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Behavioral models of hydrothermal regulation in anurans: A field study in the Atlantic Forest, Brazil

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Abstract

Anurans rely on different microhabitats and microclimates for hydro-thermoregulation, which is an important physiological property for locomotor performance, body temperature, water flux, and pathogen defense. We ask whether microhabitat scouting is related to physiological states, specifically if microhabitat choice benefits the production of behavioral fever (e.g., increasing body temperature by selecting warmer places) or sickness behavior (e.g., inactivity and anorexia). We investigated how thermoregulatory behaviors affect hydro-thermoregulation in anurans, using agar models as a sampling unit, simulating four behaviors related to behavioral fever and sickness behavior. We compared the models' hydro-thermal balance in two different environments (wet and transitional forests). We showed that the wet forest was warmer and more stable than transitional forest and the agar models in the wet forest were warmer. Still, the four behavioral simulations exhibited similar average temperatures during the five-day study period in both forests. However, the apathetic behavior models had the lowest daily temperatures. The percentage hydration across models was higher in the wet forest, and daily variation in water loss for each behavioral simulation was higher in the transitional forest. Nevertheless, when limiting the analysis to the warmest times (1000–1600h), apathetic behavior models lost less water than the others in both forests. We showed that different forests provide microclimatic variation for understory anurans, but different thermoregulatory behaviors may lead to similar body temperatures. Therefore, the Atlantic Forest offers different microclimates and thermoregulation options for anurans, as some species could develop behavioral fever while others demonstrate sickness behavior.

Abstract in Portuguese is available with online material.

KEYWORDS
agar model, behavioral fever, behavioral simulations, body temperature, operational temperatures, percentage hydration, sickness behavior, thermal gradients

1 | INTRODUCTION

One definition of microhabitat is a pool of sub-areas within more extensive ones that are used as a home range by individuals of a species and characterized by a set of resources and environmental conditions (Leopold, 1933). This notion relates to the structural complexity of certain biomes that exhibit diverse arrays of vegetation physiognomies, offer resources of diverse ecological value to a given species, and reveal context-specific physical conditions. These factors confer distinct characteristics to microhabitats and define their associated and multidimensional microclimates (Michel & Winter, 2009). Among the many traits of microclimate, humidity and temperature seem particularly important to understand the ecology of small ectothermic vertebrates. However, the concept of microclimate is multi-scalar and its application is context-specific. For many analyses, particularly those associated with physiological ecology, the scale that matters most relates to body size and may deviate substantially from macroclimatic counterparts (Angilletta Jr., 2009; Gates, 1980). For example, the thermal components of climate, when acting at a body-size scale, may influence physiological, behavioral, and ecological performance (Huey et al., 2012; Pincebourde et al., 2016; Potter et al., 2013). These relationships are particularly complex in amphibians, which are terrestrial animals that have small body size. Many physiological characters are related to temperature at both evolutionary and individual levels (Brattstrom, 1979). Further, hydrothermal balance is integrated with locomotor performance (Titon et al., 2010), and body temperature, water flux, and microhabitat selection are interconnected (Cruz-Piedrahita et al., 2018). Thus, microclimatic conditions in habitats such as the forest floor, above-ground vegetation, or sheltered spaces likely influences both body temperature and water flux in amphibians (Navas, 1996; Thorson & Svihla, 1943).

Free-ranging amphibians have behavioral options for microhabitat use; so, it is worth asking whether their navigation follows rules related to physiological states. Some experimental work suggests this may be the case. For example, anuran hydrothermal regulation shifts with dietary conditions (Lillywhite et al., 1973; Witters & Sievert, 2001), reproductive status, and immune challenges (Saad, 1988; Zapata et al., 1992). These experimental alterations in microclimatic conditions provide evidence that amphibians are equipped with sensing capacities that can influence navigation rules, but do not support that such rules become a priority under natural conditions. This crucial difference matters when discussing emerging diseases, particularly if a behavioral strategy is considered an active defense against pathogens. During the acute phase of an anuran immune response, for example, an animal will seek warmer places in order to fight infection and develop behavioral fever (Harden et al., 2015; Rakus et al., 2017). Some frog species exposed to simulated infection do enhance body temperature (Hutchison, 1981; Rowley & Alford, 2013) and produce behavioral fever, so it is logical to predict immunological advantages if this response is exhibited under natural conditions (Bicego & Branco, 2002; Karavlan & Venesky, 2016; Sauer et al., 2019; Woodhams et al., 2003). However, the temperatures selected by amphibians in thermal gradients do not necessarily result from positive thermotaxis, a response presumably required to produce behavioral fever in the field (Navas et al., 2021). Fever is also not the only possible behavioral response to pathogens. An alternative is apathy (sickness behavior), a syndrome characterized by inactivity and lethargy that may lead to starvation and loss of body mass (Hart, 2010). Notably, fever in endothermic vertebrates is mediated by temperature-enhancing metabolic paths and may develop simultaneously with sickness behavior (Harden et al., 2015; Hetem et al., 2008). Whether fever can co-occur with sickness behavior in ectothermic tetrapods is not clear; achieving behavioral fever in the field would likely require active navigation, rather than inactivity, through a thermal landscape. Indeed, Llewellyn et al. (2011) showed that the primary responses in individuals of *Rhinella marina* after simulated infection with injection of LPS (bacterial lipopolysaccharides) in the laboratory are reduced activity and refuge seeking, rather than heat-seeking behaviors. In contrast, individuals of congeneric *R. icterica* select higher temperatures in thermal gradients when similarly treated, compared with untreated toads (Moretti et al., 2018).

Because tradeoffs between apathy and behavioral fever in the field remain conjectural, they deserve empirical exploration, particularly in humid forested environments where thermal gradients at ground level may be narrow. We propose that behavioral fever can be fully realized in the field only if (a) positive thermotaxis becomes a dominant behavioral drive; (b) no other behavioral drive interferes with fever seeking; and (c) the environment provides variation in temperature compatible with enhancing body temperature through behavior. Our focus in this study is the third premise, which differs from the other two in imposing an ecological constraint rather than an intrinsic trait. Little is known about how free-ranging amphibians explore thermal landscapes, although heliothermic basking (Tattersall et al., 2006) or opportunistic thermoregulation under rocks occur in some taxa. Importantly, effective thermoregulation requires thermal opportunity (Lillywhite, 1975; Navas, 1996) and must not promote dehydration. Microsites combining heat and humidity may be dispersed, given that elevated temperatures may reduce soil moisture, but little empirical evidence exists on this postulate. This is a relevant aspect of amphibian ecology in structurally complex habitats such as tropical forests, characterized by a low incidence of direct sunlight in dense patches of the understory (Nicotra et al., 1999). These are characteristics of a biome known for its remarkable frog diversity, the Atlantic Forest (Haddad et al., 2013).

In this article, we report on whether a small region of the Atlantic Forest provides a heterothermic landscape compatible with enhancing body temperature while preserving hydration for small terrestrial anurans. Our study consists of a behavioral simulation conducted in the field to test whether understory anurans in the Southeastern Atlantic Forest encounter thermal opportunities sufficient to generate behavioral fever through modified behavior. We predict that the low thermal heterogeneity of the understory environment limits the scope of behavioral thermoregulation and thus constrains effective behavioral fever. In parallel, we explore hydrothermal tradeoffs and tradeoffs between apathy and behavioral fever.

2 | METHODS

2.1 | Study site

The Atlantic Forest is a biome dominated by the dense Ombrophilous Forest located along the Atlantic ridge. It is particularly influenced by rainfall distribution, latitude, altitude, seasonality, and humid air masses that traverse the continent from the ocean (Oliveira-Filho & Fontes, 2000; Scudeller, 2002). Our study was conducted in the Parque Estadual Intervales (PEI), an Integral Conservation Unit of the Atlantic Forest located in the municipalities of Iporanga, Ribeirão Grande, and Sete Barras, all of them located near the coastal mountains of Southeastern Brazil (24°12′–24°25′ S; 48°03–48°30′ W; 800–900 m a.s.l.). The average annual rainfall is 4216.2 ± 245.5 mm (Pizo & Oliveira, 2000), and different permanent water bodies exist. The Park is ecologically diverse for anurans (cryptozoic, rheophilic, rupicolous, terrestrial, and arboreal microhabitats) and includes a growing list of species (Bertoluci & Rodrigues, 2002; Haddad et al., 2013).

We compared two forested environments that differ in physical features and anuran assemblages. One was mature Atlantic Forest located along the east path of Morro da Anta (24° 27′20 43″ S 48° 40′.6″ W, 800 m a.s.l.) and referred to as a "wet forest" in our study. This environment features a dense canopy with tall trees covered by abundant epiphytes and bromeliads, and an understory covered by a litter layer (Ortega-Chinchilla, personal observation). During the day, the solar radiation is intercepted by the canopy, and understory temperature increases are minimized (Renaud & Rebetez, 2009). The second environment was young secondary forest adjacent to open areas and referred to as "transitional forest" (24° 27′29 4″ S 48° 41′.30 W, 772 m a.s.l.). This site includes medium-sized trees adjacent to a large cleared area that once served as a quarry.

2.2 | Operational temperatures

We measured operational temperatures across transects in the two forest environments, wet and transitional. Here, operational temperatures indicate temperatures of inanimate objects comparable with real frog species in size and shape. We used agar models shaped after the species *Physalaemus olfersii* (agar model protocol can be found in Navas & Araujo, 2000). We chose this species given its average size, according to Haddad et al. (2013). The models measured about 4.3 cm SVL and weighed about 10 g. A tetrad, the sampling unit for both operational temperatures and behavioral simulations, was composed of four sensor-fit agar models, each one representing a behavior (see next section). The tetrads used to collect operational temperatures were fitted with a 170 cm HOBO® Data Logger (U12-008) sensor programmed to record the temperature every 15 min (see sampling details in Figure 1a). To start a sampling cycle of operational temperature, we selected sites based on typical substrates for understory frogs taken from real observations of active frogs, including *Physalaemus olfersii* (e.g., fallen logs, leaf litter, and flat rocks or hard surfaces). The distance between tetrads was from 5 to 7 m, and the distance between models within a tetrad was based on sensor length (about 250 cm). We collected operational temperature data continuously over the sampling period using eight tetrads, all of which were set to collect data for five consecutive days. We sampled wet forest first (October 23 to 27, 2016), then transitional forest (October 29 to November 2, 2016). Given this sequential protocol, our data included an uncontrolled source of temporal variation in temperature and humidity in the form of rain. We refer to this variation when pertinent, but it was not quantified formally.

2.3 | Temperature and water balance of behavioral simulation agar models

We used agar models as proxies for anurans and evaluated the impact of simulated behaviors on body temperature and water balance. We compared models that were behaviorally manipulated by hand according to algorithms that simulated a strong drive toward behavioral fever, apathy, an intermediate response, and a control. This approach was performed for each forest type over the five-day periods identified above.

To record the temperature of agar models used for behavioral simulation, we used eight tetrads without sensors. The procedure for tetrad placement was similar to that described for operational temperatures, but each model within a tetrad was initially placed within 5 cm of another to ensure similar initial thermal conditions. These tetrads were left undisturbed overnight, and no manipulation occurred after 2000 h. On the next day, at 0600 h, we measured the surface temperatures of models and immediately applied the corresponding behavioral rule (see below). We repeated these steps for each tetrad in sequence until all models were in place, which took about 20 min. This procedure was performed hourly until 2000 h, at which point the four models were regrouped for the night. We used a Raytek Pro Infrared Thermometer placed 2 cm above a model's surface to measure model temperature. To measure variation in the water content for these behavioral simulations, we recorded the mass of each model at 0600 h just after measuring temperature and repeated this every 2 h. We used a portable balance (A&D Newton EJ-123, 0.01 g accuracy) and calculated water loss rates from the difference between the initial model mass and mass measured at each subsequent 2-h period. We express water loss as a percentage of maximum hydration, with negative values representing water loss (i.e., the model lost water between one weighing and the previous), while positive values represent water gain. The closer the result is to zero, the less water is gained or lost.

The behavioral simulations (translated as model-movement steps, see Figure 1b) were defined as: (a) strong behavioral fever (SBF); (b) apathy behavior (AB); (c) single thermoregulatory event (STE); and (d) control model (CO). After the temperature data were collected at 0600 h, three of the agar models (SBF, AB, and STE) were moved immediately, each one according to their corresponding protocol (SBF: Warmest Neighboring Site, AB: closest shelter, mainly small burrows, or accumulations of leaf litter; STE: Alternative Warmest Neighboring site). One hour later, at about 0700 h, and hereafter hourly, a similar procedure was repeated, but only the SBF model required movement (see details in Figure 1b).
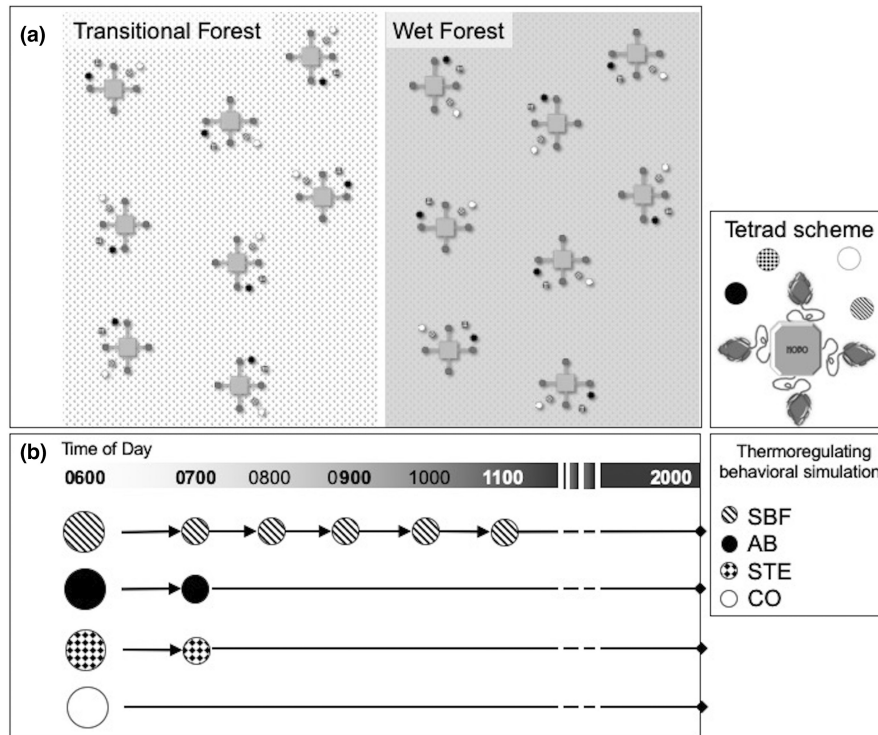
FIGURE 1 Sampling scheme. (a) Operational temperatures measured with tetrads across transects and replicated eight times at each forest landscape. Each tetrad component was an agar frog model fitted with a HOBO® data logger (U12-008) sensor programmed to record the temperature every 15 min. (b) Scheme of thermoregulatory behavioral simulations during the fieldwork. Model SBF (strong behavioral fever) simulated a sick frog with active thermoregulating; model AB (apathy behavior) simulated a sick frog with the defense behavior of seeking refuge; model STE (single thermoregulatory event) simulated a sick frog with only one episode of thermoregulation; and model CO was the control, simulating a null condition of movements. The arrows show the number of movements of each model at different times during the day. These movements were performed for 5 days in each forest (wet and transitional). The temperature of all models was measured every hour and models were weighed every 2 h from 6 h until 20 h.

The selections of Warmest Neighboring Site and Alternative Warmest Neighboring Site were based on thermograms of soil surface collected with a FLIR TG165 Thermal Imaging Thermometer (emissivity set to 0.95). Preliminary tests had shown that the overall soil temperatures would not approach the upper thermal tolerance typical of tropical frogs.

2.4 | Statistical analysis

Analyses were performed in R (3.5.1, R Core Team, 2018). For all operational temperature analysis, we used one-way ANOVAs. First, we calculated the 5-day average operational temperature of the tetrads, to check if data were comparable. In particular, we wanted to analyze possible thermal gradient within each forest or lack of homogeneous temperature across the transects. This analysis was performed for each of the forests separately. We also used the five-day mean temperature of the tetrads of each forest to compare thermal profiles between the forests. To verify if operational temperatures differed among days in each forest, we compared the daily mean temperature of the tetrads using one-way ANOVA followed by Tukey post hoc comparisons.

To compare the impact of behavioral rules on both temperature and water loss, we calculated the overall mean temperature obtained from each model across sampling days, applying a Repeated Measure ANOVA.

3 | RESULTS

3.1 | Operational temperatures in wet and transition forest

Overall, the operational temperature of the tetrads, pooled over the five sample days, were comparable both in the wet forest (mean = 18.10°C; $SD = 1.19$; ANOVA $F_{(7,112)} = 0.63$, $p = .73$) and in the transition forest (mean = 16.88°C; $SD = 2.78$; ANOVA $F_{(7,112)} = 0.18$, $p = .99$), thus ruling out that a thermal gradient affected the chosen transect. When we compared the aggregated five-day mean operational temperature between forests, the wet forest was warmer (ANOVA $F_{(1,2558)} = 225.34$, $p < .001$), and more stable (Levene test, $F_{(1,2558)} = 3.56$, $p = .014$) than the transitional forest.

In each forest, the operational temperatures varied among days (wet forest, ANOVA $F_{(4,1275)} = 271.14$, $p < .001$; transitional forest,

TABLE 1 Operational temperature (°C) over time in each forest environment.

	Wet Forest				Transitional Forest			
Day	Mean (°C)	SD	Variance	Tukey pos hoc	Mean (°C)	SD	Variance	Tukey pos hoc
1	16.67	1.08	1.17	a	13.91	0.96	0.92	a
2	18.18	1.18	1.40	b	14.53	0.85	0.73	b
3	18.19	0.37	0.13	b	16.86	1.53	2.35	c
4	19.17	1.05	1.10	c	19.24	1.89	3.58	d
5	18.26	0.50	0.25	b	19.85	1.62	2.63	e

Note: The operational temperatures varied among days in the wet forest (ANOVA $F_{(4,1275)} = 271.14$, $p < .001$) and in the transitional forest (ANOVA $F_{(4,1275)} = 1284.15$, $p < .001$). Significant differences across days are shown by different letters (Tukey's post hoc < 0.001). Day 1 and Day 4 differed from the other 3 days in wet forest. All days differed in the transitional forest.

TABLE 2 Temperature of behavioral simulation models (°C) within each forest environment.

	Wet Forest			Transitional Forest		
Thermoregulatory behavioral simulations	Mean (°C)	SD	Variance	Mean (°C)	SD	Variance
Strong behavioral fever	17.96	1.52	2.32	17.08	3.01	9.10
Apathy behavior	17.37	0.94	0.89	16.64	2.37	5.65
Single thermoregulatory event	17.87	1.31	1.72	16.92	2.94	8.68
Control	17.88	1.27	1.63	16.83	2.95	8.70

Note: The four behavioral simulations exhibited similar average temperature in the wet forest (repeated measure ANOVA $F_{(31,1248)} = 1.32$, $p < .11$) and in the transitional forest (repeated measure ANOVA $F_{(31,1248)} = 0.13$, $p = 1$).

ANOVA $F_{(4,1275)} = 1284.15$, $p < .001$, see Table 1). In the wet forest, days one and four differed from the other three days (Tukey post hoc comparisons $p < .001$), with the first day being the coldest and the fourth day being the warmest. Regarding the transitional forest thermal profile, we observed an increasing thermal gradient, with the first day being the coldest and temperatures increasing steadily until the last and warmest day. All days differed from each other with respect to average temperature in the transitional forest (Tukey post hoc comparisons $p < .001$, Table 1).

3.2 | Thermal profile and temperature variation of behavioral simulation models across forests

Daily temperatures of the behavioral models also varied across days within each forest type (wet Forest, Repeated Measure ANOVA $F_{(4,1260)} = 268$, $p < .001$; transitional forest, Repeated Measure ANOVA $F_{(4,1260)} = 907$, $p < .001$). When all data on behavioral models were analyzed as a block, independent of day and time of day, the four behavioral simulations exhibited similar average temperature (Table 2) both in the wet forest (Repeated Measure ANOVA $F_{(31,1248)} = 1.33$, $p < .11$) and in the transitional forest (Repeated Measure ANOVA $F_{(31,1248)} = 0.13$, $p = 1$). When data were analyzed as hourly distributions of mean temperatures over time of day, as in Figure 3, patterns differed across forest types. In the wet forest, the agar models simulating apathic behavior (AB) had the lowest daily temperatures, followed by STE, CO, and SBF (warmest) treatments

(Repeated Measure ANOVA $F_{(21,1248)} = 2.2$, $p < .001$, Figure 2a). In the transitional forest, the thermal profile was comparable among tetrads (Repeated Measure ANOVA $F_{(21,1248)} = 0.27$, $p = .99$, Figure 2b). When comparing the thermal profiles of agar models between 1000h and 1600h of the hottest day, the agar models simulating apathy behavior (AB) exhibited the lowest daily temperatures in both forests (Repeated Measure ANOVA $F_{(9,112)} = 71.3$, $p < .001$, Figure 3a,b).

3.3 | Water balance of behavioral simulation models across forests

The percentage hydration across models was lower in the wet forest (mean = −0.38%, $SD = 2.36$) than in the transitional forest (mean = −0.15%, $SD = 2.50$), independent of which behavior was simulated (ANOVA, $F_{(1;2556)} = 5.785$, $p = .01$). When focusing on the behavioral responses, we did not observe differences in the hydration state between behavioral simulations in any forest environment (wet forest, ANOVA repeated measures $F_{(31,1248)} = 0.175$, $p = 1$; transitional forest, ANOVA of repeated measures, $F_{(31,1248)} = 0.12$, $p = 1$, Table 3). Daily variation in water loss in each behavioral simulation in the transitional forest (Levene test, $F_{(3,1275)} = 3.56$, $p = .014$) was higher than wet forest (Levene test, $F_{(3,1275)} = 0.05$, $p = .99$). Nevertheless, when data were analyzed as hourly distributions of percentage hydration over time of day, patterns differed across forest types. In the wet forest, the agar
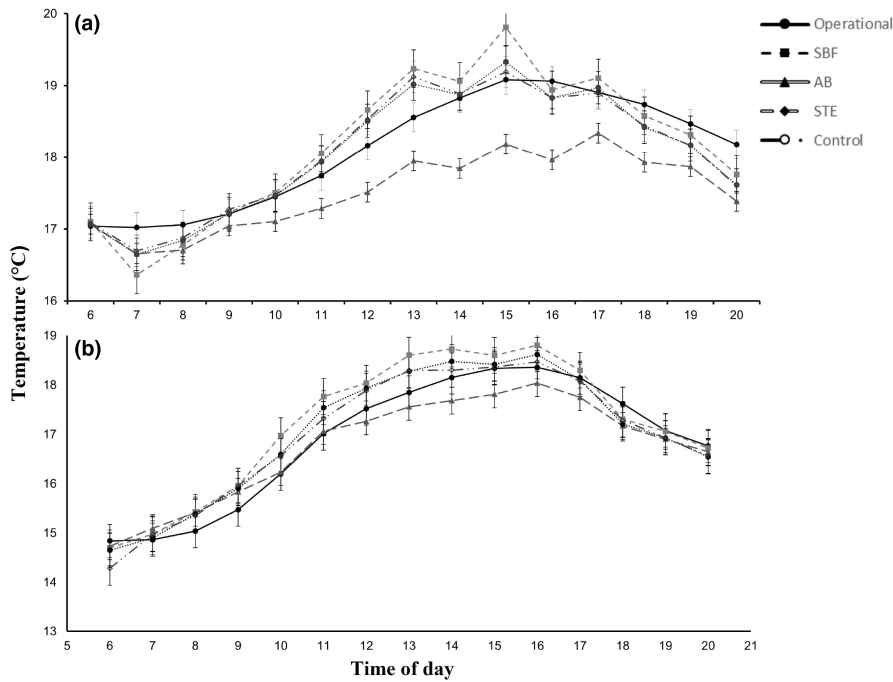
FIGURE 2 Profiles of the mean operational temperatures and behavioral simulation (strong behavioral fever SBF, apathy behavior AB, single thermoregulatory event STE, and control CO) temperatures recorded throughout the daily study periods over 5 days within the wet (a) and transitional (b) forests. The temperatures of the behavioral models differed in the wet forest (repeated measure ANOVA $F_{(21,1248)} = 2.2$, $p < .001$, Figure 2a) but not in the transitional forest (repeated measure ANOVA $F_{(21,1248)} = 0.27$, $p = .99$, Figure 2b). Error bars are included in the figure.
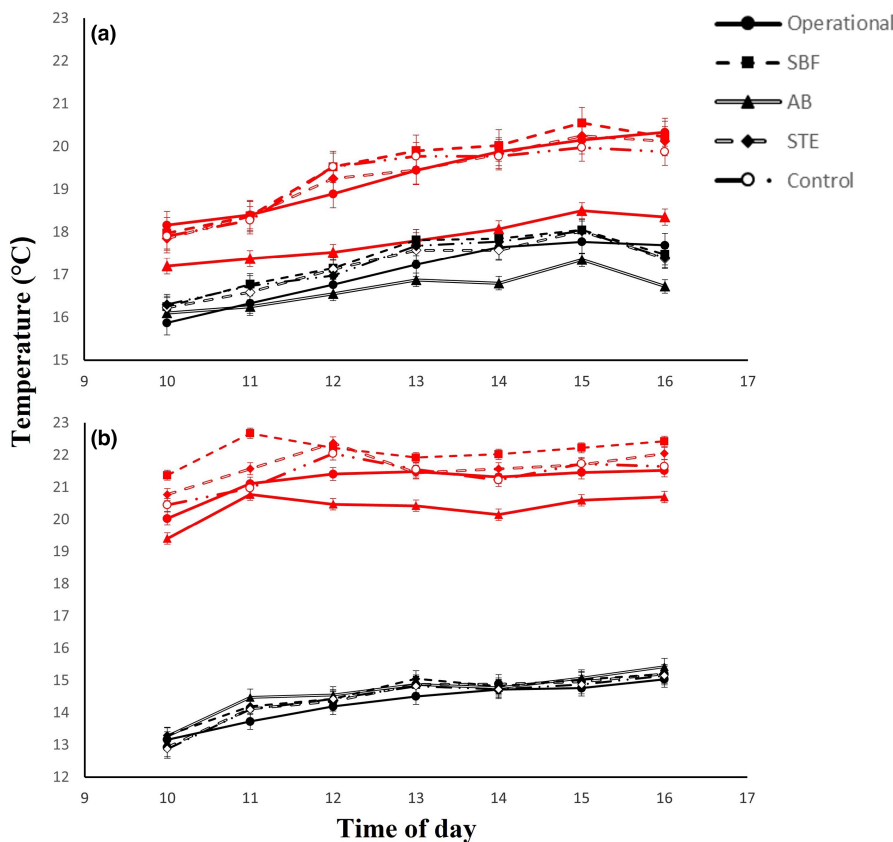

FIGURE 3 Profiles of the mean operational temperatures and behavioral simulation (strong behavioral fever SBF apathy behavior AB, single thermoregulatory event STE, and control) temperatures recorded from 1000 to 1600 h during the hottest (red lines) and coldest days (black lines) in wet (a) and transitional (b) forest environments. The agar models simulating apathy behavior (AB) exhibited the lowest daily temperatures in both forests (repeated measure ANOVA $F_{(9,112)} = 71.3$, $p < .001$). Error bars are included in the figure.

models did not exhibit differences in the hydration state (Repeated Measure ANOVA $F_{(9,112)} = 0.10$, $p = .96$). However, in the transitional forest, between 1000 h and 1600 h of the hottest day, the agar models simulating apathy behavior (AB) exhibited the lowest daily water loss (Repeated Measure ANOVA $F_{(9,112)} = 9.13$, $p < .001$, Figure 4a,b).

4 | DISCUSSION

Our study assesses the potential for hydrothermal regulation of amphibians according to different behavioral responses simulated using agar models. The types of forests studied provide some microclimatic variation for understory frogs, but this is limited so that

TABLE 3 Percentage of hydration of behavioral simulation models within each forest environment.

Thermoregulatory behavioral simulations	Wet Forest			Transitional Forest		
	Mean (%)	SD	Variance	Mean (%)	SD	Variance
Strong behavioral fever	−0.48	2.26	5.10	−0.15	2.84	8.05
Apathy behavior	−0.45	2.34	5.46	−0.18	1.59	2.52
Single thermoregulatory event	−0.32	2.34	5.47	−0.05	2.97	8.84
Control	0.04	2.50	6.25	−0.23	2.40	5.74

Note: No differences in the hydration state between behavioral simulations was observed in any forest environment (wet forest, ANOVA repeated measures $F_{(31,1248)} = 0.175$, $p = 1$; transitional forest, ANOVA of repeated measures, $F_{(31,1248)} = 0.12$, $p = 1$).The mean represents the data of the 5 days of sampling.

FIGURE 4 Profiles of the percentage hydration of behavioral simulations (strong behavioral fever SBF apathy behavior AB, single thermoregulatory event STE, and control) recorded from 1000h to 1600h during the hottest day in wet (a) and transitional (b) forest environments. The agar models did not exhibit differences in the hydration state in the wet forest (repeated measure ANOVA $F_{(9,112)} = 0.1014$, $p = .96$). The apathy behavior (AB) models exhibited the lowest daily water loss in the transitional forest (repeated measure ANOVA $F_{(9,112)} = 9.13$, $p < .001$). Error bars are included in the figure.
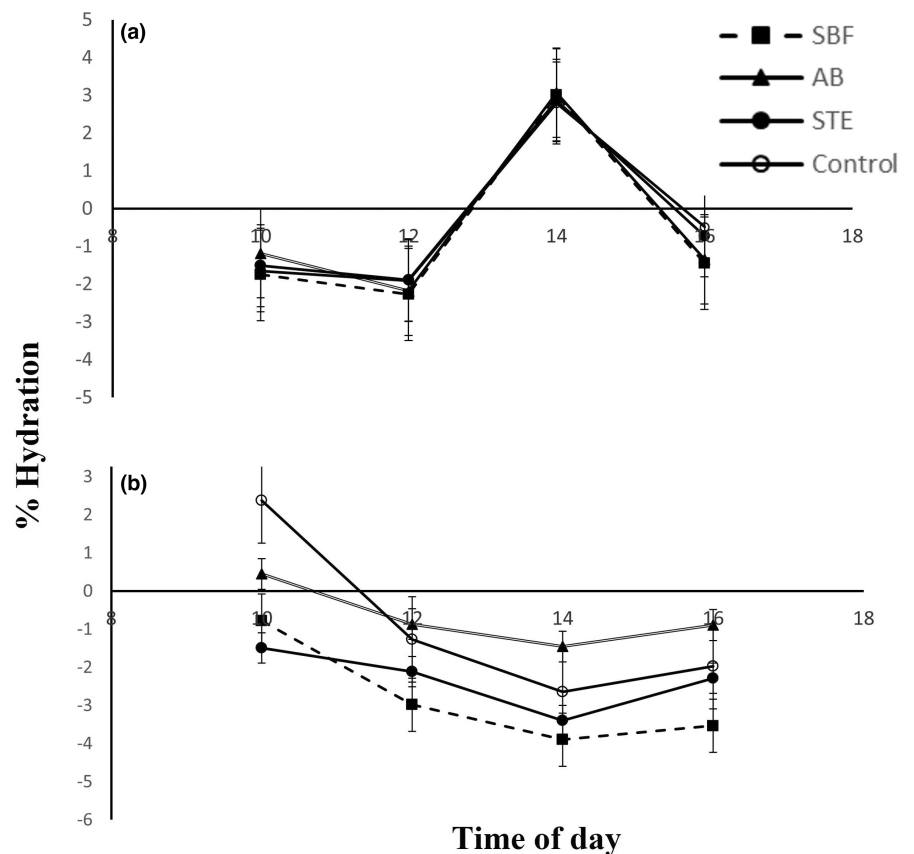
dramatically different thermoregulatory behaviors may lead to similar body temperatures. Therefore, we found support for our prediction, that behavioral fever may be a difficult behavioral drive to realize for wet and transition forest counterparts.

4.1 | Operational temperature profiles of wet and transitional forests

The wet forest offers habitats with higher temperatures and less variation compared to the transitional forest, as documented in previous reports (Hupfer & Kuttler, 1998), and known sources of microclimatic variation include altitude, structure, fragmentation, micro-topography, and time of day (Root & Schneider, 1995). The edge of a transitional

forest is exposed to greater solar radiation and a pool of factors favoring particular profiles of microclimatic variation (Camargo & Kapos, 1995). As a caveat, we did not sample both forest types simultaneously, so our study ignores temporal heterogeneity across day sequences.

4.2 | Thermal profile and temperature variation of behavioral simulations

The mean model temperature derived from different simulations of thermoregulatory behavior was similar in both forest types. It seems, then, that this forested environment presents, at the floor level, patterns of thermal variation incompatible with enhancing body temperature above average values. This is true even when models

behaved as to search repeatedly for the warmest spot nearby. It seems, then, those environmental considerations matter when inferring possible field behavior based on the behavioral responses of amphibians in thermal gradients (Feder, 1982). Amphibians infected with bacteria, fungi, and viruses may select warmer temperatures in thermal gradients in the laboratory (Bicego & Branco, 2002; Karavlan & Venesky, 2016; Moretti et al., 2018; Sauer et al., 2019), but this occurs in highly heterothermal settings that prevent dehydration and where thermoregulation is possible at a low metabolic cost. Thus, experiments in thermal gradients do not suffice as evidence of analogous field responses, a topic that remains neglected. Barrile et al. (2021) reported that free-ranging toads (*Anaxyrus boreas boreas*) experimentally infected with the fungus *Batracochytrium dendrobatidis* (*Bd*) selected more open microhabitats with higher temperatures under natural field conditions. This means that a selection of temperatures requires substantial thermal heterogeneity and affects the host–pathogen relationship in infected anurans (Richards-Zawacki, 2009; Rowley & Alford, 2013).

The simulation of apathy behavior (AB) had the lowest daily temperatures in both forests compared with the other behavioral simulations, a pattern even more evident when comparing data at the time (1000 h – 1600 h) and day of highest temperatures. The AB model represents sickness behavior with reduced activity and shelter seeking, which can be considered viable for anurans. According to Llewellyn et al. (2011), *Rhinella marina* reduces activity under simulated infection and instead seeks shelter; body temperature would increase only because daily temperatures affect the shelter's temperature. Thus, sickness behavior could be a dominant behavioral response over the behavioral fever, as shown for the endemic Brazilian frog, *Proceratophrys boiei* (Cabanzo-Olarte & Navas, *in preparation*). Individuals of this species exhibited sickness behavior predominantly over behavioral fever when in a simulated infection condition in a thermal gradient. Therefore, thermoregulation in the field leading to fever requires a thermotactic behavioral drive and thermal opportunity, two conditions that are not necessarily independent. However, remaining sheltered and inactive (sickness behavior response) may be the best option to allocate energy to an immune response (Hart, 1988), decrease pathogen load, or reduce predation risk (Lefcort & Blaustein, 1995).

4.3 | Hydric variation in behavioral simulations across forests

The field conditions in this study included relatively stable temperatures, and thermoregulation did not strongly correlate with dehydration. Nevertheless, when limiting the analysis to just the warmest times recorded (1000–1600 h, hottest day), apathetic behavior models (AB) lost less water than the other models in the transitional forest. The AB models, like the other models, lost little water in the wet forest on the hottest day perhaps because physical characteristics that directly alter microclimates, such as tall vegetation, dense canopy, and high humidity, maintain low variation of microclimatic

conditions in the understory (Geiger, 1965; Hidore & Oliver, 1993) in contrast to transitional forest. Models in the transitional forest exhibited a lower percentage of dehydration relative to wet forests, perhaps due to increased overall temperature during the last two days of sampling in the wet forest.

Since an increase in environmental temperature can directly influence hydration, these anurans must decide how to increase body temperature or conserve water. In the laboratory, high intensity and sustained locomotor performance in anurans are compromised at lower hydration levels (Moore & Gatten, 1989; Titon & Ribeiro, 2017). However, verification of this compensation is challenging to document in the field. We expected that models with behavior that enhances temperature would lose more water than sickness behavior, where models were often left in moist locations. However, our results did not support this prediction, as there was no difference in water balance between the models. If we consider that this result from the agar models does, in fact, correspond to anurans living in the wild, we can conclude that in humid forests, such as the Atlantic Forest, where there is little thermal variation in microhabitats, ensuring hydro-regulation would be less of a constraint.

We may assume that behavioral thermoregulation by amphibians in the field may be compromised by the demands of water regulation relative to temperature regulation (Brattstrom, 1979). However, according to our results from the Parque Estadual Intervales (PEI), this balance between water storage and thermoregulation was not compromised for our behavioral simulations, and individuals might move across the two forests without the tradeoff of hydro-regulation (Rozen-Rechels et al., 2019). This is important since it may allow amphibians to move freely and allocate energy to feeding, reproduction, and growth (Lillywhite et al., 1973).

5 | CONCLUSIONS

We show that behavioral fever and apathy may not be fully compatible with hydro-thermoregulation, which might be explained by the narrow temperature gradients available in the field. This means that a feverish state could not be developed in a more thermally homogeneous forest. In this scenario, an apathy behavior is more likely since hydration would not be affected. A tropical forest, like the Atlantic Forest, ideally provides different microclimates with different thermoregulation options for amphibians. In the context of disease, these possibilities for different hydro thermoregulation options matter, as some amphibian species could produce behavioral fever, while others could exhibit sickness behavior. Thus, investigating how the thermal environment in nature can help in these choices of different microhabitats is very important to understand these behaviors, which have been studied primarily in the laboratory. In the laboratory, conditions are more regulated and often ignore the thermal heterogeneity that exists inside a forest. Some research shows that the choice of higher temperatures affects hydro-regulation, however this was not what we found in our study. Despite the knowledge generated

by these laboratory experiments, we need to understand amphibian habitat selection under more natural conditions. We hope that future studies that combine experiments using these two strategies (strong behavioral fever and apathy behavior) in both laboratory and field conditions can elucidate the relevance of these parameters, especially if infectious disease is relevant.

CONFLICT OF INTEREST

The corresponding author confirms on behalf of all authors that there have been no involvements that might raise the question of bias in the work reported or in the conclusions, implications, or opinions stated.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in the Dryad Digital Repository: https://doi.org/10.5061/dryad.t76hdr83v (Ortega-Chinchilla et al., 2022).

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