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Modeling warming predicts a physiological threshold for the extinction of the living fossil frog *Calyptocephalella gayi*

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A B S T R A C T

Global climate change will have a greater impact on ectotherms in tropical and subtropical communities than at higher latitudes, because ambient temperatures are closer to the upper thermal limits of species. Amphibian species are highly dependent on external weather conditions, and the effect of global warming on these has been evaluated recently. The Great Chilean frog (*Calyptocephalella gayi*) is an endemic, monotypic species and genus whose conservation status is considered Vulnerable because of high extraction pressure for human consumption, lack of regulatory measures and comprehension by its consumers. Their populations have also declined due to the loss and destruction of their habitats. *C. gayi* has not been considered as an object of physiological study, so this large species is not known as one that can adapt to current environmental changes. In this study we analyze the thermoregulatory capacity and thermal efficiency of *C. gayi* to determine its potential for climatic adaptation. The results indicate that this species is strictly a thermal-conformer; its thermal efficiency and its ability to withstand high temperatures allow it to sustain itself under a climate change scenario, however, it has thermal constraints that do not allow it to withstand temperatures greater than 30 °C. By modeling its ontogenetic conditions mathematically, we project that the larvae are not in danger, although there is a group of around 4% which is very close to 30 °C, which is the highest temperature recorded for the species. However, about 40% of subadults and approximately 47% of adult frogs will not survive the change of ~7 °C projected for the following 85 years, which will affect future generations.

1. Introduction

Humans are altering the Earth's climate, and consequently the functioning of living organisms at all levels (Pounds et al., 2006; Sinervo et al., 2010; Lobos et al., 2013; Cavanaugh et al., 2015). Among the unfortunate results of this change is the extinction of many species of amphibians. Detection of the effects of heating as a direct producer of extinction has led to complex analyses of global temperatures (Stuart et al., 2004; Sinervo et al., 2010). One approach is to focus on organisms whose current extinction rates exceed the expected alteration of habitat and amphibians are an ideal model for this. Thousands of species have declined and hundreds are on the brink of extinction or already extinct (Blaustein et al., 1994; Houlahan et al., 2000; Collins and Storfer, 2003). Scientists have speculated that rising temperatures and changes in weather patterns could compromise the survival of many other species, and studies have shown a direct relationship between global warming and the disappearance of about 65 species of amphibians in Central and South America (McCallum, 2007). Pounds et al. (2006) recognizes that the existing effect of climate change would promote infectious disease and erode biodiversity, but it is indisputable that a synergy of many factors is producing these changes (Brook et al., 2008; Mayhew et al., 2008).

Climate change (i.e. global warming) may happen too fast for some species that fail to adapt and/or can exacerbate existing threats such as land clearing, agriculture and pollution; biological traits that make species susceptible to change and species with restricted habitats are particularly vulnerable to extinction (Lips et al., 2008). Climate-related hypotheses predict the decline of amphibians in warm years, since changes in temperature-related variables often influence the dynamics of a habitat. When temperatures rise, climate fluctuations can cross critical thresholds for certain species, so it is expected that temperature is more deadly because it heats the Earth (Zippel, 2010). Many species of amphibians are being impacted by these changes, for example due to changes in the activity of reproduction (Kusano and Inoue, 2008) or in their distributions (Seimon et al., 2007).

Calyptocephalella gayi, the "Great Chilean frog" is the largest amphibian that lives in Chile. It measures about 15 in. and lives for 10–15 years (Cei, 1962). This species has been listed as a species of particular

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Table 1
Mean ± SD of Body mass (Bm, g), Critical thermal maximum (CT_{max}), upper thermal tolerance (WT) and Thermal Time Constant (τ) estimate to ambient and lab temperature by *Calyptocephalella gayi*. n is the sample size.

Age	n	Bm	CT_{max}	WT (ambient)	WT (air)	WT (external)	τ
Larvae	13	25.61 ± 6.77	31.95 ± 1.31	10.45 ± 2.02	3.52 ± 2.90	8.91 ± 2.42	3.36 ± 0.31
Subadults	11	349.25 ± 21.51	31.26 ± 0.47	8.34 ± 2.90	2.83 ± 0.48	8.22 ± 5.73	15.47 ± 2.28
Adults	12	680.37 ± 53.28	30.36 ± 0.51	7.81 ± 3.87	2.93 ± 0.49	7.32 ± 4.88	13.77 ± 1.35
Total	36	141.68 ± 231.17	31.59 ± 1.24	9.51 ± 3.12	3.16 ± 1.27	8.55 ± 4.01	8.56 ± 5.98

interest from both ecological and historical perspectives. *C. gayi* inhabits a wide range of distribution ranging from Coquimbo (29°S) to Puerto Montt (41°S), in lagoons, lentic streams and wetlands that range from semi-desert to very humid environments (Cei, 1962). Throughout this distribution larval stages are spatially segregated within the water column; larvae utilize the still and shallower waters (which are warmer), while subadults and adults immerse themselves in the water column in search of colder temperatures (Cei, 1962; Vidal MA, pers. obs.). Correa et al. (2008) suggested that this species is associated with Australian species, related to the existence of the supercontinent Gondwana (Gómez et al., 2011). The Gondwanan legacy could also explain the marked morphological divergence between *Calyptocephalella* and *Telmatobufo* (sister group), which may be relicts of a more widely distributed and diverse lineage in the past. The living genus *Calyptocephalella* is known from Eocene deposits of Patagonia, where it was associated with crocodilian remains, suggesting the prevalence of mesic conditions at the time (Cei, 1979). The living Chilean frog *C. gayi* is almost identical to the fossil frogs of the same genus. According to Schaeffer (1949), *Calyptocephalella* have an antiquity going back to the Tertiary from 65 million years to 1.7 million years (Cei, 1979), whose fossil record includes the early Eocene, the early Oligocene and Late Miocene from Argentina (Báez and Gasparini, 1979). The relictual nature of its present distribution may be linked to the increasing desiccation of Patagonia in post-Eocene times, which is related to the uplift of the Andes and the concomitant drastic reduction of aquatic habitats and changes in their water chemistry rather than to the westward retrocession of temperate austral forests per se (Gómez et al., 2011).

The loss of habitat along with the intrinsic characteristics of species have been associated with increasing extinctions and declines of species around the world; this is phylogenetically selective in that small-bodied species are less vulnerable to declines or extinction (Cardillo, 2003; Cardillo and Bromham, 2001). Furthermore, there may be trade-offs between different traits; for example, larger species such as *C. gayi* may have an advantage in greater mobility and energetic efficiency, but do not have high reproductive rate or high population densities (Sodhi et al., 2008). From the thermal behavior point of view, Castañeda et al. (2006) showed that the larvae of *C. gayi* are affected by the environmental temperature. In fact, when they are raised to high temperatures (26 °C versus 16 °C), larval weight decreases rapidly due to an increase in metabolism. *C. gayi* is a large species that undergoes drastic changes of heat or energy as it moves from an aquatic to a terrestrial environment during feeding (Cei, 1962). Moreover, one of the most visible features of the transformation of *C. gayi* is in body mass (Veloso, 1977). This implies that the effect of thermal inertia (viewed from the perspective of the rate at which heat is transferred) plays a key role in maintaining behavioral or physiological thermoregulatory mechanisms (Pough and Gans, 1982). During ontogenetic development *C. gayi* acquires a greater body size from the larva to the adult, which would change the ability to gain or lose heat (Labra and Vidal, 2003). If we consider that the different stages of development of this species are differentially affected by the environment because water dependence is reduced in the later stages of development, it is plausible to assume that certain stages will suffer more with a change in global climate (Pounds et al., 2006). In this study we analyzed the thermoregulatory strategies in different stages of development of *C. gayi*, with the aim of estimating the best allometric equations that explain the level of dependence of these variables on certain environmental conditions, and thus understand adaptations that could allow the survival of the species in a catastrophic scenario.

2. Materials and methods

2.1. Lab conditions

36 specimens of *C. gayi* from Santiago, Linares and Trehuaco were collected, which were separated into three ontogenetic stages (Table 1): a) 13 larvae of stage 39–41 (Gosner, 1964), b) 11 subadults and c) 12 adults. Individuals were transported to the Laboratory of Ecology and Evolution of the Universidad del Bío-Bío and kept in aquaria with an average of 14 L:10D and fed ad libitum. Few individuals were captured because this species is categorized as Vulnerable in Chile and thus its capture is regulated. This study was approved by authorization n° 3798 of the Agricultural and Livestock Service (SAG in Spanish). For all experiments, data from three different localities were considered as independent for the three ontogenetic states.

2.2. Heating rate and upper thermal tolerance

Before each experiment individuals were weighed (g), then we determined the heating rate. Heating rates were measured as described previously by Labra et al. (2001), Labra and Bozinovic (2002) and Sanabria et al. (2003). Frogs were placed individually in glass vessels of 800 ml volume in a conventional refrigerator at an initial temperature of 12 °C. Once this temperature was reached, individuals were transferred to a thermoregulated Barnstead bath at 37 ± 0.5 °C. During the experiments, body temperature T_b was recorded with a laser thermometer every 30 s, starting measurements at 15 °C and ending the experiment when it reached 31 °C. In the case of the larvae, at the moment of recording the body temperature they were captured with a conical basket (5 g), which allowed taking the larva from the anterior region, and then were raised slowly. Once the larva was on the surface but in contact with the water, the body temperature was recorded with a laser thermometer. The heating rate of each individual was transformed to a Thermal Time Constant (τ). This is derived from the slope of linear regression of ln ($T_b - T_a$) as a function of time, where the air temperature T_a was constant at 37 °C. The slope of the linear regression is given by $-0.4343/\tau$ according to Cossins and Bowler (1987). To relate the thermal time constant to body mass (m), we estimated the Pearson correlation coefficient (r) (Sokal and Rohlf, 1995). To determine whether there are significant differences between different age groups, temperatures were compared using a nonparametric Kruskal-Wallis test in the Statistica 7.0 program.

Before determining the upper thermal tolerance (WT), the critical thermal maximum (CT_{max}) for each individual was first determined. CT_{max} is defined as the temperature at which an individual loses its normal operating capacity, so the individual becomes physiologically unstable (Labra et al., 2001; Labra and Vidal, 2003). This value was entered into the equation $WT = CT_{max} - T_a$. In this experiment, CT_{max} is considered as a behavior change presented by individuals during the experiment at high temperatures without actually overheating them, considering that frogs are more