

# Is thermal sensitivity affected by predation risk? A case study in tadpoles from ephemeral environments

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## Abstract

Changes in environmental temperature may induce variations in thermal tolerance and sensitivity in ectotherm organisms. These variations generate plastic responses that can be analyzed by examining their Thermal Performance Curves (TPCs). Additionally, some performance traits, like locomotion, could be affected by other factors such as biological interactions (e.g., predator–prey interaction). Here, we evaluate if the risk of predation modifies TPCs in Mendoza four-eyed frog (*Pleurodema nebulosum*, Burmeister, 1861) and Guayapa's four-eyed frog (*Pleurodema guayapae*, Barrio, 1964), two amphibian species that occur in ephemeral ponds in arid environments. We measured thermal tolerances and maximum swimming velocity at six different temperatures in tadpoles under three situations: control, exposure to predator chemical cues, and exposure to conspecific alarm cues. TPCs were fitted using General Additive Mixed Models. We found that curves of tadpoles at risk of predation differed from those of control mainly in thermal sensitivity parameters. Our work confirms the importance of biotic interactions have in thermal physiology.

## KEYWORDS

anura, ecophysiology, ectotherms, predation, tadpoles, thermal biology

## 1 | INTRODUCTION

Temperature is a key factor involved in the development and performance of organisms, conditioning how they interact with biotic and abiotic environmental variables (Dunson & Travis, 1991; Tattersall et al., 2012; Vasseur & McCann, 2005). These interactions and the responses of organisms to thermal environment changes can be analyzed by exploring thermal sensitivity and thermal tolerance, and by modeling thermal performance curves, a type of thermal reaction norm (Angilletta, 2009; Huey & Kingsolver, 1989; Huey & Stevenson, 1979). Thermal performance curves (TPCs) describe the nonlinear relationship between biological rate processes (such as locomotor activity, growth, and metabolic rates) and temperature (Angilletta, 2009; Huey & Kingsolver, 1989; Huey & Stevenson, 1979). TPCs are functions

that grow until performance is maximal (optimal temperature;  $T_o$ ) and then, decrease rapidly (Huey & Stevenson, 1979). The upper and lower limits of thermal tolerance define two points at which performance is zero: Critical Thermal Maximum (CTmax) and Critical Thermal Minimum (CTmin; Angilletta, 2009). In thermally variable environments, organisms may display physiological plasticity, which is expressed in modifications of their TPCs such as the height, the position of the optimal temperature and the thermal breadth of performance (or amplitude of the curve). Within that breadth, organisms may perform “best” (B95, 95% of the maximum performance or optimal temperature range) or “well” (B80, 80% of the maximum performance or thermal performance breadth; Angilletta, 2009; Gunderson & Stillman, 2015; Taylor et al., 2021; Tejedo et al., 2012). Moreover, plasticity in TPCs can be described through variations in their shape, including vertical shifts (i.e.,

higher-lower performance), horizontal shifts (higher-lower optimal temperature) or even nonlinear modes defined by the generalist-specialist trade-off hypothesis, which implies a correlated lower maximal performance with wider thermal breadths (Huey & Kingsolver, 1989; Izem & Kingsolver, 2005). However, the costs that ecological interactions imposed on TPCs of organisms are not usually considered (Mitchell & Angilletta Jr., 2009).

Species interactions in ectotherms, such as competition and predation, are also influenced by temperature modifying activity rates, increasing encounter rate and escape velocity, among other variables (Dayton & Fitzgerald, 2001; Dell et al., 2014; Eck et al., 2014; Gilman et al., 2010; Mitchell & Angilletta Jr., 2009; Wellborn et al., 1996). Additionally, predator-prey interactions certainly impose costs that will influence thermal adaptation, modifying the thermal physiology of prey and therefore the TPCs (Gvoždík & Boukal, 2021). For example, the presence of predators may induce wider TPCs in prey, that is, predators induce prey to modify their thermal habitat by showing broader temperature preference ranges (as the case of thermal generalists; Gvoždík et al., 2013; Mitchell & Angilletta Jr., 2009). Moreover, thermal tolerances are parameters possibly involved in predator-prey dynamics (Pintanel et al., 2021). Previous research indicated a broadly extended mismatch in higher tolerances in a predator-prey model with dragonfly naiads and amphibians; the model predicts higher tolerances and consequently better performance at higher temperatures in predators than in prey (Pintanel et al., 2021). This is a relevant point to consider in future climate change scenarios.

Among ectotherms, anuran tadpoles are known to be good models for analyzing thermal sensitivity and thermal tolerance (Burggren & Warburton, 2007; Taylor et al., 2021), either because of their sensitivity to temperature as ectotherms or because of their ease of captive rearing, with locomotion being one of the traits used for estimating performance at different temperatures (Angilletta, 2009). Moreover, in anurans, swimming plays a fundamental role in survival, allowing them to escape from predators: they can either remain still, avoiding detection, or take the risk of being detected by escaping at full speed (Arendt, 2009; Dayton et al., 2005; Katzenberger et al., 2014; Perotti et al., 2016; Watkins, 1996).

In semiarid environments, as an adaptation to the ephemeral nature of ponds, tadpoles spend a long time foraging to grow as fast as possible, at the expense of becoming more susceptible to predation (Newman, 1992). In addition, in these ponds, trophic condition and interspecific interactions of top predators are affected by high temperatures due to a constraint on the activation energies (Dell et al., 2014; Katzenberger et al., 2021). Additionally, there is evidence that many aquatic animals have the ability to recognize risk from conspecific injury cues (Mirza et al., 2006; Pollock et al., 2003; Pueta & Perotti, 2016; Wisenden & Millard, 2001; Wisenden et al., 1999). The production of alarm cues by prey (kairomones) is essential to recognize potential predation (Brown et al., 1970; Gvoždík & Boukal, 2021). Interactions such as predator-prey through the presence of alarm

cues have shown nonconsumptive effects on prey, such as a reduced population growth in aphids (Nelson et al., 2004) or a modification of prey's TPCs indicated by an increase in CTmax and optimal temperature, as is the case of *Hyla versicolor* (Katzenberger et al., 2014). Therefore, this interplay between predator and prey should be considered when evaluating thermal sensitivity and thermal tolerance.

The Dry Chaco ecoregion is an extensive seasonal forest in South America (Bucher, 1982; Cabrera & Willink, 1973). Many amphibians breed in ephemeral ponds, which are highly exposed to sunlight, thereby imposing harsh conditions on amphibian tadpoles, which are vulnerable to high temperatures and pond desiccation (Duarte et al., 2012; Lescano & Miloch, 2023; Tejedo et al., 2012; Zak & Cabido, 2002). In these environments, Mendoza's four-eyed frog (*Pleurodema nebulosum*, Burmeister, 1861) and Guayapa's four-eyed frog (*Pleurodema guayapae*, Barrio, 1964) coexist with the Llanos Frog (*Lepidobatrachus llanensis*, Reig & Cei, 1963), the main predator present in the puddles. According to the "thermal game model," predation risk should affect selection of thermal patches by prey, and impose costs that certainly affect thermal adaptation (Mitchell & Angilletta Jr., 2009). Here, we studied the thermal physiology of tadpoles of two anuran species of the genus *Pleurodema* to determine whether risk cues can affect thermal sensitivity and thermal tolerances. We hypothesize that prey tadpoles exposed to risk cues from predators and alarm cues from congeners will alter their thermal physiological responses and presumably the use of thermal patches, as postulated by the "thermal game model." We expect that, in the presence of risk cues, tadpoles of these anuran species: (i) will increase their upper thermal tolerance (CTmax); (ii) will increase optimal temperature ( $T_o$ ) by modifying TPCs horizontally, since CTmax and  $T_o$  tend to be co-adaptive traits (Angilletta, 2009; Huey et al., 2009); (iii) will increase their maximum performance (measured as swimming velocity), modifying TPCs vertically, since tadpoles are exposed to high temperatures and predation risk (Arendt, 2009; Dayton et al., 2005; Van Buskirk & McCollum, 2000); and (iv) will show TPCs with wider performance ranges (broad thermal breadths), behaving as thermal generalists according to the thermal game model.

## 2 | MATERIAL AND METHODS

### 2.1 | Study species

To address the hypothesis, we analyzed the thermal physiology of two closely related species of anuran tadpoles, *P. nebulosum* (Mendoza's four-eyed frog) and *P. guayapae* (Guayapa's four-eyed frog) (Faivovich et al., 2012). *P. guayapae* is endemic to the Dry Chaco, whereas *P. nebulosum* occupies a wider area encompassing ecoregions of different physiognomy (although it is most common in

arid regions); therefore, it is exposed to a large range of thermal variation in their environments (Ferraro & Casagrande, 2009; Roig et al., 2009). Adults of both species have explosive breeding events in temporary ponds filled with rainwater during summer (Ceï, 1980). Since these water bodies have short duration, tadpoles tend to metamorphose quickly, which is an advantage, considering the harshness of the habitat and the short hydroperiod (Di Tada, 1999; Lescano & Miloch, 2023; Lescano et al., 2015; Valetti et al., 2014).

Field sampling was conducted on Las Toscas, Córdoba province, Argentina (30°10'20.24"S, 64°52'52.56"W). Water bodies where tadpoles occur are characterized by saline ponds with high conductivity (1.61 mS/cm) and a short hydroperiod (10 days) (Lescano & Miloch, 2023). Amphibian communities in the area involve assemblies of few species and predators in pools are scarce, with the macrophagous tadpoles of *L. llanensis* being one of the main aquatic predators and highly tolerant to heat (CTmax registered for the species reaches 44.7°C, the highest of all studied Chacoan amphibian species; Carroll, 1996; Duarte et al., 2012; Pers. Obs.). We collected eggs of both *Pleurodema* species and *L. llanensis* tadpoles in December 2019. Egg masses were transported to the laboratory (Centro de Zoología Aplicada-FCEFYN-UNC, Córdoba, Argentina) to be raised in outdoor mesocosms (one plastic pool of 200 L per species). Mesocosms were filled with dechlorinated water, covered with a plastic mesh to avoid predation and exposed to natural photoperiod (i.e., about 15/9 h dark/light photoperiod). After hatching, tadpoles were fed ad libitum with a mixture of boiled lettuce and fish food (Shulet®). When tadpoles reached stages 28–30 (Gosner, 1960), a subset of individuals of each species was randomly selected and each individual was put in a plastic container to perform thermal tolerance and thermal sensitivity trials under three treatments of predation risk. After thermal trials, tadpoles were photographed in lateral view with a reference scale (0.1 mm) to obtain their snout-vent length (SVL) and to determine the developmental stage (Gosner, 1960).

## 2.2 | Study design

In previous studies of sister species of *Pleurodema*, we determined that tadpoles strongly react to chemical cues from injured conspecifics due to fear of being eaten (alarm cues; Pueta et al., 2016; Pueta & Perotti, 2016). Therefore, we expected sign of risk, such as direct predator chemical cues or alarm cues, to affect the thermal physiology of tadpoles. To test the effects of predation risk on the thermal physiology of *Pleurodema* tadpoles, we exposed them to cues that represent real risks such as: (i) Alarm cues, created using crushed tadpoles (Pueta et al., 2016), and (ii) Predator cues, predator feces, obtained by feeding larvae of *L. llanensis* with the tadpole prey, *P. nebulosum* or *P. guayapae*. The experiment consisted of a factorial design, with three treatments: the two risk treatments and a control treatment with water without alarm or predator cues (hereafter control), as detailed below. We perform trials with 10 replicates per treatment (predator cues, alarm cues, control) for thermal tolerances

(CTmax and CTmin) totalling 60 replicates (individual tadpoles) per species, and 120 tadpoles of both species. 12 replicates per treatment (predator cues, alarm cues, control) were performed to determine thermal sensitivity (swimming velocity as a function of temperature) totalling 36 replicates (individual tadpoles) per species, that is, 72 tadpoles of both species.

## 2.3 | Thermal tolerance

We measured thermal tolerances to evaluate our first prediction, find thermal limits to shape TPCs and then, evaluate the remaining predictions. Before the determination of thermal tolerance limits, tadpoles of *P. nebulosum* and *P. guayapae* were acclimated in an incubator chamber (Semedic FT 290; Semedix) with controlled temperature and photoperiod (25°C ± 0.5°C; 15 L:9 D) for 4 days, as in previous studies (Bonino et al., 2020; Perotti et al., 2018) to allow animals acclimate and stabilize their critical temperatures (Allen et al., 2012; Brattstrom, 1968; Turriago et al., 2023). Acclimation temperature was determined based on previous experience and considering the average water temperature in the natural environment, to prevent larvae stress (Perotti et al., 2018; Tejedo et al., 2012). During the last 48 h of pretest acclimation, we added every 12 h, 1 mL of predator cues, alarm cues or water to predator cue, alarm cue and control treatments, respectively. We estimated thermal tolerances (CTmax and CTmin) by employing the dynamic method of Hutchison (1961). Each trial, started at a fixed temperature (25°C), and CTmax was determined as the temperature at onset of spasms in tadpoles (Bonino et al., 2020; Lutterschmidt & Hutchison, 1997). Trials were performed by heating tadpoles in a water bath with a fast ramping rate (0.5°C ± 0.1°C per minute) controlled by a Thermo-circulator device (TU-20D). This fast rate was selected to avoid “hardening” effects and for comparative purposes (Lutterschmidt & Hutchison, 1997; Perotti et al., 2018; Rezende et al., 2011; Tejedo et al., 2012). CTmin was determined using the same procedure; cooling tadpoles at a rate of 0.5°C ± 0.1°C per minute and checking when they stopped moving (Lutterschmidt & Hutchison, 1997). When tadpoles reached CTmax or CTmin, they were quickly removed from the bath (hot or cold, respectively) and placed in a container with water at 25°C, to recover motility.

Since in small-sized aquatic ectotherms, body temperature is similar to water temperature, we measured only the water temperature and considered this value as individual temperature (Bakken, 1992; Lutterschmidt & Hutchison, 1997).

Finally, we measured CTmax in eight *L. llanensis* tadpoles (see Supporting Information S1 for details).

## 2.4 | Thermal sensitivity

To assess predictions ii, iii and iv, related to variations in TPCs and their parameters (B80, B95,  $T_o$ , and  $V_{max}$ ), we measured swimming velocity (locomotor performance) at different temperatures to

generate the TPCs. Following Bonino et al. (2020), we used a rectangular acrylic aquarium (0.43 m long  $\times$  0.07 m wide  $\times$  0.06 m high) as a “racetrack.” The swimming trials were performed at six different discrete water temperatures (test temperatures). These temperatures were selected within the critical thermal limits determined for the study species: 15°C, 20°C, 25°C, 30°C, 35°C, and 40°C. Before the trials, tadpoles were acclimated in individual buckets (300 mL) for 2 days under the same conditions as those used in thermal tolerance trials. During acclimation, we added cues (predator cues, alarm cues, and water in the control) to each of the 12 tadpoles from each treatment during 48 h.

Before the swimming trials, tadpoles were maintained at each one of the six mentioned test temperatures in individual thermal water bath containers (200 mL) for 2 h and 1 mL of predator cues, alarm cues, or water was added to the containers and racetrack, depending on the treatment. Then, tadpoles were placed at one extreme of the racetrack and gently stimulated to swim with a plastic rod. Swimming velocity trials were performed on consecutive days by randomly assigning two temperatures per day with a 2 h interval to allow tadpoles to rest. Swimming velocity of tadpoles was determined by recording tadpoles with a video camera GoPro Hero Silver at 25 frames per second, with narrow mode to reduce lens distortion. We estimated maximum swimming velocity at each temperature through video analysis using Tracker V5.0.1 (Copyright © 2018 Douglas Brown, <https://physlets.org/tracker/>).

## 2.5 | Data analysis

Variations in thermal tolerances (prediction i) were compared between species and among treatments within species with ANOVA including size (SVL) of tadpoles as a covariate.

To evaluate variations in TPC shapes and thermal sensitivity parameters (predictions ii, iii and iv), we fit thermal performance curves with Generalized Additive Mixed Models (GAMMs) using the “mcgv” package (Pedersen et al., 2019) in R software (R Development Core Team, 2020). Maximal swimming velocity ( $V_{max}$ ) was the response variable, whereas temperature, species and treatment were considered fixed effect factors, and size was included as a covariable, since thermal parameters are often related to body size (Claunch et al., 2021). We also considered each tadpole as a random effect factor and then the mixed effects structure with Akaike Information Criterion (AICc). The GAMM approach allowed us to fit the nonlinear relationship between temperature and  $V_{max}$  with a smoother function, while also considering interindividual variability (Cecchetto et al., 2020). We estimated species' thermal physiological parameters optimal temperature ( $T_o$ ), maximal performance ( $V_{max}$ ), and thermal breadth (B80 and B95), from the TPCs, using CTmax and CTmin as the extreme values (see Bonino et al., 2020 for details) of the TPCs, where performance is zero. To evaluate the effect of risk cues (predator and alarm cues) on thermal parameters, we performed ANOVAs considering,  $V_{max}$  and performance ranges (B80 and B95) as dependent variables and then we performed a Tukey post hoc test.

## 2.6 | Ethical statement

Ethical clearance was obtained from the Institutional Commission for Use and Care of Laboratory Animals (Acta no. 21/2018, CICUAL, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba). Animals were captured with authorization from the Wildlife Service of the Province of Córdoba (Permit #1580260111-821), signed by Dr. Paula Moggi, Director of the Wildlife Service of the Province of Córdoba.

## 3 | RESULTS

### 3.1 | Thermal tolerance

Considering all treatments together, the comparison of CTmax between *P. guayanae* and *P. nebulosum* (mean  $\pm$  SE 45.21°C  $\pm$  0.40°C and 45.00°C  $\pm$  0.40°C, respectively), did not show significant differences between species (ANOVA,  $F_{1,53} = 3.81$ ;  $p = 0.06$ ; see Supporting Information S1: Table S1). Mean  $\pm$  SE of CTmin differed significantly between species (ANOVA,  $F_{1,57} = 133.72$ ;  $p < 0.05$ ), with *P. nebulosum* showing lower values (5.00°C  $\pm$  0.77°C) than *P. guayanae* (5.90°C  $\pm$  1.06°C). Regarding thermal tolerances within species, CTmin and CTmax of *P. guayanae* did not vary among treatments; however, CTmin values were significantly different among treatments for *P. nebulosum* (Table 1). A Tukey honestly significant difference (HSD) post hoc test showed that CTmin was higher in tadpoles exposed to alarm cues than in control tadpoles (Supporting Information S1: Table S2). Mean  $\pm$  SE of CTmax recorded in *L. llanensis* was 45.64°C  $\pm$  0.36°C (Supporting Information S1: Table S3).

### 3.2 | Thermal sensitivity

According to the comparison of AICc model fit improved when interindividual variation was considered by including individuals as a random effect (i.e., mixed structure models; see Supporting Information S1: Table S4). The GAMM fit for the TPCs showed a significant effect of the smoothing term on temperature ( $F_{2,99,3} = 307.96$ ,  $p < 0.05$ ) and the covariable size showed statistical significance ( $p < 0.05$ ). There was a significant difference between species in GAMM fits ( $F_{6,27,6.85} = 12.12$ ,  $p < 0.001$ ; see Supporting Information S1: Table S4). The model for *P. guayanae* explained 95% of deviance, whereas the *P. nebulosum* model explained 92.4% of the deviance (see Supporting Information S1: Table S5). TPC fits with GAMMs showed that the TPCs of treatments (predator cues and alarm cues) were significantly different from TPCs in both species (Figure 1, and Supporting Information S1: Table S5).

The comparison of thermal parameters among treatments within species showed similar trends in *P. guayanae* and *P. nebulosum* (Table 2).  $V_{max}$  showed to be significantly different among treatments in each species (Table 2) and control tadpoles showed the highest  $V_{max}$  value in both species. Tukey HSD post hoc test

**TABLE 1** Means  $\pm$  SE for thermal tolerances (CTmax and CTmin) for *Pleurodema* species and treatments (Control, Predator cues and Alarm cues). Shown are the resulting ANOVAs comparing among treatments for both *Pleurodema* species with SVL as a covariate.

|                             | Control          | Predator cues    | Alarm cues       | ANOVA table |         |         |             |
|-----------------------------|------------------|------------------|------------------|-------------|---------|---------|-------------|
|                             |                  |                  |                  | df          | F value | p Value |             |
| <i>Pleurodema guayapae</i>  |                  |                  |                  |             |         |         |             |
| CTmax                       | 45.32 $\pm$ 0.36 | 45.20 $\pm$ 0.56 | 45.10 $\pm$ 0.23 | Treatment   | 2       | 0.58    | 0.57        |
|                             |                  |                  |                  | SVL         | 1       | 0.1     | 0.76        |
|                             |                  |                  |                  | Residuals   | 24      |         |             |
| CTmin                       | 6.39 $\pm$ 1.40  | 5.78 $\pm$ 0.61  | 5.52 $\pm$ 0.91  | Treatment   | 2       | 22,194  | 0.13        |
|                             |                  |                  |                  | SVL         | 1       | 18,495  | 0.19        |
|                             |                  |                  |                  | Residuals   | 26      |         |             |
| <i>Pleurodema nebulosum</i> |                  |                  |                  |             |         |         |             |
| CTmax                       | 45.13 $\pm$ 0.25 | 44.88 $\pm$ 0.52 | 44.98 $\pm$ 0.40 | Treatment   | 2       | 0.78    | 0.47        |
|                             |                  |                  |                  | SVL         | 1       | 0.09    | 0.77        |
|                             |                  |                  |                  | Residuals   | 23      |         |             |
| CTmin                       | 4.62 $\pm$ 0.46  | 4.91 $\pm$ 0.93  | 5.53 $\pm$ 0.61  | Treatment   | 2       | 40,665  | <b>0.03</b> |
|                             |                  |                  |                  | SVL         | 1       | 14,342  | 0.24        |
|                             |                  |                  |                  | Residuals   | 25      |         |             |

Note: Significant results appear in bold ( $p < 0.05$ ).

indicated different performance between risk treatments (alarm cues vs. predator cues) in both species (Table 3). Thermal breadths (B80 and B95) were significantly different among control and both risk cues in both species (see Tukey HSD post hoc test in Table 3), with wider performance breadth (B80) in risk treatments. However, the thermal breadth B95 was narrower when tadpoles were exposed to risk cues.  $T_o$  of tadpoles in the control treatment was similar in both species; however, considerably higher values for this parameter were observed in both species when they were exposed to risk treatments, particularly when they received alarm cues (Table 2). *P. guayapae* tadpoles exposed to alarm cues showed  $T_o$  between 3°C and 4°C higher than control treatment. Similarly, *P. nebulosum* showed  $T_o$  between 2.6°C and 3.3°C higher in risk treatments than in the control treatment (Table 2).

## 4 | DISCUSSION

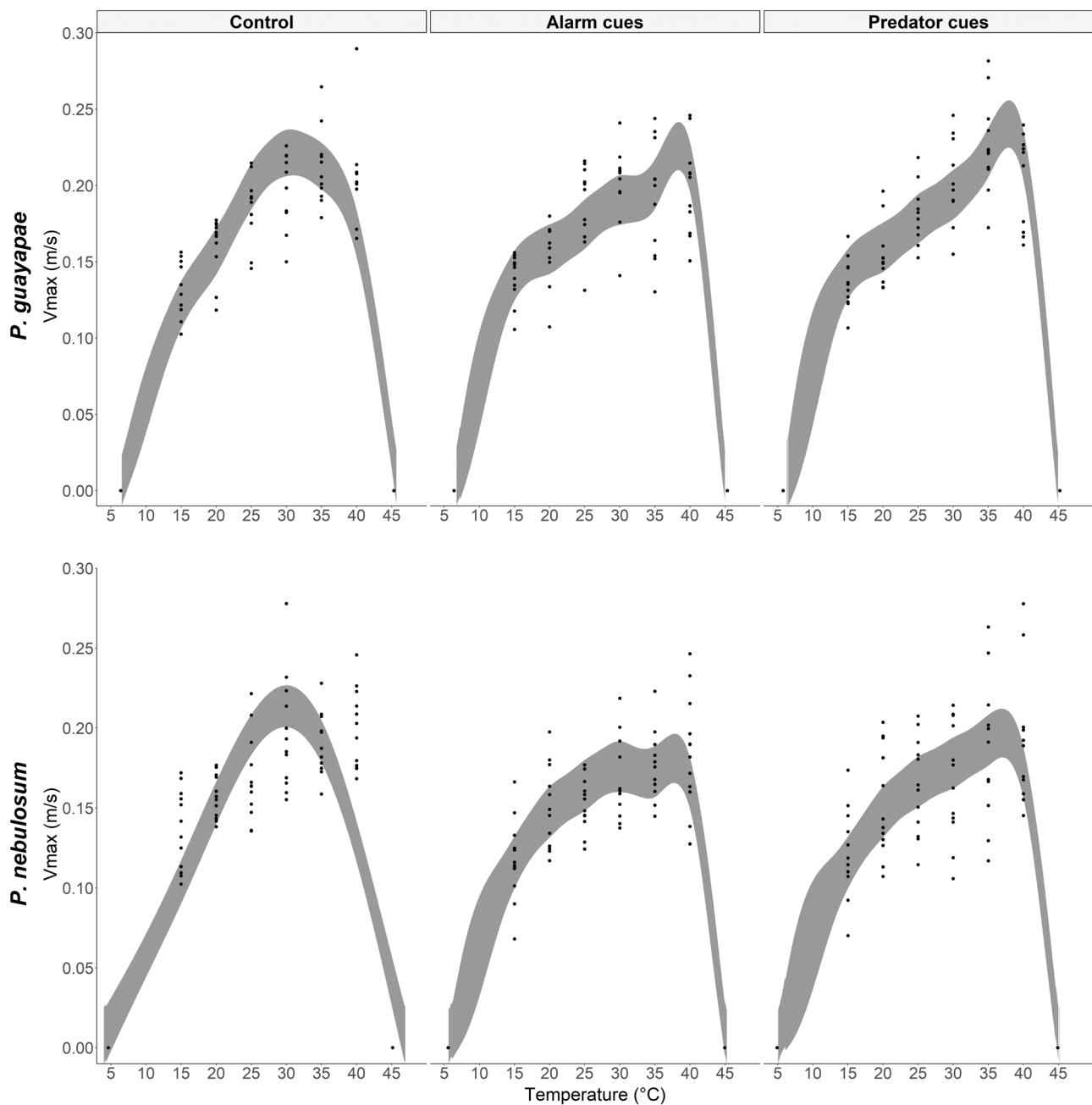
*P. guayapae* and *P. nebulosum* showed high CTmax values (Table 1), similar to the maximum tolerance limits observed in subtropical anurans from related habitats and latitudes (Duarte et al., 2012). However, contrary to previous studies in which CTmax was affected by the presence of predator cues (Katzenberger et al., 2014), we found no effect of predator cues and alarm cues on the maximum tolerances of these two *Pleurodema* species. Moreover, the predator *L. llanensis* showed similar CTmax to that of both *Pleurodema* species. The high values of CTmax found in these three species are consistent with the extremely high temperatures recorded in the puddles where they occur (40°C, Pers. Obs.). Due to the increasing temperature of

warm aquatic environments, ectothermic organisms are exposed to a range of temperatures that exceed optimal conditions for physiological processes, facing plasticity constraints (Gunderson & Stillman, 2015; Somero, 2010). In molecular terms, heat has destabilizing effects on membranes while it worsens enzymatic function (Angilletta, 2009; Herrando-Pérez et al. (2020); Bennett et al., 2021), which could set a limit across species. These factors may explain the high thermal tolerance limits we found in these amphibian species which, as ectotherms, depend on the habitat characteristics (Duarte et al., 2012; Sanabria et al., 2021).

There was no mismatch between predator and prey tadpoles at the higher limit of tolerances since both *Pleurodema* species showed similar CTmax to that of *L. llanensis*. These results contrast with those summarized by Pintanel et al. (2021) in a predator-prey system (dragonfly-tadpoles) and other examples cited there (Coombes & Bale, 2014; de Mira-Mendes et al., 2019; Franken et al., 2018; Hughes et al., 2010; Monaco et al., 2016). Then, the analysis of thermal tolerances in predator-prey interactions might show contrasting results, depending on the specific characteristics of predators, which ultimately affects the dynamic of this interaction (Pintanel et al., 2021). A possible explanation to our results might be that the three species are already experiencing temperatures close to their CTmax, considering that the ephemeral puddles where they are present are highly exposed to sunlight and elevated temperatures (Cei, 1955; Lescano et al., 2018; Sanabria et al., 2021).

As predicted, when both species were exposed to risk cues, the shape of the TPCs showed an increase in the optimal temperatures, along with changes in the performance curves (Figure 1, Table 2). *P. guayapae* showed higher optimal temperatures, almost 3°C higher





**FIGURE 1** Thermal performance curves of maximum swimming velocity (m/s) as a function of temperature ( $^{\circ}\text{C}$ ) for *Pleurodema guayanae* and *Pleurodema nebulosum* for each risk treatment (Control, alarm cues and predator cues), with global smoothing line obtained after Generalized Additive Mixed Model.

than the control, whereas in *P. nebulosum* optimal temperatures were  $2.6^{\circ}\text{C}$  and  $3.3^{\circ}\text{C}$  higher in predator cues and alarm cues treatments than in control tadpoles. Nevertheless, contrary to predicted by the hotter is better hypothesis (Angilletta et al., 2010), in treated tadpoles  $V_{\text{max}}$  of experimentally treated tadpoles was the same as or even lower than that in control tadpoles, suggesting thermal adaptation to the particular environmental conditions that amphibians face in these hot environments. Katzenberger et al. (2014) found that  $T_{\text{o}}$  was higher when tadpoles of *Hyla versicolor* are exposed to predator cues than in control tadpoles, and proposed that such changes might help

tadpoles to manage predation threats under warmer environmental conditions. Elevated  $T_{\text{o}}$  may allow tadpoles to explore warmer thermal patches that could be more favorable, in comparison to preferences displayed by predators (Gvoždík et al., 2013); however, more empirical tests are necessary to corroborate this prediction. The studied *Pleurodema* species have a short time to reach metamorphosis (Lescano & Miloch, 2023). Thus, their higher  $T_{\text{o}}$  observed when tadpoles are exposed to several predatory risks is a potential advantage, since it allows them to allocate energy and accelerate metabolic processes such as development and growth, reaching

|                             | Control      | Predator cues | Alarm cues   | F     | p Value         |
|-----------------------------|--------------|---------------|--------------|-------|-----------------|
| <i>Pleurodema guayapae</i>  |              |               |              |       |                 |
| B80                         | 14.56 ± 0.34 | 16.47 ± 0.55  | 18.37 ± 0.58 | 158.8 | <b>&lt;0.05</b> |
| B95                         | 6.83 ± 0.14  | 4.03 ± 0.01   | 3.46 ± 0.12  | 2388  | <b>&lt;0.05</b> |
| Vmax                        | 0.23 ± 0.01  | 0.23 ± 0.01   | 0.22 ± 0.01  | 5.253 | <b>&lt;0.05</b> |
| T <sub>o</sub>              | 34.93        | 37.89         | 38.44        | -     | -               |
| <i>Pleurodema nebulosum</i> |              |               |              |       |                 |
| B80                         | 11.53 ± 0.43 | 13.03 ± 1.22  | 17.58 ± 1.73 | 76.77 | <b>&lt;0.05</b> |
| B95                         | 5.14 ± 0.16  | 4.65 ± 0.24   | 4.1 ± 0.19   | 80.71 | <b>&lt;0.05</b> |
| Vmax                        | 0.22 ± 0.01  | 0.21 ± 0.02   | 0.19 ± 0.01  | 12.47 | <b>&lt;0.05</b> |
| T <sub>o</sub>              | 34.53        | 37.14         | 37.89        | -     | -               |

Note: Significant results appear in bold ( $p < 0.05$ ).

**TABLE 3** Post hoc Tukey honestly significant difference (HSD) results comparing thermal sensitivity parameters among treatments.

| Parameter                   | Contrast   | Estimate | SE   | df | t ratio | p Value         |
|-----------------------------|------------|----------|------|----|---------|-----------------|
| <i>Pleurodema guayapae</i>  |            |          |      |    |         |                 |
| B80                         | Control-AC | -3.99    | 0.15 | 29 | -27,271 | <b>&lt;0.05</b> |
|                             | Control-PC | -2.02    | 0.15 | 29 | -13,986 | <b>&lt;0.05</b> |
|                             | AC-PC      | 1.97     | 0.14 | 29 | 13,674  | <b>&lt;0.05</b> |
| B95                         | Control-AC | 3322     | 0.04 | 29 | 88,751  | <b>&lt;0.05</b> |
|                             | Control-PC | 2773     | 0.04 | 29 | 74,987  | <b>&lt;0.05</b> |
|                             | AC-PC      | -0.55    | 0.04 | 29 | -14,898 | <b>&lt;0.05</b> |
| Vmax                        | Control-AC | 0.96     | 0.45 | 30 | 2144    | 0.10            |
|                             | Control-PC | -0.46    | 0.45 | 30 | -1033   | 0.56            |
|                             | AC-PC      | -1427    | 0.45 | 30 | -3177   | <b>0.01</b>     |
| <i>Pleurodema nebulosum</i> |            |          |      |    |         |                 |
| B80                         | Control-AC | -6.86    | 0.26 | 32 | -26,452 | <b>&lt;0.05</b> |
|                             | Control-PC | -2.32    | 0.26 | 32 | -8933   | <b>&lt;0.05</b> |
|                             | AC-PC      | 4.54     | 0.25 | 32 | 18,360  | <b>&lt;0.05</b> |
| B95                         | Control-AC | 1.03     | 0.08 | 33 | 12,700  | <b>&lt;0.05</b> |
|                             | Control-PC | 0.49     | 0.08 | 33 | 6,043   | <b>&lt;0.05</b> |
|                             | AC-PC      | -0.54    | 0.08 | 33 | -6,657  | <b>&lt;0.05</b> |
| Vmax                        | Control-AC | 2.99     | 0.60 | 33 | 4,990   | <b>0.05</b>     |
|                             | Control-PC | 1.41     | 0.60 | 33 | 2,350   | 0.06            |
|                             | AC-PC      | -1.58    | 0.60 | 33 | -2,640  | <b>0.03</b>     |

Note: Control, alarm cues (AC), predator cues (PC). Significant results are shown in bold ( $p < 0.05$ ).

advanced stages to leave dangerous ponds filled with predators and begin terrestrial life as soon as possible.

*P. nebulosum* tadpoles exposed to predator and alarm cues showed slightly lower values of Vmax than those of control tadpoles. One possible explanation may be attributed to the reduction of

**TABLE 2** Means ± SE of control, alarm cues and predator cues for thermal sensitivity parameters (B80, B95, T<sub>o</sub> and Vmax). ANOVA results comparing among treatments for both *Pleurodema* species.

activity to avoid predation, as it had previously been observed in other *Pleurodema* species (Perotti et al., 2016). Whether this is a direct response to predation risk, a decision to conserve energy or some other physiological constraint needs more empirical support. Furthermore, we observed that when exposed to risk cues, both species changed their performance breadths: they decreased their optimal temperature range (B95), suggesting that risk situations may affect optimal efficiency; however, both species increased the B80 range, showing a broader performance. The lower Vmax and wider B80s observed in tadpoles exposed to predator and alarm cues than those of control tadpoles suggest a “generalist-specialist” trade-off, although more data analyses are required to confirm this hypothesis (Gilchrist, 1995; Angilletta et al., 2010; Izem & Kingsolver, 2005; Richter-Boix et al., 2015). Nevertheless, the evidence of wide TPCs in these two *Pleurodema* tadpoles when they are at risk of predation (in comparison with controls) partially agree with the thermal game model proposed by Mitchell and Angilletta Jr. (2009), in which predators modify their prey's TPCs, favoring thermal generalists.

The General Additive Mixed Models allowed us to improve the model fit through the inclusion of interindividual variation by adding individuals as a random effect. It has been recognized that there is a source of variation in reaction norms provided by phenotypic plasticity at the individual-level in several taxonomic groups (Nussey et al., 2007; Artacho et al. 2013, Cecchetto et al., 2020). It has become increasingly clear that the traditional approach of treating all individuals as homogeneous and assuming they respond equally to environmental variables can lead to biased results and even erroneous conclusions. This implies that additional factors, such as genetic variation, metabolic rate, or developmental history may also contribute to the observed interindividual differences in tadpole swimming ability. Ignoring such variations could result in an incomplete understanding of the underlying mechanisms that drive the observed patterns. Here, we support the assumption that interindividual variation needs to be considered when comparing TPCs between treatments or species.

Finally, our study contributes to the general knowledge of thermal physiology with the inclusion of the biological interactions occurring in ponds. We provide evidence of the thermal biology of two subtropical tadpole species in the context of their interaction with predators. We found that predation risk can alter the thermal physiology of tadpoles, by modifying their optimal (B95) and performance (B80) ranges, testing with different signals of predation risk (predator cues and alarm cues from conspecifics), and we determined that the thermal physiological responses of prey varied as a function of the risk signal. When thermal physiological responses are evaluated to predict the vulnerability of organisms, considering variables such as prey and predator behaviors mediated by temperature can be relevant (Mitchell & Angilletta Jr., 2009), especially since swimming velocity undoubtedly plays an important role in predator–prey interactions.

## 5 | CONCLUSION

Understanding how ectotherms respond to variations in environmental temperature is crucial for predicting their future responses. In this work, we contribute to the understanding the behavior of thermal parameters (thermal sensitivity and tolerances) within the context of predator–prey dynamics. We found that prey modify thermal sensitivity parameters when they are at risk of death. However, the relationship between biotic interactions and physiological responses is still very uncertain (de Mira-Mendes et al., 2019; Katzenberger et al., 2014).

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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