



Variation of thermal parameters in two different color morphs of a diurnal poison toad, *Melanophryniscus rubriventris* (Anura: Bufonidae)



Eduardo A. Sanabria^{a,*}, Marcos Vaira^{b,d}, Lorena B. Quiroga^c, Mauricio S. Akmentins^{b,d}, Laura C. Pereyra^{b,d}

^a CONICET, Instituto de Ciencias Básicas, Facultad de Filosofía Humanidades y Artes, Universidad Nacional de San Juan, Avenida José Ignacio de la Roza 230 (O), 5400 San Juan, Argentina

^b Centro de Investigaciones y Transferencia de Jujuy (CIT-JUJUY) CONICET, Universidad Nacional de Jujuy, Av. Bolivia 1711 (4600), Jujuy, Argentina

^c Departamento de Biología, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de San Juan, San Juan, Argentina

^d San Salvador de Jujuy, Argentina Instituto de Bio y Geociencias del NOA, Universidad Nacional de Salta, Salta, Argentina

ARTICLE INFO

Article history:

Received 29 November 2013

Accepted 29 January 2014

Available online 4 February 2014

Keywords:

Melanophryniscus rubriventris

Melanization

Coloration

Critical thermal maximum

Field body temperature

Heating rate

ABSTRACT

We study the variation in thermal parameters in two contrasting populations Yungas Redbelly Toads (*Melanophryniscus rubriventris*) with different discrete color phenotypes comparing field body temperatures, critical thermal maximum and heating rates. We found significant differences in field body temperatures of the different morphs. Temperatures were higher in toads with a high extent of dorsal melanization. No variation was registered in operative temperatures between the study locations at the moment of capture and processing. Critical thermal maximum of toads was positively related with the extent of dorsal melanization. Furthermore, we founded significant differences in heating rates between morphs, where individuals with a high extent of dorsal melanization showed greater heating rates than toads with lower dorsal melanization. The color pattern-thermal parameter relationship observed may influence the activity patterns and body size of individuals. Body temperature is a modulator of physiological and behavioral functions in amphibians, influencing daily and seasonal activity, locomotor performance, digestion rate and growth rate. It is possible that some growth constraints may arise due to the relationship of color pattern-metabolism allowing different morphs to attain similar sizes at different locations instead of body-size clines.

© 2014 Published by Elsevier Ltd.

1. Introduction

Many studies addressing the adaptive significance or ecological implications of the coloration in amphibians have focused on how the variation translates into predator avoidance (Hoffman and Blouin, 2000; Rudh and Qvarnström, 2013; Toledo and Haddad, 2009). Aposematic anurans that are chemically defended by sequestered alkaloids advertise their defenses to predators via warning signals that are most often bright colors combined with black patterns (Amézquita et al., 2013; Bonansea and Vaira, 2012; Mochida, 2009). Empirical studies show that predators avoid attacking such patterned models, implying that appearance promotes the recognition of undesirable prey (Noon and Comeault, 2009). However, positive results for the significance of particular color patterns as warning signals do not rule out other functions of

coloration (Stevens and Ruxton, 2012). If selection influences the evolution of color traits, there may be conflicting pressures that modulate color expression resulting in a compromise in the final coloration pattern (Mochida, 2011). Conspicuousness of the coloration pattern is known to exhibit considerable phenotypic variation in amphibians, suggesting that color expression may be influenced by many selection pressures (Rudh and Qvarnström, 2013). In some cases, the coloration pattern may be a compromise between several functions (i.e. warning signals, thermoregulation, crypsis, immune system function, mate recognition) although the complexity of these interactions makes it difficult to fully understand the functional significance of coloration (Stevens and Ruxton, 2012).

The Yungas Redbelly Toad, *Melanophryniscus rubriventris*, is an endemic anuran of the Yungas Andean forest, distributed in northwestern Argentina and southern Bolivia. This species exhibits a geographic color polymorphism with the presence of marked variation in the extent of bright coloration. Populations vary from uniform olive color to black dorsal patterns, while others exhibit a vivid red dorsum (Bonansea and Vaira, 2012). Despite huge differences

* Corresponding author. Tel.: +54 264 4232249.

E-mail addresses: sanabria.eduardoa@gmail.com (E.A. Sanabria), marcos.vaira@gmail.com (M. Vaira), quirolalb@gmail.com (L.B. Quiroga), mauriakme@gmail.com (M.S. Akmentins), laureech@gmail.com (L.C. Pereyra).

in bright coloration, all populations had similar amounts of skin alkaloids and consequently were suspected to be equally distasteful, constituting a case of aposematic polymorphism (Garraffo et al., 2012). What maintains this discrete polymorphism in these aposematic populations is an untested question in this species.

Identifying functional differences among color morphs seems central to understand the ecological or evolutionary significance of the maintenance of such discrete color polymorphisms in this species. Physiological functions of coloration besides the visual warning function should be further explored and might reveal other mechanisms that are critical for understanding color pattern variation (Hegna et al., 2013). Some frogs are known to vary in their efficiency of thermoregulation depending on their coloration, where darker individuals experimentally exposed to the sun reach higher body temperatures than lighter individuals (Vences et al., 2002). Therefore, two individuals with differences in dorsal melanization, but with similar conditions (e.g. posture, microhabitat) can reach thermal equilibrium at different times (Porter and Gates, 1969).

The aim of this study was to relate the observed color variation of the brightest and darkest color morphs of *M. rubriventris* with their thermoregulatory efficiency. Thus, we studied the variation in thermal parameters in two contrasting populations of Yungas Redbelly Toad with different discrete color phenotypes by comparing field body temperatures, critical thermal maximum and heating rates.

2. Methods

2.1. Study area and species

We conducted our study during February 2013, in two localities from Jujuy province, northwestern Argentina (Fig. 1): Angosto de Jaire (S 24.0097; W 65.3817; altitude: 1665 m a.s.l) and Abra Colorada (S 23.6812; W 64.9122; altitude: 1722 m a.s.l), in coincidence with the reproductive period of the species. Both study areas support well-structured montane forests with high vegetation species richness and vegetation density, but are slightly disturbed because of former clearings and the occasional presence of livestock. The entire distribution of this species is upland areas (1000 to 2000 m a.s.l) of a typical subtropical humid montane forest (Ecoregion of Southern Andean Yungas *sensu* Olson et al., 2001).

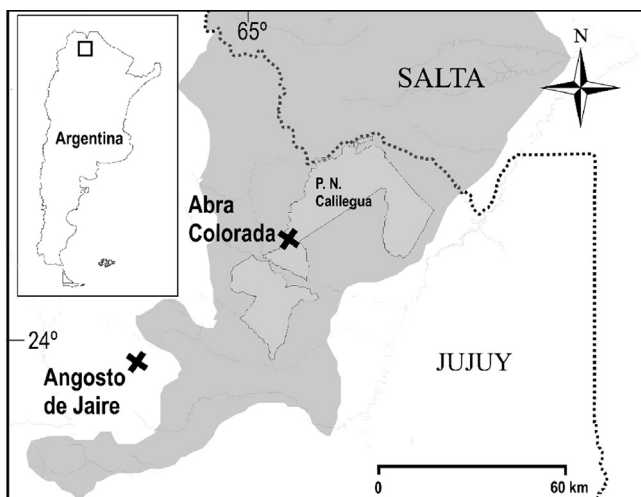


Fig. 1. Location of the studied populations of *Melanophryniscus rubriventris* in Jujuy province, NW Argentina. The larger gray shaded area is the Las Yungas Biosphere Reserve; the small gray area is the National protected area (P.N.).

M. rubriventris is a small diurnal aposematic toad, that is relatively common in primary and secondary montane forests of NW Argentina and breeds after heavy rains from November to February (Vaira, 2005). Breeding activities involve large aggregations with short and explosive reproductive events that vary highly in numbers among years where toads use shallow temporary ponds to lay eggs (Vaira, 2005). This species shows marked geographic variation in dorsal coloration with a variety of discrete morphs, from bright orange coloration covering the head, shoulders, and flanks over a black background; to a complete olive to black dorsal coloration (see Bonansea and Vaira (2012) for more detailed morph descriptions). We selected individuals from two populations differing mainly in the extent of black patches (hereafter referred as dorsal melanism).

A total of 34 toads were captured by hand and measured during a single day at each site (12 from Angosto de Jaire, and 22 from Abra Colorada).

2.2. Determination of the extent of dorsal melanization

Before taking body temperature measurements (see below), every specimen was photographed in the field from the dorsal view with a digital camera (Nikon® D80, Japan) with normal lens (Sigma® 50 mm, Japan). Those images were then imported into the public domain image processing program ImageJ 1.47 (Rasband, 2012) to calculate the extent of black dorsal skin. Images were converted to grayscale of 8 bits, and outlines of the entire body area and black areas were drawn using manual threshold settings. The extent of the dorsal melanization was then calculated from outline areas and determined as percentages of the total area of the dorsal view of toad. Individuals used in the study were well within the range of variation observed in source populations (Bonansea and Vaira, 2012). Individuals from Angosto de Jaire were characterized as high dorsal melanization (HM), while the individuals from Abra Colorada were characterized as low dorsal melanization (LM) (Fig. 2).

2.3. Determination of field body temperature and environmental parameters

Body temperature (T_b) of free-ranging toads was measured at the time of capture (catheter probes TES TP-K01) with a digital thermometer TES 1312 (TES Electrical Electronic Corp., Taipei, Taiwan, ± 0.1 °C). Toads were gripped by a forelimb to minimize heat exchange during manipulations and a thermocouple was

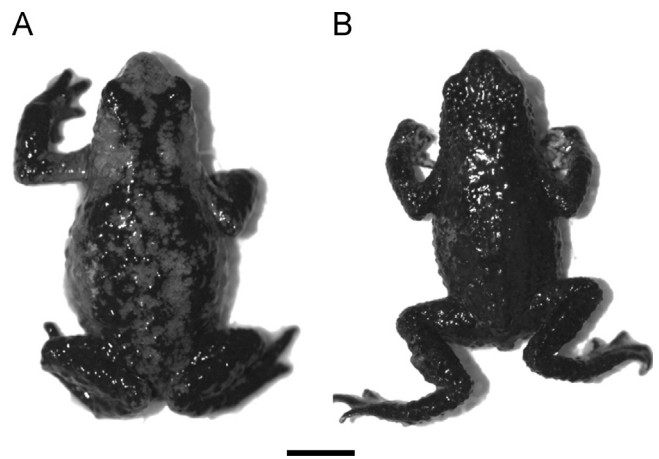


Fig. 2. Two males of *Melanophryniscus rubriventris* showing different amount of dorsal melanization. (A) Typical toad with a low dorsal melanization (LM) from Abra Colorada, (B) Typical toad with a high dorsal melanization (HM) from Angosto de Jaire, Jujuy, Argentina. Scale bar = 1 cm.

inserted 1 cm into the cloaca. At the same site of capture, we also recorded the microenvironment temperature, the substrate temperature (T_s) (TES TP-K03 substrate probe), and air temperature (T_a) at 1 cm above the soil surface (TES TP-K02 gas probe). Also, we measured the snout-vent length of toads (SVL) to the nearest 0.1 mm using digital calliper, and weighed them to the nearest 0.001 g on an electronic weighing scale.

In order to assess ambient temperature ($^{\circ}\text{C}$) throughout the sampled period, we deployed two data loggers at each sampling site (iButton DS1922L, Maxim, USA) that record temperature conditions every five minutes. We considered the ambient temperature at the moment of capture as the average temperature obtained from both loggers.

2.4. Determination of critical thermal maximum

Captured toads were transferred to the laboratory to measure their thermal tolerances. We measured the critical thermal maximum of 26 toads (12 from Angosto de Jaire, and 14 from Abra Colorada) that were acclimated for two days at 25°C within their natural photoperiod. Critical thermal maximum (CT_{\max}) was determined following Hutchison (1961). Individual toads were placed in glass containers (15×20 cm) with wet paper towels at ambient temperature ($25 \pm 2^{\circ}\text{C}$). An infrared lamp (150 w, Yinko, China) was placed at 45 cm above the toads, and the temperature was raised at a constant rate of $\sim 1^{\circ}\text{C min}^{-1}$. When toads showed muscle spasms, we measured the cloacal temperature as an estimator of the CT_{\max} . Toads were then placed in a water-filled container at ambient temperature immediately following CT_{\max} to enable recovery. Toads used to determine the CT_{\max} were used only for these tests.

2.5. Determination of heating rate

The heating rate comparison between toads with a different extent of dorsal melanization was conducted in pairs (six pairs was analyzed). For each test we selected a toad from each population (HM and LM) and of similar body weights (less than 0.5 g difference). We anesthetized the animals in a solution of benzocaine 20%. We then fixed the thermocouple in the cloaca of each toad that was connected to a data logger (PP 222; Pico logic, UK). An infrared lamp (150 w, Yinko, China) was placed at 45 cm above the toads and the temperature was raised at a rate of $\sim 1^{\circ}\text{C min}^{-1}$. The tests started at ambient temperature ($\sim 20^{\circ}\text{C}$) and heating rate was measured at 1-second intervals ending when toads reached the CT_{\max} . All toads recovered at the end of trials.

Warming rate (WR) was calculated as: $\text{HR} (^{\circ}\text{C s}^{-1} \text{ cm}^2)^{-1} = (T_n - T_0)/t/A$; Where: HR=heating rate; T_n =temperature at the end of test; T_0 =initial temperature of the test ($\sim 20^{\circ}\text{C}$); t =time in seconds to reach T_n ; A =extent of dorsal melanization.

2.6. Data analysis

We tested the null hypothesis that there is no effect of the extent of dorsal melanization on the variations of field body temperatures, critical thermal maximum and heating rates of tested individuals. We found that some variables departed from normality, and could not be transformed to achieve normality. Therefore, to compare all statistical relationships, we ran non-parametric tests. Spearman rank correlations were run to test for associations between thermal parameters. We applied the Mann–Whitney U -test to compare thermal parameters between populations. We relativized the variables, dividing each variable into their co-variables. We show the covariates used to relativize the variable when informing the statistical test applied. All data exploration and analysis was performed using the statistical software PAST version 9.4 (Hammer, 2001).

3. Results

The dorsal melanism of *M. rubriventris* between populations showed significant differences (Mann–Whitney: $U = -3.9$; $p < 0.001$). HM toads presented a mean extent of dorsal melanization of $93\% \pm 1.3\%$, whereas LH toads presented a mean of $71\% \pm 2.1\%$ (Fig. 2).

The T_b of the toads from both populations was significantly different (Mann–Whitney: $U = 54$; $p < 0.01$; Cov: T_a and T_s). The HM toads presented a T_b mean value of $22 \pm 0.4^{\circ}\text{C}$ and the LM toads presented a T_b mean value of $19.8 \pm 0.3^{\circ}\text{C}$. The environmental temperatures at the moment of capture in both populations did not show significant differences (Mann–Whitney test: $U = 1357$; $p = 0.76$). Mean temperature from Jaire was $19.2 \pm 0.4^{\circ}\text{C}$ and Abra Colorada was $18.4 \pm 0.4^{\circ}\text{C}$ (Fig. 3).

The CT_{\max} was significantly different between HM and LM toads (Mann–Whitney: $U = 6$; $p < 0.001$, Cov: dorsal melanization). The HM toads presented a CT_{\max} mean value of $35.5 \pm 0.4^{\circ}\text{C}$ and the LH toads presented a CT_{\max} mean value of $33.0 \pm 0.4^{\circ}\text{C}$. Also, we found a positive correlation between the CT_{\max} and extent of dorsal melanization of toads ($r_s = 0.55$, $p < 0.004$) (Fig. 4).

Finally, the WR showed differences between morphs (Wilcoxon pairs test: $Z = 2.20$, $p < 0.02$). The HM individuals increased their body temperature at a rate of $0.0048 \pm 0.0006^{\circ}\text{C s}^{-1} \text{ cm}^{-2}$, whereas LM toads increased their body temperatures at a rate of $0.0036 \pm 0.0006^{\circ}\text{C s}^{-1} \text{ cm}^{-2}$.

4. Discussion

We found significant differences in field body temperatures of the different morphs. Toads with a high extent of dorsal melanization had higher field body temperatures and no variation in optimal temperature was registered between study locations at the moment of capture and measurement. Also, critical thermal maximum of toads was positively related with the extent of dorsal melanization. Furthermore, we showed significant differences in heating rates between morphs, where individuals with a high extent of dorsal melanization showed greater heating rates than toads with lower dorsal melanization.

Extent of melanization in adult frogs and toads has been linked to thermoregulation, but also to predator avoidance (Clusella-Trullas et al., 2007; Stevens and Ruxton, 2012; Rudh and Qvarnström, 2013).

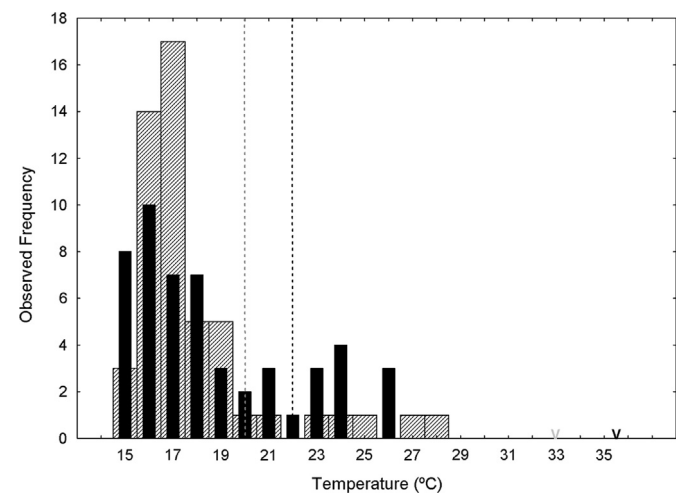


Fig. 3. Distribution of frequencies of environmental temperatures during study period, both distributions did not show significant differences between Abra Colorada (Grey bars) and Angosto de Jaire (Black bars). T_b mean values for toads from LM (grey dotted line) and HM (black dotted line) showed significant differences, as well as the CT_{\max} from HM (head arrow black) and LM (head arrow grey).

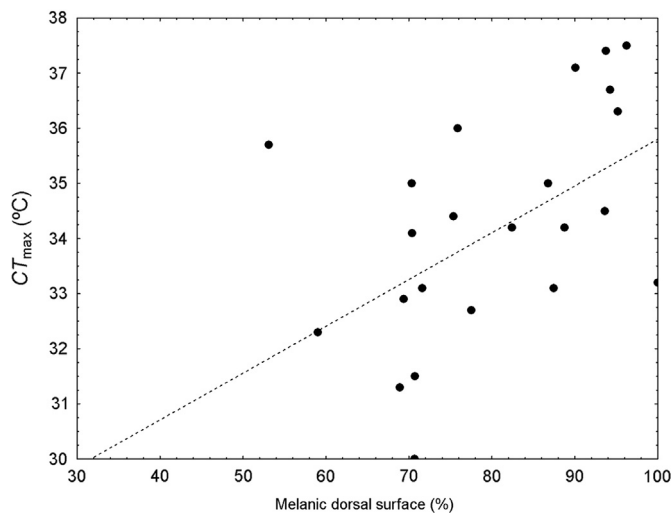


Fig. 4. Correlation between CT_{max} and extent of dorsal melanization on individuals of *Melanophryniscus rubriventris* from Jujuy, Argentina.

A body with increased melanistic pattern is more efficiently heated by solar radiation, while a lighter body may aid to avoid overheating (Clusella-Trullas et al., 2007). Moreover, disruptive patterns in the form of contrasting color patches conceal the outlines of the body reducing detection by potential predators (Stevens and Cuthill, 2006). Warning signals in poison frogs are commonly dominated by a pattern of bright colors (usually red and black) (Stevens and Ruxton, 2012). Therefore, it is possible to consider these factors as non-mutually exclusive when explaining the intraspecific color variation in *M. rubriventris*. Multiple selection pressures may be acting on the coloration patterns of different populations and trading off among signaling, concealing, and thermoregulatory roles. So, intraspecific phenotypic color variation could be a consequence of differences in environmental and ecological factors (e.g. climatic conditions and predation pressures) at a geographical scale.

Color pattern-thermal parameter relationships observed in the studied populations of *M. rubriventris* can also influence the activity patterns and body size of individuals. Body temperature is a modulator of physiological and behavioral functions in amphibians, influencing daily and seasonal activity, locomotor performance, digestion rate, and growth rate (Lillywhite et al., 1973; Marvin, 2003; Haramura, 2007). Since increased amount of dorsal melanization in Yungas Redbelly Toads may allow them not only to warm faster but also maintain higher body temperatures (Tanaka, 2009), they can achieve longer daily activity periods and therefore explore more areas or extend time in search of potential couplings and/or food. On the other hand, warming faster may prevent increased exposure time to predation in less conspicuous morphs. *M. rubriventris* is considered mainly diurnal and very mobile with most of their reproductive activity taking place during daylight over the entire breeding season (Vaira, 2005). However, we repeatedly registered events of breeding activity at night in several populations (unpublished data). Feeding patterns and prey-searching behavior is virtually unknown for this species. However, diet composition studies showed that this species can be considered an active forager searching for food (Bonansea and Vaira, 2007; Quiroga et al., 2011) and possible differences in length of daily foraging cycle and extent of area of foraging were also noticed (Garraffo et al., 2012).

Body size clines were observed among 21 species of the genus *Melanophryniscus* at a large spatial scale, showing a Bergmannian pattern where larger toads were associated with cooler environments. However, the same analysis at the intraspecific level showed some inconsistencies suggesting that other factors might

influence Bergmannian-like clines at the species level (Bidau et al., 2011). Individuals of *M. rubriventris* from HM and LM populations tested in our study were of similar body sizes, but showed significant differences in field body temperatures, heating rates, and critical thermal maximum. It is possible that some growth constraints may arise due to the relationship of color pattern-metabolism allowing different morphs to attain similar sizes at different locations instead of body-size clines due to temperature.

Our findings, showing that morphs of aposematic toads differ in the amount of dorsal melanization and differ substantially on their thermoregulatory efficiency emphasizes the need for better comprehension of the complex role of coloration (see Hegna et al., 2013). Understanding how variation in coloration relates to whole-organism performance in an integrated manner (i.e. under multiple selection pressures) is key to understanding the role and maintenance of discrete colour morphs in a species or population.

Acknowledgments

We acknowledge C. García and V. Palchetti for their assistance in field work. Special thanks to R. Rhymer and anonymous reviewer for the critical review and to for correction of English that improved this work. This project was partially supported by a PICTO-UNJu grant # 153 and the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

References

- Amézquita, A., Castro, L., Arias, M., González, M., Esquivel, I.C., 2013. Field but not lab paradigms support generalisation by predators of aposematic polymorphic prey: the *Oophaga histrionica* complex. *Evol. Ecol.* 27, 769–782.
- Bidau, C.J., Martí, D.A., Baldo, D., 2011. Inter- and intraspecific geographic variation of body size in South American redbelly toads of the genus *Melanophryniscus* Gallardo, 1961 (Anura: *Bufo*idae). *J. Herpetol.* 45, 66–74.
- Bonansea, M.I., Vaira, M., 2007. Geographic variation of the diet of *Melanophryniscus rubriventris* (Anura: *Bufo*idae) in northwestern Argentina. *J. Herpetol.* 41, 231–236.
- Bonansea, M.I., Vaira, M., 2012. Geographic and intrapopulation variation in colour and patterns of an aposematic toad, *Melanophryniscus rubriventris* (Amphibia, Anura, *Bufo*idae). *Amphibia-Reptilia* 33, 11–24.
- Clusella-Trullas, S., van Wyk, J.H., Spotila, J.R., 2007. Thermal melanism in ectotherms. *J. Therm. Biol.* 32, 235–245.
- Garraffo, H.M., Andriamaharavo, N.R., Vaira, M., Quiroga, M.F., Heit, C., Spande, T.F., 2012. Alkaloids from single skins of the Argentinian toad *Melanophryniscus rubriventris* (Anura, *Bufo*idae): an unexpected variability in alkaloid profiles and a profusion of new structures. *SpringerPlus* 1, 1–15.
- Hammer, Ø., Harper, T., Ryan, P.D., 2001. PAST: paleontological statistics software package for education and data analysis. *Paleontol. Electron.* 4, 1–9.
- Haramura, T., 2007. Microhabitat selection by tadpoles of *Buergeria japonica* inhabiting the coastal area. *J. Ethol.* 25, 3–7.
- Hegna, R.H., Nokelainen, O., Hegna, J.R., Mappes, J., 2013. To quiver or to shiver: increased melanization benefits thermoregulation, but reduces warning signal efficacy in the wood tiger moth. *Proc. R. Soc. London, Ser. B* 280 (20122812) <http://dx.doi.org/10.1098/rspb.2012.2812>.
- Hoffman, E.A., Blouin, M., 2000. A review of colour and pattern polymorphisms in anurans. *Biol. J. Linn. Soc.* 70, 633–665.
- Hutchison, V.H., 1961. Critical thermal maxima in salamanders. *Physiol. Zool.* 2, 92–125.
- Lillywhite, B.H., Licht, P.Y., Chelgren, P., 1973. The role of behavioral thermoregulation in the growth energetics of the toad, *Bufo boreas*. *Ecology* 54, 375–383.
- Marvin, G., 2003. Aquatic and terrestrial locomotor performance in a semiaquatic plethodontid salamander (*Pseudotriton ruber*): influence of aquatic temperature, thermal acclimation and body size. *Copeia* 2003, 704–713.
- Mochida, K., 2009. A parallel geographical mosaic of morphological and behavioural aposematic traits of the newt, *Cynops pyrrhogaster* (Urodela: *Salamandridae*). *Biol. J. Linn. Soc.* 97, 613–622.
- Mochida, K., 2011. Combination of local selection pressures drives diversity in aposematic signals. *Evol. Ecol.* 25, 1017–1028.
- Noonan, B.P., Comeault, A.A., 2009. The role of predator selection on polymorphic aposematic poison frogs. *Biol. Lett.* 5, 51–54.
- Olson, D.M., Dinerstein, E., Wikramanayake, E.D., Burgess, N.D., Powell, G.V.N., Underwood, E.C., D'Amico, J.A., Itoua, I., Strand, H.E., Morrison, J.C., Loucks, C.J., Allnutt, T.F., Ricketts, T.H., Kura, Y., Lamoreux, J.F., Wettengel, W.W., Hedao, P., Kassem, K.R., 2001. Terrestrial ecoregions of the world: a new map of life on Earth. *Bioscience* 51, 933–938.

- Porter, P.W., Gates, D.M., 1969. Thermodynamic equilibria of animals with environment. *Ecol. Monogr.* 39, 227–244.
- Quiroga, M.F., Bonansea, M.I., Vaira, M., 2011. Population diet variation and individual specialization in the poison toad, *Melanophryniscus rubriventris* (Vellard, 1947). *Amphibia-Reptilia* 32, 261–265.
- Rasband, W., 2012. Image J, version 1.47. Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, (<http://imagej.nih.gov/ij/>).
- Rudh, A., Qvarnström, A., 2013. Adaptive coloration in amphibians. *Semin. Cell Dev. Biol.* <http://dx.doi.org/10.1016/j.semcdb.2013.05.004>
- Stevens, M., Cuthill, I.C., 2006. Disruptive coloration, crypsis and edge detection in early visual processing. *Proc. R. Soc. London, Ser. B* 273, 2141–2147.
- Stevens, M., Ruxton, G.D., 2012. Linking the evolution and form of warning coloration in nature. *Proc. R. Soc. London, Ser. B* 279 (417–426).
- Tanaka, K., 2009. Does the thermal advantage of melanism produce size differences in color-dimorphic snakes? *Zool. Sci.* 26, 698–703.
- Toledo, L.F., Haddad, C.F.B., 2009. Colors and some morphological traits as defensive mechanisms in Anurans. *Int. J. Zool.* 2009, 1–12.
- Vaira, M., 2005. Annual variation of breeding patterns of the toad, *Melanophryniscus rubriventris* (Vellard, 1947). *Amphibia-Reptilia* 26, 193–199.
- Vences, M., Galán, P., Vieites, D.R., Puente, M., Oetter, K., Wanke, S., 2002. Field body temperatures and heating rates in a montane frog population: the importance of black dorsal pattern for thermoregulation. *Ann. Zool. Fenn.* 39, 209–220.