BRIEF COMMUNICATION

Geography, Temperature, and Water: Interaction Effects in a Small Native Amphibian

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ABSTRACT

Amphibian locomotor capacity is strongly linked to temperature and hydration. However, organisms in nature experience covariation of multiple environmental factors, and thus to better understand the effects of thermal and hydric conditions on physiological performance, it is critical not only to experimentally disentangle them but also to incorporate potential interactive effects due to geographic variation. To this end, we selected two populations of the small amphibian *Pleurodema thaul* inhabiting highly contrasting temperatures and precipitation regimens. With these two populations, we evaluated the thermal and hydric sensitivities of locomotor performance. For both factors, performance increased with temperature as well as with hydration level, although performance reached a plateau between 25° and 30°C. In addition, the influence of dehydration on performance was independent of the temperature at which it was tested. Our results also showed that the population from the warmer environment has lower sensitivity of locomotor performance to dehydration, probably as a consequence of thermal adaptation, although further studies might be required to fully understand this.

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Keywords: Chile, dehydration, four-eyed frog, performance, temperature.

Introduction

Environmental temperature (T_a) and water availability are critical factors influencing most aspects of organismal biology, from the basic conditions for biochemical reactions to the abundance and distributional limits of species (Angilleta 2009; McKinley et al. 2018; Sears et al. 2016). In addition, both factors are important agents of selection, driving the evolution of phenotypic traits that allow organisms to cope with particular combinations of environmental conditions (Rozen-Rechels et al. 2019).

Although T_a is considered to be the factor with the largest impact on the physiology and ecology of most of Earth's biodiversity (Angilletta 2009), ectotherms adjust the temperature they select in their environment depending on the available water and on their particular resistance to water loss (Angilletta 2009). In this context, the highly permeable skin of amphibians makes them extremely sensitive to changes in both T_a and moisture levels (Tracy et al. 2014; Mitchell and Bergmann 2016; Anderson and Andrade 2017). Nevertheless, the impact of future climate warming on the thermal tolerance and sensitivity of ectotherms has received much more attention than the potential impact of climate warming on the water budget and tolerance to dehydration of organisms (Huey et al. 2012; Gunderson and Stillman 2015; Pinsky et al. 2019). In addition, although it is known that T_a and hydric state have profound impacts on overall amphibian locomotor performance (Titon et al. 2010; Prates et al. 2013; Mitchell and Bergmann 2016; Anderson and Andrade 2017), those impacts are not always direct (i.e., a reduced locomotor performance with lower T_a's and/or higher dehydration; e.g., Prates et al. 2013). That is, interactive effects between them usually occur, and therefore both factors should be simultaneously considered to understand the general influence each might have on performance (Navas et al. 2008; Williams et al. 2008; Titon et al. 2010; Anderson and Andrade 2017).

Ectothermic species with broad geographical distributions usually display extensive phenotypic variation (Lardies et al. 2004, 2010; Hoffmann and Sgro 2011; Barría et al. 2014, 2017; Gaitán-Espitia et al. 2014). This variation is especially pervasive along geographic clines (e.g., latitude) and reflects, at least partially, the capacity of populations to adapt to their environmental conditions but in particular to T_a, which has been considered to be a main

agent of selection (Fox and Czesak 2000; Ayrinhac et al. 2004; Kingsolver et al. 2004; Mitchell-Olds et al. 2007). Notwithstanding the importance of T_a as a driver of phenotypic change, organisms in nature experience covariation of multiple environmental factors. Therefore, to better understand the effects of thermal and hydric conditions on physiological performance, it is critical not only to experimentally disentangle them but also to incorporate potential interactive effects due to geographic variation.

To this end, we use the four-eyed frog, *Pleurodema thaul*, as our study model. This is a small terrestrial amphibian species endemic to Chile and Argentina, with a distributional range of more than 2,500 km from the Atacama Desert to Aysén and from the Pacific coast to 2,700 m asl (Correa et al. 2007). In its extensive latitudinal distribution, *P. thaul* occupies a broad diversity of biomes, from highly arid in the north to the Valdivian rain forest in the south (Werner et al. 2018). From its large latitudinal range, we selected two populations with highly contrasting environmental temperatures and precipitation regimens (table 1): Carrera Pinto, an oasis in the Atacama Desert that represents the northern limit of the distribution, and El Caulle, about 1,500 km south of Carrera Pinto, in the middle of the Valdivian rain forest and near the center of the current distribution of the species (Barría and Bacigalupe 2017; Bacigalupe et al. 2018). Although these populations of *P. thaul* experience daily and seasonally contrasting patterns in temperature and precipitation, water availability is not different between them, mainly because the desert population inhabits an oasis with a permanent pond. Frogs in the desert are active and aboveground 365 days a year, retreating to the pond to cool off or to hydrate on a daily basis during the nonreproductive period. For these two populations, we evaluated the thermal and hydric sensitivities of locomotor performance by measuring maximum jumping speed. This is a well-known trait of ecological relevance to amphibians in general (Navas et al. 2007), and for *P. thaul* in particular, it represents the primary mode of locomotion, predator escape, and foraging. On the basis of previous evidence suggesting that there is geographic variation in performance (Barría and Bacigalupe 2017) and plasticity in the parameters of the thermal performance curve (Ruiz-Aravena et al. 2014; Barría and Bacigalupe 2017), we hypothesize that (i) performance will similarly decrease with temperature in both populations (i.e., the interaction of temperature and population is not significant) and (ii) the population from the desert will have lower sensitivity of locomotor performance to dehydration (i.e., the interaction between population and hydration).

Material and Methods

Populations and Laboratory Maintenance

We collected 41 individuals of *Pleurodema thaul* in December 2014 at Carrera Pinto (−27.10788, −69.90726), an oasis in the Atacama Desert where the northernmost population of the species inhabits (Correa et al. 2007; fig. 1). We collected 27 individuals in November 2014 from El Caulle (−40.65074, −72.17262), approximately in the center of the current distribution of the species and 1,500 km south of Carrera Pinto (fig. 1). All individuals were transported to the Universidad Austral de Chile (Valdivia)

Table 1: Climatic information from 1985 to 2005 for the localities used in this study

	Carrera Pinto	El Caulle
T_{max} (°C)	19.6 ± .01	13.0 ± .01
T_{min} (°C)	7.4 ± .01	4.8 ± .01
T_{mean} (°C)	13.1 ± .01	8.5 ± .01
Precipitation (mm)	21.3 ± .19	2,291.0 ± .03
RH (%)	48.6 ± .06	82.6 ± .03

Source. http://simulaciones.cr2.cl.
Note. Data are presented as mean ± SD. T_{max} = annual mean maximum temperature; T_{min} = annual mean minimum temperature; T_{mean} = annual mean temperature; RH = relative humidity.

within 2 or 3 d of capture and were housed in terraria (length × width × height: 40 cm × 20 cm × 20 cm) provided with a cover of moss, vegetation, and a small bowl filled with water. Animals were maintained at a temperature of 18° ± 1°C with a photoperiod of 12L : 12D and were fed once a week with mealworms (*Tenebrio molitor* larvae) and Mazuri (St. Paul, MN) gel diets. All measurements were conducted according to current Chilean law, and the protocols we used were approved by the Committee on the Ethics of Animal Experiments of the Universidad Austral de Chile.

Experimental Design and Performance

After 1 mo at maintenance conditions, individuals were randomly assigned to three different measurement temperatures: 20°C (Carrera Pinto, $N = 14$; El Caulle, $N = 9$), 25°C (Carrera Pinto, $N = 14$; El Caulle, $N = 9$), and 30°C (Carrera Pinto, $N = 13$; El Caulle, $N = 9$). At each temperature, the performance of each individual was measured when fully hydrated (100% body mass) and when hydrated at 90% and 75%–80% of their initial body mass. Every day, we measured performance at a randomly chosen combination of measurement temperature and hydration level, after which individuals were allowed to rest for 3 d at maintenance conditions. The order of hydration treatments was completely randomized among individuals.

To standardize hydration levels before performance trials, individuals were maintained for 45 min in bioclimatic chambers at the particular measurement temperature of the individual in hermetic cases with approximately 10 mm of water. We confirmed that each individual reached the desired body temperature by measuring its dorsal temperature using a UEi INF155 Scout 1 infrared thermometer (for details, see Ruiz-Aravena et al. 2014). Individuals were patted dry with a paper towel and stimulated to urinate before trials through manual pressure of their lower abdomen, and their hydrated body mass (i.e., 100% hydration) was recorded with a Shimadzu TX323L electronic balance (Shimadzu, Kyoto, Japan). In order to obtain the different hydration treatments, we used a manual hair dryer in its cool mode (average speed: 3.5 m/s; 20°C) for 30 s to 1 min until individuals reached the desired hydrated percentage of their initial body mass (i.e., 90% and 75%–80%). We confirmed that each individual was still at the desired body temperature using an infrared thermometer, after which performance was immediately measured.
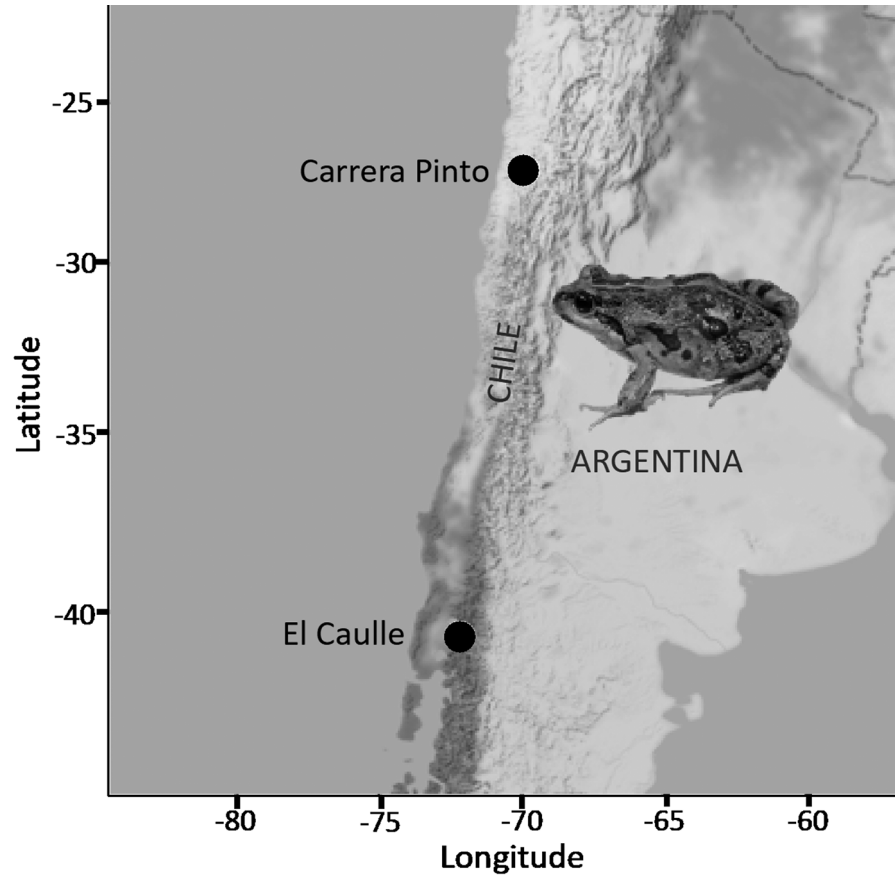
Figure 1. Geographic distribution of *Pleurodema thaul*.

Performance trials were run within the bioclimatic chamber at the specific measurement temperature (20°, 25°, or 30°C) on a metallic track (length × width × height: 75 cm × 12 cm × 20 cm). Each individual was allowed to explore the track for a couple of minutes before it was positioned on one end of the track. Its velocity was registered with an Anytime XL-018 professional timer stopwatch. It was motivated to jump-run by gentle touching of its dorsal-caudal body region until it reached the other end of the track. Performance was measured as the time needed for an individual to reach the end of the track and was measured twice per individual at each hydration level, with measurements of the same individual at the same hydration level 15 min apart. The individual performance at that hydration level was the average of the two measurements. Body size was obtained after each trial using a Shimadzu TX323L electronic balance, while body length was obtained using a digital calliper to correct velocities by each individual's length. Body mass did not significantly change during the whole period of testing ($\chi_2^2 = 1.403$, $P = 0.496$).

Statistical Analyses

We used a linear mixed modeling approach employing restricted maximum likelihood to evaluate the effects of population, hydration levels, and temperature while taking into account that we had repeated measurements for individuals (i.e., at each temperature, individual performance was measured at different hydration levels). For all analyses, temperature, hydration, and population were set as fixed factors, and the identity of each individual was included as a random factor. P values for fixed effects were obtained using the package lmerTest (Kuznetsova et al. 2017) with type III sums of squares based on Satterthwaite's approximation for denominator degrees of freedom. We performed analyses only with second-order interactions in order to better disentangle the effects of the fixed factors studied. Velocity was $\log_{10}$ transformed to meet normality assumptions. Statistical analyses were performed using the lme4 package (Bates et al. 2015) implemented in R version 3.4.2 (R Development Core Team 2013) with α at 0.05.

Results

Overall descriptive statistics for velocity, body mass, and repeatability of performance trials are presented in table A1. The interactions involving measurement temperature were both nonsignificant, albeit marginally (temperature × hydration: $F_{4,\,120.88} = 2.010$, $P = 0.097$; temperature × population: $F_{2,\,67.88} = 2.473$, $P = 0.092$). However, the interaction between population and hydration levels was highly significant ($F_{2,\,120.68} = 11.298$, $P < 0.001$) and resulted mainly from differences at the lowest hydration level,
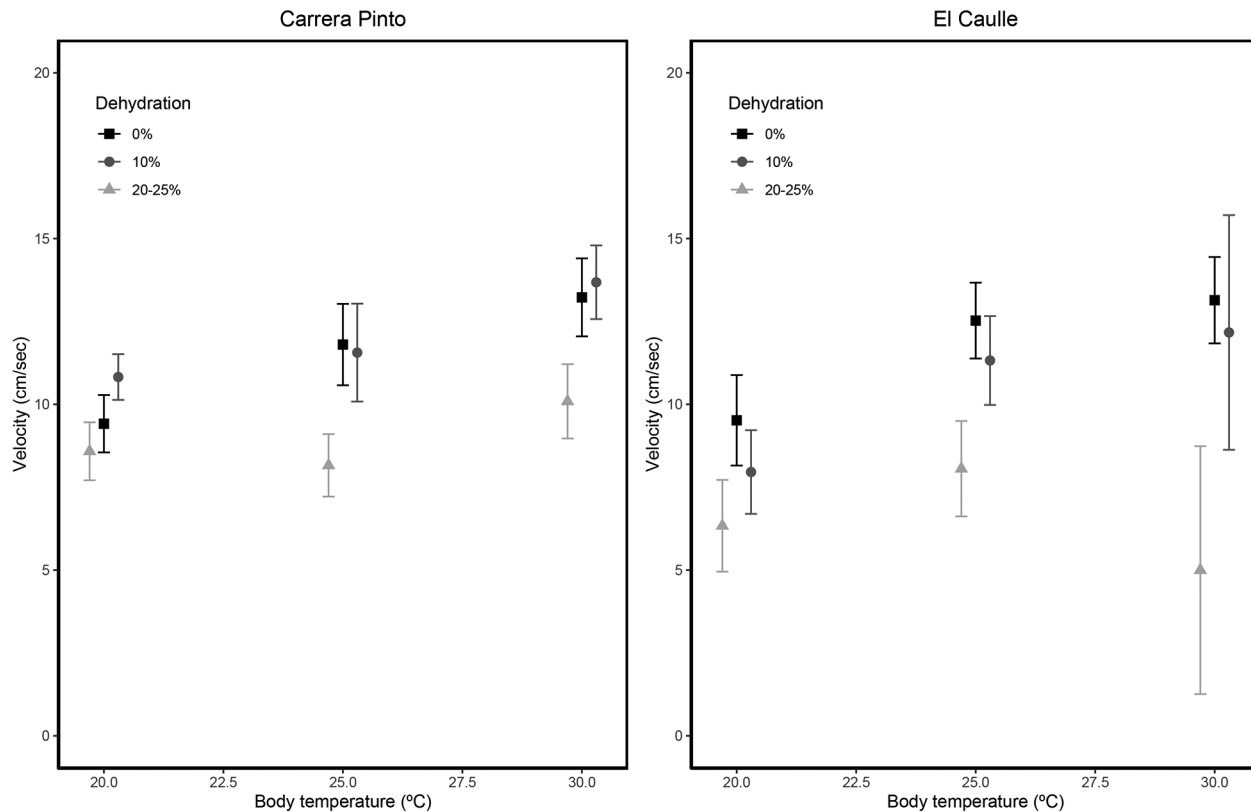
Figure 2. Locomotor velocity (cm/s) at three different measurement temperatures (20°, 25°, and 30°C) and at three hydration levels (fully hydrated [100% body mass], 10% dehydrated, and 20%–25% dehydrated) for two populations (Carrera Pinto: warmer; El Caulle: cooler) of *Pleurodema thaul*. Means are plotted using some jitter. Error bars show 95% confidence intervals. See "Material and Methods" for further details. A color version of this figure is available online.

where, in particular, performance was greatly reduced in individuals from El Caulle (fig. 2; table A1). In addition, performance increased with measurement temperature ($F_{2, 78.47} = 10.123$, $P < 0.001$) but only from 20° to 25°C, as it did not differ between 25° and 30°C (fig. 2).

Discussion

As has been reported for other terrestrial anurans, both temperature and hydration affected the locomotor performance of *Pleurodema thaul* (Titon et al. 2010; Prates et al. 2013; Mitchell and Bergmann 2016; Anderson and Andrade 2017). For both factors, performance increased with temperature as well as with hydration level, although performance reached a plateau between 25° and 30°C (Ruiz-Aravena et al. 2014). In addition, the influence of dehydration on performance was independent of the temperature at which it was tested (fig. 2). Although similar results have been reported for *Rhinella granulosa* (Prates et al. 2013), an interaction between hydration and temperature seems to be more prevalent in the literature. In particular, it has been shown that the drop in performance is more pronounced at lower hydration levels and higher temperatures (Preest and Pough 1989; Titon et al. 2010; Anderson and Andrade 2017), and thus this potential trade-off might be problematic if frogs are actually de-

hydrated in nature. This seems unlikely for our populations, as one inhabits an oasis with a permanent pond that is used daily by frogs to cool off and/or hydrate (Carrera Pinto) while the other in is the middle of a temperate rain forest (El Caulle). Therefore, this might explain why the interaction was not statistically significant. Nevertheless, as animals can reach similar levels of performance at various combinations of temperature and hydration levels (Walvoord 2003), behaviorally selecting lower temperatures and/or reducing activity levels can be used as a strategy to minimize the loss of performance (Tracy et al. 1993; Anderson and Andrade 2017) when water availability is compromised.

Our results also show that the population from the desert (Carrera Pinto) has lower sensitivity of locomotor performance to dehydration, in agreement with our predictions. In fact, although performance in both populations was reduced as hydration decreased, the drop in performance at the lowest hydration level was higher in individuals from El Caulle (fig. 2). To the best of our knowledge, this pattern of geographic variation in the effect of dehydration has been reported only at the species level (Beuchat el al. 1984; Titon et al. 2010). For example, for three *Rhinella* species, Titon et al. (2010) showed that the effect of hydration levels varied between species mostly because of their different seasonal patterns of reproductive activity, which determine the temperature at which they maintain their locomotor activity. That is, the

species show a pattern of thermal adaptation, and thus the effect of dehydration is more pronounced on those species inhabiting cooler environments but measured at higher temperatures. A similar argument can be made in our case, as these populations of *P. thaul* inhabit markedly different thermal environments, although possibly not very dissimilar hydric ones. Indeed, we have previously shown that their thermal tolerances and selected body temperatures are shifted toward higher values in Carrera Pinto (Barría and Bacigalupe 2017), which might be indicative of local adaptation to local thermal regimes, something that has been extensively reported for species broadly distributed (e.g., Angilletta et al. 2010; Gaitán-Espitia et al. 2014; Barría et al. 2018). However, we should also note that although individuals from both populations were sampled during the same dates and were maintained at the same laboratory conditions before the start of the experiments, these factors are insufficient for us to properly infer that the differences we found were because of local adaptation (Garland and Adolph 1994; Blanquart et al. 2013). Overall, these results predict that the El Caulle population should have higher rates of evaporative water loss and lower rates of water uptake or much more precise behavioral patterns associated with microhabitat selection.

Although a great body of literature has been produced on the impact of future climate warming on the thermal tolerance and sensitivity in ectotherms (e.g., Huey et al. 2012; Gunderson and Stillman 2015; Pinsky et al. 2019), our results strongly suggest that the impact of that warming on the overall water budget should not be left out in order to make better predictions regarding the fate of most ectothermal species (e.g., Titon et al. 2010; Prates et al. 2013; Mitchell and Bergmann 2016; Anderson and Andrade 2017). As temperature and water usually show interactive effects between them, evaluating their joint role in organism performance is therefore critical.

In summary, our results show that a decrease in both temperature and hydration negatively affect performance; the reduction was similar in both populations when considering temperature but was higher in individuals from El Caulle (southern population) when the dehydration level was highest, which might be a consequence of a thermal adaptation pattern. Nevertheless, further studies will be necessary to fully disentangle both effects.

Acknowledgments

APPENDIX

Table A1: Descriptive statistics (mean $\pm$ SD) for velocity and body mass for each combination of measurement temperature and dehydration level (0%, 10%, and 20%–25%)

Locality, temperature (°C)	0%	10%	20%–25%	0%	10%	20%–25%
	Velocity (cm/s)			Body mass (g)		
Carrera Pinto:						
20	9.41 ± 1.66	10.82 ± 1.81	8.58 ± 2.46	2.38 ± .38	2.36 ± .37	2.63 ± .33
25	11.80 ± 2.12	11.56 ± 3.27	8.16 ± 2.01	2.28 ± .46	2.36 ± .47	2.43 ± .51
30	13.23 ± 3.10	13.68 ± 3.48	10.09 ± 2.50	2.88 ± 1.02	2.74 ± .91	2.88 ± .093
El Caulle:						
20	9.52 ± 3.53	7.96 ± 4.37	6.34 ± 4.88	7.69 ± 4.02	7.54 ± 3.95	7.74 ± 4.10
25	12.53 ± 2.49	11.32 ± 3.59	8.05 ± 3.14	3.96 ± .74	3.87 ± .73	3.92 ± .66
30	13.14 ± 3.57	12.17 ± 7.98	5.00 ± 3.18	4.84 ± 2.60	4.91 ± 2.71	5.14 ± 2.72
	Repeatability			N		
Carrera Pinto:						
20	.218	.429	.487	14	14	14
25	.05	**.552**	.128	14	14	14
30	**.870**	.357	**.680**	13	13	13
El Caulle:						
20	.438	.593	**.853**	9	9	9
25	.223	.515	**.776**	9	9	9
30	.203	.523	**.880**	9	9	9

Note. Velocity means reported here are the same as those depicted in figure 2. Repeatability estimates between velocities registered 15 min apart were calculated as Pearson correlation coefficients. Estimates in boldface type are significant at 0.05.

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