

Behavioural thermoregulation in a Neotropical frog is detectable only when physiological integrity allows movement

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Abstract

Neustonic Neotropical anurans remain understudied in tests of thermal sensitivity. Yet, this hampers our understanding of how frogs might respond to climate change. Here, we quantified the thermal sensitivity of jumping performance and tested for behavioural thermoregulation in 60 *Pseudis limellum* (Hylidae) from the Brazilian Pantanal, in a 170 × 55 cm arena with a 20–40 °C thermal gradient. We recorded response time, first-jump distance, and total jumping distance, and analysed them with a Bayesian hurdle log-normal model for distances and a right-censored Weibull accelerated-failure-time model for response time. Eight individuals (13 %) did not jump within the 90 s window, with non-response concentrated at the cold (≤ 20 °C: 43 %) and hot (32–36 °C: 25 %) extremes. Total jumping distance was concave in final temperature, peaking at a ‘parking temperature’ $T_{\text{park}} = 27.2$ °C. Relative hind-limb length did not improve predictive performance in any model. We compared each observed final temperature with a two-dimensional Pearson random-walk null; the combined test rejected the geometric null, with the signal concentrated in cold and hot starting bands. Behavioural thermoregulation in *P. limellum* is expressed only when thermal conditions permit locomotion: at the extremes, individuals are simultaneously most likely to need to leave and least able to do so. The species’ narrow thermal niche centred on ~ 27 °C makes it vulnerable to the projected 4–7 °C warming of the Pantanal by the end of the century.

Blinded methods

Trials were conducted by a single observer (CCG) and could not be blinded to the experimental treatment (gradient position), because the gradient itself defined the treatment. Data entry and quantification of jumping distances and response times were performed on the same trial sheet, with no separation between data-collection and treatment-assignment roles. This trade-off is intrinsic to single-observer field thermal-gradient experiments; we believe it has not biased our results because the response variables are physically measured (cm and seconds) rather than scored, and because the focal inference (T_{park} and the geometric-null comparison) is derived from the joint distribution of observed final positions, not from the experimenter’s qualitative judgement.

Significance Statement

Many animals ‘choose’ the temperatures they experience by moving through their environment, a behaviour called thermoregulation. Whether this strategy still works at the extremes of a species’ thermal tolerance has been hard to test directly. We exposed 60 small Neotropical frogs (*Pseudis limellum*) from the Brazilian Pantanal to a controlled 20–40 °C thermal gradient and asked whether they actively move toward favourable temperatures. They do, but only when their muscles still let them. At the coldest and hottest starting positions, nearly half of the frogs did not move within our 90 s observation window, while those that did move concentrated their final positions around 27 °C. Behavioural thermoregulation in this species is conditional on the physiological capacity that thermoregulation is meant to maintain, a finding with direct relevance to projected Pantanal warming.

Introduction

Behavioural thermoregulation is the main mechanism by which terrestrial and semi-terrestrial ectotherms approach their thermal optima, while avoiding the metabolic and ecological costs of unfavourable body temperatures (Angilletta 2009). The classical cost–benefit model of Huey and Slatkin (1976) formalises this as a decision rule in which animals trade the energetic, time, and exposure costs of moving against the physiological and fitness benefits of body-temperature regulation. The framework remains foundational and has been extended in several directions: to capture the asymmetric costs of overheating in increasingly hot environments (Vickers et al. 2011); to formalise the joint optimisation of body temperature and water balance in terrestrial and semi-terrestrial taxa (Rozen-Rechels et al. 2019); and to and to link, in the field, thermoregulatory effectiveness, locomotor performance and survival (Wild et al. 2025). Across taxa, recent work continues to confirm the broad qualitative predictions of the cost–benefit logic, such as in habitat-management contrasts that change microclimatic costs (Neel et al. 2018) and in the strong covariation between thermoregulatory accuracy and seasonal habitat structure (Neel et al. 2021). Yet, most of the empirical work that has driven the modern theory has been done on basking lizards (Vickers 2023). Amphibians, despite being among the most diverse and most thermally constrained vertebrate groups, remain comparatively understudied as behavioural thermoregulators.

Two related, but distinct concepts dominate ectotherm thermal biology: the thermal performance curve (TPC), which describes how an organism’s physiological output depends on body temperature under controlled conditions (Bodensteiner et al. 2020; Rezende and Bozinovic 2019), and behavioural thermoregulation, which describes how animals position themselves along a thermal landscape to influence their realised body temperature. The two concepts answer different questions and demand different experimental designs. TPC estimation requires holding individuals at fixed temperatures while measuring performance, so that the entire functional relationship between temperature and capacity can be reconstructed (Angilletta 2006). Conversely, behavioural thermoregulation tests instead require a heterogeneous thermal environment in which the animal is free to choose where to be, so that the realised body temperature reflects an active choice, rather than an imposed condition (Hertz et al. 1993; Vickers and Schwarzkopf 2016). When the two designs are mixed, as happens whenever a thermal gradient is used both to expose animals to specific starting temperatures and to allow them to relocate, the resulting data answer the behavioural question well, but only partially constrain the underlying TPC (Laterza-Barbosa et al. 2025). For species in which the main interest is whether and how individuals choose their body temperature in heterogeneous environments, a gradient design with movement is the appropriate primary test.

Despite recent advances, knowledge of amphibian thermal biology is taxonomically and geographically uneven. A recent phylogenetically informed imputation of heat tolerance across more than five thousand amphibian species estimated that empirical thermal-tolerance values are still missing for roughly 40% of described amphibians, with a strong bias toward temperate and terrestrial taxa (Pottier et al. 2025). Tropical lowland amphibians are simultaneously over-represented in vulnerability projections, due to narrow thermal safety margins and tight coupling between body and microhabitat temperature

(Nowakowski et al. 2017), and under-represented in the experimental literature that informs those projections (Pottier et al. 2025). Within the Neotropics, microhabitat-explicit behavioural thermoregulation has now been documented for fossorial salamanders (Giacometti et al. 2024), for diurnal rainforest frogs that consistently underfill the warm end of their thermal niches (Klinges et al. 2024), for terrestrial toads with seasonal thermoregulatory adjustments (Anderson et al. 2018), and for the high-altitude aquatic frog *Telmatobius culeus* (Muñoz-Saravia et al. 2018). However, for semi-aquatic Neotropical hylids that inhabit the water–air interface, the equivalent tests are essentially absent: most aquatic-frog thermal work has used the fully aquatic *Xenopus* (Pipidae), whose swimming-dominated locomotor mode and stable aquatic microhabitat differ markedly from the jumping-dominated locomotion of taxa that live on floating vegetation. This is a non-trivial gap, because frogs at the water–air interface are exposed to a thermal regime that combines the wide diel amplitude of an open-water surface with the locomotor demands of a terrestrial-style ballistic jump.

The Neotropical paradoxical-frog clade (Hylidae: Pseudae, *sensu* Turazzini et al. 2023; *Pseudis* and *Lysapsus* of Aguiar et al. 2007) offers a well-suited system for testing behavioural thermoregulation in this neglected niche. Comparative osteology of the Pseudae clade indicates a paddle-like autopodium and modified pelvic architecture associated with a primarily aquatic-surface lifestyle (Turazzini et al. 2023), but field observations show that locomotion in *Pseudis* consists of short, consecutive jumps along the floating mat, rather than sustained swimming, with diving reserved as a final escape response (Brandão et al. 2003; Garda et al. 2007). Anuran jumping morphology is a phylogenetically conserved trait whose performance covaries strongly with microhabitat across the order (Citadini et al. 2018; Moen 2019), but the relevant performance has rarely been measured along a thermal gradient in aquatic-surface taxa. The combination of (i) a thermally exposed neustonic niche, (ii) a discrete and easily quantified locomotor mode, and (iii) an active population in the Pantanal, a wetland projected to warm by 4–7°C and lose 30% of dry-season precipitation by 2100 (Marengo et al. 2015), makes *P. limellum* an informative system for testing behavioural thermoregulation under realistic conditions.

Here, we report a fully Bayesian analysis in which 60 *P. limellum* were individually exposed to a 20–40°C thermal gradient and allowed to choose their final position over a 90 s observation window. We measured three response variables: response time, first-jump distance and total jumping distance and posed three alternative hypotheses for how these responses should covary with initial and final temperature (Fig. 1). Under the active-escape hypothesis (Fig. 1a), individuals released at thermally hostile starting temperatures would be most motivated to leave and would therefore travel the greatest total distance, producing a U-shaped relationship between total jumping distance and initial temperature. Under the alternative physiological-limitation hypothesis (Fig. 1b), the same individuals would also be most motivated to leave, but the locomotor system would itself be impaired at the thermal extremes, producing the opposite ∩-shaped relationship, in which the largest displacements occur at intermediate starting temperatures. Crucially, both hypotheses converge on the same prediction once the response is plotted against *final* temperature (Fig. 1c): a parking response in which the realised distribution of stopping temperatures is concentrated around a focal value, regardless of where the animal began. To separate genuine thermal choice from the geometric expectation that any walker

released at one end of a bounded gradient will tend to drift toward the centre, we simulated each individual's expected behaviour under a two-dimensional Pearson random-walk null model with reflective boundaries (Vickers and Schwarzkopf 2016). Treating the binary decision to move and the continuous magnitude of movement as separable components of the same response (*via* a hurdle log-normal family) and accounting for right-censoring of response times (*via* a Weibull accelerated-failure-time model with explicit censoring), we ask whether *P. limellum* expresses behavioural thermoregulation in this gradient, whether the signal is restricted to individuals released at thermal extremes, and whether the realised parking temperature is consistent with the temperature regime observed at the species' field habitat.

Material and Methods

Study species and sampling

Pseudis limellum (Cope 1862) is a small Neotropical hyliid (mean snout–vent length $\approx$ 1.85 cm; Garda et al. 2007) that inhabits floating vegetation at the surface of open ponds, marshes, and oxbow lakes across central and southern South America (Garda et al. 2007). Despite being closely associated with the water surface, individuals locomote primarily by jumping rather than swimming: short consecutive jumps along the floating mat are the species' main escape, foraging, and dispersal behaviour, with diving used only as a final escape response (Brandão et al. 2003; Nauwelaerts et al. 2005; Garda et al. 2007). The species is therefore best described as neustonic (aquatic-surface-dwelling) rather than fully aquatic. Water temperatures recorded at the ponds inhabited by *P. limellum* in the Brazilian Pantanal range from 26 to 32°C across the day–night cycle (Garda et al. 2007), and predation pressure comes mainly from wolf spiders, water snakes, and giant water bugs (Garda et al. 2007; Landgref-Filho et al. 2019).

We captured 60 adults of *P. limellum* in September 2018 by visual encounter survey at night around ponds at the Pantanal Field Station (19° 34' S, 57° 00' W; SIRGAS 2000) of the Federal University of Mato Grosso do Sul, in Corumbá, Mato Grosso do Sul, central Brazil. Nocturnal capture exploits the period when individuals are calling and less prone to evasion (Garda et al. 2007). Each captured frog was placed in a plastic bag with water and floating macrophytes from its source pond and transported less than 1 km to the field laboratory, where it was held overnight under ambient temperature and humidity until the following morning trial. No mortality occurred during transport, holding or trials; all 60 individuals were returned to their capture pond after the experiment.

Experimental arena and thermal gradient

We built a rectangular arena from porcelain tiles measuring 170 × 55 × 70 cm (length × width × height; see Fig. S1). A thermal gradient along the long axis was generated by suspending a 150 W infrared lamp at $\sim$ 50–60 cm above the floor near one short end (the hot end) and placing crushed ice at the opposite end (the cold end), producing surface temperatures ranging from $\sim$ 20°C at the cold end to $\sim$ 40°C at the

hot end. Fixed thermocouples connected to a digital thermometer (Instrutherm 6802 II; Resolution: 0.1 °C) positioned at both ends of the arena were used to monitor gradient stability. To control for the well-documented interaction between hydration and locomotor performance in anurans (Mitchell and Bergmann 2016; Ortega et al. 2022), we kept a 1 cm layer of water across the arena floor; the layer was added at ambient temperature at the start of each session and replenished throughout the day by the melting ice.

Surface temperature at the intended starting position of each frog was measured immediately before the trial using a handheld infrared thermometer (Benetech® GS320; distance-to-spot ratio: 12:1; resolution: 0.1 °C from 1 m; emissivity $\epsilon = 0.95$). If the local surface temperature did not fall within the target band, additional ice was added and the trial was deferred until the gradient stabilised. This per-trial verification ensured that the recorded initial temperature corresponded to the actual substrate temperature at the moment of release. The infrared lamp remained on continuously throughout each session, and the cold end was checked periodically and replenished with ice as needed.

Trial protocol

Trial protocol

All trials were conducted between 09:00 and 10:00 local time, within the species' diurnal activity window (Garda et al. 2007). Each frog was tested in a single trial. Single-trial designs avoid both within-individual learning effects and motor fatigue between exposures to contrasting thermal regimes, but they preclude estimation of intra-individual repeatability (Careau et al. 2014); this trade-off is revisited in the Discussion.

The initial position of each frog along the gradient was assigned at random, and the realised starting temperature recorded as described above. The frog was then placed inside an opaque restriction grid for 10 min, a period intended to allow thermal equilibration with the substrate, rather than physiological acclimation in the strict sense (Stevenson 1985). The grid was wide enough to permit the experimenter's arm and the stimulus device to enter at the end of the equilibration period but prevented the frog from moving away from its assigned position. After the equilibration period, the grid was removed and a mechanical stimulus was applied: the same experimenter (CCG) approached the frog with the tip of a slender ~ 30 cm wooden stick and lightly touched its body once. If the frog did not jump within 30 s, the stimulus was repeated, for a maximum of three stimuli and a maximum observation window of 90 s per individual.

For each frog, we recorded three response variables: (i) response time (s), measured with a digital stopwatch from the first stimulus to the first jump; (ii) first-jump distance (cm), measured with a measuring tape between the starting and landing locations of the first jump; and (iii) total jumping distance (cm), the straight-line distance between the starting position and the final resting position after the 90 s observation window. Individuals that did not jump within the observation window were recorded with `response_time = 90` (right censored), `first_jump_distance = 0` and `total_jumping_distance = 0`; eight

of the 60 individuals fell in this category. Surface temperature at the final position (henceforth final temperature) was recorded with the same infrared thermometer immediately after the trial.

After each trial, we measured snout–vent length (SVL) and hind-limb length (LL = femur + tibiofibula) of each frog with a digital calliper (to the nearest 0.01 mm). Relative hind-limb length (LL / SVL) was computed as a body-size-corrected morphological covariate.

Statistical analysis

All analyses were carried out in R 4.6.0 (R Core Team 2026) under the Bayesian framework implemented in the brms package (Bürkner 2017) with the cmdstanr backend. Reporting follows Kruschke (2021), Davis and Kay (2023) and Gerstner et al. (2017).

Response structure

Inspection of the empirical distribution of the three response variables showed that the eight non-responders produced (i) right-censored response times (the 90 s upper bound is the observation limit, not the actual reaction time) and (ii) structural zeros in both distance variables (first_jump_distance = 0 and total_jumping_distance = 0 co-occur with response_time = 90). Non-responses concentrated at the cold ($\leq 20^{\circ}\text{C}$, 43% non-response) and hot ($32\text{--}36^{\circ}\text{C}$, 25%) ends of the gradient, suggesting two distinct physiological mechanisms (cold-induced torpor and heat-induced muscular failure) for failing to jump. Both features make a hurdle process: separating the binary decision to move from the continuous magnitude of movement, and an explicit censoring model preferable to a naïve log-Gaussian or transformation-based approach.

Models

We fitted three Bayesian generalised non-linear models, one per response variable. *Total jumping distance* (focal model, hereafter M_T) was modelled with a hurdle log-normal family. The continuous (μ) component related log-distance to final temperature via a second-degree orthogonal polynomial, plus relative hind-limb length, while the hurdle (π) component modelled the probability of *not* jumping as a second-degree polynomial of the initial temperature. *First-jump distance* was modelled analogously, with a hurdle log-normal family and second-degree polynomials of initial temperature on both the μ and π components. *Response time* was modelled as a Weibull accelerated failure-time (AFT) process with right-censoring at 90 s, with a second-degree polynomial of initial temperature, plus relative hind-limb length on the log-mean (see Fig. S9). For all three models, a second-degree polynomial on temperature was preferred over a flexible smooth: exploratory analyses showed that all relationships were unimodal and modest in magnitude ($R^2 \leq 0.30$), and a non-parametric smooth would have been over-parameterised for $n = 60$.

Following Gelman et al. (2008), we used weakly informative priors centred at zero on the log scale. For both hurdle log-normal models, slope coefficients received $N(0, 2)$ priors on the log scale (corresponding to 95% prior mass on multiplicative effects within a factor of $\sim e^2 \approx 7.4\times$), the log-distance intercept received $N(3, 1)$ (corresponding to a 95% prior on the conditional median between ~ 2.7 and ~ 148 cm), the logit-hurdle intercept received $N(0, 2)$, and the residual log-scale standard deviation received a half-Student- $t(3, 0, 2.5)$ prior. For the Weibull AFT model, slopes received $N(0, 1)$ on the log-time scale, the log-baseline intercept received $N(3, 1)$ (~ 7 – 55 s median baseline) and the shape parameter received a $\text{Gamma}(2, 1)$ prior consistent with both decreasing and increasing hazard functions. All priors were validated via prior predictive simulation, confirming that the implied prior predictive distributions covered the observed data range without placing mass at biologically impossible values (Fig. S7).

Each model was fitted with four chains of 4,000 iterations each (1,500 warm-up, 2,500 sampling, 10,000 post-warm-up draws per model), with $\text{adapt_delta} = 0.95$. Convergence was assessed by $\hat{R} \leq 1.01$, bulk and tail effective sample sizes $> 1,000$ (Vehtari et al. 2021), no divergent transitions, and visual inspection of trace plots for all population-level parameters (Fig. S5).

Model adequacy was evaluated through four complementary checks for each model: (i) overlay of the observed and predicted densities restricted to the jumping subset; (ii) match between the observed and posterior-predicted proportion of zeros; (iii) match between the observed mean–standard-deviation joint distribution and the posterior-predicted joint distribution; and (iv) match between the observed and predicted means stratified by initial-temperature band (cold, cool, warm, hot). Grouping the predictive check by *temperature band* rather than by *response status* avoids conditioning the diagnostic on the outcome variable itself (Fig. S3 and S4).

Robustness of each posterior to the choice of priors was evaluated via power-scaling sensitivity diagnostics (Kallioinen et al. 2024). Posteriors that remained stable across prior power-scaling values of $\alpha \in \{0.8, 1, 1.25\}$ were considered likelihood-dominated (Fig. S7). For each response variable, we additionally fitted a reduced model omitting relative hind-limb length and compared the two with approximate leave-one-out cross-validation (PSIS-LOO-CV; Vehtari et al. 2017) implemented in the *loo* package (Vehtari et al. 2025). Pareto- k diagnostics were inspected to confirm reliability of the importance sampling (Fig. S6). For every fixed-effect parameter we report the posterior median with 95% equal-tailed CrI, the probability of direction (pd; Makowski et al. 2019) – the posterior mass on the side of the mode – and the proportion of the 95% posterior within a region of practical equivalence (ROPE; Kruschke 2018). We adopted ROPE bands of ± 0.10 log-units on the log-distance and log-time scales ($\approx 10\%$ proportional change) and ± 0.18 logit-units on the hurdle scale (≈ 5 percentage points around an immobility baseline of 0.5, Table S2). The probability of concavity for each polynomial term was computed directly from the posterior in *brms*.

From the posterior of M_T we derived the *parking temperature* (T_{park}): the value of final temperature at which the expected total jumping distance is maximised, by computing the argmax of the posterior

expected response over a fine temperature grid (0.25°C resolution) and reporting the posterior median with 95% CrI.

Geometric null model for thermal choice

Because the gradient itself constrains the achievable final temperatures of a frog jumping from any given starting position, a non-trivial portion of the observed concentration of final temperatures around T_{park} could arise from arena geometry alone, independent of any behavioural preference. To separate geometric drift from active thermoregulation, we simulated a null distribution of final temperatures expected under random-walk movement in the absence of thermal preference, following the individual-based null-model approach proposed by Vickers and Schwarzkopf (2016) for ectotherms. For each frog that jumped, we performed $B = 2,000$ Monte Carlo replicates of a two-dimensional Pearson random walk (Pearson 1905; Codling et al. 2008) constrained to the 170×55 cm arena floor, with reflective boundaries on both axes. The starting x-coordinate was set to the position corresponding to the frog's observed initial temperature (assuming a linear gradient), the starting y-coordinate was sampled uniformly from the arena width, the number of jumps per replicate equalled the empirical estimate $\text{round}(\text{total_distance}/\text{first_jump_distance})$ for that individual, and each jump amplitude was sampled with replacement from the empirical distribution of observed first-jump lengths, with jump direction drawn from a uniform circular distribution. The simulated final x-coordinate was mapped back to temperature and compared with the observed final temperature. The frog-level deviation $|T_{\text{final}} - T_{\text{park}}|$ was compared with the null deviation distribution to produce a per-individual P -value, and the per-individual P -values were combined into a global test using Fisher's combined probability method (Fisher 1948; Heard and Rubin-Delanchy 2018) and reported separately by starting band. A Quarto dynamic document with all code used and the data are publicly archived on Zenodo (<https://doi.org/10.5281/zenodo.20723427>).

Results

Realised starting (initial) surface temperatures spanned 17.0–42.5°C and final surface temperatures spanned 11.5–42.4°C, indicating that the thermal gradient was effective throughout the operational range and that frogs moved both downstream and upstream along the gradient during the 90 s observation window. Of the 60 individuals, 52 jumped within the observation window and eight remained immobile for the full 90 s and were treated as right-censored for response time and as structural zeros for the two distance variables. Non-response was strongly concentrated at the cold ($\leq 20^\circ\text{C}$: 6 of 14, 43%) and warm-to-hot (32–36°C: 1 of 4, 25%) extremes of the gradient, with no immobility recorded in any of the intermediate bands.

All models converged with no divergent transitions. Potential scale reduction factors were $\hat{R} \leq 1.00$ for every population-level parameter, with bulk effective sample sizes $\geq 7,000$ and tail effective sample sizes $\geq 6,500$ across all chains. Posterior predictive checks (Table S1, Fig S3 and S4) showed that the

predicted conditional density on jumpers, the predicted proportion of zeros, and the joint posterior predictive mean–standard-deviation distribution all bracketed the observed values for each model. The predicted means stratified by initial-temperature band (cold, cool, warm, hot) also tracked the observed band means.

The hurdle component of the focal model M_T , the probability of failing to jump as a function of initial temperature, yielded a baseline log-odds of -1.92 (95% CrI $[-2.70, -1.22]$; $pd = 1.0000$; 0% in ROPE), corresponding to a baseline probability of immobility of approximately 0.13 at the mean initial temperature. The linear and quadratic polynomial terms of T_{init} on the hurdle scale had posterior medians of -2.19 (95% CrI $[-5.52, 1.04]$; $pd = 0.906$) and 1.25 (95% CrI $[-2.07, 4.53]$; $pd = 0.773$), respectively (Table S2). Although the credible intervals on both polynomial terms include zero, the posterior $P(\text{immobile})$ curve (Fig. 2d) rises to approximately 0.22 (95% CrI $[0.05, 0.45]$) at the cold end and ~ 0.12 at the hot end of the gradient, qualitatively matching the observed band-wise frequencies of immobility.

Response time among the 52 jumpers was modelled with a Weibull accelerated-failure-time process with right-censoring at 90 s for the 8 non-responders ($n_{\text{uncensored}} = 52$, $n_{\text{censored}} = 8$). The shape parameter posterior was 0.56 (95% CrI $[0.44, 0.69]$), well below 1, indicating a strongly decreasing hazard: individuals that jumped tended to react quickly after stimulus, whereas individuals that delayed beyond the first few seconds were progressively less likely to jump at all, a pattern consistent with treating the 90 s threshold as a true censoring boundary rather than a long but finite response time. The log-mean intercept was 4.84 (95% CrI $[0.73, 8.96]$; $pd = 1.000$; 0% in ROPE). The linear and quadratic polynomial terms of T_{init} were -2.16 (95% CrI $[-4.84, 0.54]$; $pd = 0.856$; 4.6% in ROPE) and 2.09 (95% CrI $[-0.51, 4.68]$; $pd = 0.843$; 5.7% in ROPE), respectively, both with credible intervals that cross zero, but with posterior mass concentrated in the direction expected by a U-shaped relationship between initial temperature and reaction time (longer responses at thermal extremes). The relative hind-limb length effect was -1.00 (95% CrI $[-4.48, 2.53]$; $pd = 0.617$; 8% in ROPE), uninformative on either direction (Table S2, Fig. 2a).

First-jump distance, conditional on the frog having jumped, ranged from 6 to 48 cm ($n = 52$, median = 23 cm). The hurdle log-normal continuous component related $\log(\text{first-jump distance})$ to a second-degree polynomial of initial temperature and to relative hind-limb length. The intercept on the log scale was 2.81 (95% CrI $[0.73, 4.94]$; $pd = 0.995$; 0.3% in ROPE). The linear and quadratic polynomial terms of T_{init} were -1.01 (95% CrI $[-2.14, 0.14]$; $pd = 0.957$; 3.2% in ROPE) and -1.08 (95% CrI $[-2.21, 0.07]$; $pd = 0.969$; 2.3% in ROPE), respectively, both with credible intervals that just cross zero, but with posterior mass strongly on the negative side, consistent with a unimodal, concave dependence of first-jump distance on initial temperature. Relative hind-limb length contributed 0.20 (95% CrI $[-1.63, 1.97]$; $pd = 0.590$; 9% in ROPE), with the credible interval evenly spanning zero (Fig. 2b, Table S2). The residual log-scale standard deviation was 0.55 (95% CrI $[0.45, 0.68]$).

Total jumping distance over the 90 s observation window, conditional on the frog having jumped, ranged from 7 to 261 cm ($n = 52$, median = 32.5 cm) and is the focal response of this study. We modelled it as a function of *final* temperature (the temperature at which the frog ultimately stopped) using a hurdle log-normal continuous component, while the hurdle component remained a function of *initial* temperature. On the log scale, the intercept was 3.20 (95% CrI [0.70, 5.72]; $pd = 0.995$; 0.3% in ROPE). The quadratic polynomial term of T_{final} was -2.33 (95% CrI [$-4.05, -0.59$]; $pd = 0.995$; 0.3% in ROPE), the only fixed-effect parameter across all three models for which the 95% credible interval excluded zero. The corresponding linear term was -0.15 (95% CrI [$-1.91, 1.61$]; $pd = 0.571$; 9.6% in ROPE), as expected for a polynomial centred near the empirical vertex. The probability that the quadratic term is negative (concave response surface) was 0.995. Relative hind-limb length contributed 0.27 (95% CrI [$-1.87, 2.42$]; $pd = 0.605$; 6.9% in ROPE). The residual log-scale standard deviation was 0.71 (95% CrI [0.58, 0.87]).

From the posterior of the continuous component, we derived the parking temperature (T_{park}) as the value of T_{final} at which the expected log-distance is maximised (Fig. 2c). The posterior median was $T_{\text{park}} = 27.2^{\circ}\text{C}$ with a 95% CrI of [19.8, 30.0] $^{\circ}\text{C}$. The relatively wide lower bound reflects sampling uncertainty under a moderate sample size ($n = 52$ jumpers) and a modest effect magnitude; the upper bound is tight against the operational maximum of the gradient.

In every model, the posterior of the relative hind-limb length coefficient included zero in its 95% credible interval, with pd values between 0.59 and 0.62. To complement these per-parameter summaries with a model-wide predictive assessment, we fitted a reduced version of each model without relative hind-limb length and compared the two with approximate leave-one-out cross-validation. The expected log pointwise predictive density (ELPD) differences (full minus reduced model) were $\Delta\text{ELPD} = -0.6$ (SE = 0.2) for M_T (total distance), $\Delta\text{ELPD} = -1.1$ (SE = 0.4) for the first-jump-distance model, and $\Delta\text{ELPD} = -0.1$ (SE = 0.3) for the response-time model. All three differences lie within ~ 1 SE of zero, indicating that relative hind-limb length provides essentially no predictive improvement once temperature is accounted for. Pareto- k diagnostics were below 0.7 for every observation in every fit (Fig. S6), confirming the reliability of the importance-sampling LOO approximation.

Power-scaling sensitivity diagnostics indicated that all three posteriors were dominated by the likelihood rather than the priors. Posterior densities for the polynomial slope coefficients of each model remained visually indistinguishable across prior scaling values of $\alpha \in \{0.8, 1, 1.25\}$, and the joint prior-likelihood sensitivity statistics did not exceed the recommended threshold for any parameter (Fig. S7). The inferences reported above can therefore be interpreted as data-driven rather than prior-driven.

The posterior of T_{park} concentrates the inference of a parking response on a specific final temperature ($\sim 27^{\circ}\text{C}$). However, the gradient itself constrains where a frog can end up when starting from a given position, so part of the observed concentration of final temperatures around T_{park} could in principle arise from arena geometry alone. To separate active thermoregulation from geometric drift, we compared the observed final temperature of each jumping frog with the null distribution produced by 2,000 Monte Carlo replicates of a two-dimensional Pearson random walk launched from the same initial position, with

empirical jump amplitudes and uniformly distributed direction (see Methods). Globally, the per-individual P -values (the proportion of null replicates whose final position lay within the observed absolute deviation from the focal temperature) were combined across the 52 jumpers using Fisher's combined probability method. The combined statistic strongly rejected the geometric null (Fisher combined $P < 0.0001$, $n = 52$).

Stratifying by initial-temperature band reveals where the signal of active thermoregulation is located (Table 1; Fig. 3). In the Cold band ($T_{\text{init}} \leq 22^\circ\text{C}$, $n = 14$), observed final temperatures fell on average 0.86°C closer to T_{park} than the geometric null predicted (observed mean $|T_{\text{final}} - T_{\text{park}}| = 3.74^\circ\text{C}$ vs null mean = 4.60°C). In the Hot band ($T_{\text{init}} > 35^\circ\text{C}$, $n = 13$), the discrepancy was even larger: observed final temperatures fell 2.05°C closer to T_{park} than the null (observed = 8.03°C vs null = 10.09°C). In contrast, the intermediate Cool ($22\text{--}28^\circ\text{C}$, $n = 15$) and Warm ($28\text{--}35^\circ\text{C}$, $n = 10$) bands produced near-perfect matches between observed and null distributions (differences of $+0.05^\circ\text{C}$ and $+0.49^\circ\text{C}$, respectively). This is exactly the pattern predicted by active thermoregulation: when frogs are released at temperatures already close to the focal value, both a random walk and an active thermoregulator end up near intermediate temperatures by geometric constraint, so the test has no discriminative power. When frogs are released at the thermal extremes, only active orientation can systematically reduce the deviation from T_{park} , and that is where the observed deviations are smaller than the null expectation.

Table 1

Stratified comparison of observed versus geometric-null deviations from the posterior median parking temperature, by initial-temperature band. Mean $|T_{\text{final}} - T_{\text{park}}|$ was computed observed per individual and averaged across null replicates. 'Observed - null' is negative when frogs reached final temperatures closer to T_{park} than expected from a geometric random walk launched at the same initial position. T_{park} posterior median = 27.2°C (95% CrI [19.8, 30.0]).

band	n	obs_mean	null_mean	diff	p_closer_mean
Cold ($\leq 22^\circ\text{C}$)	14	3.74	4.60	-0.86	0.33
Cool ($22\text{--}28^\circ\text{C}$)	15	3.14	3.09	0.05	0.49
Warm ($28\text{--}35^\circ\text{C}$)	10	4.90	4.41	0.49	0.56
Hot ($> 35^\circ\text{C}$)	13	8.03	10.09	-2.05	0.28

Discussion

We found that behavioural thermoregulation in *Pseudis limellum* is conditional on physiological capacity for movement. Under the cost-benefit framework (Huey and Slatkin 1976, Vickers et al. 2011), behavioural thermoregulation is a decision rule that weighs the energetic and survival costs of moving against the benefits of body-temperature regulation. Our model of total jumping distance recovered the predicted concave dependence on final temperature, with high posterior support and a posterior median parking temperature of $T_{\text{park}} = 27.2^\circ\text{C}$ (95% CrI [19.8, 30.0]). This concentration of final temperatures

around T_{park} is not an artefact of the arena geometry: the two-dimensional Pearson random-walk null model (Vickers and Schwarzkopf 2016) was rejected globally and, most informatively, *only* in the cold ($\Delta = -0.9^{\circ}\text{C}$) and hot ($\Delta = -2.1^{\circ}\text{C}$) starting bands. Frogs released in the intermediate Cool and Warm bands produced final temperatures statistically indistinguishable from random drift, exactly as expected when the starting position is already near the focal temperature. Active thermal choice is therefore real, but its signature only emerges when the thermal environment forces a non-trivial decision.

This finding goes against our original prediction. We had hypothesised, following the standard escape-motivation argument, that total jumping distance would follow a U-shaped function of initial temperature: frogs at the extremes would travel furthest as they tried to leave thermally hostile patches. Instead, total jumping distance followed an inverted-U ($\cap$ -shaped) function: individuals at the extremes travelled *less*, not more. The mechanism is well established in the comparative literature on anuran burst performance: whole-animal jumping is strongly temperature-sensitive within the active range (Hirano and Rome 1984; Knowles and Weigl 1990; Navas et al. 1999), with sharp declines at both the cold and hot tails of the thermal performance curve (Whitehead et al. 1989; Vidal et al. 2024). Elastic energy storage modulates but does not erase this temperature dependence, because warming itself controls how much energy the muscle can load into the elastic system (Navas et al. 1999, Mendoza et al. 2023). Our hurdle component reinforces the same point: 43% of individuals released at $\leq 20^{\circ}\text{C}$ did not jump at all within the 90 s observation window, and a further 25% failed to respond at $32\text{--}36^{\circ}\text{C}$. Failure to move at the extremes can be seen as a physiological constraint. Behavioural thermoregulation in *P. limellum* is gated by the very physiological capacity that thermoregulation is meant to maintain: when conditions force the decision, the actuator that would implement it is already compromised.

The estimated parking temperature of $\sim 27^{\circ}\text{C}$ falls in the lower part of the daily thermal envelope reported for *P. limellum* ponds in the field ($26\text{--}32^{\circ}\text{C}$; Garda et al. 2007), and below the daytime maxima of the dry season. Two interpretations are mutually consistent. First, the species' jumping-performance optimum is closer to morning–dawn water temperatures than to the afternoon thermal regime, which suggests that the trade-off favouring early-day activity recorded in the field (Garda et al. 2007) has a direct locomotor underpinning. Second, the comparatively narrow posterior of T_{park} , a 95% credible interval of $\sim 10^{\circ}\text{C}$, is consistent with the recent evidence of intra- and interspecific variability in thermal performance among neotropical anurans (Vidal et al. 2024) and with the population-level differentiation in optima in *Limnodynastes peronii* (Wilson 2001). Frogs in general select moisture conditions that prevent dehydration, even when these sacrifices jumping performance (Mitchell and Bergmann 2016). In our arena, the constant 1 cm water layer factored out that trade-off by design, isolating the thermal axis. The realised T_{park} we estimate is therefore a relatively pure thermal-performance optimum, not a compound thermo-hydric optimum. Under field conditions, where evaporative water loss is unconstrained, the locally chosen microhabitat temperature may well sit cooler than 27°C , a hypothesis that future field thermography could test.

Placing this result in a comparative context highlights how poorly the aquatic-surface niche is sampled in the thermal-biology literature. Most experimental work on anuran burst locomotion has used terrestrial

ranids, hylids, and a few Australian or Chilean taxa (Hirano and Rome 1984; Knowles and Weigl 1990; Whitehead et al. 1989; Wilson 2001; Vidal et al. 2024), with the fully aquatic *Xenopus* (Pipidae) the only other widely studied aquatic model (Herrel and Bonneaud 2012; Padilla et al. 2019). *Pseudis* is neither terrestrial nor fully aquatic: it inhabits the air–water interface on floating vegetation, locomoting by short consecutive jumps rather than by sustained swimming (Brandão et al. 2003; Nauwelaerts et al. 2005; Garda et al. 2007). The pelvic and hind-limb morphology of the genus is adapted to short, ballistic jumps on a fluid substrate (Soliz and Ponssa 2016, Soliz et al. 2017), closer to the mechanics of terrestrial ranid jumping than to the propulsion mechanics of *Xenopus*. Our results extend the comparative window: the species behaves more like a temperature-sensitive ranid, than like a tropical *Xenopus tropicalis* (Herrel and Bonneaud 2012), with a relatively narrow optimum and steep declines at the extremes. The recent comparative effort in Chilean anurans (Vidal et al. 2024) and in Caribbean *Eleutherodactylus* (Crespo-Martínez et al. 2025) is beginning to populate the Neotropical end of the thermal-performance dataset. This study adds the first measurement for an aquatic-surface hylid.

Three limitations qualify these inferences and outline the design improvements that would tighten them. First, each frog was tested only once, which precludes estimation of intra-individual repeatability. This design choice deliberately avoided fatigue and learning across thermal contrasts, but it also means that the dispersion captured by our population-level inference is a mixture of true between-individual variance and within-individual stochasticity (Careau et al. 2014). Mark-recapture or sequential-arena designs with multiple trials per individual would partition the two sources. Second, our 60 frogs were sampled from a single Pantanal population in a single season; the population-level differentiation documented by Wilson (2001) and Vidal et al. (2024) implies that T_{park} may shift along latitudinal and microclimatic gradients. A multi-population replication is the natural next step. Third, our test was acute: thermal-acclimation effects, which can broaden the thermal performance curve in some anurans (Ruiz-Aravena et al. 2014) but not others (Padilla et al. 2019; Knowles and Weigl 1990), were not assessed. Pairing the present acute design with an acclimation experiment would resolve whether the narrow optimum we report reflects an evolved trait of the species or a transient state of acutely tested animals. The linear-gradient assumption of the arena was also not directly verified at intermediate positions, although the empirical range of measured final temperatures (11.5–42.4°C) suggests the gradient operated as intended.

The relevance of these results is reinforced by the characteristic asymmetry of ectotherm thermal performance curves. Performance typically increases with body temperature up to an optimum but declines steeply once body temperature exceeds that optimum (Huey et al. 2012). In amphibians, this thermal axis is coupled with water balance: warmer temperatures increase evaporative water loss and can accelerate dehydration-induced declines in locomotor performance (Greenberg and Palen 2021). Our results are consistent with this combined thermal–hydric constraint. Even under a hydrated experimental arena, frogs released at the warmest starting temperatures showed a high frequency of non-response and reduced locomotor output. Under field conditions in the Pantanal, where high temperatures are accompanied by evaporative water loss and variable access to moist refugia, the functional cost of overheating may therefore be stronger than the acute thermal effect estimated in our hydrated arena.

Climate projections for the Pantanal converge on an annual mean warming of 4–7°C by 2071–2100 and a ~ 30% reduction in dry-season precipitation (Marengo et al. 2015), with recent process-based niche modelling estimating that more than 80% of amphibian species in the Upper Paraguay River Basin will lose suitable habitat over the same horizon (Neves et al. 2025). For a species whose jumping-performance optimum sits at ~ 27°C and whose locomotor system shuts down at temperatures already well within the projected daytime range, this trajectory is concerning on two reinforcing fronts: the optimum will fall below operative temperatures more often, and the windows during which behavioural thermoregulation is even possible will shorten. Three lines of future work would directly strengthen the predictive use of this study. First, replicating the experiment across multiple populations spanning the species' latitudinal range would parameterise the population-level component of T_{park} (Wilson 2001, Vidal et al. 2024). Second, complementing the gradient design with classical thermal-performance-curve estimation at fixed temperatures would allow direct calibration of the gating function we infer from the hurdle component. Third, factorial designs that cross temperature with hydration state would integrate the present results with the moisture-dominated decision rule (Mitchell and Bergmann 2016). This is a particularly important integration for a species, like *P. limellum*, that lives at the air–water interface and may face simultaneous thermal and hydric stressors as the Pantanal becomes drier and warmer.

Generative AI Use Disclosure

This section follows the AI disclosure for Improved Transparency (AIdIT) framework proposed by Drobniak et al. (2026), the COPE position on authorship and AI tools (COPE 2023), and the Springer Nature editorial policy on AI use in scholarly publishing (Springer Nature 2024). No AI tool is listed as an author of this manuscript.

We used the following AI engines in the present study:

- Claude Opus (Anthropic), v. 4.7 (May 2026).
- Undermind (undermind.ai), accessed on 28 May 2026, for a structured literature review of the temperature-and-frog-jumping literature.

Areas of generative AI usage:

- **Formal analysis × generation.** Claude Opus generated R code for the Bayesian hurdle log-normal and Weibull AFT models in *brms*, for the two-dimensional Pearson random-walk null simulation, for the prior and posterior predictive checks, for the power-scaling prior-sensitivity analysis, and for the bayestestR-equivalent direct-from-draws computation of probability of direction and ROPE.
- **Formal analysis × refinement.** Claude Opus refined existing R code through iterative debugging (e.g. correcting a ROPE-percentage double-scaling error, fixing a syntax error in the DAG, replacing a 1D random walk with a 2D Pearson random walk to better match arena geometry).
- **Validation × generation.** Claude Opus identified appropriate validation approaches (prior predictive checks, posterior predictive checks stratified by initial-temperature band rather than by response

status, Pareto-*k* diagnostics for PSIS-LOO-CV).

- • **Visualisation × generation.** Claude Opus generated R / Python code for Figs. 1–3, the directed acyclic graph (DAG) of the Bayesian model architecture, and the supplementary diagnostic figures.
- • **Writing × generation.** Claude Opus drafted initial versions of the Introduction, Methods, Results and Discussion sections under explicit human direction; all factual content (numerical results, citations, biological claims) was verified by the human authors against the underlying analysis output (*analise_v2.html*) and primary sources before inclusion in the present version of the manuscript.
- • **Writing × refinement.** Claude Opus suggested copy edits for style and concision; final wording was decided by the human authors.
- • **Writing × comparisons.** Undermind generated a structured literature map of the temperature-and-frog-jumping debate (Drobniak et al. 2026 'comparisons' category), which informed the construction of Sections 1–2 of the Introduction and the comparative paragraphs of the Discussion. Every reference suggested by Undermind was retrieved and read by the human authors before being cited.

Per the COPE (2023) and Springer Nature (2024) positions: the authors declare that they have verified and approved all content generated or modified by the AI tools used in this manuscript, and that the human authors retain full responsibility for the scientific content, the interpretation of the results, the citation accuracy, and the conclusions drawn. The use of AI in this paper followed the ethical regulations of all funders and host institutions. No generative AI images were used, in compliance with Springer Nature (2024). All analytical pipeline that produces the results reported in the manuscript is archived on Zenodo (<https://doi.org/10.5281/zenodo.20723427>).

The disclosure provided in this section is also intended to comply with the requirements (Portaria CNPq nº 2.664) of the Brazilian National Council for Scientific and Technological Development (CNPq), which funded DBP under grant #407318/2021-6. Specifically, the CNPq policy requires explicit declaration of generative AI tools used at any stage of the research – conception, writing, data analysis or submission – specifying the tool and the purpose, and full author responsibility for the final content, including for any plagiarism or inaccuracy generated by the AI tool. Both requirements are met by the present disclosure and by the human-oversight workflow described above.

Declarations

Author contributions

Diogo B. Provete: Conceptualization (Contributing); Methodology (Equal); Software (Lead); Formal analysis (Lead); Validation (Lead); Data curation (contributing); Visualization (Lead); Project administration (Equal); Funding acquisition; Writing – original draft (Equal); Writing – review & editing (Equal). **Carolina Cunha Ganci:** Conceptualization; Methodology (Equal); Investigation (Lead); Data curation (Lead); Project administration (Equal); Writing – original draft (Equal); Writing – review & editing

(Equal). **Zaida Ortega:** Conceptualization (Lead); Methodology (Equal); Investigation (Equal); Resources (Lead); Supervision (Lead); Validation (Equal); Funding acquisition; Writing – review & editing (Equal).

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Competing interests. The authors declare no competing financial or non-financial interests in the last three years.

Ethics approval. All field collection and animal handling followed Brazilian regulations under collecting permits (SISBio # 61697-1 and #63653) and was approved by the Animal Use Ethics Committee of UFMS (CEUA-UFMS).

Data and code availability. The raw data file and the fully reproducible Quarto analysis pipeline used to produce every figure, table, and statistical result are archived on Zenodo (10.5281/zenodo.20723426), together with reproduction instructions.

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Figures

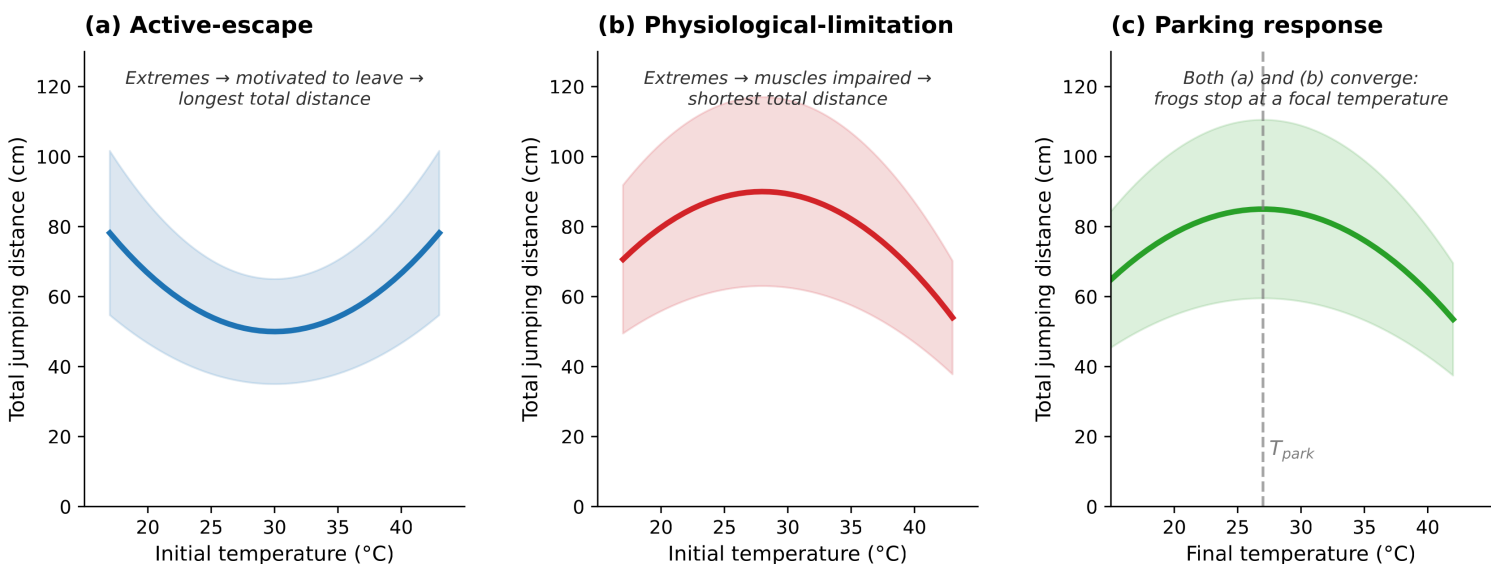


Figure 1

Conceptual hypotheses for the relationship between jumping performance and gradient temperature in *Pseudis limellum*. (a) Active-escape hypothesis: individuals released at thermally hostile starting temperatures are most motivated to leave, producing a U-shaped relationship between total jumping distance and initial temperature. (b) Physiological-limitation hypothesis: the same individuals are most motivated to leave but their locomotor system is impaired at the thermal extremes, producing the opposite $\cap$ -shaped relationship in which the largest displacements occur at intermediate starting temperatures. (c) Parking response: both hypotheses converge on the same prediction when the response is plotted against final temperature – a concave function with a focal maximum (T_{park}) regardless of starting position. Lines: hypothesised central tendency. Shaded bands: hypothesised uncertainty (illustrative).

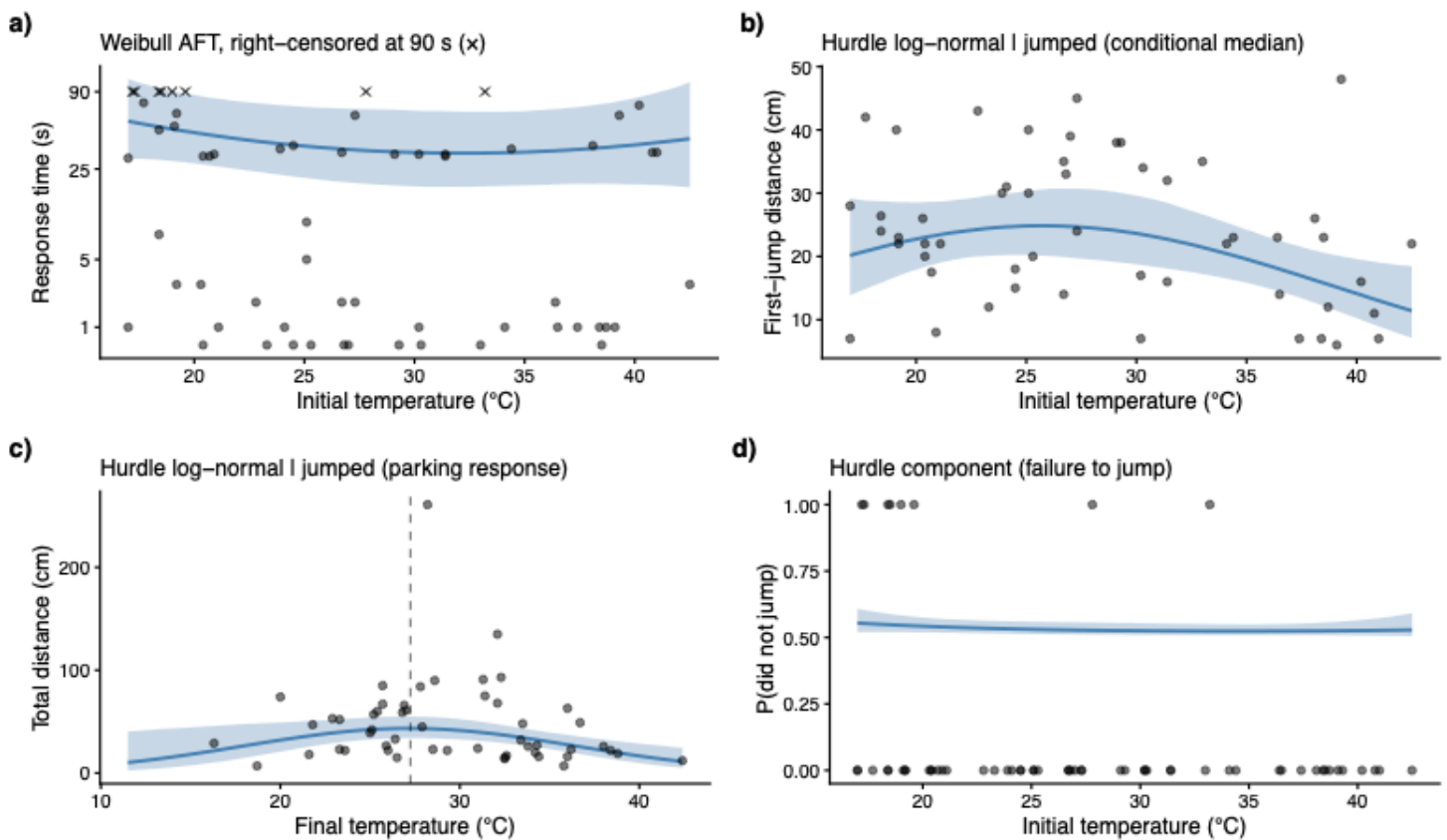


Figure 2

Bayesian fits of the three response variables. (a) Response time (s) as a function of initial temperature (°C), modelled as a Weibull accelerated-failure-time process with right-censoring at 90 s ($n_{\text{uncensored}} = 52$, $n_{\text{censored}} = 8$; censored values shown as crosses at the 90 s threshold; y-axis on log_{1p} scale). (b) First-jump distance (cm) as a function of initial temperature (°C), modelled with the continuous (log-normal) component of a hurdle log-normal model conditional on jumping ($n = 52$). (c) Total jumping distance (cm) as a function of *final* temperature (°C), modelled with the continuous component of a hurdle log-normal model conditional on jumping ($n = 52$); the vertical dashed line marks the posterior median parking temperature $T_{\text{park}} = 27.2$ °C. (d) Probability that an individual did not jump within the 90 s

observation window, as a function of initial temperature (°C) – the hurdle component of the model in panel (c) (n = 60). In all panels, dots represent observed data, the solid line is the posterior median fit and the shaded band the 95 % equal-tailed credible interval. Posterior median fits in (b) and (c) are back-transformed to the original (cm) scale via $\exp(\mu)$; the hurdle component in (d) is back-transformed to the probability scale via the inverse logit.

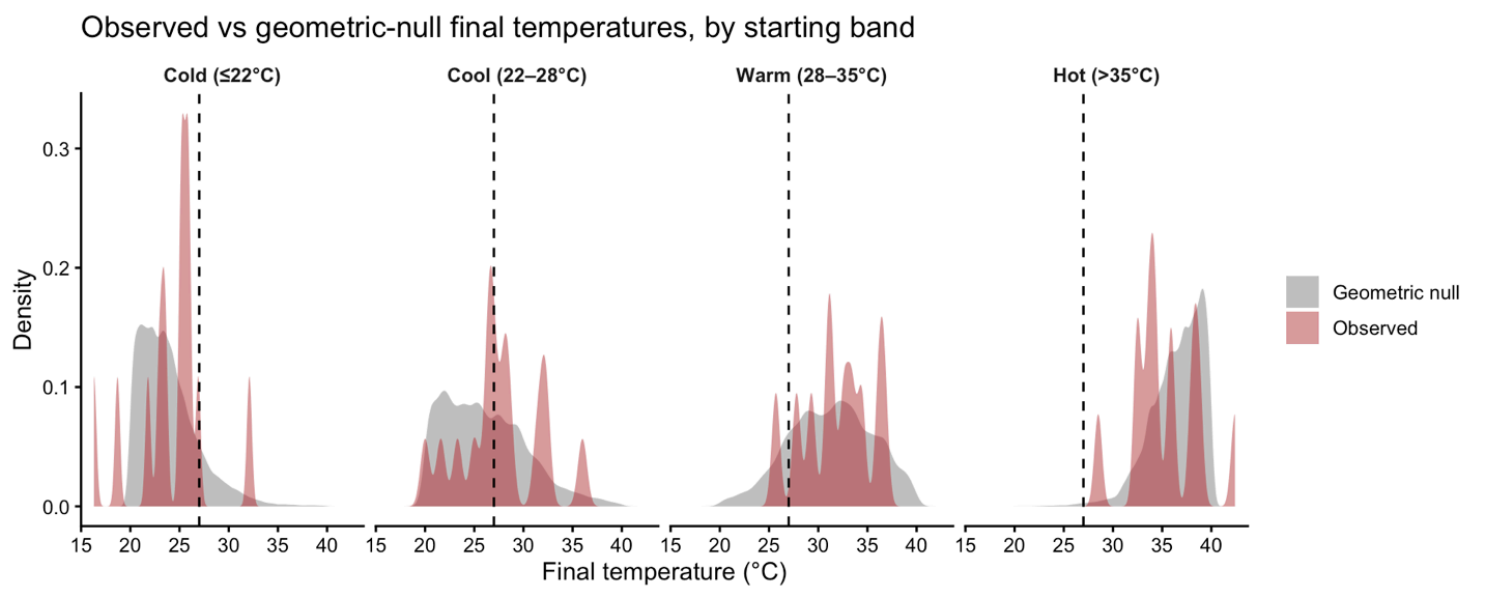


Figure 3

Observed versus geometric-null final-temperature distributions, faceted by initial-temperature band. Red densities: empirical distribution of final temperatures (T_{final}) for jumping frogs in each starting band. Grey densities: null distribution of T_{final} . The vertical dashed line marks the posterior median parking temperature $T_{\text{park}} = 27.2^{\circ}\text{C}$. Active thermoregulation predicts a tighter clustering of the red density around T_{park} than the grey density only in the Cold and Hot bands, where geometric drift alone would not reach T_{park} .

Supplementary Files

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- [Supplementaryv1.docx](#)