Seasonal Changes in the Thermal Tolerances of *Odontophrynus occidentalis* (BERG, 1896) (Anura: Cycloramphidae)

Eduardo A. Sanabria*,[1], Lorena B. Quiroga[2] & Adolfo L. Martino[3]

[1] Laboratorio de Investigaciones Andrológicas de Mendoza, Instituto de Histología y Embriología de Mendoza, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Centro de Investigaciones en Ciencia y Técnica de Mendoza, CONICET, Mendoza, Argentina.

[2] Departamento de Biología, Facultad de Ciencias Exactas Físicas y Naturales, Universidad Nacional de San Juan. Av. Jose Ignacio de la Rosa y Meglioli, 5400 San Juan, Argentina.

[3] Cátedra de Ecología, Departamento de Ciencias Naturales, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto. Ruta Nacional N° 36 - km 601, (X5804BYA) Río Cuarto, Argentina.

* Corresponding author: sanabria.eduardoa@gmail.com

ABSTRACT. We studied the thermal tolerances of *Odontophrynus occidentalis* during the dry and wet seasons of the Monte Desert in San Juan Province, Argentina. This toad had differences in CT_{max} between dry and wet seasons, with CT_{max} values being greater in the wet season (Austral summer). Operative temperature, body temperature, and environmental maximal temperature were related to CT_{max} suggesting seasonal acclimatization in *O. occidentalis*. Additionally, the CT_{max} recorded for *O. occidentalis* was 36.1 ºC, and the maximum ambient temperature measured during the toads' activity time was 37 ºC. The CT_{min} of *O. occidentalis* was 4.1 ºC while the minimum environmental temperature recorded was 7.2 ºC. The wide tolerance range observed and the relationship between tolerance limits and the environmental extremes indicate that seasonal acclimatization is an effective mechanism by which toads can raise their thermal tolerance. This is a highly relevant adaptation allowing them to survive in the challenging conditions of the Monte Desert.

KEY WORDS: Amphibia; Argentina; Monte Desert; Seasonal Acclimatization; Thermal Tolerance

INTRODUCTION

All organisms possess some capacity to modify their behavioral and physiological characteristics in response to environmental temperature (ANGILLETTA, 2009). The thermal extremes minimum and maximum (critical temperatures) are defined as "the thermal point at which locomotory activity becomes disorganized and an animal loses its ability to escape from conditions that will promptly lead to its death" (COWLES & BOGERT, 1944). The critical thermal maximum (CT_{max}) and critical thermal minimum (CT_{min}) are widely used to define the range of thermal tolerances of animals within their environments (DOUGHTY, 1994; HUEY & STEVENSON, 1979; STEBBINS & COHEN, 1995). In amphibians, thermal tolerances are influenced by hydration (CLAUSSEN, 1969; POUGH & WILSON, 1970),

nutritional status (CUPP, 1980), photoperiod (HUTCHISON, 1961; HUTCHISON & KOSH, 1964; HUTCHISON & FERRANCE, 1970), melatonin (ERSKINE & HUTCHISON, 1982), fatigue (BURKE & POUGH, 1976) and relative humidity (SANABRIA et al., 2012). Also, daily and seasonal fluctuations in environmental temperatures affect variation in the thermal tolerances of many species of ectotherms (ROME et al., 2002). Finally, life-history parameters and reproductive activity also influence temperature tolerances. For example, male toads (*Rhinella arenarum*) have higher CT_{max} in the reproductive season when they are active during the day than in the post-reproductive season when they are active only at night (SANABRIA & QUIROGA, 2011a). This variation in thermal tolerances, being associated with local climatic variation (daily or seasonal) and the organism's natural history, is an

important form of physiological acclimatization (IUPS Thermal Commission, 2003).

Physiological strategies such as acclimatization enable animals to compensate for, and thus avoid, the consequences of thermal extremes, which can be lethal or cause permanent damage to cells, membranes, and proteins (Brattstrom & Lawrence, 1962). It is possible that animals do not tolerate temperatures above 50 °C (Kay, 1998) through biochemical restrictions imposed by the denaturation of proteins, as the upper limit that proteins can tolerate before denaturation is between 45–50 °C. All the mechanisms above are associated with critical temperature extremes (Huey & Stevenson, 1979; Hill et al., 2006) and influence the survival of ectotherms during brief periods of exposure to potentially lethal temperatures (Brattstrom & Lawrence, 1962).

The thermal tolerances and acclimatization abilities of South American amphibians – especially those inhabiting deserts – have rarely been studied. We investigated the thermal tolerances of a population of the toad *Odontophrynus occidentalis* in the Monte Desert of San Juan, Argentina. We compared the thermal tolerances (CT_{max} and CT_{min}) of *O. occidentalis* between the dry and wet season. In addition, we tested the relationship that CT_{max} and CT_{min} have with operative temperatures (T_e) and several abiotic characteristics of the environment. We hypothesized that thermal tolerances of *O. occidentalis* toads change between dry and wet season, and that the thermal tolerance parameters (CT_{max} and CT_{min}) are related to abiotic parameters because the toads of the desert experience an acclimatization process during the year.

MATERIAL AND METHODS

Study site

The Quebrada de Las Flores is localized 55 km east of the city of San Juan, Caucete Department, San Juan Province, Argentina (31.70079 °S, 68.09506 °W, elevation: 800 m). The small saline stream (7710 mS cm^{-1}) that runs through this canyon fluctuates dramatically in rates of daily and seasonal flow. The dominant plants in the area are *Deuterocohnia longipetala*, *Larrea cuneifolia* and *L. divaricata*, *Prosopis* sp., *Typha dominguensis* and *Baccharis salicifolia*. Quebrada de Las Flores lies within the Monte phytogeographic province, a region characterized by an arid climate with a mean annual temperature of 17.3 °C, a mean maximum temperature of 25.7 °C, a mean minimum temperature of 10.4 °C, and a mean annual precipitation of 89 mm falling primarily in summer (Cabrera, 1976).

Measuring of Field Body Temperatures and Environmental Parameters

We compared the thermal biology of toads during the wet and dry seasons from November 2008 to October 2009. The wet season has heavy rains from December to March and the dry season lasts from April to November. We collected individuals haphazardly using the technique of visual encounter (Heyer et al., 2001).

We measured the body temperatures (T_b) of surface-active adult toads ($N = 35$) at the time of capture with a digital thermometer (± 0.1 °C; TES 1312, TES Electrical Electronic Corp., Taipei, Taiwan) by inserting probes (28 AWG type-K catheter probes, TES TP-K01) ~1 cm into the cloaca of each toad. T_bs were measured from August to November (dry season) and December to March (wet season). Thereafter, we measured toad body sizes (snout–vent length, SVL) to the nearest mm with a transparent ruler.

Environmental data, including the maximum and minimum temperature (T_{max}, T_{min}), effective heliophany (number of hours of light in the field), and relative humidity (RH) for the study period were obtained from the Instituto Nacional de Técnicas Agropecuarias (INTA), Pocito, San Juan.

Estimating Critical Thermal Maximum and Minimum

Immediately after capture, toads were transported to the laboratory to measure their

thermal tolerances; the measurements were made on 25 toads (9 for the dry season and 16 for the wet season). Critical thermal maximum (CT_{max}) was determined using the methods described by HUTCHINSON (1961). Individual toads were placed in a glass container (15 x 30 cm) with 300 ml of water at ambient temperature (25 ± 2 °C). A catheter probe (TES TP-K01) was inserted ~1 cm into the cloaca to measure T_b. The catheter probe was fixed into the cloaca with a drop of instant glue (La Gotita, Poxipol®, Buenos Aires, Argentina). The toads were kept in captivity for 2 d during which the probes detached on their own. An electric heating mantle raised the toad's temperature at a rate of approximately 1 °C min⁻¹. The righting reflex was checked at regular intervals of two minutes after the toads showed a voluntary escape behavior when being turned on their backs. When the righting reflex was lost, CT_{max} was recorded.

After the measurement of the critical temperature (CT_{max}), the toads were placed in individual terrariums (40x20x30) with free access to water and food at ambient temperature. After 72 h, we measured the CT_{min}. To determine CT_{min}, we followed the same procedure described above for CT_{max} except that we placed the glass container inside a larger plastic container containing water and ice, which reduced the toad's temperature at a rate of approximately 1 °C min⁻¹. The T_b at which the righting reflex was lost was recorded as the animal's CT_{min}.

All measurements were taken at night, since the natural activity period of this toad species is nocturnal.

Construction and Calibration of Plaster Operative Temperature Models

Four plaster models were constructed using methods described by Tracy et al. (2007). We sacrificed two adult male *O. occidentalis* (mean SVL = 52 ± 0.5 mm), by injecting an overdose of anesthesia (2.5 ml of 2% xylocaine and 2% lidocaine HCl, AstraZeneca Labs, Bs. As., Argentina) into their lymphatic sacs. Toads were fixed in their water-conservation posture with 10% formaldehyde (formalin) for 3 d. Thereafter, the toads were placed in acetic silicone (Dow Corning, Bs. As., Argentina) to create a flexible matrix. This matrix was filled with a preparation of plaster (30% plaster of Paris and 70% water, by volume) to obtain replicas of the specimens. To prevent dehydration, a water source was connected to the models by inserting a silicone tube (outer and inner diameters 4 mm and 2 mm, respectively) into the left side of the toad model until the core was reached and the tube was connected to a 1-L plastic bottle filled with distilled water. Capillary action kept the models hydrated. In addition, we inserted a thermistor from a data logger (Hobo H8, Onset Computer Corporation, USA) into the models to record their temperature every 5 min.

The four models were calibrated by comparing them to one male toad (SVL=53 mm). This toad was anesthetized with 0.5 ml of 2% xylocaine and 2% lidocaine HCl (AstraZeneca Labs, Buenos Aires, Argentina). The calibration was performed under field conditions T_e models remained in the field for 3 d mo⁻¹. T_e (sensu: BAKKEN, 1992) was recorded every 5 min. We only used T_e measurements that were recorded during the daily activity periods of the toads.

Data Analyses

We calculated the mean and standard error for all thermal data, and analyzed relationships using non-parametric Spearman correlations. We applied the Mann–Whitney U test to evaluate differences between the dry and wet seasons and to evaluate the differences in thermal parameters between the species studied. We used non parametric statistical analysis, because the data were not normally distributed and there was no homogeneity in the variance. The PAST version 9.4 (HAMMER, 2001) statistical packet was used for statistical analysis.

RESULTS

Calibrations of Plaster Operative Temperature Models

We obtained significant regression coefficients ($r^2 = 0.98$, $P = 0.00001$) for the comparisons of model and toad temperatures for the calibration. The slight difference in temperature between the model and the T_b of the toad used for calibration (0.4 ± 0.2 ºC) was not statistically significant ($U = 191.5$, $P > 0.81$).

Operative Temperatures and Environmental Parameters

The mean of T_e was measured as 18.31 $\pm$ 0.4 ºC (range: 14.5–21.3 ºC). The mean environmental thermal maximum (T_{max}) reached 30.5 $\pm$ 0.8 ºC (range: 14.4–37.0 ºC) and the mean environmental thermal minimum (T_{min}) 15.6 $\pm$ 0.8 ºC (range: 7.2–21.0 ºC). The effective heliophany had a mean of 10.6 $\pm$ 0.3 h (range: 6.8–12.4 h) and the mean relative humidity was 55.9 $\pm$ 0.73% (range: 47–64%).

Relationship Between Thermal Tolerances and Environmental Parameters

The mean tolerance range of *O. occidentalis* was 30.17 $\pm$ 0.26 °C (wet season) and 30 $\pm$ 0.56 °C (Dry season), not showing any significant

differences between seasons ($U = 40.5$, $P > 0.1$). CT_{max} was significantly higher in the wet (36.1 $\pm$ 0.2 ºC) than in the dry seasons (34.1 $\pm$ 0.3 ºC) ($N = 25$, $U = 10.5$, $P < 0.00004$; Fig. 1). CT_{max} was significantly correlated to T_b ($r = 0.45$, $P < 0.02$; Fig. 2) as well as to T_e ($r = 0.53$, $P < 0.006$; Fig. 2), T_{max} ($r = 0.44$, $P < 0.02$; Fig. 3) and T_{min} ($r = 0.45$, $P < 0.02$; Fig. 3). No relationship was found with the other variables such as SVL ($P > 0.3$) or effective heliophany ($P > 0.3$) and RH ($P > 0.2$).

The CT_{min} of *O. occidentalis* was significantly higher in the wet (4.9 $\pm$ 0.1 ºC) than in the dry seasons (4.1 $\pm$ 0.4 ºC) ($N = 35$, $U = 54$, $P < 0.002$; Fig. 4). Also CT_{min} was significantly correlated with T_b ($r = 0.41$, $P < 0.01$) but no significant relationship was found between CT_{min} and SVL ($P > 0.1$), T_{min} ($P > 0.15$), T_e ($P > 0.12$), T_{max} ($P < 0.3$), effective heliophany ($P > 0.9$), or RH ($P > 0.2$).

DISCUSSION

Odontophrynus occidentalis showed a different CT_{max} in the dry compared to the wet seasons, and the CT_{max} values were greater in the wet season (Austral summer). In addition, T_e, T_b, T_{max}, and T_{min} were significantly related to CT_{max}. This suggests that the acclimatization process (HUTCHISON & MANESS, 1979) could be mainly
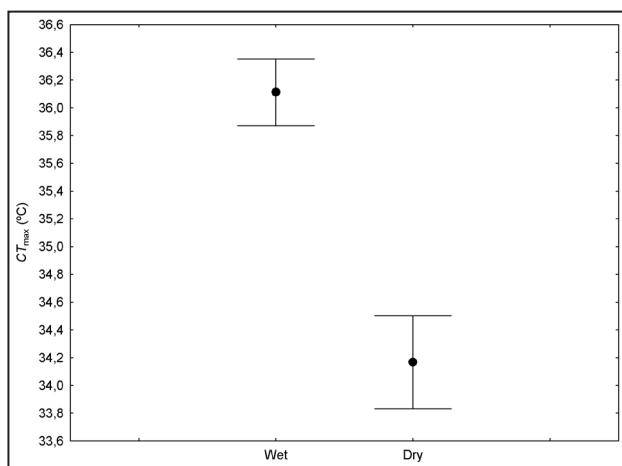

Fig. 1. – Variation of critical thermal maximum between wet and dry season, for *Odontophrynus occidentalis* from the Monte Desert of Argentina.
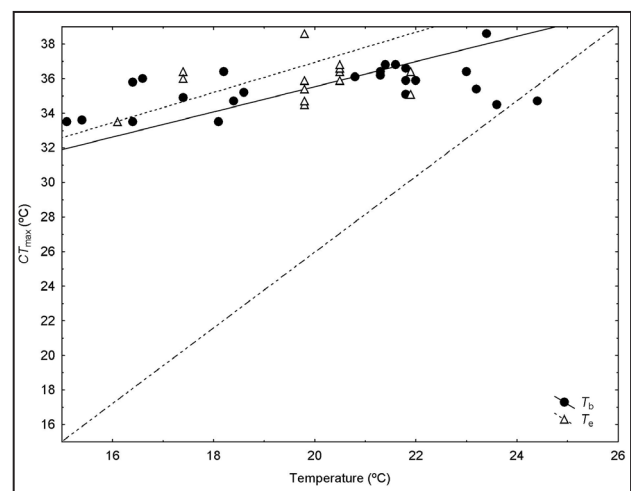

Fig. 2. – Relationship between the critical thermal maximum and body (white dots) and operative (black dots) temperatures for *Odontophrynus occidentalis* from the Monte Desert of Argentina.

regulated by environmental conditions. The four variables (T_e, T_b, T_{max}, and T_{min}) have an important role in the acclimatization of the toads. Thermal tolerances (CT_{min} and CT_{max}) vary with changes in local weather conditions where amphibians develop (BRATTSTROM, 1968; HUTCHISON, 1961; HUTCHISON & KOSH, 1964; HUTCHISON & FERRANCE, 1970; ROME et al., 2002) and with the physiological condition of the animals (BURKE & POUGH, 1976; CLAUSSEN, 1969; CUPP, 1980; ERSKINE & HUTCHISON, 1982). It is likely that the changes in temperature between the dry and wet seasons are the environmental cues stimulating seasonal thermal acclimatization in *O. occidentalis*. *Rhinella arenarum* at the same latitude exhibits an acclimatization process, where the climatic parameters are a principal modulator (SANABRIA et al., 2012). In contrast, changes in photoperiod have been suggested to be the principal acclimatization cue for other ectotherms such as salamanders, turtles, and the frog *Lithobates pipiens* (HUTCHISON & KOSH, 1964; HUTCHISON, 1961; HUTCHISON & FERRANCE, 1970). Photoperiod affects the acclimation of some amphibians, especially anurans with diurnal activity that gain heat energy via basking (BRATTSTROM, 1968; DUELLMAN & TRUEB, 1986). Apparently, in adults of *O. occidentalis*, which are nocturnal, photoperiod is probably not significant for acclimatization,

because there was no association between effective heliophany and thermal tolerances. However, the tadpoles of *Odontophrynus occidentalis* have a long larval period prior to metamorphosis with diurnal activity. This tadpole shows changes in the thermal parameters in relation to the photoperiod. Apparently, in this condition, the photoperiod is a modulator of acclimatization and confers more tolerance to environmental temperature with an increase in hours of light (SANABRIA & QUIROGA, 2011b).

The critical thermal minimum has not been widely investigated in amphibians, although there have been a few anecdotal studies (DUELLMAN & TRUEB, 1986). We found the CT_{min} of *O. occidentalis* to be significantly different between wet and dry seasons. Our data furthermore allows us to calculate the thermal tolerance range of the species in the dry and wet seasons. *O. occidentalis* showed no significant differences in mean values of thermal ranges during the wet and dry seasons. Ecologically, the thermal tolerance range is considered as the safety margin of a species, allowing it to escape extreme temperatures (ANGILLETTA, 2009; HUEY & STEVENSON, 1979). Species that have wide thermal tolerance ranges are generally associated with environments that have strong seasonal fluctuations in temperature (NAVAS et al., 2008), such as deserts (WARNER, 2004).
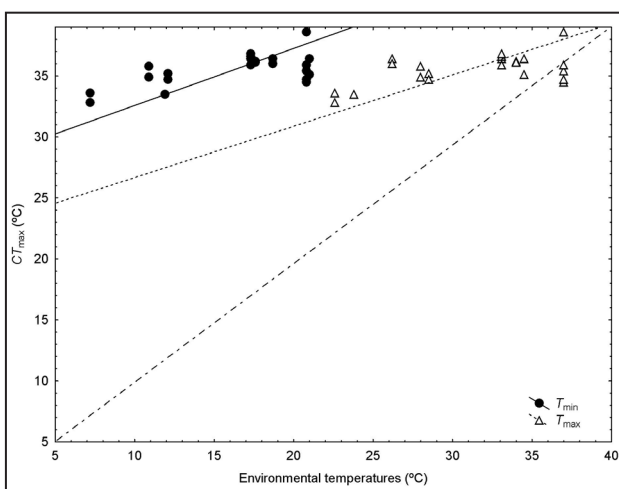

Fig. 3. – Relationship between the critical thermal maximum and environmental thermal maximum (black dots) and minimum (white dots) for *Odontophrynus occidentalis* from the Monte Desert of Argentina.
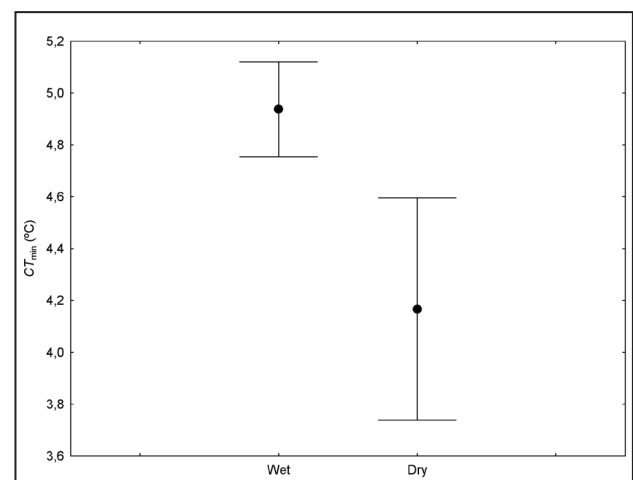

Fig. 4. – Variation of the critical thermal minimum between the wet and dry season for *Odontophrynus occidentalis* from the Monte Desert of Argentina.

Studies of desert-dwelling anuran larvae, where high temperatures are common, have proven that these taxa possess high thermal tolerances (BROWN, 1969; CUPP, 1980; HOPPE, 1978; ULTSCH et al., 1999; WHITFORD, 2002). In contrast, tropical frogs living in aseasonal environments do not have to undergo acclimatization because temperatures are relatively constant throughout the year (DUELLMAN & TRUEB, 1986; NAVAS, 1997). Having wide thermal tolerance ranges allows a species to quickly acclimatize to extreme temperatures and thus avoid the generation of heat shock proteins and the energetic costs of their production (ANGILLETTA, 2009; HILL et al., 2006).

The toads in this study were found to have extreme temperature tolerances close to daily minimum (T_{min}) and maximum (T_{max}) environmental temperatures. This indicates that the species studied here became adapted to extreme daily temperature fluctuations. The wide thermal range detected and the relationship with the environmental parameters (acclimatization) is an efficient mechanism by which toads can raise thermal tolerance (BRATTSTROM, 1963). However, further studies are required to understand the relationship between the thermal extremes of amphibians of the desert and the environmental parameters that influence the thermal physiology of these species. Additional studies are needed to understand the relationship between the thermal tolerances of this desert amphibian and the environmental parameters that influence its thermal physiology.

ACKNOWLEDGMENTS

REFERENCES

ANGILLETTA MJ JR (2009). Thermal adaptation a theoretical and empirical synthesis. Oxford University Press, Oxford.

BAKKEN GS (1992). Measurement and application of operative and standart operative temperatures in ecology. American Zoology, 32:194-216.

BRATTSTROM BH (1963). A preliminary review of the thermal requirements of amphibians. Ecology, 44:238-255.

BRATTSTROM BH (1968). Thermal aclimation in anuram amphibians as a function of latitud and altitude. Comparative Biochemistry and Physiology, 24:93-111.

BRATTSTROM BH & Lawrence P (1962). The rate of thermal acclimation in anuran amphibians. Physiological Zoology, 35:148-156.

BROWN HA (1969). The heat resistance of some anuran tadpoles (Hylidae and Pelobatidae). Copeia, 1969:138–147.

BURKE ME & POUGH FH (1976). The role of fatigue in temperature resistance of salamanders. Journal of Thermal Biology, 1:163-167.

CABRERA AL (1976). Regiones fitogeográficas de la República Argentina. In: ACME (Ed.), Enciclopedia Argentina de Agricultura y Jardinería, Buenos Aires, Argentina.

CLAUSSEN D (1969). Thermal aclimatation in ambystomatid salamanders. Comparative Biochemistry and Physiology, 58A:333-340.

COWLES RB & BOGERT CM (1944). A preliminary study of the thermal requirements of desert reptiles. Bulletin of the American Museum of Natural History, 83:261-296.

CUPP P (1980). Thermal tolerance of five salientian amphibians during development and metamorphosis. Herpetologica, 36:234-244.

DOUGHTY P (1994). Critical thermal minima of garter snake (*Thamnophis*) depend on species and body size. Copeia, 1994:537-540.

DUELLMAN WE & TRUEB L (1986). Biology of Amphibians. McGraw-Hill Publ. Co., Baltimore, Maryland, USA.

ERSKINE DJ & HUTCHISON VH (1982). Reduced thermal tolerance in an amphibian treated with melatonin. Journal of Thermal Biology, 7:121–123.

HAMMER Ø, HARPER T & RYAN PD (2001). PAST: Paleontological Statistics Software Package for

Education and Data Analysis. Paleontología Electrónica, 4(1):1-9. http://palaeo-electronica.org/2001_1/past/issue1_01.htm.

HEYER WR, DONNELLY MA, McDIARMID RW, HAYEK LC & FOSTER MS (2001). Medición y monitoreo de la diversidad biológica, métodos estandarizados para anfibios. Editorial Universitaria de la Patagonia, Universidad Nacional de la Patagonia San Juan Bosco, Trelew, Chubut, Argentina.

HILL R, WYSE G & ANDERSON M (2006). Fisiología Animal. Editorial Médica Panamericana, Madrid, España.

HOPPE DM (1978). Thermal tolerance in tadpoles of the chorus frog Pseudacris triseriata. Herpetologica, 34:318-321.

HUEY RB & STEVENSON RD (1979). Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. American Zoologist, 19:357- 366.

HUTCHISON VH (1961). Critical thermal maxima in salamanders. Physiological Zoology, 2:92-125.

HUTCHISO VH & KOSH RJ (1964). The effect of photoperiod on the critical thermal maxima of painted turtles (Chrysemys picta). Herpetologica, 20:233-238.

HUTCHISON VH & FERRANCE MR (1970). Thermal tolerances of Rana pipiens acclimated to daily temperature cycles. Herpetologica, 23:1-8.

HUTCHISON VH & MANESS JD (1979). The role of behavior in temperature acclimation and tolerance in ectotherms. American Zoologist, 19:367-384.

HUEY RB & STEVENSON RD (1979). Integrating thermal physiology and ecology of ectotherms: a discussion of Approaches. American Zoologist, 19:357-366.

IUPS Thermal Commission (2003). Glossary of terms for thermal physiology, 3rd ed.. Journal of Thermal Biology, 28:75-106.

KAY I (1998). Introduction to animal physiology. BIOS Scientific publisher limited. Ferst Publisher, UK.

NAVAS CA, GOMES FR & CARVALHO JE (2008). Review: Thermal relationship and exercise physiology in anuran amphibians: integration and evolutionary implications. Comparative Biochemistry and Physiology, 151:344-362.

NAVAS CA (1997). Thermal extremes at high elevations in the Andes: physiological ecology of frogs. Journal of Thermal Biology, 22:467-477.

POUGH FH & WILSON RE (1970). Natural daily temperature stress, dehydration, and acclimation in juvenile Ambystoma maculatum (Shaw) (Amphibia: Caudata). Physiological Zoology, 43:194-205.

ROME L, STEVENS D & JOHN-ADLER B (2002). The influence of temperature and thermal acclimatation on physiological function. In: Feder M. & Burggeren W. (eds), Environmental physiology of the amphibians:183-205.

SANABRIA EA & QUIROGA LB (2011a). Thermal parameters changes in males of Rhinella arenarum (Anura: Bufonidae) related to reproductive periods. International Journal of Tropical Biology, 59:347-353.

SANABRIA EA & QUIROGA LB (2011b). Change in the thermal biology of tadpoles of Odontophrynus occidentalis from the Monte desert, Argentina: Responses to photoperiod. Journal of Thermal Biology, 36(5): 288-291.

SANABRIA EA, QUIROGA LB & MARTINO A (2012). Seasonal Changes in the Thermal Tolerances of the Toad Rhinella arenarum (Bufonidae) in the Monte Desert of Argentina. Journal of Thermal Biology, 37:409-412.

STEBBINS RC & COHEN WN (1995). Temperature caracteristics. In: A natural history of amphibians:89-101, Princeton University Press.

IUPS THERMAL COMMISSION (2003). Glossary of terms for thermal physiology, 3rd ed. Journal of Thermal Biology, 28:75–106.

TRACY RC, BETT G, TRACY RC & CHRISTIAN KA (2007). Plaster models to measure operative temperature and evaporative water loss of amphibians. Journal of Herpetology, 41:597-603.

ULTSCH GR, BRADFORD DF & FREDA J (1999). Physiology coping with the environment. In: McDiarmid R.W. & Altig R. (eds), Tadpole: The Biology of Anuran larvae: 202-210, University of Chicago Press, Chicago, Illinois, U.S.A.

WARNER TT (2004). Desert Meteorology. Cambridge University Press. New York, USA.

WHITFORD W (2002). Ecology of Desert Systems. Elsevier Academic Press, London.