



# Preliminary Antecedents on the Selected Temperature ( $T_{sel}$ ) of *Rhinella spinulosa* Wiegmann 1834 (Anura: Bufonidae) from the Andean Environments of Chile

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## Research Article

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## Abstract

The ambient temperature is a decisive factor in many activities of poikilothermic organisms, since it influences the correct development of their vital processes, which implies the existence of an optimal temperature range. The present study describes selected temperatures ( $T_{sel}$ ) of *R. spinulosa* in adult individuals. For this purpose, adults of this species were acclimatized at 10°C and then subjected to a thermal gradient was used in the laboratory. The mean  $T_{sel}$  in the studied individuals was  $23.38 \pm 2.65^\circ\text{C}$ , with a range from  $17.73^\circ\text{C}$  to  $28.93^\circ\text{C}$ . Future surveys are suggested to determine the habitat thermal quality and thermoregulatory precision.

**Keywords:** Amphibians; Selected Temperature; Thermoregulation

## Introduction

The global distribution and abundance of amphibians is affected by environmental temperature [1,2]. Temperature has been considered as a selective factor influencing the survival, growth and spread of these organisms [3]. However, in spite of being ectotherms, amphibians have been able to adapt to and inhabit a wide spectrum of thermal climates, including high latitudes, deserts and mountains, characterized by extreme temperatures [4]. Even so, changes in ambient temperature resulting from global warming can shorten the time frame for biological activities such as foraging and reproduction [5-7] causing the animals to spend more time in their shelters [8,9].

To reach their preferred body temperatures, organisms can strategically move between different microhabitats, alter their activity times, adjust posture and body orientation, or take advantage of retreat sites [10-14]. Most amphibians regulate temperature by selection of microhabitats during the day and night, thus maintaining an optimum temperature ( $T_o$ ) range [15]. Amphibians in mountainous areas optimize the body temperature ( $T_b$ ) by exposing themselves to the sun, process called heliothermy, or by contact with the substrate, process called tigmothermy [16-19], which allows them to obtain a body temperature similar to that of the environment, that is, to maintain a thermoconformist behavior [3]. However, the time spent on this behavior

will depend on an individual's ability to tolerate water loss [20,21] and the necessity of being on a humid substrate to avoid desiccation [22,23].

Natural environmental gradients strongly influence the variation patterns of species, giving rise to great phenotypic diversity in populations, as ambient conditions vary among sites or in controlled experimental conditions under gradients of a particular factor [24,25]. Comparison of populations exposed to different environments allows us to investigate the extent to which selective pressures lead to adaptive responses and to identify the geographic scale in which these phenomena occur [26].

The experiments called common garden are used to determine whether geographically separated populations respond similarly to warming [27]. This helps to understand whether these responses are produced by local adaptation or by phenotypic plasticity [28].

One way of assessing the restrictions imposed by thermoregulation under natural conditions is to determine the temperatures that organisms voluntarily use for thermoregulation in ideal, unrestricted conditions. These temperatures are called selected body temperatures or  $T_{sel}$  and are usually established under laboratory conditions using a thermal gradient [29,30]. The selection of this temperature may vary over time due to the high plasticity of amphibians in terms of  $T_b$ ; their ability to acclimate allows them to move between thermal gradient extremes [31].

Mass and body size are variables that significantly affect individual thermoregulation [32]. Larger individuals have greater thermal inertia or resistance to temperature changes and heat and cool more slowly than smaller individuals, because they have less exposed surface area [33].

*Rhinella spinulosa* Wiegmann 1834 is an amphibian that is present in Bolivia, Peru, Argentina and Chile and inhabits heterogeneous thermal environments; in Chile it is found at latitudes from 18° to 41° S [34], but it is absent between 25° and 31° S [35]; it thus has a discontinuous distribution on the western slope of the Andes Range. This species inhabits areas near streams, lakes and wetlands at altitudes from 1000 to 4600 m and has been the subject of numerous studies, showing geographic variation in morphological, ecological, genetic and physiological traits [36-46]. It has a strong geographic structure and low levels of gene flow determined by low vagility and physiological limitations [41,42].

*Rhinella spinulosa* is more active during warmer periods of the year [47]. It has physiological tolerance to water and temperature stress, which allows it to live at higher elevations [18,37,48]. Thermal studies indicate that *R. spinulosa* uses a

variety of strategies to control body temperature [16-18]. Adults exhibit heliothermic behavior during the morning, which also helps them to improve digestion of prey caught the night before [49]. During the rest of the day, the species has tigmothermic behavior, using damp and shaded sites that reduce the loss of water by evaporation [17]. Larvae and postmetamorphics are active diurnally, while adults are nocturnal, staying hidden under rocks during the day [50]. Adults are more terrestrial than aquatic [47], unlike the postmetamorphics who prefer to stay close to water bodies [45].

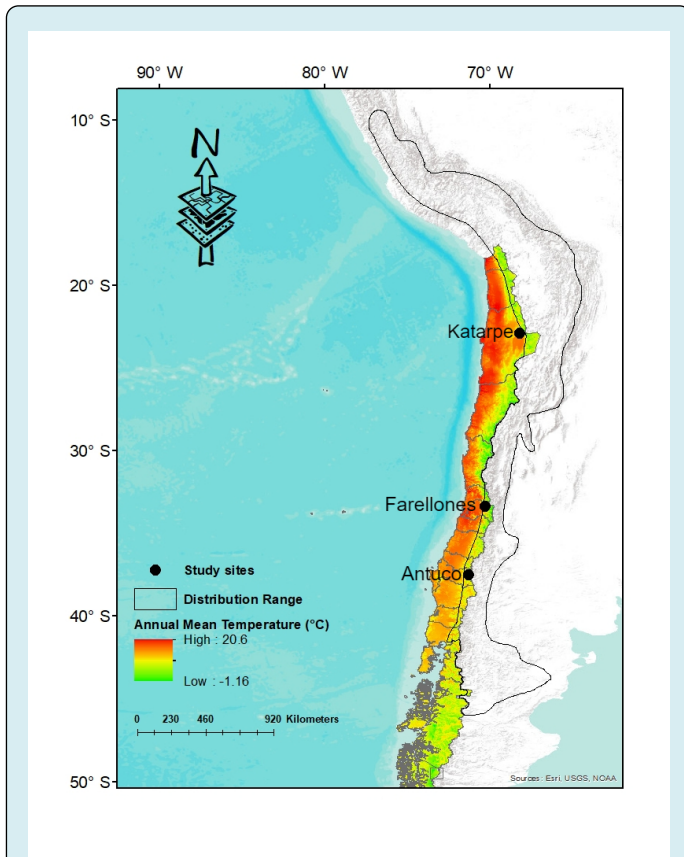
The present study aims to determine temperature preferences ( $T_{sel}$ ) in *R. spinulosa* species and establish differences between localities. These results are complementary to those published by the authors in Alveal, et al. [51] where the thermal tolerance of the species was analyzed. Alveal, et al. [51] determined that the species tolerates a wide range of temperatures, so it is considered eurythermic. In addition, significant differences were observed between the northern locality versus the central and southern ones in terms of  $CT_{max}$ , establishing that individuals from the north tolerate higher temperatures. Both,  $T_{sel}$  and thermal tolerance are generally accepted as central to characterize the thermal biology of ectothermic organisms [52].

## Materials and Methods

During the spring and summer of 2012 and 2013, three localities on the western slope of the Andes range were visited. These sites are representative of different climatic zones throughout of the range latitudinal of the species in Chile: Katarpe, Antofagasta region: 22°53'22.26''S-68°12'53.73''W, 2466 m, Farellones, Metropolitan region: 33°21'27''S-70°18'14''W, 2392 m and Antuco, Bio Bio region: 37°28'32''S-71°19'10''W, 1409 m (Figure 1), with an annual average temperature of 12.7°C, 7.5°C and 5.9°C, respectively. In these areas the annual mean temperature decreases in relation to latitude ([www.worldclim.org](http://www.worldclim.org)) which implies a different thermal environment in each population.

Captures were made manually using the "Protocol for the control of infectious diseases in amphibians during field surveys" [53]. A total of 19 males adult individuals were used for the experiment, three from the north, four from the center and 12 from the south. The individuals were maintained in the Laboratorio de Ecofisiología y Conservación de Herpetozoos at the Universidad de Concepción in terrariums (40 x 42 x 35 cm) and provided with conditions similar to those of their habitats, maintaining humidity with water containers to prevent dehydration. Individuals were subjected to a temperature of acclimatization of 10 °C for a period of two weeks, which corresponds approximately to the average environmental temperature of the three localities under

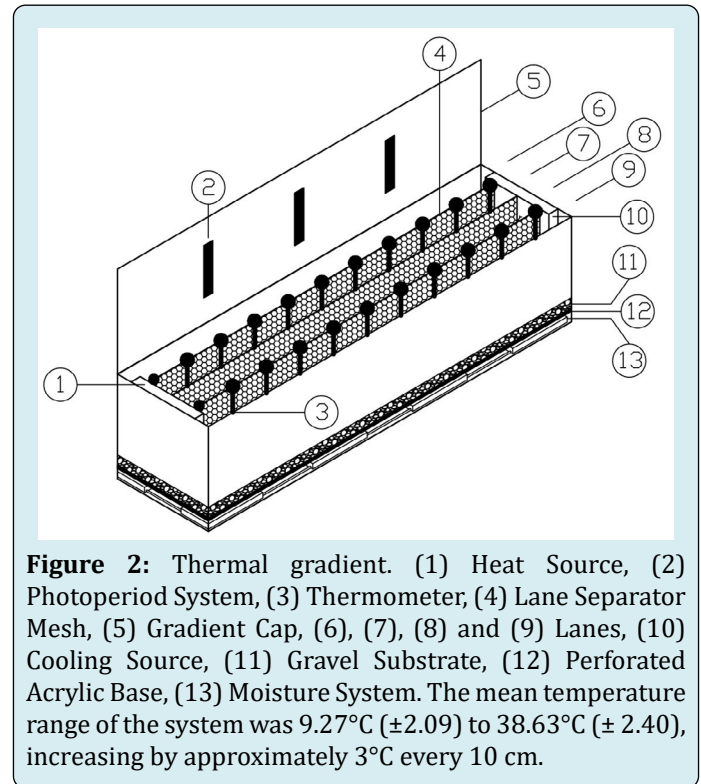
study. A photoperiod regime of 10H light and 14H dark was used, which resembles the photoperiod of its activity period. They were fed *ad libitum* with earthworms (*Lumbricus terrestris* Linnaeus 1758) and larvae of *Chilecomadia moorei* Silva 1915 and *Tenebrio molitor* Linnaeus 1758. Individuals were marked with elastomers in the ventral zone and intermembrane of hind limbs for identification, according to the Northwest Marine Technology protocol [54]. Prior to each experiment, the snout-vent length (SVL, mm) was measured with a digital caliper with 0.01 mm precision, and mass (g) using a digital balance with 0.01 g precision.



**Figure 1:** Locations of *R. spinulosa* in Chile. In circle, localities for the study of  $T_{sel}$ . In line without filling, distribution range of the species. In color gradient, mean annual temperature.

To determine the  $T_{sel}$  in the laboratory, an artificial temperature gradient was used. The system of thermal preference used consisted of a heat-sealed metal structure 195 cm long, 60 cm wide and 56 cm high; the internal space was divided into four lanes of about 15 cm each using a porous metal mesh. The base was made of transparent acrylic, perforated and covered with gravel. As the level of dehydration of individual anurans affects the performance [55], a constant moisture system was provided underneath which maintained the ambient humidity at approximately

60%. The gradient cover consisted of a transparent acrylic surface attached to a photoperiod system. The thermal gradient heated at one end and cooled at the other, providing a range of temperature in air and soil. The mean temperature range of the system was  $9.27^{\circ}\text{C} (\pm 2.09)$  to  $38.63^{\circ}\text{C} (\pm 2.40)$ , increasing by approximately  $3^{\circ}\text{C}$  every 10 cm (Figure 2). The laboratory thermal gradient allowed the animals access to a wide range of environmental temperatures without being affected by other variables such as predators, prey and a number of other factors that could limit the field [56].



**Figure 2:** Thermal gradient. (1) Heat Source, (2) Photoperiod System, (3) Thermometer, (4) Lane Separator Mesh, (5) Gradient Cap, (6), (7), (8) and (9) Lanes, (10) Cooling Source, (11) Gravel Substrate, (12) Perforated Acrylic Base, (13) Moisture System. The mean temperature range of the system was  $9.27^{\circ}\text{C} (\pm 2.09)$  to  $38.63^{\circ}\text{C} (\pm 2.40)$ , increasing by approximately  $3^{\circ}\text{C}$  every 10 cm.

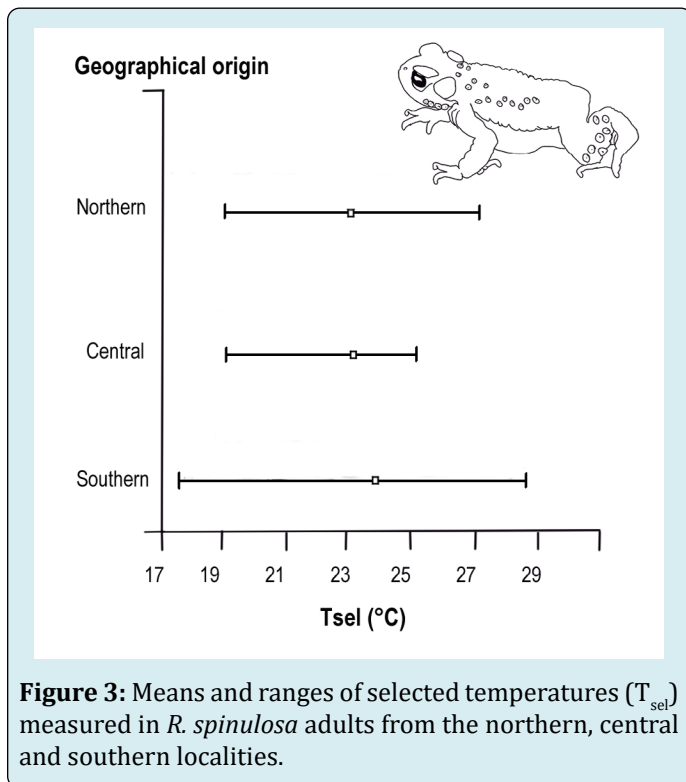
Data collection was performed simultaneously for four individuals, one in each lane, where it was not possible for them to come into contact. The animals were placed in the center of each lane and  $T_b$  dorsal temperature with a Digital Infrarrojo Laser ( $380^{\circ}\text{C}/^{\circ}\text{F}$ ) Fluke thermometer was recorded at the beginning and in three subsequent moments: 5, 6 and 7 hours after starting the experiment. The mean of these three values corresponds to the  $T_{sel}$  of the organisms [57]. By measuring with an infrared thermometer it was not necessary to handle the animal, which reduces stress and preserves natural behaviors and eliminates the risk of altering the subject's  $T_b$  [56].

The statistical support was tested in Infostat version 2020e. Kruskal Wallis tests were performed to show significant differences in the parameters mass, size and  $T_{sel}$  among the three localities. A nonparametric ancova analysis was also used, using mass and size as  $T_{sel}$  covariates.

## Results

*Rhinella spinulosa* size and body mass varied significantly among localities. Analysis by locality showed a significantly larger size of individuals from the central locality (northern:  $71.97 \pm 0.28$  mm; central:  $89.63 \pm 4.50$  mm, southern:  $75.63 \pm 4.01$  mm; K-W:  $H=10.07$ ,  $P=0.006$ ), which also had higher body mass compared to the other sites analyzed (northern:  $45.33 \pm 7.77$  g; central:  $69.18 \pm 6.75$  g, southern:  $55.15 \pm 11.87$  g. K-W:  $H=7.72$ ,  $P=0.021$ ).

The individuals from the three localities preferred  $T_{sel}$  above  $23^{\circ}\text{C}$  ( $T_{sel}$  northern:  $23.87 \pm 2.52^{\circ}\text{C}$ ,  $T_{sel}$  central:  $23.16 \pm 2.13^{\circ}\text{C}$ ,  $T_{sel}$  southern:  $23.34 \pm 2.88^{\circ}\text{C}$ ) (Figure 3); however, there were no significant differences between localities (K-W:  $H=0.45$ ;  $P=0.80$ ). Therefore, as there were no differences between the three locations, the mean  $T_{sel}$  of *R. spinulosa* for this study was  $23.38 \pm 2.65^{\circ}\text{C}$  (range:  $17.73^{\circ}\text{C}$ - $28.93^{\circ}\text{C}$ ). On the other hand, the non-parametric Ancova analysis established that there were no significant differences in  $T_{sel}$  using mass ( $F=1.70$ ;  $P=0.19$ ) and size ( $F=2.47$ ;  $P=0.12$ ) as covariates.



**Figure 3:** Means and ranges of selected temperatures ( $T_{sel}$ ) measured in *R. spinulosa* adults from the northern, central and southern localities.

## Discussion

The regulation of body temperature in ectotherms has an important impact on their physiological and behavioral processes [3]. The selected temperature ( $T_{sel}$ ) is the body temperature at which physiological processes such as

digestion, swimming, and locomotion are optimized [58,59].  $T_{sel}$  can be modified in response to environmental parameters and/or physiological state so understanding the results of experiments with amphibians in thermal gradients is crucial for proper inference in studies of evolution, ecological climate change, niche modeling, and emerging diseases, among others [60,61].

In our study, *R. spinulosa* shows to select a wide range of temperatures when subjected to a thermal gradient, which is consistent with its eurythermic behavior, since it tolerates critical maximum temperatures above  $36^{\circ}\text{C}$  and critical minimum temperatures below  $0^{\circ}\text{C}$  [50,51]. This antecedents confirm that it is a thermogeneralist species, a characteristic that allows the species to survive in adverse microclimatic conditions [51].

$T_{sel}$  studies in the genus *Rhinella* for the adult stage show a temperature range fluctuating between  $21^{\circ}\text{C}$  to  $28^{\circ}\text{C}$  [52,60-64] so our results are within the range described for the genus.

For *R. spinulosa*, previous works only address  $T_{sel}$  in larval and postmetamorphic stages, which show a  $T_{sel}$  around  $25^{\circ}\text{C}$  [38,50,65]. The results of the present study are consistent with these  $T_{sel}$  showing a difference of  $2^{\circ}\text{C}$  less, which could be attributed to the nocturnal habits of adults.

The effect of activity periods on  $T_{sel}$  is an interesting aspect to address in thermal studies that could explain why adults subjected to a wide thermal supply select temperatures higher than the ambient temperature of their respective habitats. Anguilletta, et al. state that anurans in cold environments do not necessarily select these same temperatures in experimental gradients, so we can hypothesize that the optimal field temperature for processes such as locomotion in *R. spinulosa* is lower than  $T_{sel}$  in the laboratory. Furthermore, it has been established that in the absence of thermoregulatory opportunities (as might occur during the night), animals experience thermoconformational behavior, allowing time to be spent on other activities such as foraging, reproduction and shelter-seeking for daily torpor [62,66]. However, for other processes, such as digestion, adults of *R. spinulosa* exhibit heliothermic behavior during the morning that helps them to improve the digestion of prey captured the previous night [49].

In a temperature gradient, individuals have greater thermal options to thermoregulate and there are no other variables that may hinder their preference such as lack of operative temperatures, risk of predation, water balance, among others [63,66,67]. Therefore, their  $T_{sel}$  will adjust to the temperature at which physiological performance is optimal [49], which allows them to compensate for thermal



conditions in captivity, selecting temperatures similar to those they would select in shelters in their respective habitats.

These toads are active during the night and quickly equilibrated with the ambient temperature; however, during daytime rest, they bury or hide in volcanic rock shelters (Personal Obs.). In addition to this, since the experimentation was during the day, the  $T_{sel}$  would correspond to that of the resting period, so the individuals thermoregulated behaviorally selecting higher temperatures than those corresponding to the ambient temperatures in the field, which allows them to maintain a high metabolic rate during their resting period, a situation that changes during their period of activity at night. This has already been described in other bufonids, such as *Anaxyrus fowleri* under natural conditions [68]. These toads are active during the night and maintain a  $T_b$  similar to  $T_a$ , but during daytime rest, they select and maintain a body temperature around 30°C, in order to maintain a high metabolic rate. Thus, it can be established that these animals thermoregulate behaviorally during their resting hours [68].

In our field studies, it has been observed that during the active season the toads spend the daylight hours in shelters made up of volcanic rock burrows and that they do not explore microhabitats with higher thermal quality, such as areas open to the sun, a situation that also occurs in other species of the genus [64]. It is presumed that these shelters maintain stable temperatures throughout the daily cycle of the species and that night temperatures there are close to  $T_{sel}$ , allowing them to reach an optimal temperature after foraging.

Our results show the need for future studies to contrast experimental results with values obtained in field conditions, such as evaluating thermal precision and thermoregulatory efficiency taking into account the habitat thermal quality.

Although the three sampling localities are widely separated from each other [35,46], the mean  $T_{sel}$  is similar in the three localities analyzed, without evidencing significant differences, which would indicate that individuals exhibit a conservative  $T_{sel}$  and thus rely mainly on behavioral thermoregulation to cope with cold and variable temperatures, since within the gradient, they tend to avoid low temperatures.

The degree to which physiological traits are phylogenetically conserved has been described primarily in reptiles, which demonstrate that lizards inhabiting different thermal environments have similar body temperatures due to behavioral thermoregulation which helps to reduce the strength of natural selection and reduce rates of trait

evolution [69-72].

However, without further background, it is not possible to confirm this response in our study. On the other hand, previous studies in *R. spinulosa* that analyzed local adaptation to temperature indicate that those originating from a highly variable environment show a broad transcriptional response to temperature change, thus possessing greater plasticity and tolerance than populations from homogeneous habitats [46].

This is how the selection of a temperature that is optimal for the organism may vary over time, as amphibians are organisms with high plasticity in terms of body temperature, whose acclimatization capacity allows them to move between thermal extremes [31] and  $T_{sel}$  should not necessarily be considered as coadapted with the optimal temperature and replicate field body temperature [73,60].

Finally, because the amphibians have a permeable skin and both aquatic and terrestrial life stages make them susceptible to changes in temperature, studies related to thermal physiology in species such as *R. spinulosa* contribute to the understanding of how amphibian species will be affected by climate change [74], which also allows for more accurate predictions of species distribution models [75]. According to Blaustein, et al. [76], species distribution models should be strengthened with laboratory and field experiments, as understanding the causal mechanisms that limit species distribution is likely to improve our ability to anticipate changes in their range in response to climate change [77].

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