Journal of Thermal Biology 93 (2020) 102721

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: http://www.elsevier.com/locate/jtherbio

Effects of dehydration on thermoregulatory behavior and thermal tolerance limits of *Rana catesbeiana* (Shaw, 1802)

Estefany Caroline Guevara-Molina *, Fernando Ribeiro Gomes, Agustín Camacho

Laboratory of Behavior and Evolutionary Physiology, Department of Physiology, Institute of Biosciences, University of São Paulo, 05508-090, Brazil

A R T I C L E I N F O

Keywords:
Thermal tolerance
Invasive species
Integration
Hydration level
Anurans

A B S T R A C T

Predicting the effects of high environmental temperatures and drought on populations requires understanding how these conditions will influence the thermoregulatory behavior and thermal tolerance of organisms. Ectotherms show proportional (fine-tuned) and all-or-none (abrupt) responses to avoid overheating. Scattered evidence suggests that dehydration alters these behavioral responses and thermal tolerance, but these effects have not been evaluated in an integrative manner. We examined the effects of hydration level on the behavioral thermoregulation and behavioral and physiological thermal limits of the "bullfrog" (*Rana catesbeiana*), a well-studied and important invasive species. To examine the effects of dehydration on proportional responses, we compared the Preferred Body Temperatures (PBT) of frogs with restricted and unrestricted access to water. To assess the effect of dehydration on all-or-none responses, we measured and compared the Voluntary Thermal Maximum (VTMax) at different hydration levels (100%, 90%, 80% of body weight at complete hydration). Finally, to understand the effect of dehydration on physiological thermal tolerance, we measured the Critical Thermal Maximum (CTMax) of frogs at matched hydration levels. PBT, VTMax, and CTMax all decreased in response to higher dehydration levels. However, bullfrogs changed their PBT more than their VTMax or CTMax in response to dehydration. Moreover, some severely dehydrated individuals did not exhibit a VTMax response. We discuss the implications of our results in the context of plasticity of thermoregulatory responses and thermal limits, and its potential application to mechanistic modeling.

1. Introduction

Global warming is causing increased temperatures and droughts across many regions of the world (Barnett et al., 2005; Bates et al., 2008). These variable and stressful climatic conditions may have important consequences on the geographical distribution, behavior, and physiological functions of animals, resulting in pervasive consequences on their life history (Malcolm et al., 2006; Post et al., 2008; Tewksbury et al., 2008; Ceballos et al., 2015). Most ectothermic animals exhibit relatively low thermal insulation and small body size, making them intrinsically less protected against changes in environmental temperatures. Also, wet skinned ectotherms such as anurans are particularly susceptible to dehydration since they exhibit high rates of evaporative water loss (Wygoda, 1984; Lillywhite, 2006). Nonetheless, amphibians exploit suitable microhabitats so as to maintain adequate thermal and water balance even at very hot and arid environments (Wygoda, 1984; Buttemer and Thomas, 2003; Tracy and Christian, 2005; Young et al., 2005; Cartledge et al., 2006; Tracy et al., 2014).

Research on how animals' thermoregulatory behavior and thermal limits respond to stressful climatic conditions (e.g. high environmental temperatures, low water availability) may help us better understand how species will adapt to changing environments (Williams et al., 2008). Ectotherms can fine-tune their body temperature using thermoregulatory behaviors by changing their body posture, basking positions, and microhabitats they inhabit (Heath, 1970; Lillywhite, 1970, 1971; Brattstrom, 1979; Nelson et al., 1984). These precise adjustments allow amphibians and other taxa to keep their body temperatures within a range of preferred body temperatures (i.e. PBT) that optimizes multiple physiological functions (Licht, 1965; Heath, 1970; Hertz et al., 1993; Angilletta et al., 2002; Tracy et al., 2010), including locomotor performance (Navas et al., 1999; Deere and Chown, 2006; Köhler et al., 2011; Mitchell and Bergmann, 2016), feeding rates and digestive efficiency (Wang et al., 2002; McConnachie and Alexander, 2004; Fontaine et al., 2018), rates of development and growth (Berger et al., 2011), and

Abbreviations: PBT, Preferred Body Temperatures; VTMax, Voluntary Thermal Maximum; CTMax, Critical Thermal Maximum.

* Corresponding author.
E-mail addresses: scarolinemolina@usp.br (E.C. Guevara-Molina), fribeirogomes@gmail.com (F.R. Gomes), agus.camacho@gmail.com (A. Camacho).

https://doi.org/10.1016/j.jtherbio.2020.102721
Received 16 February 2020; Received in revised form 11 August 2020; Accepted 1 September 2020
Available online 8 September 2020

reproduction (Navas and Bevier, 2001; Symes et al., 2017). However, if environmental temperatures increase and elevate body temperatures above the animals' preferred body temperatures, they will often move away abruptly to seek shelter from the heat source or pant (e.g. Cowles and Bogert, 1944; Heath, 1970). Thus, experimentally, this Voluntary Thermal Maximum (VTMax) can be measured as the body temperature that induces an individual to move away from a heating device (e.g. Camacho et al., 2018, see methods). However, this variable has rarely, if ever, been evaluated in anurans. If behavioral thermoregulation responses (i.e. moving to maintain body temperature around PBT or below VTMax) are not effective, individuals may reach their physiological thermal tolerance, typically represented by their Critical Thermal Maximum (CTMax, Cowles and Bogert, 1944). At this temperature, locomotor function is disrupted, and animals die from heat shock (Cowles and Bogert, 1944; Rezende et al., 2014).

Higher environmental temperatures also lead to higher rates of evaporative water loss, which together dehydrate and impair the performance of many organisms, including anurans (Moore and Gatten, 1989; Preest and Pough, 1989; Plummer et al., 2003). Anurans show dynamic changes in their hydration level, swiftly losing body water and rehydrating, or rapidly cooling down through bodily water evaporation (Wolcott and Wolcott, 2001; Prates and Navas, 2009; Tracy et al., 2010; Anderson et al., 2017). However, if access to water is limited, dehydration is inevitable (Tracy et al., 2014). Dehydration may lead to lower rates of bodily water loss (Anderson et al., 2017), decrease in cutaneous gas exchange (Burggren and Vitalis, 2005), drying and stiffening of the integument, and a reduced efficiency of cooling by evaporation (Lillywhite, 1971). Thus, both thermoregulatory traits (e.g. PBT, VTMax) and thermal tolerance (CTMax) may be altered by dehydration (Mitchell and Bergmann, 2016; Anderson and Andrade, 2017). In addition, other aspects of experimental measurements may affect these traits, and thus need to be accounted for during studies of thermal tolerance. For example, the PBT and CTMax of different ectotherms are sensitive to heating rate, initial body temperature (Lutterschmidt and Hutchison, 1997; Terblanche and Chown, 2007), and body mass (Ribeiro et al., 2012). Since the PBT, VTMax, and CTMax are key parameters of mechanistic models of species distribution (NicheMapper, Nowakowski et al., 2017), understanding the principles governing these parameters should provide the basis for more realistic models of climatic restrictions for anurans. Thus, there is a need for comprehensive studies assessing how behavioral thermoregulation (i.e. fine-tuned and all-or-none responses) and physiological thermal limits interact with dehydration, while accounting for methodological factors.

To conduct such a study, we examined the American bullfrog, *Rana catesbeiana,* which is an easily obtained and locally abundant species. Bullfrogs are generally found in deep, permanent bodies of water (Wright and Wright, 1949; Fuller et al., 2011), but juveniles may occupy temporary ponds and are seasonally affected by hot temperatures and drought. This species is widely distributed across North America (Both et al., 2011; Quiroga et al., 2015) and has been widely introduced around the world (Jennings and Hayes, 1985). Here, we test if dehydration lowers the PBT, VTMax, and CTMax of *R. catesbeiana* while controlling for other factors (such as, heating rate, start body temperature, body mass).

2. Materials and methods

2.1. Collection and maintenance of individuals

Between June and November 2017, we commercially obtained 128 juvenile individuals of *Rana catesbeiana* from the Santa Clara Frog Pond (Santa Isabel municipality, São Paulo, Brazil). Frogs were kept in the vivarium of the Physiology Department of the Institute of Biosciences, University of São Paulo, Brazil. Individuals were kept in plastic containers that was 19 cm high by 33 cm long for 2–3 days before taking their measurements. All frogs had access to water, shelter, and were exposed to a photoperiod of 13 h of light and 11 h of darkness (13L: 11D). The temperature of the vivarium ranged between 21 and 24 °C, similar to temperatures at the pond where animals were obtained. For all experiments, body temperature was registered every 10 s by attaching a thin T-type thermocouple (model 5SRTC/1 mm in diameter, omega ®) to the groin of each individual with surgical tape. We initially placed thermocouples in the frogs' cloaca. However, the instruments were easily displaced, so we compared temperatures taken from the cloaca and groin and found that they both varied in the same way as a function of time (See Supplementary S1). Thus, we report the values taken from the groin in our analyses. The thermocouples were factory calibrated and connected through a FieldLogger PicoLog TC–08 to a computer. All the experiments were conducted in an acclimatized room under controlled temperature and relative humidity conditions (mean: 18.5 °C, 67.1%, N = 34). Since American bullfrog juveniles exhibit extensive diurnal/crepuscular activity (Stebbins, 2003), we made all thermal measurements during the day, when behavioral thermoregulation was easier to measure due to the existence of stronger thermal gradients in nature (Geiger, 1965). Each individual was measured using different thermal indices in order to avoid residual effects of previous experiments and cross contamination. Animals were fed cockroaches immediately after the experiments and were euthanized two days after measurements were taken by decapitation following sedation of individuals with a solution of Benzocaine, 0.1g/L. Individuals who died 24 h after the experiments were not included in the analyses. The ethics committee of the Biosciences Institute at the University of São Paulo approved all procedures for animal handling and euthanasia (CEUA N° 289/2017).

2.2. Hydration levels of individuals

To hydrate bullfrogs, we placed them in a small container with water *ad libitum* for 1 h, right before the start of the experiment. Then, we emptied the bladder of each individual by slightly pressing its pelvic waist to expel the urine. We then weighed them to obtain their 100% hydrated body mass. All individuals were 100% hydrated before measuring PBT. However, before the measurement of VTMax and CTMax, we separated frogs into three groups of 15 individuals, corresponding to different hydration levels (100%, 90%, and 80% of their fully hydrated weight). To obtain frogs at 90% and 80% hydration levels, we placed fully hydrated, previously weighed frogs inside mesh bags in front of a fan, and weighed them every 5–10 min until obtaining the desired hydration level (e.g. Titon and Gomes, 2017).

2.3. PBT measurements

Four artificial gradients were constructed with rectangular plastic containers (19 cm width by 60 cm long). The gradients had an acrylic lid with a thin opening in the middle 1 cm in diameter. This opening was not wide enough to cause thermal variation across the gradient but it prevented condensation of water in the gradients. The thin opening of the acrylic lid allowed for the passage of the thermocouple wire and for the displacement of the individuals without affecting the recording of measurements. A 1 mm thick aluminum sheet 14 cm wide by 56 cm long was placed on the lower part of each container. Foam paper on top of the aluminum sheet helped absorb circulating moisture. This aluminum sheet was heated from below at one end with a 60 W incandescent bulb and cooled with frozen gel bags at the other end. To corroborate that the gradients offered a sufficient range of temperatures for individuals, we estimated those temperatures by placing eight gypsum models imitating the shape and size of the frogs within each thermal gradient. The models were separated from one another by a distance of 6–7 cm and were distributed along the gradient. Each model had a T-type thermocouple attached to it to record the temperatures along the gradients. The temperature of each model within each gradient was recorded every 10 s for 90 min between 10:30–12:00 h, rendering an average temperature of

20 °C (sd: 10, range: 10.38–42.32 °C; 4320 records) (See Supplementary S2).

To assess the effect of dehydration, time, and access to water on the PBT of bullfrogs, we created two experimental groups: The control group (CG) had unrestricted access to water while the water-restricted group (WRG) did not have access to water inside the gradient during measurements. For the CG, 14 petri dishes 6 cm in diameter were filled with water at room temperature ensuring constant access to water (Fig. 1A). Every day for two weeks in November 2017, we measured the PBT in groups of four individuals per day (two in CG, two in WRG), totaling in 32 individuals altogether (16 in the CG and 16 in the WRG). All experiments began between 10:00–11:00 h. After the lamps of the four gradients were switched-on and the frozen bags were located, we placed each individual on each gradient and started the recording of their body temperatures. At that time, individuals began to explore the gradient before choosing a location. Since thermal gradients took 20 min to stabilize, we did not include the body temperature data recorded within the first 20 min of the experiment. Once the recording of body temperatures began, the body mass of each individual was also recorded every 30 min. To do this, the recording of body temperatures was stopped, and each individual was weighed (without removing the thermocouple) on a previously calibrated balance that was located next to the gradients. Once each individual was weighed, it was placed back in the middle of its respective gradient. Again, individuals began to explore the gradient before choosing a location. We waited 5 min before resuming the recording of their body temperatures to avoid data affected by handling. Whenever one of the WRG individuals reached 80% of their initial hydration level, all of the individuals from both experimental groups had their body masses recorded for the last time and the experiment ended. Fully hydrated CG and WRG individuals had similar initial body masses (CG: mean: 14.28 g, sd: 3.89, range: 7.99–22.79 g, N = 16; WRG: mean: 13.56 g, sd: 3.54, range: 8.58–23.51 g, N = 16).

Later, for each body mass measurement, we calculated the average of body temperatures recorded 5 min before each body mass measurement. Thus, for each individual, we could associate a value of hydration level

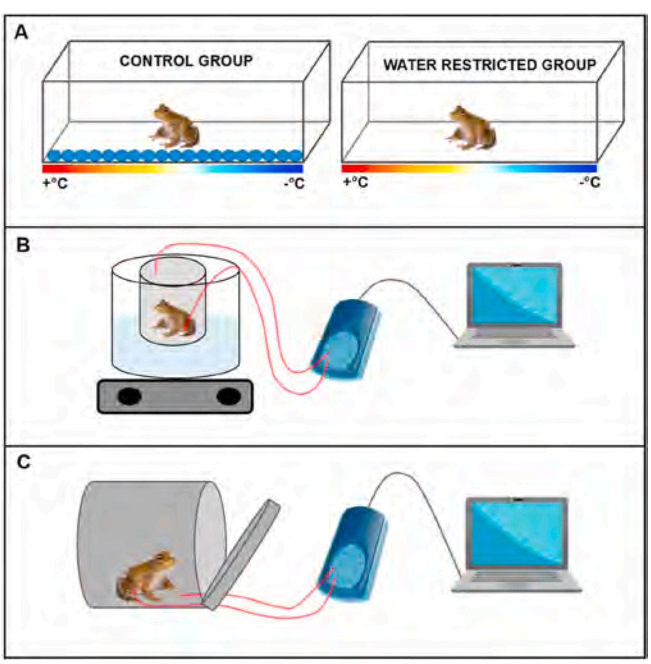

Fig. 1. Scheme of the machinery used for thermoregulatory behavior measurements and thermal limits in *Rana catesbeiana*.
(A) Thermal gradients used for measuring the PBT. (B) A thermal bath for CTMax measurement method. (C) A can-system for measuring the VTMax. The range of temperatures offered by the thermal gradients was between 10.38 and 42.32 °C (mean = 20 °C; sd: 10; 4320 records).

with the respective body temperature. That procedure rendered several (3–4) repeated measures per individual that we used to test the effect of hydration on the preferred body temperature.

2.4. CTMax measurements

The effect of dehydration on CTMax was assessed in July 2017. The mean initial standard body mass of all individuals before applying hydration conditions was 21.36 g (sd: 5.21, range: 12.06–29.11 g, N = 15) for the 100% hydrated group; 19.10 g (sd: 2.75, range: 12.06–29.11 g, N = 15) for the 90% hydrated group, and 37.64 g (sd: 10.49, range: 15.99–54.49 g, N = 15) for the 80% hydrated group.

For this experiment, frogs were heated inside an aluminum container covered with an acrylic lid within a thermal bath. A T-type thermocouple was placed inside the aluminum container to register surface temperature and sample the heating rate of individuals (0.41 °C/min, Fig. 1B). The heating rate was controlled with a dimmer connected to the power source. The average initial body temperature of individuals was 20 °C (sd: 1.87, range: 17.21–23.80 °C, N = 45) and the aluminum container was 19.39 °C (sd: 2.17, range: 14.38–22.50 °C, N = 45). All experiments began between 10:00–16:00 h. Each individual was heated in the thermal bath until it attempted to escape. Thereafter, the frog was turned belly up using forceps to check for its righting response. This procedure was repeated every 30 s until the individual lost the righting response by showing tremor in their legs, panting, exhaustion, red legs, or a combination of any of these responses. At that time, the individual's body temperature was recorded and considered its CTMax, and it was immediately weighed and cooled off in water at room temperature.

2.5. VTMax measurements

We measured the VTMax in another 15 individuals per hydration level in August 2017. The mean initial standard body mass of frogs was 21.31 g (sd: 6.10, range: 13.67–33.56 g, N = 15) for the 100% hydrated group; 8.61 g (sd: 1.28, range: 6.62–11.77 g, N = 15) for the 90% hydrated group; and 13.00 g (sd: 1.72, range: 10.08–15.42 g, N = 15) for the 80% hydrated group. All experiments began between 10:00–16:00 h. All individuals were independently heated within a metallic cylindrical container, wrapped in a thermal resistance for homogeneous heating (Fig. 1C). A T-type thermocouple was placed inside the container and adhered to the surface to register temperature and sample the heating rate of individuals (0.30 °C/min, Fig. 1C). The heating rate was again controlled with a dimmer connected to the power source. The container had a half-opened, easily movable plastic lid so that the individual could exit the box at will (Fig. 1C). The average initial body temperature of individuals was 20.03 °C (sd: 1.38, range: 17.31–23.47 °C, N = 37) and the metallic cylindrical container was at 21.19 °C (sd: 1.16, range: 19.50–24.42 °C, N = 37). When each frog left the box, its body temperature was recorded and this was considered its VTMax. We also measured its final body mass and took it to a container with water at room temperature to recover. The interior of the heating container provided a refuge for frogs during measurements. Thus, it is safe to assume that for the typical duration of the experiments, frogs remained in their containers, until they were forced to leave (e.g. increasing the temperature inside the container).

During the measurements of CTMax and VTMax, we calculated the rates of bodily water loss as the difference between initial weight (in each hydration level) and final weight divided by the duration of the test for each individual to see if initial hydration level affected their body water loss rates.

2.6. Statistical analysis

We fitted linear mixed-effects models in R (Vr. 3.5.0 R Core Team, 2018; lme4 package, "lmer" function; Bates et al., 2015) to test whether hydration level (measured every 30 min), group (CG, WRG), and time (fixed factors) affected the PBT of bullfrogs. We included the identity of individuals as the only random effect in the model and compared Akaike information criterions (AIC) of five models including and excluding fixed effects and their interactions. Differences in AIC over three units were considered statistically significant (Wang and Qun, 2006). Because we did not find significant differences between the body masses of the individuals of the CG and WRG ($p = 0.589$, $df = 30$, t–test = 0.546), our models did not include body mass.

During the analyses of CTMax and VTMax, we compared generalized least squares models including initial body mass, heating rate, and initial body temperature with models not including them (i.e. hydration level only). In some instances, AIC scores did not allow for clear distinction among competing models, so we applied a model averaging procedure (Symonds and Moussalli, 2010) using the MuMIn package in R (Barton, 2016) to estimate the effects of hydration state accounting for these factors. This approach allows a formal estimation of dependent variable values, integrating the effects of the factors included in the most plausible models. Finally, the effect of hydration level on water loss rates during CTMax and VTMax assays was tested using a one-way ANOVA test. The resulting plots were made in SigmaPlot Vr. 11.0.

3. Results

3.1. Effects of dehydration on PBT

The average PBT for the CG was 28.51 °C (sd: 0.42, range: 17.59–36.47 °C, N = 16), whereas the average PBT of the WRG was 22.69 °C (sd: 0.42, range: 14.83–33.66 °C, N = 16) (Fig. 2A). For each frog, the PBT measurement period lasted for approximately 1 h and 40 min. All individuals survived 24 h after experiments.

The five constructed models showed that model V had the lowest AIC value with a minimum of 133 units of difference with the other models, and reflects an interaction between time in the gradient, hydration level and experimental group (CG and WRG) in the thermoregulatory behavior of *R. catesbeiana* (Table 1, Supplementary S3). CG animals maintained higher temperatures and hydration levels for longer periods than water restricted animals (Fig. 2A and B).

3.2. Effects of dehydration on CTMax

The average CTMax was 36.82 °C for 100% hydrated individuals (sd: 0.77, range: 35.60–38.95 °C, N = 15), 35.50 °C for 90% hydrated individuals (sd: 0.80, range: 34.11–37.14 °C, N = 15), and 34.63 °C for 80% hydrated individuals (sd: 0.41, range: 34.01–35.46 °C, N = 15). Our model selection approach showed models including different combinations of the factors (hydration level, heating rate, start body temperature and initial body mass) predicted CTMax better than the intercept (See Supplementary S4). Because the best three models (with the lowest AIC) had differences below three AIC units among them, we applied model averaging to these three models to estimate the effects of hydration levels and the other factors. The average of the three best models (See Supplementary S4) showed that CTMax was affected by hydration level and the heating rate of individuals, but not by the start body temperature and body mass (Fig. 3A and B; Table 2). These models show an average increase of 0.07° in CTMax per every 1% in hydration level. Heating rate had a much larger effect (1.3° per 1% increase). We also found significant effects of hydration level on rates of water loss during CTMax experiments. Hydrated individuals lost water faster than

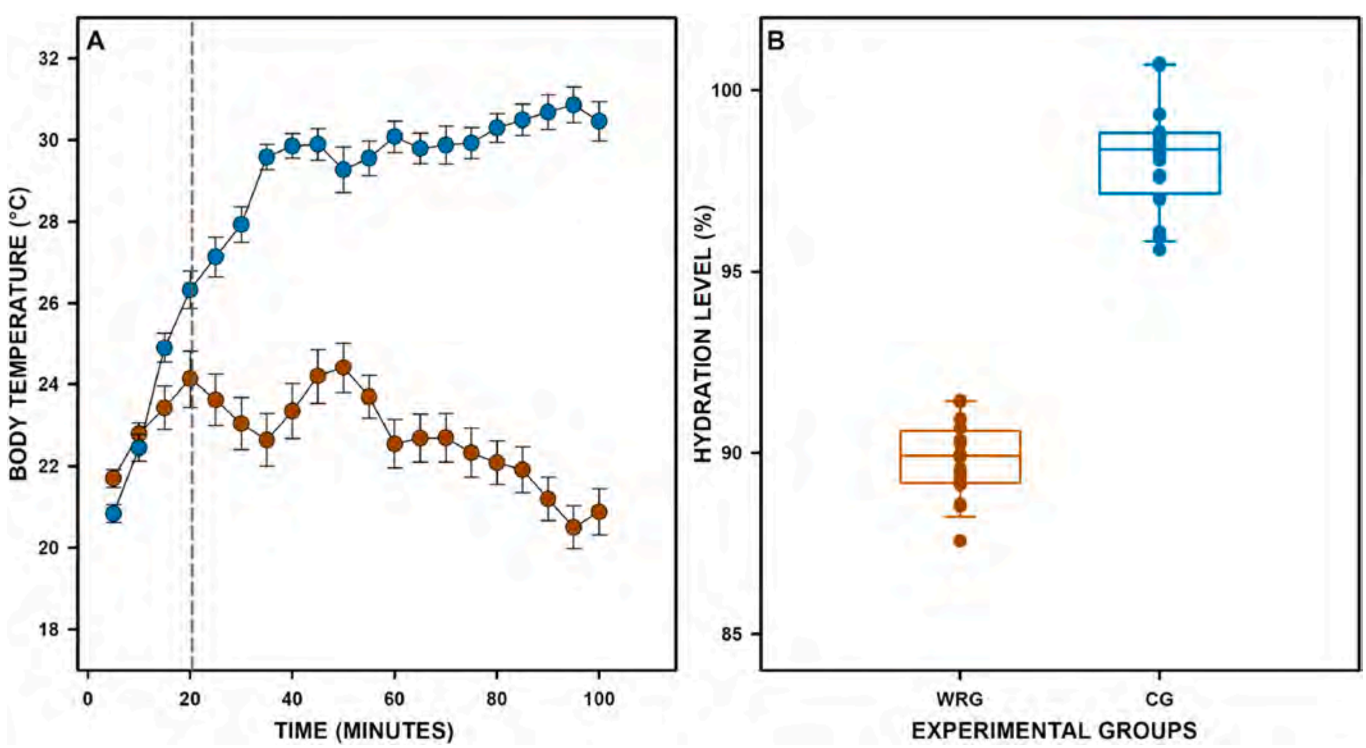

Fig. 2. Body temperatures of *Rana catesbeiana* kept in thermal gradients under water restricted (WRG, 16 individuals) and control (CG, 16 individuals) conditions.
(A) Dots represent the average body temperature every 5 min
for all individuals within experimental groups, and bars indicate standard error of data. Blue dots are for control group and brown dots are for water restricted group. Both experimental groups started with similar body temperatures but, as the time passes, WRG individuals started exhibiting lower body temperatures. The data shown before the dotted line correspond to temperatures selected by the individuals before the thermal gradients had already stabilized and that were not used in the analyses. (B) shows an averaged hydration level per individual (i.e. a single dot for each one), resulting from each body mass recorded every 30 min
(i.e. around 3–4 body mass recording per individual) during the measurements of PBT. Blue box and dots are for control group and brown box and dots are for water restricted group. Whiskers represent standard deviation of data.

Table 1
Effect of time, hydration level and group (WRG, CG) on the preferred body temperatures of *Rana catesbeiana*.
Model V was chosen as the best explanatory model for having the lowest AIC value = 1077.889 and 10 degrees of freedom. More detailed results are shown in Supplementary S3.

Model	Variable	Value	Std.Error	t-value	p-value	df	AIC-value
I	Intercept	25.176	0.561	44.88	<0.001	3	1268.539
II	Intercept	19.82771	5.30915	3.735	0.0002	4	1269.768
	Hydration level	0.05632	0.0556	1.013	0.3121		
III	Intercept	28.0924	0.3184	88.24	<0.001	4	1210.192
	Group	-5.9433	0.4605	-12.9	<0.001		
IV	Intercept	2.3	0.6292	36.547	<0.001	4	1223.714
	Time	2.275	0.003271	7.262	<0.001		
V	Intercept	2.278	3.378	6.744	0.0754	10	1077.889
	Hydration level	-2.054	0.399	-6.042	0.6818		
	Group	-1.992	3.736	-5.331	<0.001		
	Time	-1.11	0.1936	-5.734	0.0774		
	Hydration level * Group	1.987	0.3761	5.284	<0.001		
	Hydration level * Time	1.16	0.00195	5.947	0.0956		
	Group * Time	0.9857	0.2059	4.788	<0.001		
	Hydration level * Group *Time	-0.01023	0.002115	-4.838	<0.001		

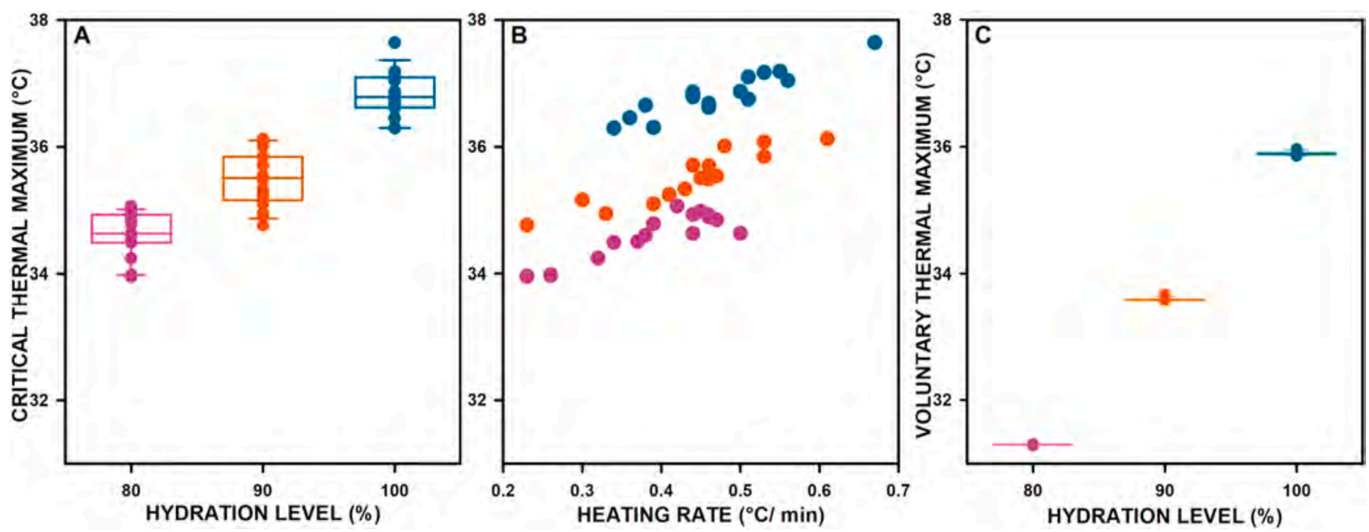

Fig. 3. Factors influencing the CTMax and VTMax of *Rana catesbeiana* at three different hydration levels (N = 15 individual per hydration level except for VTMax at hydration level 80%, N = 7).
(A) Shows the relationship between the three hydration levels with the CTMax. (B) Shows the relationship between the heating rate and CTMax of individuals at different hydration levels. While CTMax changed associated to hydration level (Panel A), it was also susceptible to variations in heating rate (Panel B). (C) Shows the relationship between the three hydration levels with the VTMax. Lines within each box represent the median, whiskers represent standard deviation of data and dots represent each individual. Colors represent hydration state (blue: 100%, orange: 90%, and purple 80%).

Table 2
Results of model averaging for best models explaining the CTMax of *Rana catesbeiana*.
Heating rate has the strongest effect on CTMax followed by hydration level. Start temperature and body mass did not show discernible effects. More detailed results are shown in Supplementary S4.

Variable	Value	Std. Error	Adjusted SE	z-value	p-value
Intercept	30.4768	2.0203	2.0563	14.821	<0.0000
Hydration level 90%	0.7486	0.2911	0.2986	2.506	0.0122
Hydration level 100%	1.454	0.4231	0.4314	3.37	0.00075
Heating rate	4.6252	1.197	1.2302	3.76	0.00017
Start body temperature	0.126	0.0934	0.0949	1.327	0.18463
Initial body mass	-0.0042	0.0099	0.0101	0.423	0.67198

dehydrated ones (F (2,42) = 20.91, $p < 0.001$). The mean duration of each assay was 35 min (sd: 8.76, range: 26–63 min, N = 45), and all individuals survived 24 h after experiments.

3.3. Effects of dehydration on VTMax

Average VTMax was higher in fully hydrated frogs (35.89 °C, sd: 0.16, range: 34.56–36.77 °C, N = 15) than in 90% hydrated (33.60 °C, sd: 0.34, range: 30.21–35.06 °C, N = 15) and 80% hydrated ones (31.29 °C, sd: 0.43, range: 30.05–33.11 °C, N = 7). Three models predicted VTMax better than the intercept but showed nonsignificant differences in AIC among them (See Supplementary S5). They included different combinations of factors (hydration level, heating rate and start body temperature). The averaged model found strong effects of dehydration on the VTMax but not of the heating rate or the start body temperature (Fig. 3C; Table 3). These models show an average increment of 0.21° in VTMax per every 1% increase in hydration level. In addition, hydrated individuals also showed higher water loss rates than

Table 3
Results of model averaging, showing the effects of factors affecting the VTMax of *Rana catesbeiana*.
Hydration level is the single most important factor. Heating rate and start body temperature did not show discernible effects. More detailed results can be consulted in Supplementary S5.

Variable	Value	Std. Error	Adjusted SE	z-value	p-value
Intercept	31.5551	1.7814	1.8272	17.27	<0.0000
Hydration level 90%	2.2653	0.5187	0.5372	4.216	<0.0000
Hydration level 100%	4.5984	0.4971	0.5155	8.92	<0.0000
Heating rate	0.1372	0.7454	0.7711	0.178	0.859
Start body temperature	-0.014	0.0802	0.0822	0.171	0.854

dehydrated ones ($F (2,34) = 16.13$, $p < 0.001$). The mean duration of assays was 41 min (sd: 10.14, range: 18–68 min, N = 37). Eight individuals in the 80% hydration group failed to exit the container and died. All the other individuals survived the 24 h observation period after the experiments.

4. Discussion

Our study aimed to understand how anurans integrate behavioral responses and thermal limits during dehydration. Bullfrogs adjusted their position in the gradient in order to maintain their body temperature within the range of their PBT. Further, they proportionally adjusted their position in the gradient in response to their hydration level, lowering their PBT as they dehydrated. For a fully hydrated wet-skinned ectotherm, maintaining high body temperature below the VTMax benefits several processes such as growth in molluscs (Díaz et al., 1996, 2000, 2011) and locomotion in anurans (Moore and Gatten, 1989; Anderson and Andrade, 2017). In turn, dehydration impairs thermal tolerance (Anderson and Andrade, 2017; this study), survival (Beuchat et al., 1984), locomotor performance (Moore and Gatten, 1989; Tingley et al., 2012; Mitchell and Bergmann, 2016), and lowers the optimal temperature for locomotion (Beuchat et al., 1984; Preest and Pough, 1989; Titon et al., 2010; Titon and Gomes, 2015, 2017). In this way, choosing lower temperatures may bring dehydrated animals closer to their lowered thermal optima. Moreover, selection of lower temperatures reduces rates of evaporative water loss (Mitchell and Bergmann, 2016; Anderson and Andrade, 2017) and thus increases the available time to find a water source before severe dehydration risks occur.

We discovered that frogs may respond to dehydration by decreasing their VTMax and that severely dehydrated individuals may still lose this response, despite being able to escape heating sources. These observations highlight the dangers of experiencing a combination of high temperatures and low hydration levels for anurans. Many wild anuran populations maintain hydration levels above 90% even in the dry season (Tracy et al., 2014). However, severely dehydrated anurans in the field may be underreported, particularly in less abundant populations, close to their climatic limits in distribution (Jessop et al., 2013). High temperatures and low water availability have been hypothesized to explain the absence of dehydration prone species in isolated forest fragments (e.g. Watling and Braga, 2015) and might impose limits to species distribution (Schwarzkopf and Alford, 2002; Brown et al., 2011; Florance et al., 2011; Tingley and Shine, 2011; Letnic et al., 2015; Titon and Gomes, 2017). Interestingly, the change of heating rates we found (0.17–0.87 °C/min) did not affect the VTMax in our study, suggesting that slow heating rates do not impair behavioral thermoregulation, as widely believed (Goldstein, 2000; Gibbons, 2002). VTMax values were consistent across heating rates and always below CTMax values. This suggests that frogs were exiting the container due to a thermal level perceived as stressful and that the CTMax was induced by temperature, rather than a response to handling. On the other hand, the heating of frogs at different hydration states may have changed relative humidity across treatments. The lid was half open on the upper side during the VTMax trials, allowing for continuous gas exchange between the room and the container, which likely minimized potential differences in humidity among treatments. However, since we did not monitor relative humidity during warming, we encourage further studies addressing the effects of relative humidity on the VTMax of frogs and other animals to investigate this more closely.

Our study highlights the importance of studying the effects of dehydration on both behavioral responses and thermal limits. In American bullfrogs, dehydration lowered the PBT more than the VTMax, and the latter more than the CTMax. Interestingly, the magnitude of PBT decrease almost doubled the response of VTMax (0.44 °C decrease in PBT per 1% of standard body mass lost, vs. 0.23 for VTMax), and both were between 3 and 6 times larger than the decrease in CTMax (and 0.07 per 1% of standard body mass lost, after correcting for heating rate). These different magnitudes make sense in the light of previous literature and our observations. When dehydrated, risks for frogs at high temperatures may multiply. Dehydration impairs locomotor performance and lowers thermal optima for locomotion, potentially decreasing their abilities to find a refuge, capture prey, or avoid predators at high temperatures (e.g. Beuchat et al., 1984; Preest and Pough, 1989; Titon et al., 2010; Titon and Gomes, 2015, 2017). Frogs might also lose the perception of thermal risk. Our study demonstrates for the first time that severely dehydrated frogs may not exhibit a VTMax, despite being able to move at such reduced hydration levels. While not leaving the container may well be interpreted as failure to thermoregulate, it makes sense for the survival of an individual sheltered within a hydrothermal refuge (i.e. under a stone in a dry pond), whose chances of survival may decrease with any increment in activity. By adjusting their PBT and VTMax more intensely than their CTMax, bullfrogs may increase their thermal safety margin when dehydrated.

These integrative and adaptive responses might also be relevant in other ectotherms that face the double jeopardy of low water availability and high environmental temperatures. While this might not often be the case for adult *R. catesbeiana*, since they inhabit permanent bodies of water, it may be for juveniles that experience water shortages when using temporary ponds (Bury and Whelan, 1984). Severe dehydration in this species (i.e. hydration levels ≤80%) makes the integument drier and stiffer, obstructing both cutaneous respiration and the evaporation of water in response to short increases in body temperature (Lillywhite, 1971; Tracy, 1976; Beuchat et al., 1984; this study). Dehydration also affects thermoregulatory behavior and thermal tolerance of more terrestrial anurans. Interestingly, toads (*Rhinella diptycha*) also lowered their PBT more than their CTMax in response to dehydration, but with less difference among them (0.13 °C/1% and 0.06 °C/1% of standard body mass lost, respectively; Anderson and Andrade, 2017). Unfortunately, the effects of hydration on thermoregulation behavior and tolerance are poorly studied and not well understood. Previous studies have made use of different methodologies and focused on the effects of other factors on thermal tolerance (e.g. acclimation, heating rate, initial temperature of individuals; Shoemaker et al., 1989; Dupré and Crawford, 1985; Crowley, 1985; Ladyman and Bradshaw, 2003; Plummer et al., 2003; Mitchell and Bergmann, 2016). Thus, we recommend further comparative and integrative studies on the interactive effects of thermoregulation, thermal tolerance, and hydration level. A better understanding of the neural bases that integrate thermoregulatory behavior and hydration levels in anurans may also help in better understanding how anurans will respond to changing climates. For example, parapineal organs in frogs respond to dehydration (Steyn, 1966). In order to understand how the integration of dehydration and body temperatures occurs, future studies might apply our protocols to measure thermoregulation under different hydration levels and hormonal or electric manipulation of these neural centers.

Our observations of different thermoregulatory responses to dehydration are also relevant for mechanistic modeling techniques, like the NicheMapper (e.g. Kearney et al., 2008; Kearney and Porter, 2009; Bartelt et al., 2010; Nowakowski et al., 2017; Oyamaguchi et al., 2018). These models use the PBT, VTMax, and CTMax of organisms to estimate water loss. However, they assume that these parameters remain constant and are independent of hydration level or heating rates. As shown before, these different responses seem to be displayed by anurans from very different habitats (semiaquatic frog and terrestrial toad). Thus, we highlight the need to couple thermoregulation, thermal tolerance, dehydration, and heating rates in mechanistic models of activity and physiological performance. Nonetheless, we acknowledge that for the specific case of American bullfrogs, dehydration might only be relevant for juveniles, which more often use shallow waters and temporary ponds (Wright and Wright, 1949).

In conclusion, we showed that the PBT, VTMax, and CTMax of American bullfrogs may change across different hydration levels. These dynamic responses should be applied to mechanistic models of activity and physiological performance to provide more realistic predictions of climatic restrictions on activity and distribution of anuran species. The macroevolutionary patterns and neurological processes that trigger and regulate these plastic responses remain unknown and warrant further investigation.

Author contributions

Estefany Caroline Guevara Molina: Conceptualization; Methodology; Validation; Formal analysis; Visualization, Investigation; Writing-Original draft preparation; Writing- Reviewing and Editing; Project administration; Funding acquisition. **Agustín Camacho Guerrero:** Conceptualization; Methodology; Investigation; Validation; Visualization; Investigation; Formal analysis; Resources; Writing-Reviewing and Editing; Supervision; Funding acquisition. **Fernando Ribeiro Gomes:** Conceptualization; Investigation; Validation; Visualization; Investigation; Writing- Reviewing and Editing; Supervision; Funding acquisition.

Funding

This work was supported by a research grant from FAPESP (Process n° 2014/16320-7). ECGM and ACG were funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brasil (CAPES)- Finance Code 001. Finally, ECGM was supported for the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (Process n° 2017/14382-3). FRG is a research fellow from the Brazilian CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico from Brazil - #302308/2016–4).

Declaration of competing interest

No competing interests are declared by the authors.

Acknowledgments

The authors thank the members of the Laboratório de Comportamento e Fisiologia Evolutiva (LACOFIE) of the Department of Physiology, Institute of Biosciences (IB), University of São Paulo (USP), for their comments and contributions in this study. Likewise, we thank Juan C. Díaz-Ricaurte and Carla Bonetti Madelaire for their collaboration in conducting experiments and maintaining animals in captivity. We also thank Braz Titon for his suggestions about statistical analysis and Emma Kimberly, Julie Jung and Brandon Güell for helping improve the grammar and writing on earlier versions of this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2020.102721.

References

Anderson, R.C., Andrade, D.V., 2017. Trading heat and hops for water: dehydration effects on locomotor performance, thermal limits, and thermoregulatory behavior of a terrestrial toad. Ecol. Evol. 7 (21), 9066–9075. https://doi.org/10.1002/ece3.3219.

Anderson, R.C., Bovo, R.P., Eismann, C.E., Menegario, A.A., Andrade, D.V., 2017. Not good, but not all bad: dehydration effects on body fluids, organ masses, and water flux through the skin of *Rhinella schneideri* (Amphibia, Bufonidae). Physiol. Biochem. Zool. 90 (3), 313–320. https://doi.org/10.1086/690189.

Angilletta, M.J., Niewiarowski, P.H., Navas, C.A., 2002. The evolution of thermal physiology in ectotherms. J. Therm. Biol. 27, 249–268. https://doi.org/10.1016/S0306-4565(01)00094-8.

Bartelt, P.E., Klaver, R.W., Porter, W.P., 2010. Modeling amphibian energetics, habitat suitability, and movements of western toads, *Anaxyrus* (= *Bufo*) *boreas*, across present and future landscapes. Ecol. Model. 221, 2675–2686. https://doi.org/10.1016/j.ecolmodel.2010.07.009.

Barnett, T.P., Adama, J.C., Lettenmaier, D.P., 2005. Potential impacts of warming climate on water availability in snow-dominated regions. Nature 438, 303–309. https://doi.org/10.1038/nature04141.

Barton, K., 2016. MuMIn: multi-model inference. R package version 1.15.6. https://CRAN.R-project.org/package=MuMIn.

Bates, B.C., Kundzewicz, Z.W., Wu, S., Palutikof, J.P., 2008. Climate change and water. In: Technical Paper of the Intergovernmental Panel on Climate Change. IPCC Secretariat, Geneva.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Software 67, 1–48. https://doi:10.18637/jss.v067.i01.

Berger, D., Friberg, M., Gotthard, K., 2011. Divergence and ontogenetic coupling of larval behavior and thermal reaction norms in three closely related butterflies. Proc. R. Soc. Lond [Biol] 278, 313–320. https://doi.org/10.1098/rspb.2010.1398.

Beuchat, C.A., Pough, F.H., Stewart, M.M., 1984. Response to simultaneous dehydration and thermal stress in three species of Puerto Rican frogs. J. Comp. Physiol. B. 154, 579–585. https://doi.org/10.1007/BF00684411.

Both, C., Lingnau, R., Santos Jr., A., Madalozzo, B., Lima, L.P., Grant, T., 2011. Widespread occurrence of the american bullfrog, *Lithobates catesbeianus* (shaw, 1802) (Anura: ranidae), in Brazil. S. Am. J. Herpetol 6 (2), 127–134. https://doi.org/10.2994/057.006.0203.

Brattstrom, B.H., 1979. Amphibian temperature regulation studies in the field and laboratory. Am. Zool. 19, 345–356. https://doi.org/10.1093/icb/19.1.345.

Brown, G.P., Kelehear, C., Shine, R., 2011. Effects of seasonal aridity on the ecology and behaviour of invasive cane toads in the Australian wet–dry tropics. Funct. Ecol. 25, 1339–1347. https://doi.org/10.1111/j.1365-2435.2011.01888.x.

Burggren, W.W., Vitalis, T.Z., 2005. The interplay of cutaneous water loss, gas exchange and blood flow in the toad, *Bufo woodhousei*: adaptations in a terrestrially adapted amphibian. J. Exp. Biol. 208 (1), 105–112. https://doi:10.1242/jeb.01349.

Bury, R.B., Whelan, J.A., 1984. Ecology and Management of the Bullfrog. US Department of the Interior, Washington, DC. Resource Publication 155.

Buttemer, W.A., Thomas, C., 2003. Influence of temperature on evaporative water loss and cutaneous resistance to water vapour diffusion in the orange-thighed frog (*Litoria xanthomera*). Aust. J. Zool. 51, 111–118. https://doi.org/10.1071/ZO02057.

Camacho, A., Rusch, T., Ray, G., Telemeco, R.S., Rodrigues, M.T., Angilletta, M.J., 2018. Measuring behavioral thermal tolerance to address hot topics in ecology, evolution, and conservation. J. Therm. Biol. 73, 71–79. https://doi.org/10.1016/j.jtherbio.2018.01.009.

Cartledge, V.A., Withers, P.C., Thompson, G.G., McMaster, K.A., 2006. Water relations of the burrowing sandhill frog, *Arenophryne rotunda* (Myobatrachidae). J. Comp. Physiol. B. 176, 295–302. https://doi.org/10.1007/s00360-005-0051-x.

Ceballos, G., Ehrlich, P.R., Barnosky, A.D., García, A., Pringle, R.M., Palmer, T.M., 2015. Accelerated modern human–induced species losses: entering the sixth mass extinction. Sci. adv 1, e1400253. https://doi.org/10.1126/sciadv.1400253.

Cowles, R.B., Bogert, C.M., 1944. A preliminary study of the thermal requirements of desert reptiles. B. Am. Mus. Nat. History 83, 261–296.

Crowley, S.R., 1985. Thermal sensitivity of sprint-running in the lizard *Sceloporus undulatus*: support for a conservative view of thermal physiology. Oecologia 66, 219–225. https://doi.org/10.1007/BF00379858.

Deere, J.A., Chown, S.L., 2006. Testing the beneficial acclimation hypothesis and its alternatives for locomotor performance. Am. Nat. 168, 630–644. https://doi.org/10.1086/508026.

Díaz, F., Ramirez, F.B., Sevilla, B.B., Farfán, C., 1996. Behavioral thermoregulation of *Bulla gouldiana* (gastropoda: opisthobranchia: cephalaspidea). J. Therm. Biol. 21, 319–322.

Díaz, F., Portilla, M.A., Sierra, E., Aguilar, M., Araujo, A.D., 2000. Preferred temperature and critical thermal maxima of red abalone *Haliotis rufescens*. J. Therm. Biol. 25, 257–261. https://doi.org/10.1016/S0306-4565(99)00032-7.

Díaz, F., Salas, A., Re, A.D., Gonzalez, M., Reyes, I., 2011. Thermal preference and tolerance of *megastrea (lithopoma) undosa* (wood, 1828; gastropoda: Turbinidae). J. Therm. Biol. 36, 34–37. https://doi.org/10.1016/j.jtherbio.2010.10.004.

Dupré, R.K., Crawford Jr., E.C., 1985. Behavioral thermoregulation during dehydration and osmotic loading of the desert iguana. Physiol. Zool. 58, 357–363. https://doi.org/10.1086/physzool.58.4.30156010.

Florance, D., Webb, J.K., Dempster, T., Kearney, M.R., Worthing, A., Letnic, M., 2011. Excluding access to invasion hubs can contain the spread of an invasive vertebrate. Proc. Roy. Soc. Lond. B. 278, 2900–2908. https://doi.org/10.1098/rspb.2011.0032.

Fontaine, S.S., Novarro, A.J., Kohl, K.D., 2018. Environmental temperature alters the digestive performance and gut microbiota of a terrestrial amphibian. J. Exp. Biol. 221 (20), 1–7. https://doi.org/10.1242/jeb.187559.

Fuller, T.E., Pope, K.L., Ashton, D.T., Welsh Jr., H.H., 2011. Linking the distribution of an invasive amphibian (*Rana catesbeiana*) to habitat conditions in a managed river system in northern California. Rest. Ecol. 19 (201), 204–213. https://doi.org/10.1111/j.1526-100X.2010.00708.x.
Geiger, R., 1965. The Climate Near the Ground Harvard University Press, Massachusetts, Cambridge.
Gibbons, W., 2002. The Legend of the Boiling Frog Is Just a Legend. Ecoviews 2007.
Goldstein, L., 2000. How to boil a live frog. Analysis 60 (2), 170–178.
Heath, J.E., 1970. Behavioral regulation of body temperature in poikilotherms. Physiol. 13, 399.
Hertz, P.E., Huey, R.B., Stevenson, R.D., 1993. Evaluating temperature regulation by field-active ectotherms: the fallacy of the inappropriate question. Am. Nat. 142, 796–818. https://doi.org/10.1086/285573.
Jennings, M.R., Hayes, M.P., 1985. Pre-1900 overharvest of California red-legged frogs (*Rana aurora draytonii*): the inducement for bullfrog (*Rana catesbeiana*) introduction. Herpetologica 94–103.
Jessop, T.S., Letnic, M., Webb, J.K., Dempster, T., 2013. Adrenocortical stress responses influence an invasive vertebrate's fitness in an extreme environment. Proc. Royal Soc. B 280 (1768), 1444. https://doi.org/10.1098/rspb.2013.1444.
Kearney, M., Phillips, B.L., Tracy, C.R., Christian, K.A., Betts, G., Porter, W.P., 2008. Modelling species distributions without using species distributions: the cane toad in Australia under current and future climates. Ecography 31, 423–434. https://doi.org/10.1111/j.0906-7590.2008.05457.x.
Kearney, M., Porter, W.P., 2009. Mechanistic niche modelling: combining physiological and spatial data to predict species' ranges. Ecol. Lett. 12, 334–350. https://doi.org/10.1111/j.1461-0248.2008.01277.x.
Köhler, A., Sadowska, J., Olszewska, J., Trzeciak, P., Berger-Tal, O., Tracy, C.R., 2011. Staying warm or moist? Operative temperature and thermal preferences of common frogs (*Rana temporaria*), and effects on locomotion. J. Herpetol. 21 (1), 17–26.
Ladyman, M., Bradshaw, D., 2003. The influence of dehydration on the thermal preferences of the Western tiger snake, *Notechis scutatus*. J. Comp. Physiol. B. 173, 239–246. https://doi.org/10.1007/s00360-003-0328-x.
Letnic, M., Webb, J.K., Jessop, T.S., Dempster, T., 2015. Restricting access to invasion hubs enables sustained control of an invasive vertebrate. J. Appl. Ecol. 52, 341–347. https://doi.org/10.1111/1365-2664.12390.
Licht, P., 1965. The relation between preferred body temperatures and testicular heat sensitivity in lizards. Copeia 4, 428–436. https://doi.org/10.2307/1440991.
Lillywhite, H.B., 1970. Behavioral temperature regulation in the bullfrog, *Rana catesbeiana*. Copeia 1970 (1), 158–168. https://doi.org/10.2307/1441983.
Lillywhite, H.B., 1971. Thermal modulation of cutaneous mucus discharge as a determinant of evaporative water loss in the frog, *Rana catesbeiana*. Z. Vgl. Physiol. 73, 84–104. https://doi.org/10.1007/BF00297703.
Lillywhite, H.B., 2006. Water relations of tetrapod integument. J. Exp. Biol. 209 (2), 202–226. https://doi.org/10.1242/jeb.02007.
Lutterschmidt, W.I., Hutchison, V.H., 1997. The critical thermal maximum: data to support the onset of spasms as the definitive end point. Can. J. Zool. 75 (10), 1553–1560. https://doi.org/10.1139/z97-782.
Malcolm, J.R., Liu, C., Neilson, R.P., Hansen, L., Hannah, L.E.E., 2006. Global warming and extinctions of endemic species from biodiversity Hotspots. Conserv. Biol. 20, 538–548. https://doi.org/10.1111/j.1523-1739.2006.00364.x.
McConnachie, S., Alexander, G., 2004. The effect of temperature on digestive and assimilation efficiency, gut passage time and appetite in an ambush foraging lizard, *Cordylus melanotus melanotus*. J. Comp. Physiol. B. 174, 99–105. https://doi.org/10.1007/s00360-003-0393-1.
Mitchell, A., Bergmann, P.J., 2016. Thermal and moisture habitat preferences do not maximize jumping performance in frogs. Funct. Ecol. 30, 733–742. https://doi.org/10.1111/1365-2435.12535.
Moore, F.R., Gatten Jr., R.E., 1989. Locomotor performance of hydrated, dehydrated, and osmotically stressed anuran amphibians. Herpetologica 1, 101–110.
Navas, C.A., James, R.S., Wakeling, J.M., Kemp, K.M., Johnston, I.A., 1999. An integrative study of the temperature dependence of whole animal and muscle performance during jumping and swimming in the frog *Rana temporaria*. J. Comp. Physiol. B. 169, 588–596. https://doi.org/10.1007/s003600050259.
Navas, C.A., Bevier, C.R., 2001. Thermal dependency of calling performance in the eurythermic frog *Colostethus subpunctatus*. Herpetologica 3, 384–395.
Nelson, D.O., Heath, J.E., Prosser, C.L., 1984. Evolution of temperature regulatory mechanisms. Am. Zool. 24, 791–807. https://doi.org/10.1093/icb/24.3.791.
Nowakowski, A.J., Watling, J.I., Whitfield, S.M., Todd, B.D., Kurz, D.J., Donnelly, M.A., 2017. Tropical amphibians in shifting thermal landscapes under land-use and climate change. Conserv. Biol. 31, 96–105. https://doi.org/10.1111/cobi.12769.
Oyamaguchi, H.M., Vo, P., Grewal, K., Do, R., Erwin, E., Jeong, N., Tse, K., Chen, C., Miyake, M., Lin, A., Gridi-Papp, M., 2018. Thermal sensitivity of a Neotropical amphibian (*Engystomops pustulosus*) and its vulnerability to climate change. Biotropica 50, 326–337. https://doi.org/10.1111/btp.12519.
Plummer, M.V., Williams, B.K., Skiver, M.M., Carlyle, J.C., 2003. Effects of dehydration on the critical thermal maximum of the desert box turtle (*Terrapene ornata luteola*). J. Herpetol. 4, 747–750. https://doi.org/10.1670/18-03N.
Post, E.S., Pedersen, C., Wilmers, C.C., Forchhammer, M.C., 2008. Phenological sequences reveal aggregate life history response to climatic warming. Ecology 89, 363–370. https://doi.org/10.1890/06-2138.1.
Prates, I., Navas, C.A., 2009. Cutaneous resistance to evaporative water loss in Brazilian *Rhinella* (Anura: bufonidae) from contrasting environments. Copeia 3, 618–622. https://doi.org/10.1643/CP-08-128.
Preest, M.R., Pough, F.H., 1989. Interaction of temperature and hydration on locomotion of toads. Funct. Ecol. 6, 693–699. https://doi.org/10.2307/2389501.
Quiroga, L.B., Moreno, M.D., Cataldo, A.A., Aragón-Traverso, J.H., Pantano, M.V., Olivares, J.P.S., Sanabria, E.A., 2015. Diet composition of an invasive population of *Lithobates catesbeianus* (American Bullfrog) from Argentina. J. Nat. Hist. 49 (27–28), 1703–1716. https://doi.org/10.1080/00222933.2015.1005711.
(Core Team), R., 2018. R: a language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. http://www.R-project.org/.
Rezende, E.L., Castañeda, L.E., Santos, M., 2014. Tolerance landscapes in thermal ecology. Funct. Ecol. 28, 799–809. https://doi.org/10.1111/1365-2435.12268.
Ribeiro, P.L., Camacho, A., Navas, C.A., 2012. Considerations for assessing maximum critical temperatures in small ectothermic animals: insights from leaf-cutting ants. PloS One 7 (2), e32083. https://doi.org/10.1371/journal.pone.0032083.
Shaw, G., 1802. General Zoology or Systematic Natural History, vol. III. Thomas Davison, London. Part 1. Amphibia.
Shoemaker, V.H., Baker, M.A., Loveridge, J.P., 1989. Effect of water balance on thermoregulation in waterproof frogs (Chiromantis and Phyllomedusa). Physiol. Zool. 62, 133–146. https://doi.org/10.1086/physzool.62.1.30160002.
Schwarzkopf, L., Alford, R.A., 2002. Nomadic movement in tropical toads. Oikos 96, 492–506. https://doi.org/10.1034/j.1600-0706.2002.960311.x.
Stebbins, R.C., 2003. Western Reptiles and Amphibians. Peterson Field Guides. Houghton Mifflin Company, Boston, New York, USA.
Steyn, W., 1966. Three eyes: wider implications of a narrow specialty. South Afr. J. Sci. 62 (1), 13.
Symes, L.B., Rodríguez, R.L., Höbel, G., 2017. Beyond temperature coupling: effects of temperature on ectotherm signaling and mate choice and the implications for communication in multispecies assemblages. Ecol. Evol. 7, 5992–6002. https://doi.org/10.1002/ece3.3059.
Symonds, M., Moussalli, A., 2010. A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. Behav. Ecol. Sociobiol. 65 (1), 13–21. https://doi.org/10.1007/s00265-010-1037-6.
Terblanche, J.S., Chown, S.L., 2007. The effects of temperature, body mass and feeding on metabolic rate in the tsetse fly *Glossina morsitans centralis*. Physiol. Entomol. 32 (2), 175–180. https://doi.org/10.1111/j.1365-3032.2006.00549.x.
Tewksbury, J.J., Huey, R.B., Deutsch, C.A., 2008. Putting the heat on tropical animals. Science 320, 1296–1297. https://doi.org/10.1126/science.1159328.
Tingley, R., Shine, R., 2011. Desiccation risk drives the spatial ecology of an invasive anuran (*Rhinella marina*) in the Australian semi-desert. PloS One 6, e25979. https://doi.org/10.1371/journal.pone.0025979.
Tingley, R., Greenlees, M.J., Shine, R., 2012. Hydric balance and locomotor performance of an anuran (*Rhinella marina*) invading the Australian arid zone. Oikos 121 (12), 1959–1965. https://doi.org/10.1111/j.1600-0706.2012.20422.x.
Titon Jr., B., Navas, C.A., Jim, J., Gomes, F.R., 2010. Water balance and locomotor performance in three species of neotropical toads that differ in geographical distribution. Comp. Biochem. Physiol. A. Mol. Integr. Physiol 156, 129–135. https://doi.org/10.1016/j.cbpa.2010.01.009.
Titon Jr., B., Gomes, F.R., 2015. Relation between water balance and climatic variables associated with the geographical distribution of anurans. PloS One. 10, e0140761. https://doi.org/10.1371/journal.pone.0140761.
Titon Jr., B., Gomes, F.R., 2017. Associations of water balance and thermal sensitivity of toads with macroclimatic characteristics of geographical distribution. Comp. Biochem. Physiol. A. Mol. Integr. Physiol 208, 54–60. https://doi.org/10.1016/j.cbpa.2017.03.012.
Tracy, C.R., 1976. A model of the dynamic exchanges of water and energy between a terrestrial amphibian and its environment. Ecol. Monograph 46 (3), 293–326. https://doi.org/10.2307/1942256.
Tracy, C.R., Christian, K.A., 2005. Preferred temperature correlates with evaporative water loss in hylid frogs from northern Australia. Physiol. Biochem. Zool. 78, 839–846. https://doi.org/10.1086/432151.
Tracy, C.R., Christian, K.A., Tracy, C.R., 2010. Not just small, wet, and cold: effects of body size and skin resistance on thermoregulation and arboreality of frogs. Ecology 91, 1477–1484. https://doi.org/10.1890/09-0839.1.
Tracy, C.R., Tixier, T., Le Nöene, C., Christian, K.A., 2014. Field hydration state varies among tropical frog species with different habitat use. Physiol. Biochem. Zool. 87, 197–202. https://doi.org/10.1086/674537.
Watling, J.I., Braga, L., 2015. Desiccation resistance explains amphibian distributions in a fragmented tropical forest landscape. Landsc. Ecol. 30, 1449–1459. https://doi.org/10.1007/s10980-015-0198-0.
Wang, T., Zaar, M., Arvedsen, S., Vedel-Smith, C., Overgaard, J., 2002. Effects of temperature on the metabolic response to feeding in *Python molurus*. Comp. Biochem. Physiol. A 133, 519–527. https://doi.org/10.1016/S1095-6433(02)00250-7.
Wang, Y., Qun, L., 2006. Comparison of Akaike information criterion (AIC) and Bayesian information criterion (BIC) in selection of stock–recruitment relationships. Fish. Res. 77 (2), 220–225. https://doi.org/10.1016/j.fishres.2005.08.011.
Williams, S.E., Shoo, L.P., Isaac, J.L., Hoffmann, A.A., Langham, G., 2008. Towards an integrated framework for assessing the vulnerability of species to climate change. PLoS Biol. 6, e325. https://doi.org/10.1371/journal.pbio.0060325.

Wolcott, T.G., Wolcott, D.L., 2001. Role of behavior in meeting osmotic challenges. Am. Zool. 41, 795–806. https://doi.org/10.1093/icb/41.4.795.

Wright, A.H., Wright, A.A., 1949. Handbook of Frogs and Toads of the United States and Canada. Cornell University Press.

Wygoda, M.L., 1984. Low cutaneous evaporative water loss in arboreal frogs. Physiol. Zool. 57, 329–337. https://doi.org/10.1086/physzool.57.3.30163722.

Young, J.E., Christian, K.A., Donnellan, S., Tracy, C.R., Parry, D., 2005. Comparative analysis of cutaneous evaporative water loss in frogs demonstrates correlation with ecological habits. Physiol. Biochem. Zool. 78, 847–856. https://doi.org/10.1086/432152.

Estefany Caroline Guevara Molina. I am interested in understanding adaptations of amphibians and reptiles through processes of phenotypic and/or adaptive plasticity in relation to the dynamics in their environments. I am mostly focused to evaluate the impacts of climatic stressful condition on behavior, physiology and conservation of amphibians.

Fernando Ribeiro Gomes. My interests and research experience are focused on the areas of Evolutionary Physiology, Animal Behavior, Ecophysiology, and Comparative Physiology.

Agustín Camacho Guerrero: I seek to understand how the environment interacts with evolving phenotypes to determine where species can live, and on the consequences of such interaction over different organizational levels of nature (i.e. Individuals' phenotypes, population abundance, species' range size, community richness, etc).