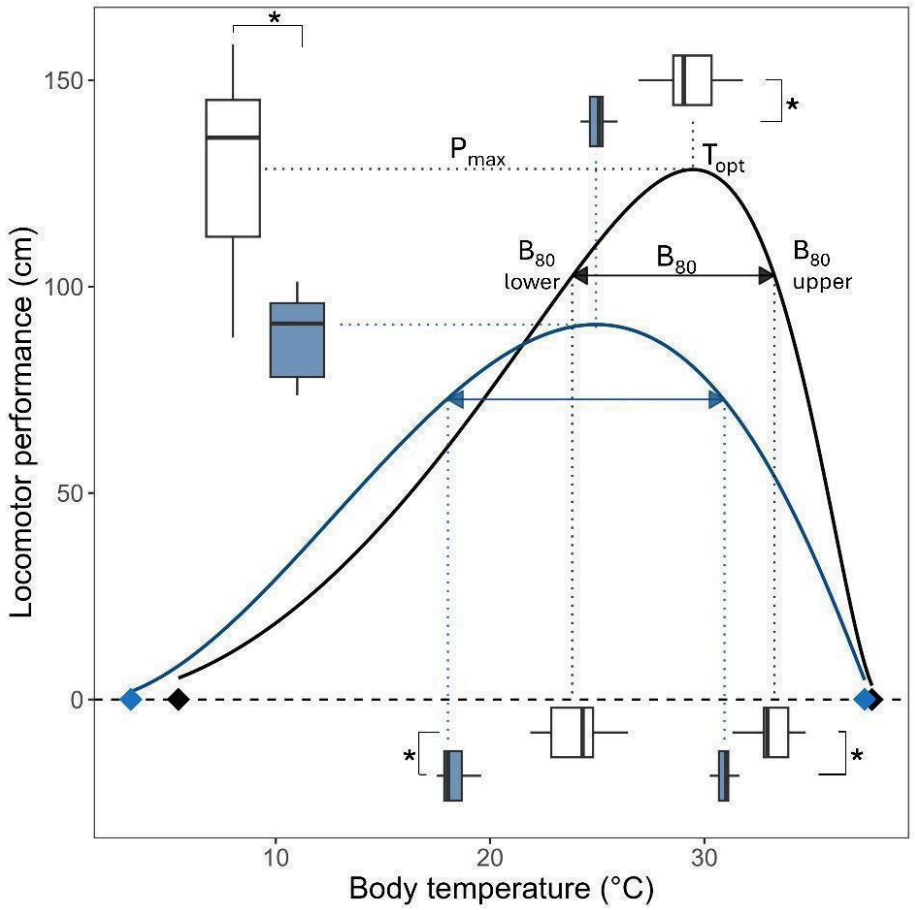
Figure 2 – Thermoregulatory indexes



Source: Prepared by the author, 2025

Figure 2. Seasonal variation in operative temperatures (T_e), accuracy of thermoregulation (d_b), environmental thermal quality (d_e) and effectiveness of thermoregulation (*E*). The lower and upper edges of the boxes correspond to the first (Q1) and third (Q3) quartiles, respectively; the horizontal lines within the boxes indicate median values, and the vertical whiskers represent the lower and upper limits of the data. Horizontal lines and asterisks indicate seasonal statistical differences ($p < 0.05$).

Figure 4 – Locomotor performance



Source: Prepared by the author, 2025

Figure 4. Thermal performance curves of *Scinax fuscovarius* jumping capacity for the cold-dry (blue) and hot-wet (black) seasons. Dotted lines indicate T_{opt}, P_{max}, and the lower and upper limits of B_{80}; horizontal arrows indicate amplitude of B_{80}. For clarity, labels are included only for the hot-wet season TPC. Boxplots summarize the interquartile thermal range for T_{opt}, P_{max}, and B_{80} limits for the cool-dry (blue boxes) and hot-wet (white boxes) seasons. Asterisks denote significant seasonal differences ($p < 0.05$) between boxplots. Diamonds at the base of the curves represent the empirically determined critical thermal limits (see Maenaka et al., 2023) used to anchor the TPCs, at zero performance.

4 DISCUSSION

Our results reveal that *Scinax fuscovarius* maintains constant thermoregulatory accuracy and hydration levels year-round, despite pronounced seasonal variation in environmental conditions. On the other hand, locomotor performance varied seasonally, showing downward shifts in performance traits during the cold-dry season, as is known to occur with its thermal preference, suggesting an integrated behavioral–physiological response to colder conditions. These adjustments indicate that *S. fuscovarius* buffers seasonal environmental constraints through a complex interplay of behavioral and physiological mechanisms that enables the species to remain active without relying on aestivating strategies typical of other Neotropical amphibians.

Operative temperatures dropped substantially during the cold-dry season, reducing overall environmental thermal quality. However, frogs were able to maintain their field body temperatures close to their seasonal T_{pref} range. Our T_{field} values closely match those reported by Maenaka et al. (2023), which corroborates the downward shift in T_{field} during the cold-dry season for this *S. fuscovarius* population. Maenaka et al. also showed that shifts in T_{field} are mirrored by similar shifts in T_{pref}, suggesting that frogs adjust their thermoregulatory thermal targets on a seasonal basis to remain within ranges consistent with prevailing environmental conditions. Our findings expand on this evidence by showing how such seasonal downward adjustment of T_{pref} effectively reduced the mismatch between T_{field} and T_{pref}, a key factor in promoting high thermoregulatory accuracy despite the seasonal decline in environmental thermal quality. Moreover, the elevated thermoregulatory effectiveness provides clear evidence that frogs did not passively follow environmental temperatures but actively avoided the lowest operative temperature conditions, further aligning T_{field} with their seasonal adjusted T_{pref}. Together, our results indicate that both the seasonal adjustment of T_{pref} and the behavioral avoidance of unfavorable operative temperatures function as complementary mechanisms sustaining high thermoregulatory accuracy in *S. fuscovarius*, thereby enabling the species to buffer seasonal declines in thermal quality and remain active year-round.

Despite the reduced environmental water availability that characterizes the cold-dry season in the region, *S. fuscovarius* maintained consistently high hydration levels in the field year-round (~98.7%). Given that this species exhibits reduced

rehydration capacity but unaltered skin resistance to evaporation during the cold-dry season (Maenaka et al., 2023), its ability to remain fully hydrated during this period suggests water conservation through behavioral means rather than physiological buffering via increased skin resistance or enhanced rehydration capacity. With no clear relationship between field hydration and microclimatic variables (T_{ar}, T_{sub}, and RH) or body mass, it appears that *S. fuscovarius* maintains hydration by staying near their moist shelters. The year-round availability of moistened shelters provided by anthropogenic water sources (ground-level drainages) at the collecting site may contribute to sustaining high hydration levels during drier periods, as observed in other anurans that exploit anthropogenic water sources across seasons (González-Bernal et al., 2016). Supporting this, nearly all frogs were found active within ~50 cm of their daytime shelters except on rainy days, when distances easily exceeded 10 m. This spatial site fidelity aligns with findings in other year-round active amphibians in seasonal climates, where bodily dehydration rates are driven primarily by shelter soil moisture rather than air temperature or humidity (Seebacher and Alford, 1999, 2002). Still, during the hot-wet season, individuals captured later at night showed lower hydration levels, indicating progressive water loss with prolonged activity. In contrast, this effect was absent in the cold-dry season, likely because frogs ceased field activity earlier (23:05 h in the cold-dry season vs. 02:23 h in the hot-wet season). In some individuals, hydration levels exceeded presumed full hydration (>100%, Fig. 3) and while the proximal cause is unclear, similar observations have been reported in other anurans (Preest et al., 1992; Walvoord, 2003). Possibly, this reflects incomplete bladder voiding at the time of capture or a transient hyperhydration following the hydration procedure (Preest & Pough, 1989; Brischoux & Cheron, 2019).

Contrary to our initial expectations, *S. fuscovarius* did not exhibit a mismatch between thermal performance and environmental temperatures during the cold-dry season. Instead, frogs shifted downward their temperature at which optimal performance was achieved compared to the hot-wet season. This response indicates that *S. fuscovarius* adjusts its locomotor thermal sensitivity *in tandem* with the lower environmental temperatures of the cold-dry season. While this adjustment certainly contributes to enabling the animals to attain appropriately high performance levels in both seasons, it should be noted that the absolute level of maximal performance (i.e., P_{max}) decreased substantially during the cold-dry season. However, even though *S.*

fuscovarius remains active year-round, its overall activity level is lower during the cold-dry season, and observed reduction in locomotor P_{max} may simply be aligned with this pattern. The shift in T_{opt} towards colder temperatures during the cold-dry season also carries the potential added benefit of decreasing the potential for evaporative water loss, a convenient response during this period. Finally, the shape of the thermal performance curve changed seasonally, revealing that, in addition to the reduction in P_{max}, there was also a broadening of performance breadth. This change is congruent with the specialist–generalist trade-off, in which broader levels of performance are associated with a lower sensitivity to temperature variation (Gilchrist, 1995, Boyles et al., 2011). From a functional perspective, this means that the functional capabilities of *S. fuscovarius* will be less affected by variations in the thermal environment during the cold-dry season.

5 CONCLUSION

In summary, our results demonstrate that *Scinax fuscovarius* employs a combination of behavioral and physiological strategies to remain active year-round, despite seasonal fluctuations in environmental temperature and water availability. By shifting both thermal preferences and performance traits to match colder conditions, and by maintaining hydration state, this species effectively mitigates the trade-offs typically associated with cold-dry periods in seasonal environments. These adjustments reduce reliance on energetically costly thermoregulation and support functional performance without compromising hydration. Altogether, our findings underscore the role of thermal and hydroregulatory seasonal adjustments as key for the animals to persist active in seasonal environments and contribute to a broader understanding of how amphibians cope with multiple environmental stressors without resorting to aestivation.

REFERENCES

ANDERSON, R.C.; ANDRADE, D.V. Trading heat and hops for water: Dehydration effects on locomotor performance, thermal limits, and thermoregulatory behavior of a terrestrial toad. **Ecology and Evolution**, v. 7, n. 21, p. 9066–9075, 26 set. 2017.

ANDERSON, Rodolfo César de Oliveira; BOVO, Rafael Parelli ; ANDRADE, Denis Vieira. Seasonal variation in the thermal biology of a terrestrial toad, Rhinella icterica (Bufonidae), from the Brazilian Atlantic Forest. **Journal of Thermal Biology**, v. 74, p. 77–83, 2018.

ANGILLETTA, M. J.; NIEWIAROWSKI, P. H.; NAVAS, C. A. The evolution of thermal physiology in ectotherms. **Journal of Thermal Biology**, v. 27, n. 4, p. 249–268, ago. 2002.

ANGILLETTA JR., M. J. Thermal Adaptation. **Thermal Adaptation: A Theoretical and Empirical Synthesis**, 29 jan. 2009.

BAGULEY, T. Standardized or simple effect size: What should be reported? British **Journal of Psychology**, v. 100, n. 3, p. 603–617, ago. 2009.

BOYLES, J. G. et al. Adaptive Thermoregulation in Endotherms May Alter Responses to Climate Change. **Integrative and Comparative Biology**, v. 51, n. 5, p. 676–690, 20 jun. 2011.

BRISCHOUX, François ; CHERON, Marion. Osmotic “cost” of reproduction in breeding male toads. **Biology Letters**, v. 15, n. 11, p. 20190689–20190689, 2019.

CARVALHO, José E; NAVAS, Carlos A ; PEREIRA, Isabel C. Energy and Water in Aestivating Amphibians. **Progress in molecular and subcellular biology**, p. 141–169, 2009.

DUELLMAN, W. E. Temporal Fluctuations in Abundances of Anuran Amphibians in a Seasonal Amazonian Rainforest. **Journal of Herpetology**, v. 29, n. 1, p. 13, mar. 1995.

FROST, Darrel R. Amphibian Species of the World: an Online Reference. Version 6.2. **American Museum of Natural History**, New York, 2025. Disponível em: https://amphibiansoftheworld.amnh.org/index.php. Acesso em: (04 nov. 2025). DOI: 10.5531/db.vz.0001.

GILCHRIST, G. W. Specialists and Generalists in Changing Environments. I. Fitness Landscapes of Thermal Sensitivity. **The American Naturalist**, v. 146, n. 2, p. 252–270, 1 ago. 1995.

GOLDBERG, J. et al. Body size variation and sexual size dimorphism across climatic gradients in the widespread treefrog Scinax fuscovarius (Anura, Hylidae). **Austral Ecology**, v. 43, n. 1, p. 35–45, 30 set. 2017.

GONZÁLEZ-BERNAL, E. et al. Toads in the backyard: why do invasive cane toads (Rhinella marina) prefer buildings to bushland? **Population Ecology**, v. 58, n. 2, p. 293–302, 16 fev. 2016.

HADDAD, C. F. B.; TOLEDO, L. F.; PRADO, C. P. A.. Scinax fuscovarius. In: HADDAD, C. F. B.; TOLEDO, L. F.; PRADO, C. P. A. **Anfíbios da Mata Atlântica**. São Paulo: Editora Neotropica, 2008. p. 177.

HERTZ, P. E.; HUEY, R. B.; STEVENSON, R. D. Evaluating Temperature Regulation by Field-Active Ectotherms: The Fallacy of the Inappropriate Question. **The American Naturalist**, v. 142, n. 5, p. 796–818, nov. 1993.

HUEY, R. B.; STEVENSON, R. D. Integrating Thermal Physiology and Ecology of Ectotherms: A Discussion of Approaches. **American Zoologist**, v. 19, n. 1, p. 357–366, fev. 1979.

HUEY, R. B. et al. Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. Philosophical Transactions of the Royal Society B: **Biological Sciences**, v. 367, n. 1596, p. 1665–1679, 7 maio 2012.

KINGSOLVER, J.; HUEY, R. B. Size, temperature, and fitness: Three rules. **Evolutionary Ecology Research**, v. 10, n. 2, p. 251–268, 1 fev. 2008.

KISS, A. C. I. et al. Seasonal metabolic changes in a year-round reproductively active subtropical tree-frog (Hypsiboas prasinus). **Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology**, v. 152, n. 2, p. 182-188, fev. 2009.

KÖPPEN, W.; GEIGER, R. Das geographische System der Klimate. In: KÖPPEN, W.; GEIGER, R. (Ed.). Handbuch der Klimatologie. **Berlin: Verlag von Gebrüder Borntraeger**, 1936. p. 44.

LERTZMAN‐LEPOFSKY, G. F. et al. Water loss and temperature interact to compound amphibian vulnerability to climate change. **Global Change Biology**, v. 26, n. 9, p. 4868–4879, 14 jul. 2020.

LITTLE, A. G.; SEEBACHER, F. Acclimation, acclimatization, and seasonal variation in amphibians and reptiles. **CRC Press eBooks**, p. 41–62, 9 jun. 2016.

MAENAKA B. M., SENZANO, L. M., ANDRADE, D. V. Seasonal variation in thermal biology and water balance in a year-round active Neotropical treefrog, Scinax fuscovarius. **Herpetologica**. v. 79, p. 155-165, 2023.

MITCHELL, A.; BERGMANN, P. J. Thermal and moisture habitat preferences do not maximize jumping performance in frogs. **Functional Ecology**, v. 30, n. 5, p. 733–742, 29 ago. 2015.

NAVAS, C. A.; ARAUJO, C. The use of agar models to study amphibian thermal ecology. **Journal Of Herpetology**, Granville, OH, v. 34, n. 2, p.330-334, jun. 2000.

OROMÍ, N.; SANUY, D.; SINSCH, U. Thermal ecology of natterjack toads (Bufo calamita) in a semiarid landscape. **Journal of Thermal Biology**, v. 35, n. 1, p.

34–40, jan. 2010.

PADFIELD, D.; O'SULLIVAN, H.; PAWAR, S. A new pipeline to fit thermal performance curves in r. **Methods in Ecology and Evolution**, v. 12, n. 6, p. 1138–1143, 25 mar. 2021.

PRADO, C. P. A.; UETANABARO, M.; HADDAD, C. F. B. Breeding activity patterns, reproductive modes, and habitat use by anurans (Amphibia) in a seasonal environment in the Pantanal, Brazil. **Amphibia-Reptilia**, [s.l.], v. 26, n. 2, p. 211-221, jan. 2005.

PREEST, Marion R; BRUST, Douglas G ; WYGODA, Mark L. Cutaneous Water Loss and the Effects of Temperature and Hydration State on Aerobic Metabolism of Canyon Treefrogs, Hyla arenicolor. **Herpetologica**, v. 48, n. 2, p. 210–219, 1992.

PREEST, M. R. ; POUGH, F. H. Interaction of Temperature and Hydration on Locomotion of Toads. **Functional Ecology**, v. 3, n. 6, p. 693, 1989.

REBOITA, M. S. et al. Entendendo o tempo e o clima na América do Sul. **Terrae Didatica**, v. 8, n. 1, p. 34, 29 jun. 2015.

ROZEN‐RECHELS, David; DUPOUÉ, Andréaz; LOURDAIS, Olivier; et al. When water interacts with temperature: Ecological and evolutionary implications of thermo‐hydroregulation in terrestrial ectotherms. **Ecology and Evolution**, v. 9, n. 17, p. 10029–10043, 2019.

RUIBAL, R.; TEVIS, L.; ROIG, V. The Terrestrial Ecology of the Spadefoot Toad Scaphiopus hammondii. **Copeia**, v. 1969, n. 3, p. 571, 29 ago. 1969.

SANABRIA, E. A.; QUIROGA, L. B.; MARTINO, A. L. Seasonal changes in the thermoregulatory strategies of Rhinella arenarum in the Monte desert, Argentina. **Journal of Thermal Biology**, v. 36, n. 1, p. 23–28, jan. 2011.

SEEBACHER, F.; ALFORD, R. A. Movement and Microhabitat Use of a Terrestrial Amphibian (Bufo marinus) on a Tropical Island: seasonal variation and environmental correlates. **Journal Of Herpetology**, [s.l.], v. 33, n. 2, p. 208, jun. 1999.

SEEBACHER, Frank ; ALFORD, Ross A. Shelter Microhabitats Determine Body Temperature and Dehydration Rates of a Terrestrial Amphibian (Bufo marinus). **Journal of Herpetology**, v. 36, n. 1, p. 69–69, 2002.

SENZANO, L. M.; BOVO, R. P.; ANDRADE, D. V. Empirical estimation of skin resistance to water loss in amphibians: agar evaluation as a non-resistance model to evaporation. **Journal of Experimental Biology**, v. 225, n. 15, 12 jul. 2022.

SILVA, Emanuel Giovani Cafofo; DELARIVA, Rosilene Luciana; AFFONSO, Igor de Paiva. Distribuição espaço-temporal de Scinax fuscovarius (Lutz, 1925) (Anura, Hylidae) em Maringá – PR, Brasil. **Revista em Agronegócio e Meio Ambiente**, v. 2, n. 3, p. 431–445, 2009.

SPOTILA, J. R.; BERMAN, E. N. Determination of skin resistance and the role of the skin in controlling water loss in amphibians and reptiles. **Comparative Biochemistry**

and Physiology Part A: Physiology, v. 55, n. 4, p. 407–411, jan. 1976.

TAYLOR, E. N. et al. The thermal ecology and physiology of reptiles and amphibians: A user's guide. **Journal of Experimental Zoology** Part A: Ecological and Integrative Physiology, v. 335, n. 1, p. 13–44, 8 jul. 2020.

TRACY, C. R. et al. Field Hydration State Varies among Tropical Frog Species with Different Habitat Use. **Physiological and Biochemical Zoology**, Chicago, v. 87, n. 2, p. 197-202, mar./abr. 2014.

VIMERCATI, Giovanni; DAVIES, Sarah J ; MEASEY, John. Rapid adaptive response to a mediterranean environment reduces phenotypic mismatch in a recent amphibian invader. **The Journal of Experimental Biology**, 2018.

WALVOORD, MARK E. Cricket Frogs Maintain Body Hydration and Temperature Near Levels Allowing Maximum Jump Performance. **Physiological and Biochemical Zoology**, v. 76, n. 6, p. 825–835, nov. 2003.