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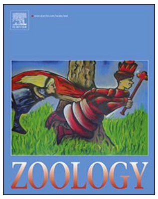

How sensitive are temperate tadpoles to climate change? The use of thermal physiology and niche model tools to assess vulnerability

María Gabriela Perotti[a,*], Marcelo Fabián Bonino[a], Daiana Ferraro[b], Félix Benjamín Cruz[a]

[a] *Laboratorio de Fotobiología, Instituto de Investigaciones en Biodiversidad y Medioambiente (INIBIOMA), CONICET-UNCOMA, Quintral 1250, Bariloche, Río Negro 8400, Argentina*

[b] *Laboratorio de Biodiversidad y Conservación de Tetrápodos, Instituto Nacional de Limnología (INALI-CONICET), Santa Fe, Argentina*

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ABSTRACT

Ectotherms are vulnerable to climate change, given their dependence on temperature, and amphibians are particularly interesting because of their complex life cycle. Tadpoles may regulate their body temperature by using suitable thermal microhabitats. Thus, their physiological responses are the result of adjustment to the local thermal limits experienced in their ponds. We studied three anuran tadpole species present in Argentina and Chile: *Pleurodema thaul* and *Pleurodema bufoninum* that are seasonal and have broad geographic ranges, and *Batrachyla taeniata,* a geographically restricted species with overwintering tadpoles. Species with restricted distribution are more susceptible to climate change than species with broader distribution that may cope with potential climatic changes in the environments in which they occur. We aim to test whether these species can buffer the potential effects of climate warming. We used ecological niche models and the outcomes of their thermal attributes (critical thermal limits, optimal temperature, and locomotor performance breadth) as empirical evidence of their capacity. We found that *Pleurodema* species show broader performance curves, related to their occurrence, while the geographically restricted *B. taeniata* shows a narrower thermal breadth, but is faster in warmer conditions. The modeled distributions and empirical physiological results suggest no severe threats for these three anurans. However, the risk level is increasing and a retraction of their distribution range might be possible for *Pleurodema* species, and some local population extinctions may happen, particularly for the narrowly distributed *B. taeniata*.

1. Introduction

Over recent decades temperature increase on a global scale has motivated researchers to study whether this warming scenario may affect biodiversity on a wide range of levels, from molecular (Hochachka and Somero, 2002) to ecosystemic (Pörtner, 2012). In relation to the interaction between climate and the physiology of organisms, different bioclimatic envelope models were developed and are often used to predict the future availability and distribution of suitable climate conditions for species (e.g. Peterson, 2001; Pearson et al., 2002; Gerick et al., 2014). Although these models provide a useful approximation of species' potential vulnerability to climate change (Pearson and Dawson, 2003), most of them contain no information on the mechanistic links between the species' current distribution ranges and climate. New mechanistic methods have recently been developed (Buckley, 2008; Kearney and Porter, 2009; Buckley and Kingsolver, 2012) and the combination of performance physiology with models provided new insights (Deutsch et al., 2008; Tewksbury et al., 2008; Huey et al., 2009; Kolbe et al., 2010; Sinervo et al., 2010; Angert et al., 2011; Thomas et al., 2012; Bonino et al., 2015a).

According to the available information, temperate and high-latitude ectotherms are less vulnerable in physiological terms to climate warming than tropical species, due to their broader thermal safety margins (Araújo et al., 2006; Deutsch et al., 2008; Tewksbury et al., 2008; Huey et al., 2009; Duarte et al., 2012; Tejedo et al., 2012; Araujo et al., 2013; Bonino et al., 2015a,b; Gutierrez-Pesquera et al., 2016). However, a recent study shows that some tropical species may not be as vulnerable to warming as previously predicted (Simon et al., 2015); Kingsolver et al. (2013) and Vasseur et al. (2014) have argued that temperate species may be the most vulnerable to climate warming, given their higher thermal variability in temperate zones (Sinclair et al., 2016). There is evidence of this in temperate organisms occurring in Patagonia, where an increase in environmental temperatures is probably not a severe threat to their upper thermal thresholds (e.g., Bonino et al., 2015b). However, it has been observed that when extreme temperatures are considered, species response differs drastically, even

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[*] Corresponding author.
E-mail address: mgperotti@comahue-conicet.gob.ar (M.G. Perotti).

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though biological mechanisms such as thermoregulation may operate to counterbalance the imposed risk of warming scenarios (Gerick et al., 2014; Buckley and Huey, 2016; Dillon et al., 2016).

Ectotherms are particularly vulnerable to climate change due to their natural dependence on environmental temperatures (Deutsch et al., 2008; Kingsolver, 2009; Bonino et al., 2011, 2015a,b). Among vertebrate tetrapods, amphibians are particularly interesting because of their complex life cycle related to different environments that may differ in temperature, since adults and larvae may occur in a range of environments. Perhaps because of this complexity, fewer studies have been carried out on this topic in anurans than in other tetrapods, especially in the case of contrasting climatic models with physiological performance (Duarte et al., 2012; Gouveia et al., 2013; Gerick et al., 2014). For this reason, available information on the thermal physiology of temperate amphibians is limited, and there are few studies that estimate climatic niches in regions characterized by harsh environmental conditions (e.g., high winds, low air temperatures and snowfall in winter) (Araújo et al., 2006; Gouveia et al., 2013; Blank et al., 2014; Gerick et al., 2014; Merilä and Hendry, 2014; Rueda Solano et al., 2016).

As mentioned before, amphibians are characterized by a complex life cycle, where the first stages of ontogeny are crucial for the persistence of an amphibian population, since they may be more vulnerable to increasing temperatures. This is because, unlike adults, tadpoles cannot thermoregulate in several ecological contexts. Most amphibians have highly permeable skin in both aquatic and terrestrial life stages, making them very sensitive to changes in temperature and precipitation (Winter et al., 2016). Small adult frogs are isothermal with water temperature; therefore, we assume that tadpoles behave isothermally with respect to their surrounding environment because of their small body size and the high thermal conductance of water (Lutterschmidt and Hutchison, 1997). The temperature of the water around tadpoles could thus be considered a good predictor of their body temperature. Despite this, tadpoles may regulate their body temperatures behaviorally (Hutchison and Dupré, 1992), by using suitable thermal microhabitats. These suitable microhabitats may be limited; for instance, when tadpoles are trapped in heated shallow ponds they are obliged to thermoconform (Balogová and Gvoždík, 2015). Thus, their physiological responses may be the result of adjusting to the local thermal extreme limits experienced in their ponds through thermal selection (Niehaus et al., 2012; Kern et al., 2015; Richter-Boix et al., 2015). In addition to long-term behavioral adjustments and thermal resistance, another important response to temperature change is the compensatory adjustment in physiological performance, also known as acclimation response (Prosser et al., 1991), which involves the ability to respond quickly to changes in the environment. A recent study suggests that acclimation decreases sensitivity to temperature and climate change in freshwater species (Seebacher et al., 2015).

We studied three anuran tadpole species that reach high latitude distribution in southern South America (Argentina and Chile): *Batrachyla taeniata* (Batrachylidae), with a distribution range restricted to *Nothophagus* forests in South America; *Pleurodema bufoninum* (Leptodactylidae), which occurs throughout most of Argentinian Patagonia (Ferraro and Casagranda, 2009); and *Pleurodema thaul* (Leptodactylidae), which has a long latitudinal range from 18°S in Chile (Capurro, 1950; Correa et al., 2010) to southern Chile and Argentina, mainly associated with forests and ecotonal zones, and with occasional records in the Patagonian steppe (Ortiz and Díaz-Páez, 2006; Ferraro and Casagranda, 2009). We studied whether these species can buffer the potential impact of climate warming by using environmental niche models (ENMs) to attain projections of the future distribution of these species. We combined these with the outcomes of their physiological thermal attributes, as evidence of their capacity to cope with future climatic conditions. A key factor in determining a species' vulnerability to climate change is to measure its ability to cope with limiting temperatures through plastic or evolutionary responses (Williams et al., 2008; Hoffmann et al., 2012). We focused on two main objectives: (1) to describe the thermal limits (CTmax = critical thermal maximum, CTmin = critical thermal minimum), the temperature at which performance is maximal (To), and the locomotor performance breadths at two levels (B80 and B95) of the anuran tadpole species studied here; and (2) to evaluate their vulnerability to heat impact through examination at distributional range level through ecological niche model (ENM) indicators. We were interested in the percentage of the projected distribution area within which these organisms may experience (i) environmental temperatures exceeding their lethal temperatures (above CTmax) in current and future scenarios, (ii) suboptimal temperatures outside their thermal performance breadth (B80), and (iii) a more conservative approach, which is at temperatures above their optimal temperature range (B95). We expect that species with a wider distribution range will show broader thermal physiology responses (broader critical thermal limits and broader locomotor performance breadths); in addition, in future climate change scenarios, species with wider distribution will not show important losses of area in terms of area percentage; finally, latitudinal distribution range will be reflected in the thermal biology of the species. *Pleurodema* species in this study correspond to the more austral species of the genus (*P. bufoninum* and *P. thaul*), and in particular, *Pleurodema* species belong to a Neotropical genus (15 species) that has a broad distribution ranging from tropical and subtropical areas to Patagonia (Faivovich et al., 2012). This broad distribution range makes both *Pleurodema* species less vulnerable to global climate change in comparison with more restricted species, such as *B. taeniata*. Finally, we explored whether the proportion of threatened areas in different climate scenarios shows differences within and among the studied species. Our main questions are: (a) Are distributional ranges related to thermal breadth limits and performance breadths in these temperate anuran tadpoles? (b) Do the species distributed at higher latitude show lower performance values than the species at lower latitudes? (c) Are the narrowly distributed species more vulnerable according to ENM? (d) Are ENM results and thermal physiology congruent?

2. Materials and methods

2.1. Anuran species

Pleurodema thaul and *P. bufoninum* (Leiuperidae) are two closely related species found only in Argentina and Chile (Ferraro and Casagranda, 2009). These species breed primarily in ephemeral and temporary habitats, although they also reproduce occasionally in permanent habitats (Jara and Perotti, 2010). On the southern edge of their range the reproductive season starts during the austral spring and extends until early summer. *P. thaul* inhabits mostly large vernal ponds and flood meadows in the forest, which vary in permanence from year to year, whereas *P. bufoninum* is a typical steppe species, inhabiting temporary and ephemeral Patagonian flood meadows called 'mallines'. Eggs of both species are deposited in gelatinous strings in water, sometimes forming globular masses among the aquatic plants (Duellman and Veloso, 1977; Cei, 1980; Jara and Perotti, 2010). *B. taeniata* (Batrachylidae) is commonly found in Valdivian forests and swamps of Chile and Argentina (Pisano, 1956; Di Castri, 1968). Reproductive events start in autumn and external fertilization takes place on land with oviposition sites in natural hollows in damp grass (Díaz et al., 1987). Eggs are laid in a cluster, usually several meters distant from available water. Development at early embryological stages is intracapsular, with parental care, and duration depends on water availability (Úbeda and Nuñez, 2006). Larvae and metamorphosis occur in the wetland approximately two months after hatching (Díaz et al., 1987) and larval stage duration may exceed a year, including overwintering tadpoles.

Tadpoles of the three species were collected with the approval of the Subsecretaria de Medioambiente of San Carlos de Bariloche City (SMA-

SCB) and the Administración de Parques Naciones (APN), Argentina (permit numbers: 41-AP-2014, APN 498 and APN 1484). This investigation was performed under the institutional animal care guidelines established by SMA-SCB and APN.

Before the trials, tadpoles were acclimated in individual 1 liter buckets for 4 days at a uniform temperature of 20 °C with a photoperiod of 12L:12D, and food was offered ad libitum. The acclimation temperature chosen was based on previous experience with these temperate tadpole species in order to prevent stressful temperatures for larvae (Tejedo et al., 2012).

2.2. *Thermal tolerance*

To determine the critical thermal maximum (CTmax) of each tadpole from each species, we used Hutchison's dynamic method (Lutterschmidt and Hutchison, 1997). Each tadpole was placed individually in a 700 ml glass bowl bath filled with tap water, then a device (thermoregulator TU-20D; Bibby Scientific/Techne, Stone, UK) was submerged into the bowl to heat and circulate the water and safely control the temperature of the liquid in the bath within precise limits (from the initial test temperature to a maximum temperature superior to the expected critical maximum temperature). The water in the bath was heated at a rate of Δ1.0 °C per minute. We chose this fast ramping rate for comparative purposes and to avoid rapid acclimation or "hardening" effects which can lead to an overestimation of CTmax (Lutterschmidt and Hutchison, 1997; Rezende et al., 2011; Tejedo et al., 2012), since the physical condition of tadpoles is less affected by potential uncontrolled factors (e.g. increased metabolism and energetic demands), which could lead to cumulative thermal stress and an underestimation of CTMax, which happens when using slower, more realistic heating rates common in nature (Rezende et al., 2011; Tejedo et al., 2012). Each trial started at a fixed water temperature of 15 °C, at which all tadpole species can be maintained for experimental purposes and which is also around the average temperature observed in nature. CTmax was the temperature of the water surrounding the tadpoles taken at the moment when tadpoles lost their righting response and the onset of spasms was registered (easily observed in all tadpole species because they exceeded 30 mm in size) (Lutterschmidt and Hutchison, 1997).

To determine the critical thermal minimum (CTmin) each tadpole was placed individually in a 100 ml glass bowl filled with tap water. Tadpoles were introduced into the refrigerator and water cooling was controlled to achieve a rate of −Δ 0.8 °C ± 0.1 per minute. Each trial started at a fixed temperature for each tadpole (15 °C) and CTmin was registered following the same protocol as CTmax measurements (Lutterschmidt and Hutchison, 1997). Once tadpoles reached CTmax or CTmin, they were removed and placed in water at 20 °C for recovery. Tested individuals were weighed to the nearest 0.01 mg and their developmental stage was determined according to Gosner (1960).

2.3. *Thermal sensitivity and locomotor performance*

Thermal traits were obtained from "thermal physiological performance curves" (TPC), which represent a continuous reaction norm where an organism's performance (i.e. locomotor ability, metabolic rate, growth) is described as a function of temperature (Huey and Stevenson, 1979). TPCs are typically convex in shape, with a central or right-skewed maximum (optimum temperature, To) and lower trait values at both lower and higher temperatures (Huey and Stevenson, 1979; Knies et al., 2006; Angilletta, 2009). The shape of the curve is described by: optimal temperature (To); the temperature at which performance is maximal (Vmax); thermal performance breadth (B80), the thermal range at which individuals' performance is at least 80% of their maximum speed; and optimal temperature range (B95), the thermal range within which individuals' performance is at least 95% of their maximum speed; CTmax (critical thermal maximum); and CTmin (critical thermal minimum) (Huey and Stevenson, 1979).

We focused on locomotion as a performance trait, assuming it has important ecological relevance and is correlated with fitness (Crowley, 1985; Bauwens et al., 1995; Bonino et al., 2015a). The data for thermal sensitivity came from tadpole swimming experiments (stages 30–38; Gosner, 1960) in a rectangular acrylic aquarium ("racetrack") 0.8 m long x 0.08 m wide x 0.12 m high with seven LED sensors and beams (spaced every 0.10 m) connected to an electronic circuit and hooked up to a computer to measure tadpole swimming speed (m/s). For each swimming race tadpoles were placed at one end of the aquarium; a glass rod was used to stimulate swimming until the entire track was completed. Tadpole swimming speed was measured between consecutive sensors (0.1 m). The trials were performed at five discrete water temperatures (5 °C, 15 °C, 25 °C, 30 °C and 35 °C) within specific CTmin and CTmax temperatures. Tadpoles were heated and cooled to the desired test temperatures individually in receptacles of 1 liter in an incubator chamber (Semedic FT 290; Semedix, Buenos Aires, Argentina). Swimming races were conducted on consecutive days, at one temperature per day, which was assigned randomly. Each specimen completed six trials in the aquarium for each of the five temperatures, following Losos et al. (2002). Thus, each tadpole swam 30 times within a period of 5 days. No more than six swimming tests per day were conducted per individual, and they were divided into three series of two trials, with an interval of at least 2 h for the tadpoles to rest. Temperature of the aquarium was checked and recorded prior to each race with a digital thermometer (Extech 421502; Extech Instruments, Waltham, MA, USA). From the six trials for each discrete temperature we retained the highest speed value recorded from all sensors, which was considered the maximum speed (m/s) at each temperature for each specimen. Once the series of speed points for each temperature/individual/species had been obtained, performance curves were constructed using CTmin and Ctmax of each species as extreme values. To select the best fit curve, we applied the Akaike criterion; this was applied to select from the equations considered adequate to describe biological curves (see Angilletta, 2006, 2009), and after this criterion the best fit was observed for the exponentially modified Gaussian model, described by Angilletta (2006) as "a familiar biological function with left skewness":

$$y = \frac{ac\sqrt{2\pi}}{2d}\exp\left(\frac{b-x}{d} + \frac{c^2}{2d^2}\right)\left[\frac{d}{|d|}\right] - erf\left(\frac{b-x}{\sqrt{2c}} + \frac{c}{\sqrt{2d}}\right)$$

where y is the modeled swim velocity for temperature x, and a, b, c, d, are the adjustment parameters for the exponentially modified Gaussian model (a, amplitude; b, center; area = $\sqrt{}(2\pi ac)$; full width at half maximum = $2\sqrt{}(2 \ln 2c)$; d, time constant exponential; constraints: $c > 0$, $d \neq 0$); "exp" and "erf" are the exponential function and Gauss error function, respectively.

To fit the model, we used the series of speed points for each individual and adjusted it using TableCurve 2D V5.01 (Systat Software Inc., San Jose, CA, USA).

We standardized the performance curve for each tadpole, obtaining the relative velocity at each temperature (instantaneous velocity at each temperature/maximum speed reached). Following this, for each individual we calculated the optimal temperature range and thermal performance breadth, defined as body temperature ranges over which the tadpole can swim at 95% (B95) or 80% (B80) of its maximum speed (Vmax), respectively (Hertz et al., 1983). B80 and B95 were the arbitrary thresholds used as descriptors of the performance curve breadths of each species. We chose these variables based on the literature, and because we consider that below the B80 threshold performance is suboptimal. Additionally, we consider B95 as a more conservative approach when estimating areas where species may experience suboptimal temperatures.

2.4. Potential distributional range of the species

We estimated the potential distributional range of the species, or more precisely, the area of current climate suitability, using the theoretical biotic-abiotic-mobility approach (BAM) of Soberón and Peterson (2005), which captures and links the geographic and environmental dimensions of species distributions (Peterson and Soberon, 2012). The BAM model has three components: the B component or biotic conditions, the A component or abiotic conditions (e.g. bioclimatic variables), and the M component, which represents the region of the world which has been accessible to the species via dispersal over relevant periods of time. Generally, the B component (i.e. food availability and absence of strong competitor species) is not included in the modeling process due to the fact that it is difficult to make an accurate spatial quantification of this data (Barve et al., 2011). This heuristic scheme assumes that stable populations of a species will be found only at the intersection of the B, A, and M components ($B \cap A \cap M$) (Soberón and Peterson, 2005).

To obtain the spatial projections we used ecological niche models (ENMs). ENMs were calibrated using a maximum entropy algorithm in MAXENT v3.3.3 K (Phillips et al., 2006) since this has been shown to be a robust method for presence-only datasets (Elith et al., 2006; Pyron et al., 2008). We obtained data on species' geographic distribution (species occurrence data) from detailed bibliographic surveys and specimens located in several museums (see information in the supplementary online Appendix). Data were filtered by removing any unreliable locality or uncertain species determination. We obtained a total of 454 species-presence localities, from Argentina and Chile, as follows: 201 for *Pleurodema thaul*, 137 for *P. bufoninum*, and 116 for *Batrachyla taeniata*.

We used 20 environmental variables (19 bioclimatic variables obtained from the global meteorological database Worldclim, http://www.worldclim.org/), and elevation at a 1 km × 1 km resolution (A component of the theoretical BAM approach). All layers were clipped to the calibration area, defined as the region of the world which has been accessible to the species via dispersal over relevant periods of time (M component of the theoretical BAM approach). We performed a pairwise Pearson correlation between the 20 variables. We selected the variables that did not show colinearity with other variables ($r < 0.75$); from this selection we obtained 10 variables, as follows: mean diurnal range, isothermality, temperature seasonality, maximum temperature of warmest month, minimum temperature of coldest month, mean temperature of driest quarter, precipitation of wettest month, precipitation of driest month, precipitation seasonality and elevation. To evaluate model performance in each species, we used the area under the curve (AUC) of the receiver operating characteristics (ROC) curve. AUC shows the proportion of correctly and incorrectly classified predictions in a range of probability thresholds (Pearce and Ferrier, 2000). Due to the limitations of the ROC curve approach mentioned by Lobo et al. (2008), we also used a partial ROC area under the curve (AUC) approach (Hijmans et al., 2005; Peterson et al., 2008). Finally, the projections obtained with MaxEnt were reclassified to convert the continuous output into a map of presence–absence (0–1) using a minimum training presence (MTP) threshold that avoids the omission of known localities on output maps.

We used this tool (ENMs) to estimate the level of vulnerability in the face of global climate change. To this end, we identified the areas within the current distribution ranges of each species (from the ENMs) in terms of what they may be exposed to in the future; for example, a maximum environment temperature (MET) that may exceed our arbitrary thresholds; the upper limit of the performance range (B80) and the upper limit of the optimum temperature range (B95) and the critical maximum temperature (CTmax), under the assumption of no dispersion of the species. That is, we can identify and compare, under present and future conditions, areas where the species may experience potential thermal stress (e.g. $\mathrm{MET} > \mathrm{B80}$ and B95) or lethal temperatures ($\mathrm{MET} > \mathrm{CTmax}$).

As an estimator of MET we used the maximum temperature of warmest month (MTWM) coinciding with the period of tadpole activity, when the three species are active, for the current year and 2070 (2060–2080) conditions, considering that this represents the maximum temperature to which these organisms may be exposed, and therefore an appropriate estimator to evaluate the possibility of heat stress. Also, we used air temperatures, assuming that temporary wetlands inhabited by our focal species perform as shallow littoral zone temperatures, which are known to be very similar to air temperatures, depending on altitude and latitude (Livingstone et al., 1999; Gerick et al., 2014). We considered the RCP 6.0 (representative concentration pathways) proposed by the IPCC (2013) since it is an "intermediate" scenario in terms of severity. Due to the uncertainty introduced by different atmosphere–ocean global circulation models (AOGCMs) for future conditions (Diniz-Filho et al., 2009; Nori et al., 2011), and with the intention of covering the range of variation among them, we selected three different AOGCMs: CCSM4 (Community Climate System Model, version 4); CGCM4 (Fourth Generation Atmospheric General Circulation Model); and HadGEM2, (Hadley Centre Global Environmental Model, version 2), which have different equilibrium climate sensitivity values. We worked with the MTWM average of these three AOGCMs.

3. Results

3.1. Thermal tolerance, thermal sensitivity and locomotor performance

The larvae of the studied anuran species varied in their thermal biology. Mean CTmax was significantly different between species (Kruskal–Wallis, $H = 24.81$, $P < 0.001$) (*Batrachyla taeniata* 35.96 °C ± 0.22; *Pleurodema bufoninum* 38.46 °C ± 0.07; *P. thaul* 36.76 °C ± 0.13). *P. bufoninum* was significantly different from *B. taeniata* and *P. thaul* in CTmax values (Dunn's method, *P. bufoninum* vs *B. taeniata* $Q = 4.57$, $p < 0.05$; *P. bufoninum* vs *P. thaul* $Q = 4.02$, $p < 0.05$), whereas *B. taeniata* and *P. thaul* showed no significant differences in their CTmax (Dunn's method, *Batravhyla taeniata* vs *P. thaul* $Q = 1.80$, $p > 0.05$).

Critical thermal minimum (CTmin) was significantly different between species (Kruskal–Wallis test, $H = 12.66$, $p = 0.002$) (*P. bufoninum* 0.31 °C ± 0.03; *P. thaul* 0.33 °C ± 0.06; *B. taeniata* 1.06 °C ± 0.04). Tadpoles of *B. taeniata* showed significant differences in thermal minimum tolerance compared to *P. bufoninum* and *P. thaul* (Dunn's method, *B. taeniata* vs *P. bufoninum* $Q = 3.01$, $p < 0.05$; *B. taeniata* vs *P. thaul* $Q = 3.44$, $p < 0.05$), while no differences between *Pleurodema* species were detected (Dunn's method, $Q = 0.22$, $p \geq 0.05$).

Mean optimal temperature (To) ranged from 21.2 °C to 25.67 °C (*P. thaul* and *B. taeniata*, respectively) and species mean maximum speed

Table 1
Summary of thermal physiological traits from the resultant performance curves of three anuran species.

Species	B80inf	B80sup	B80range	B95inf	B95sup	B95range	To	Vmax
Batrachyla taeniata	17.7 ± 1.26	31.33 ± 0.70	13.63 ± 0.58	22.07 ± 1.26	28.72 ± 0.95	6.65 ± 0.32	25.67 ± 1.14	0.26 ± 0.02
Pleurodema thaul	13.02 ± 0.77	28.58 ± 0.70	15.55 ± 0.25	17.26 ± 0.86	24.95 ± 0.82	7.69 ± 0.15	21.2 ± 0.87	0.17 ± 0.01
Pleurodema bufoninum	12.93 ± 0.76	30.21 ± 0.46	17.27 ± 0.36	17.64 ± 0.82	26.16 ± 0.64	8.52 ± 0.21	22.02 ± 0.76	0.27 ± 0.02

Table 2
Mean maximum speed (± SD) for each species corresponding to each fixed temperature (approximately, 5, 15, 25, 30, 35 °C) in the swimming performance trials.

	Species		
	B. taeniata	***P. bufoninum***	***P. thaul***
Fixed temperature °C	Speed (m/s) ± SD	Speed (m/s) ± SD	Speed (m/s) ± SD
5	0.119 ± 0.034	0.239 ± 0.124	0.094 ± 0.031
15	0.253 ± 0.105	0.258 ± 0.135	0.114 ± 0.049
25	0.233 ± 0.076	0.319 ± 0.111	0.124 ± 0.032
30	0.248 ± 0.131	0.263 ± 0.128	0.133 ± 0.054
35	0.168 ± 0.067	0.218 ± 0.082	0.101 ± 0.031

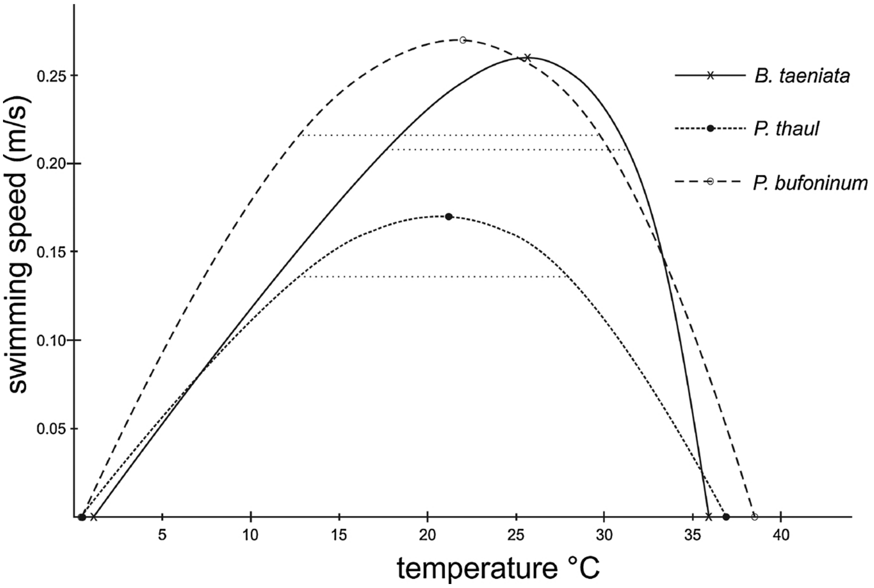

Fig. 1. Thermal performance curves for the swimming speed of tadpoles of *B. taeniata*, *P. thaul* and *P. bufoninum* from the fitted model. Dotted horizontal lines indicate the thermal performance breadths (B80); empty circle, filled circle, and cross show the optimum temperature (To) for each species, respectively.

(Vmax) was 0.17 m/s for *P. thaul*, 0.26 m/s for *B. taeniata* and 0.27 m/s for *P. bufoninum* (Table 1).

The species with the narrowest distribution range (*B. taeniata*) showed the narrowest performance breadth and a relatively higher optimum temperature compared to both of the more widely distributed *Pleurodema* species (Table 2; Fig. 1). Thermal performance breadth (B80) was significantly different between species (*B. taeniata* vs *P. bufonium* Mann–Whitney test U = 3.50, $p < 0.001$*; B. taeniata* vs *P. thaul* t-test, t = –3.53, p = 0.001*; P. bufonium* vs *P. thaul* Mann–Whitney test U = 24.5, p = 0.002); however, lower and upper performance limits showed differences depending on the species comparisons. The lowest value of B80 was observed in *B. taeniata* and the highest in *P. bufoninum* (Table 1). *B. taeniata* was significantly different from the *Pleurodema* species at B80 lower limit (*B. taeniata* vs *P. bufonium* t-test, t = 3.12, p = 0.006; *B. taeniata* vs *P. thaul* t-test, t = 3.31, p = 0.003), while only *B. taeniata* and *P. thaul* showed a significant difference in their upper limit (*B. taeniata* vs *P. thaul* t-test, t = 2.79, p = 0.009) (Table 1). The optimal temperature range (B95) also showed significant differences between species. Tadpoles of *B. taeniata* had a narrower B95 range and were different from *Pleurodema* species (*B. taeniata* vs *P. bufonium* t-test, t = –4.65, $p < 0.001$*; B. taeniata* vs *P. thaul* t-test, t = –3.35, p = 0.002) (Table 1). Optimal temperature (To) was different in *B. taeniata* and *Pleurodema* species (*B. taeniata* vs *P. bufoninum* t-test, t = 2.59, p = 0.01; *B. taeniata* vs *P. thaul* t = 3.01, p = 0.005), the former species showing the highest To value of the three species (Table 1).

In addition, the species showed variation in maximum speed (Vmax). *P. thaul* presented the lowest speed (Table 1) and was significantly different than the other two species (*P. thaul* vs *P. bufoninum*, Mann–Whitney test, U = 7.0, p = 0.001; *P. thaul* vs *B. taeniata* U = 54.0, p = 0.03).

3.2. Distributional range of the species and vulnerability to global climate change

We found AUC values higher than 0.90 for the three species studied here (*B. taeniata* 0.96 ± 0.01; *P. bufoninum* 0.92 ± 0.01; *P. thaul* 0.95 ± 0.01). Furthermore, the statistics of Partial Roc were highly significant ($p < 0.001$), indicating high reliability of the models. The projected distributions for current and future emission scenarios are shown in Fig. 2. We analyzed the distributional indicators of vulnerability and we found no areas where these anurans would experience lethal temperatures (none of the temperatures exceeded CTmax), neither in current nor in future conditions. However, we found that the three species might experience chronic, non-lethal, potentially suboptimal temperatures, because some areas and/or percentages of the current species distribution range are under suboptimal temperatures; that is, above the upper limits of B95 (Table 3). Moreover, B95 upper limits appear to be exceeded particularly for *P. bufoninum* (Table 3). Considering the cut-off threshold of upper B80, for a future, warmer scenario (2070), the areas under potentially suboptimal temperatures (higher than the B80 upper limit) were between three and five times larger than the areas observed under current temperature scenarios, depending on the species. Moreover, B95 upper limits seem to be particularly suboptimal for *P. bufoninum* (Table 3).

Considering the known localities where each one of the species occurs, we observed two different patterns of vulnerability, depending on the species and temperature scenarios (Table 3). Under current

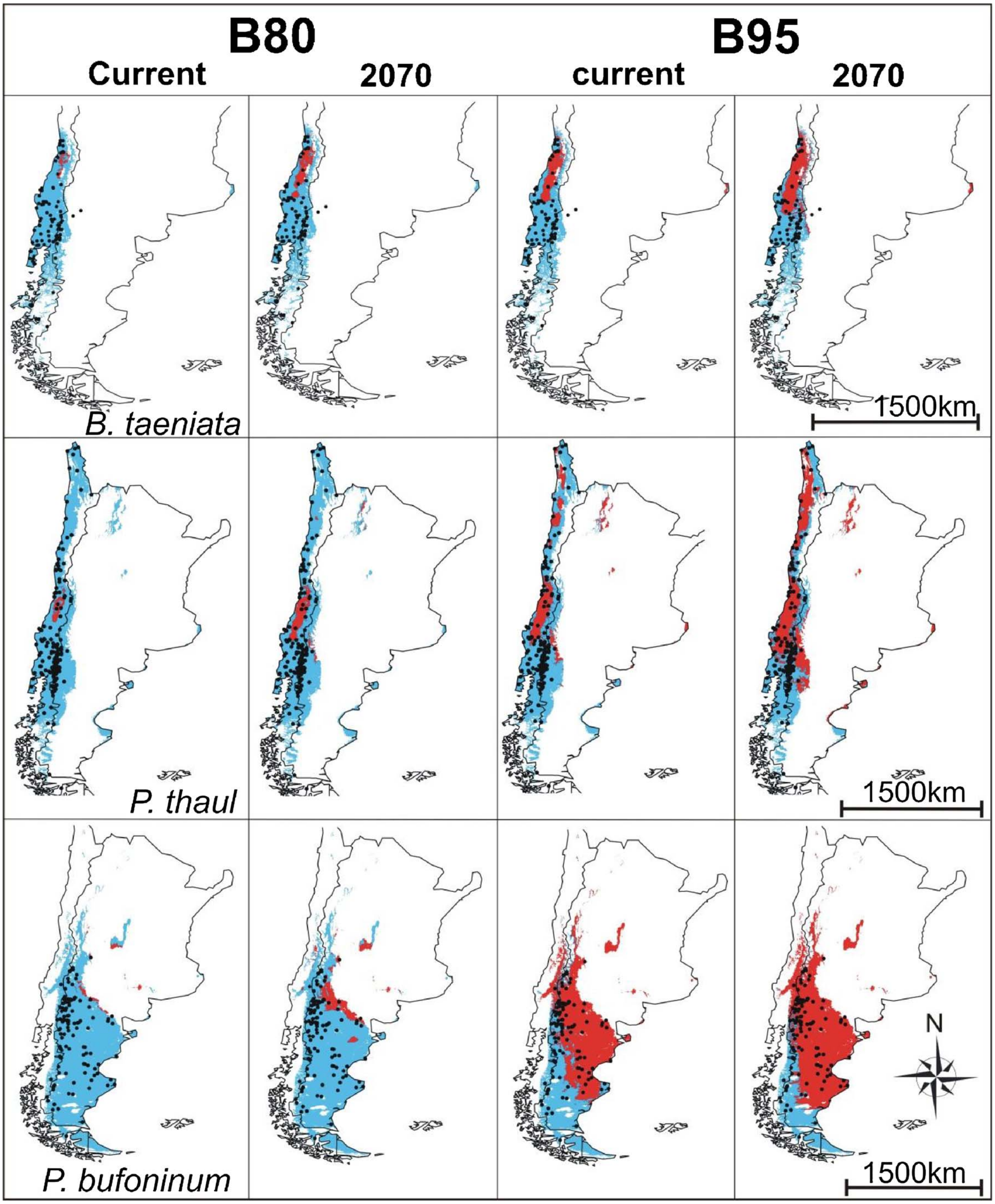

Fig. 2. Maps showing projected distributions (painted areas) of B. taeniata, P. thaul and P. bufoninum and their areas under thermal stress (red-painted areas) in current and future (2070) conditions considering two stress thresholds: B80 (upper limit of the thermal performance breadth) and B95 (upper limit of the optimal temperature range). Black dots indicate locality records.

conditions only *P. bufoninum* showed a substantial percentage of localities where temperatures above the optimum range (70.7%) may be experienced; in contrast, *P. thaul* and *B. taeniata* showed a small percentage of localities that may affect their optimum range (around 13% up to B95 limit). However, when we examined thermal conditions for future warmer scenarios, *P. thaul* and *B. taeniata* seemed to be more affected, with the highest increase in the percentage of localities affected by suboptimum temperatures (Table 4).

4. Discussion

How organisms adapt to their thermal environment is important in defining the phenology and distribution of many species (Gaston, 2003; Danks, 2007; Bozinovic et al., 2011). In particular, in the context of global warming, ectotherms need compensatory responses (behavior, plasticity, or adaptation) and/or need to access new habitats that provide relief from extreme changes in operative temperatures in order to survive these effects.

The tadpoles of the species studied here show different ways of dealing with climate in this temperate region; on the one hand, the species with a wide distribution range (*P. bufoninum* and *P. thaul*) show broader performance curves. For example, *P. thaul* has an extended latitudinal distribution occurring from an isolated oasis in the Northern Mediterranean desert in Chile to the southern hyperoceanic climate of the temperate rainforests of southern Chile and Argentina (Correa et al., 2007), embracing altitudinal and latitudinal ranges that could imply some ecological plasticity (Cei, 1962). Thus, both *Pleurodema* species that show broad performance breadths are less sensitive to temperature changes and have a large temperature difference between their critical thermal limits. On the other hand, the southern and narrowly distributed *B. taeniata* (see Correa et al., 1854) showed a narrower thermal performance curve.

With respect to locomotor performance, we observed that thermal sensitivity varied according to tadpole species. *B. taeniata* showed narrower performance and optimal temperature breadths (B80 and B95, respectively) at relatively high temperature values for both thermal ranges (B80 ~ 18–31 °C; B95 ~ 22–29 °C). In contrast, *Pleurodema* species showed broader B80 and B95 ranges (Fig. 1; Table 1), but at similar or even lower temperatures than *B. taeniata* (see *P. thaul* in Fig. 1). These results suggest that the species with broader physiological plasticity − in terms of swimming speed − may have achieved wider distributions (*Pleurodema*) compared to species with narrower physiological plasticity (*Batrachyla*). Additionally, broader performance breadths are expected to cause a corresponding loss of the peak at the thermal optimum (Huey and Kingsolver, 1989). Although our results are not conclusive, the three species studied here seem to follow different strategies to counteract thermal heterogeneity. Each species shows a particular distributional range and geographic characteristics that may lead to the observed differences in our results. It is possible that the biogeographic origin of each genus (*Pleurodema* and *Batrachyla*) plays a role in these different ways of coping with abiotic components of the environment. Although there is no detailed biogeographic study, it is possible that because we observe that the basal clades of *Pleurodema* occur today mainly in subtropical zones in the north of Argentina and Uruguay, such as *P. guayapae*, *P. nebulosum*, *P. bibroni* and *P. cordobae* (Faivovich et al., 2012), this genus may have originated in warmer environments; in contrast, *Batrachyla* belongs to a clade of species that mainly occur in cold climate environments, such as Alsodidae (*sensu* Blotto et al., 2013). However, it is interesting that the swimming speed of *P. thaul* is slower than that of *B. taeniata*, which also performs at higher temperatures, suggesting specialized thermal physiology for swimming in the latter species.

With regard to the activity patterns and phenology of the studied species, *B. taeniata* starts its reproductive season earlier, at the onset of winter, and produces overwintering tadpoles (Díaz et al., 1987), probably due to the harshness of the environment where this species clade evolved. This extended larval cycle is also confined to the same pond during at least one complete year, undergoing severe thermal variation between winter and summer (winter pond mean temperature ~ 2.5 °C and summer pond mean temperature ~ 14 °C). Furthermore, as mentioned before, *B. taeniata* tadpoles seem to be thermal specialists (narrow thermal breadth) and appear to be adapted for swimming in warmer conditions, which is apparently suitable for growth and development during the available short warm season. In addition, the thermal ability (faster speed at high temperatures) observed in *B. taeniata* may be interpreted as a mechanism for maintaining burst swimming velocity, allowing these species to better exploit resources and also escape predators during the time of the season when food and predators are present (Angilletta, 2009; Katzenberger et al., 2014; Richter-Boix et al., 2015), without the cost of maintaining constant, higher metabolic rates to maximize assimilation and growth. In the case of *Pleurodema* species, compensatory shifts such as latitudinal clines could counteract thermal changes (Laugen et al., 2003; Orizaola et al., 2010; Muir et al., 2014), and this could match with a “jack of all temperatures and master of none” hypothesis, as expected for low diversity and temperate communities or assemblages, whereas in diverse

Table 3
Potential distribution area (percentage) under current and warming stressful scenarios, considering out of the performance range (upper B80), out of the suboptimum (upper B95), and under lethal temperatures (upper CTmax tolerance limits).

Current scenario							**Future scenario (2070)**						
	Total area	**% area > CTmax**	**area > B80**	**%**	**area > B95**	**%**	**% area > CTmax**	**area > B80**	**%**	**% increase**	**area > B95**	**%**	**% increase**
B. taeniata	239336	0	7196	3.0	43968	18.37	0	30016	12.5	9.5	81808	34.18	15.81
P. thaul	664832	0	17766	2.7	144877	21.79	0	56465	8.5	5.8	306891	46.16	24.37
P. bufoninum	835788	0	15134	1.8	519188	62.12	0	75206	9.0	7.2	651582	77.96	15.84

Table 4
Number and percentage of known localities for each species' presence under stressful temperatures in two different scenarios: current and warmer (2070). N = number of known localities.

	Current scenario					Future scenario (2070)				
	N	N > limitB80	% > upper limit	N > limitB95	% > upper limit	N	N > limitB80	% > upper limit	N > limitB95	% > upper limit
B. taeniata	101	1	1.0	14	13.9	101	10	9.9	30	29.7
P. thaul	191	3	1.6	25	13.1	191	8	4.2	86	45.0
P. bufoninum	133	3	2.3	94	70.7	133	7	5.3	114	85.7

and subtropical community specialists are expected. There are exceptions, however, such as that observed in an anuran assemblage in the dry Chaco of Bolivia (Schalk et al., 2016). Performance curves are linked to locomotor speed, which is considered an indicator of survival, and therefore of fitness (Christian and Richard, 1981; Hertz et al., 1983; van Berkum, 1988; Jayne and Bennett, 1990); for example, swimming capacity in tadpoles might be relevant to escape from potential predators. However, it would be inappropriate to consider this sole physiological trait as the only response that explains relationship to geographic range amplitude. Indeed, even the same trait may vary during the ontogeny of a species, and other traits should also be considered in order to make solid assumptions as to the fitness of a species (Sinclair et al., 2016). Thus, the link between thermal physiology, distribution and fitness remains unclear and more data are needed. Moreover, the presence of competitors or predators can prevent species from persisting in a region where abiotic conditions (e.g. temperature) would otherwise permit persistence (Sexton et al., 2009). There is evidence of this in anuran tadpoles, where predators alter the thermal physiology by increasing CTmax, optimum temperature and thermal performance curves (Katzenberger et al., 2014). Thus, despite the apparent simplicity of tadpole biology, there are multiple factors (abiotic, biotic and interactions) to consider if we expect a conclusive answer.

According to the ENMs results, none of the studied species experience or will experience lethal temperatures (T° > CTmax) at their larval stages in relation to the MTWM values obtained for present and future (2070) scenarios. Furthermore, the studied species occur in fluctuating environments with temperatures somewhat lower than the temperature at which swimming performance is maximal. In this case they may center thermal preferences at a temperature below the body temperature that maximizes instantaneous performance (as an indicator of fitness), and the "suboptimal is optimal" hypothesis could be applicable in these organisms (Martin and Huey, 2008).

The modeled species distributions, combined with empirical physiological results, enable us to deduce the responses and climatic conditions of these three temperate amphibians in future climate-warming scenarios. Our results suggest that these three Patagonian anurans will not be drastically affected in their distributional ranges in future scenarios. However, these species may suffer, to a certain extent, some problems in populations where temperature may compromise them. For example, current MTWM shows higher temperature values than these thermal ranges, at least in part of their distributional range. Moreover, the number of localities and the proportion of distribution under suboptimal temperatures will consistently increase for the three species in the scenario of the year 2070. Thus, although they will not experience lethal temperatures, the potential risk of chronic exposure to suboptimal temperatures is unknown. This is particularly important when we consider the suboptimal temperatures above the upper limits of the performance ranges. It should be noted that overheating is more dangerous for species than exposure to low temperatures, especially because of the asymmetry of the performance curves, where the performance drops abruptly at temperatures above the optimum temperature. Our results are not conclusive enough to determine whether local extinctions of certain populations of any of the studied species might be possible, with the consequent retraction of distributional range; however, we are aware that the threat level is increasing in the context of climate change. A recent study on Neotropical anurans showed that because of differential species-specific susceptibility to *Batrachochrytrium* infection, human activity and climate warming, negative impacts are likely to be experienced by some anuran species, including *P. thaul* (Bacigalupe et al., 2017).

A recent study on tropical amphibians at high altitude in the Andes (*Telmatobius marmoratus*, *Rhinella spinulosa*, and *Pleurodema marmoratum*) showed that these organisms may be plastic across habitats, sites and years, coping with dramatic changes due to global climate change and expanding to new ecological niches (Seimon et al., 2017). In a similar way, the range expansion successfully achieved by cane toads in southern Australia may be due to their ability to rapidly adjust phenotypic traits to novel challenges (Kolbe et al., 2010; Amiel et al., 2011). We recognize that the estimation of thermal performance of a population level subset in *Pleurodema* species may differ from the species level estimation when we consider the large distributional range of *Pleurodema* compared to *Batrachyla*. However, the evidence observed in amphibians at high Andean altitudes (Seimon et al., 2007; Seimon et al., 2017), and the range expansion of cane toads in Australia, allow us to infer that different mechanisms could be operating on each of the species or genus studied here. Contrary to our expectations, both *Pleurodema* species were found to be more vulnerable to potentially higher temperatures when considering ENMs in future scenarios (Fig. 2). This might be explained by the fact that these species seem to be more sensitive to high temperatures than *B. taeniata,* according to their performance curves. Also, the areas showing risk in future scenarios in Fig. 2 correspond to the dryer habitats in the distribution range of these *Pleurodema* species, where shallow waters may be lost due to desiccation.

In addition to the above factors, changes during dry seasons could put selective pressure on amphibian larvae. Increasing temperature in the future may constrain tadpole performance; e.g., wetlands with short hydroperiods could force larvae to accelerate development resulting in small-sized metamorphs, with consequences for the survival of subsequent life stages (O'Regan et al., 2014). In this regard, there is evidence that Patagonian populations of *P. bufoninum* and *P. thaul* demonstrate local adaptation to the selective pressure imposed by wetland loss by evaporation, showing faster development and smaller size at metamorphosis (Perotti et al., 2011). On the other hand, the development and survivorship of *B. taeniata* embryos and tadpoles is highly dependent on fall precipitation (Úbeda and Nuñez, 2006). More information is therefore necessary to test how performance mediated by temperature (a potential negative synergic effect) is altered in drying ponds.

Finally, we know that the incorporation of more empirical data is necessary for the distribution models (e.g. intraspecific trait variation; population shifts as responses to abiotic variables, ontogeny, and other traits), in order to understand more clearly how species counteract the challenges imposed by the environment (Kearney and Porter, 2009; Moran et al., 2016; Singer et al., 2016). But we are confident that our data, combining environmental niche modeling and empirical physiological results, similar to previous studies (Singer et al., 2016 and reference therein) may help to understand how temperate amphibian species will cope with future scenarios of increasing temperature.

5. Conclusions

We found that these three temperate anuran species have different ways of coping with the temperate climate they are exposed to. *Pleurodema* species show broader performance curves, allowing them to occur over a broad geographic range. In contrast, *Batrachyla taeniata* seems to combine different mechanisms, such as an extended larval cycle (overwintering tadpoles), which expose tadpoles to severe thermal variation between winter and summer, which may be counterbalanced by high performance at warmer temperatures. *Pleurodema* species belong to a widely distributed genus, where basal clades that today occur in subtropical zones may have originated in warmer environments (Faivovich et al., 2012). *Batrachyla,* on the other hand, belongs to a small clade of species mainly occurring in cold climate environments (Patagonia) inhabiting a more restricted region in the temperate forest of austral South America (Rabanal and Núñez, 2008). The present information suggests that the biogeographic origin of each genus may have played a role in the expression of the different ways these species cope with thermal environmental characteristics. Our findings from modeled distributions, combined with empirical physiological results, suggest that these three anuran species will not be drastically affected in their distributional ranges. However, we are aware that the threat level is increasing in the context of climate change, and this may lead to possible retractions of the distributional range of some species (*Pleurodema*), and perhaps local extinction of some populations, particularly those with a narrow distribution range and a strong dependence on hydroperiod beyond temperature change, as is the case for *Batrachyla taeniata*.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.zool.2018.01.002.

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