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Quantitative Determination of the Minimum Body Size for Photo-identification of *Melanophryniscus montevidensis* (Bufonidae)

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***Abstract.*—Toads of the genus *Melanophryniscus* possess unique color patterns on the belly, which allows for individual recognition. Photo-identification has proven to be an efficient non-invasive technique to identify individuals for this genus. However, such color patterns are absent in newly metamorphosed individuals. We studied the development of the ventral coloration pattern and evaluated its persistence in *Melanophryniscus montevidensis* to determine the minimum size and age at which use of the color pattern is a trustworthy (i.e., as stable as in adults) identification method for this species. From spawns raised in the field, we obtained eight metamorphs and maintained them in semi-natural conditions to photograph their bellies. We visually analyzed the images to establish the stabilization point of the color pattern. Using the software Wild-ID, we calculated the similarity score between the images from the stabilization point with sets of images before and after stabilization. Similarity scores of adults from previous studies did not differ significantly from the scores of juveniles after the pattern stabilized, but they did differ significantly from the scores of juveniles compared to themselves at least 70 d before stabilization. The color pattern developed progressively and stabilized at a median of 220 d after metamorphosis, with a maximum snout-vent length of 13.2 mm, which we considered the minimum size for photo-identification purposes. Although we observed ontogenetic and individual variation, the pattern remained unchanged since just before the first year of age. Taking into account the threshold size we determined, photo-identification is a suitable method for ecological studies of this species.**

Key Words.—age; color pattern; non-invasive methods; ontogeny

Introduction

The Neotropical genus *Melanophryniscus* comprises 29 species distributed from central-southern Brazil to central-eastern Argentina, including Bolivia, Paraguay, and Uruguay (Amphibian Species of the World: an Online Reference. 2016. Version 6.0. American Museum of Natural History, New York, USA. Available from http://research.amnh.org/herpetology/amphibia/index.html. [Accessed 25 January 2016]). The dorsal pigmentation of these small toads is generally not colorful. However, most of them possess a conspicuous color pattern on the belly (and sometimes on the hands and feet). The black, brown, or green background with red, yellow, white, green, or orange spots inspires their common name of red belly toads (Mebs et al. 2007; Caorsi et al. 2014). When threatened, *Melanophryniscus* individuals exhibit a defensive behavior, the unken reflex, which includes displaying the bright coloration on their palms and belly (Toledo and Haddad 2009). Neither the color pattern nor the associated unken reflex are apparent during the first days after metamorphosis in some species of this genus (Langone et al. 2008; Caorsi et al. 2014), or in toads belonging to the genus *Bombina* (Löhner 1919).

In Uruguay, there are six species of *Melanophryniscus*, and three are globally listed as threatened. One of them is *Melanophryniscus montevidensis* (International Union for the Conservation of Nature [IUCN] global: Vulnerable; IUCN national: Critically Endangered; Carreira and Maneyro 2015; IUCN, Red List of Threatened Species. 2016. Available from http://www.iucnredlist.org/ [Accessed 15 April 2016]). This is a small sized (adult snout-vent length [SVL]: 20–30 mm) coastal species, which exploits psammophilic vegetation (vegetation from sandy soils), and breeds in ephemeral ponds after heavy rains (Maneyro and Carreira 2012; Pereira and Maneyro 2016a). Zank et al. (2014) recommended population-level studies for this species, and non-invasive marking techniques like photo-identification are the most advisable approaches for threatened species.

Elgue et al. (2014) tested the accuracy of using photo-identification for adult individuals of *M. montevidensis* (mainly black with red and yellow spots) and it seems to perform well as an identification method. Using this method to identify individuals has no effect on the health and behavior of the animal, lasts for the duration of the study, and has minimal cost (Ferner 2007). As in other species of the genus, newly metamorphosed *M. montevidensis* may not possess the characteristic color pattern. Furthermore, juveniles are difficult to find and there are no records of juvenile recaptures using photo-identification (Pereira and Maneyro 2016b). Some information about the appearance of the ventral patterns in young juvenile toads is available in the literature (Gollman and Gollman 2011; Caorsi et al. 2014), but our study is the first to quantify the development time of the color pattern and estimate the minimum body size at which the color pattern stabilizes and becomes useful for valid photo-identification. For *M. montevidensis* we predicted that the color patterns would develop progressively during the first year, which may violate the assumption of consistent appearance of the mark if researchers use photo-identification for early life stages.

Materials and Methods

On February 2014, during a breeding event, we captured nine amplectant pairs in a temporary pond located 1 km away from La Riviera, Rocha (34°32'42.40"S, 54°19'34.04"W; 3–10 m above sea level). This locality is within Paisaje Protegido Laguna de Rocha, part of the national system of protected areas (Dirección Nacional de Medio Ambiente [DINAMA] 2010). To obtain spawns, we kept the amplectant pairs for 24 h in plastic cages containing water and vegetation from the pond. Later, we placed the spawns in separate *in situ* plastic enclosures and monitored them until tadpoles reached metamorphosis. To count the age of the larvae until front limb emergence, the day of oviposition was considered day 0.

From 19 to 25 March, we randomly selected four metamorphs (Gosner stage 42; Gosner 1960) from two different amplectant pairs. We reared these eight individuals inside individual 6-l plastic containers (30 cm × 15 cm × 13 cm) in an open room exposed to the natural photoperiod and temperatures (mean 15.7 ± [SD] 3.7° C). We periodically added soil and grass collected from the border of the pond as a source of invertebrate food and shelter. We supplied water from the pond daily to maintain humidity. When the pond dried up, we mixed water from a nearby water body with distilled water to emulate the original pond (mean conductivity = 64.3 ± 37.3 µS cm-1, n = 81; mean pH = 6.2 ± 0.4, n = 81; pH and conductivity meter, Hanna® Instruments, Limena, Padua, Italy). During the sixth month, we offered ants (*Solenopsis* sp. and *Linepithema humile*; Formicidae: Myrmicinae and Dolichoderinae, respectively) to increase the size and variety of food items for the toads. However, some toads rejected the ants, especially *Solenopsis* sp., and died before the end of the first year. We deposited all but one of the deceased toads in the scientific collection of Facultad de Ciencias, Universidad de la República (Montevideo, Uruguay); siblings from the spawn of couple 1: ZVCB 23365–23367; siblings from the spawn of couple 2: ZVCB 23368–23371.

We took photographs of the belly and pelvic patch every 2 d after front limb emergence during the first 70 d and then monthly for the lifespan of each animal (range, 200–400 d), using a Coolpix L810 camera (16.0 MP; Nikon, Tokyo, Kantō, Japan). Because animals were small at the beginning (median = 5.8 mm SVL), we photographed them in a drop of water through a plastic dish. We included a ruler for scale in the picture frames and held the distance and lighting constant to standardize images and facilitate taking digital measurements. We used the software Micrometrics® SE Premium (ACCU-SCOPE, Commack, New York, USA) to measure the SVL.

We predicted that the color patterns would stabilize at some point during the first year. Before that point, we suspected that the images would have low resemblance. However, when comparing the image at stabilization with the last picture of the animal, the level of resemblance should be similar to that reported for adults (see Elgue et al. 2014). To test this, we created a database of 4,523 images documenting the developmental sequence of the color pattern of each animal. We chose 109 photos (one photo of each animal every 10 d until day 63 [from day 3 after front limb emergence] and one photo each month after day 63). We used the same procedure as Elgue et al. (2014). We cropped images to show only the ventral pattern, pelvic patch, and axillar marks, and modified the brightness and contrast to improve the visibility of the color patterns whenever necessary. Separately, we visually inspected the sequence of images of each animal to determine the image at which the color pattern stabilized. We defined a color pattern as stable when it stopped changing and retained its main contours and number of spots. One animal died before stabilization and we excluded it from analysis.

We used the software Wild-ID (Dartmouth College, Hanover, New Hampshire, USA) to select the best images for visual confirmation of recapture. The software identified the top-ranked 20 photos that matched the analyzed one (Bolger, D.T., B. Vance, T.A. Morrison, and H. Farid. 2011. Wild ID user guide: pattern extraction and matching software for computer-assisted photographic mark. Available from http://envs.dartmouth.edu/people/douglas-thomas-bolger

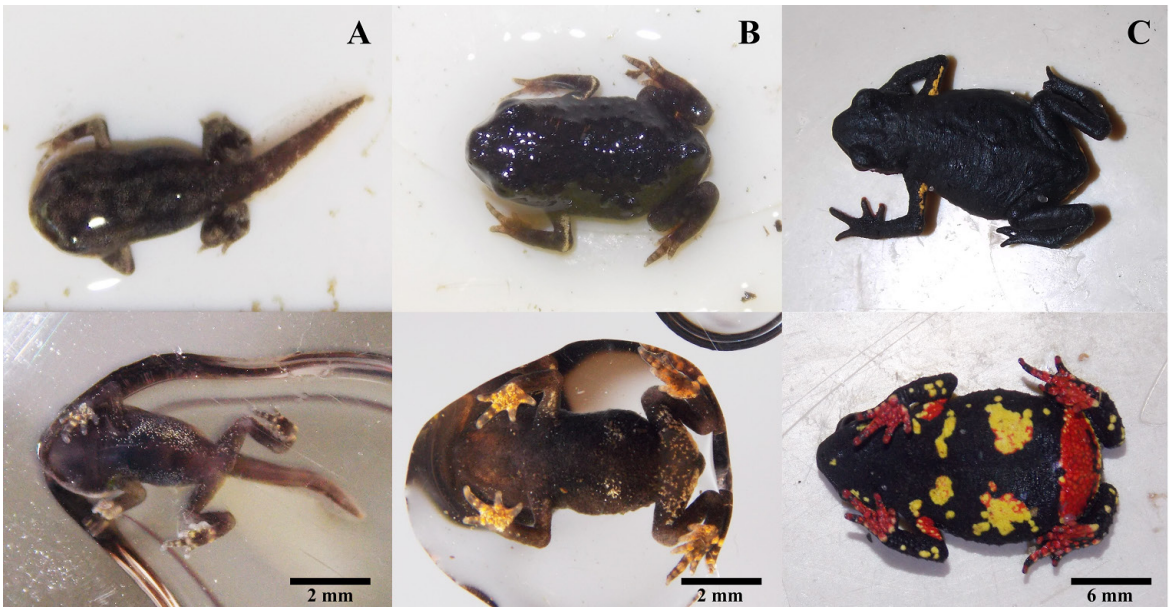

Figure 1. Dorsal (top) and ventral (bottom) views of *Melanophryniscus montevidensis* at different stages: A) newly metamorphosed individual (i.e., front limb emergence; Gosner stage 42), B) 13 d after front limb emergence (ZVCB-23367), C) adult photographed in the field. Image brightness and contrast were adjusted. (A and B photographed by Cecilia Bardier; C photographed by Nicolás Martínez).

[Accessed 20 March 2016]). Others have successfully used this software to identify individuals of species belonging to the *Melanophryniscus* genus (Abadie 2012; Caorsi et al. 2012; Elgue et al. 2014). Wild-ID computes a resemblance score for each pair of images (i.e., qualifies the similarity among individuals) from zero to one; the higher the score, the higher the similarity between individuals (Bolger, D.T., B. Vance, T.A. Morrison, and H. Farid. 2011. *op. cit*.).

To test our prediction, we contrasted four sets of similarity scores. Three sets were the scores derived by Wild-ID when we compared photographs of each juvenile (n = 7) taken at the time of ventral color pattern stabilization with photographs of itself taken at a median of 100 d before, a median of 70 d before, and the last photograph of itself (taken at a median of 70 d after stabilization). The fourth set was the similarity scores of photographs of recaptured adults from field data (n = 18) taken at a maximum of 14 mo apart, from Elgue et al. (2014). This last set was included to decide which levels of similarity scores of juveniles were within the variation of adult scores, already considered valid for photo-identification by Elgue et al. (2014).

Because of the small sample sizes, we used the Kruskal-Wallis test in statistical software R (The R Foundation for Statistical Computing. 2002. R. Available from https://www.r-project.org/foundation/ [Accessed 10 February 2015]) to analyze the differences among these four sets of scores. We tested for significant differences among sets of scores using a multiple comparison Dunn post hoc test available in the R package PMCMR, which applies the Holm P-value adjustment method (Pohlert, T. 2016. Calculate Pairwise Multiple Comparisons of Mean Rank Sums. Version 4.1. Available from https://cran.r-project.org/web/packages/PMCMR/index.html [Accessed 12 April 2016]). We considered the median SVL at which the color pattern stabilized as the minimum body size at which the photo-identification is a reliable marking method if two conditions were met: adult scores and the scores after the stabilization of the color pattern did not differ significantly, and adult scores and the scores before the stabilization of the color pattern did differ significantly.

Results

Our study subjects completed metamorphosis (i.e., front-limb emergence; Gosner stage 42) after a median of 23 d after oviposition (range, 19–25 d). Unlike the black dorsum and colorful ventral pattern of adults, the dorsal and ventral coloration of the newly metamorphosed toads was grayish-brown and slightly transparent in the ventral region (Fig. 1). Dorsal darkening occurred gradually and reached the characteristic black coloration around the tenth day after front limb emergence (Fig. 1). The ventral color patterns developed progressively by the accumulation of small spots (Fig. 2). The age at which we considered the color pattern stable varied among juveniles (Table 1), median = 220 d after front limb emergence (range, 153–344 d). Although the

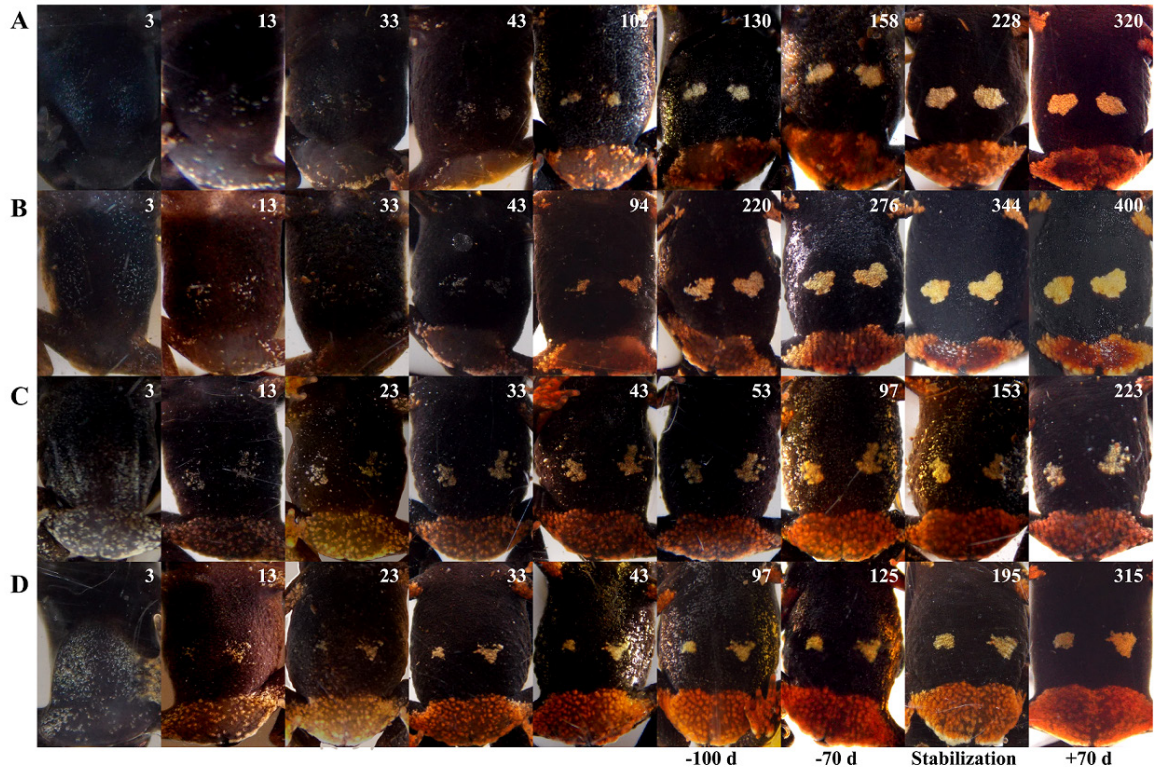

Figure 2. Developmental sequence of the color pattern of four juvenile toads (*Melanophryniscus montevidensis*) 1-23365 (A), 1-n.a. (B), 2-23370 (C), and 2-23371 (D). The first number of each animal indicates the amplectant pair, the second number is the catalog number in the scientific collection of Facultad de Ciencias, Universidad de la República (ZVCB). Top right numbers are the ages (d after front limb emergence. -100 d and -70 d are the images of the animals at a median of 70 and 100 d before the stabilization of the pattern, respectively. Stabilization represents the first images with stable patterns. +70 d are the last images of each animal (median = 70 d after stabilization of the pattern). Image brightness and contrast were adjusted to facilitate comparisons. (Photographed by Cecilia Bardier).

hands, pelvic patch, and axillar spots developed the red coloration in early stages (range, 20–30 d), the color pattern in the belly developed first in yellow, and the red coloration appeared later over the yellow spots (Fig. 2). During manipulation for a photo session, one juvenile (ZVCB 23368) was the first to display unken reflex, 34 d after front limb emergence.

When we made multiple comparisons of the sets of juvenile similarity scores and the set of adult scores (recaptures from Elgue et al. 2014), the four groups of Wild-ID scores differed significantly ($\chi^2 = 20.15$, df = 3, $P < 0.001$; Fig. 3). The adult scores and the scores of juveniles after the color pattern stabilized did not differ significantly ($P > 0.05$). There were significant differences among the adult scores and the scores obtained from comparisons at a median of 70 and 100 d before stabilization ($P \leq 0.05$). However, there were no differences between the scores obtained from comparisons at median = 70 d before and comparisons at median = 70 d after stabilization ($P > 0.05$). Once we verified that the requirements of the scores were fulfilled, we determined that the color pattern stabilized with a median SVL = 8.8 mm (range, 8.2–13.3 mm), which is the minimum body size at which the photo-identification is reliable in this species (Table 1).

Discussion

Our observations established a point at which the color patterns stabilize in young juveniles of *M. montevidensis*. The similarity scores before juveniles reach pattern stabilization do not resemble the adult re-capture scores. At earlier time intervals, the scores of toads compared to themselves were low and lacked statistical differences among age groups. This supports our prediction that the color patterns develop gradually. The low scores after stabilization and the lack of differences between the scores just before and just after the stabilization provide further evidence for gradual development of the pattern. However, the low scores and lack of differences also challenge our determination of the pattern stability of juveniles, and photo-identification techniques should be applied judiciously taking into account the considerations below.

Changes in the color pattern may occur, especially in early juvenile stages (e.g., Gollmann and Gollmann

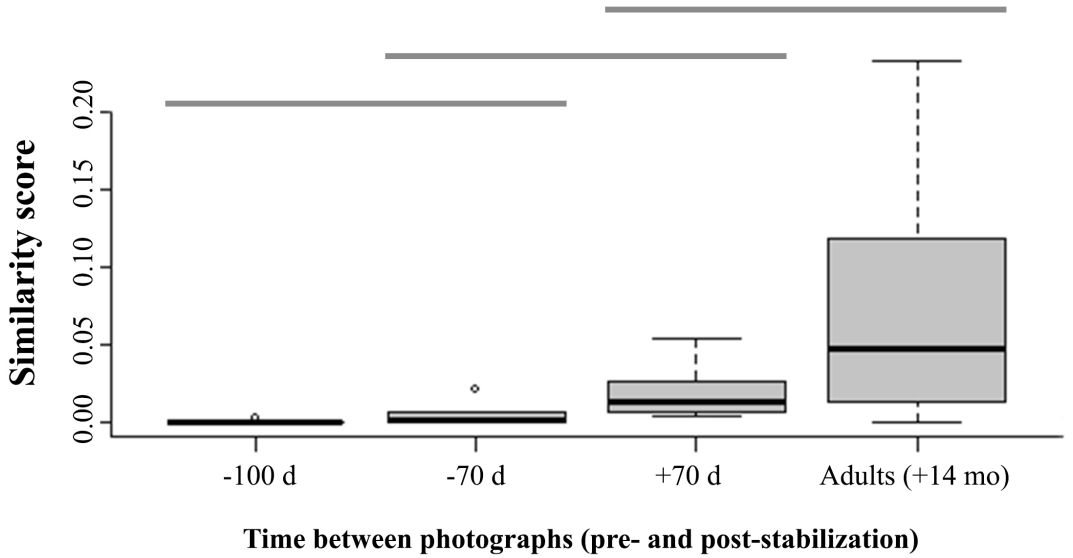

Figure 3. Boxplots of similarity scores comparing photographs of juvenile toads (Melanophryniscus montevidensis, n = 7) taken when the ventral color pattern stabilized with images taken at median = 100 d (-100 d) and 70 d (-70 d) prior to stabilization, and median = 70 d after stabilization (+70 d). Adults (+14 mo) refers to similarity scores comparing images of recaptured adult toads (n = 18) taken within a maximum of 14 mo apart and reported by Elgue et al. (2014). Similarity scores not joined by horizontal bars are significantly different from each other ($P \leq 0.05$; Dunn's *post hoc* comparisons).

2011). When slight variations in the pattern occur, photo-identification can still be applied, but visual methods combined with the use of visual-assisted software are more advisable than exclusive use of automated software (Gollmann and Gollmann 2011; Bendik et al. 2013). In addition, the image quality is important because high resolution, in-focus, and constant distance images are less prone to mismatches than low quality images (Gollmann and Gollmann 2011; Bendik et al. 2013).

We expected later changes in juvenile coloration because we observed stable coloration patterns when only yellow spots were present whereas adults generally have both red and yellow spot coloration. We found that the red coloration appeared later and on top of the yellow spots (i.e., not at the edges), and thus did not alter the main outline of the pattern. The software Wild-ID is based on an algorithm that recognizes and compares Key Points Independent from the Scale (Lowe 2004). It does not consider coloration and needs visual assistance to confirm matches (Bolger, D.T., B. Vance, T.A. Morrison, and H. Farid. 2011. *op. cit*.). Therefore, the later appearance of the red coloration would not affect the recognition using the Wild-ID software in subsequent recaptures.

Table 1. Ages (number of days after front limb emergence) and sizes of *Melanophryniscus montevidensis*. Age of image -100 d and Age of image -70 d refer to photographs from a median of 70 and 100 d before stabilization of the pattern, respectively. Age of stabilization refers to the first image with a stable pattern. Age of image +70 d refers to the final image (median time = 70 d after stabilization). Wild-ID stabilization scores are the similarity scores comparing the first image with a stable pattern to the final image. For Animal I.D., the first number indicates parental pair; the second is the catalog number in the scientific collection of Facultad de Ciencias, Universidad de la República (ZVCB). One catalog number was unavailable (noted as 1-n.a.).

Animal I.D.	Age of image -100 d	Age of image -70 d	Age of stabilization	SVL at the age of stabilization (mm)	Age of image +70 d	Wild-ID stabilization scores
1-23365	130	158	228	8.55	320	0.0395
1-23366	153	195	279	11.10	347	0.0088
1-n.a.	220	276	344	13.24	400	0.0134
1-23367	122	150	220	8.95	312	0.0045
2-23368	53	102	158	8.48	228	0.0535
2-23369	—	—	—	—	200	—
2-23370	53	97	153	8.18	223	0.0035
2-23371	97	125	195	8.86	315	0.0140

Although juvenile similarity scores after pattern stabilization resembled adult scores, adult scores were low (generally < 0.2) and juvenile scores were even lower (generally < 0.05). Other studies reported low similarity scores from field photographed recaptures using Wild-ID, especially when the image quality was low and when animals experienced weight changes (i.e., allometric variations; Bendik et al. 2013; Morrison et al. 2016). Differences between the adult and juvenile scores may be a result of the manner in which we photographed the juveniles versus adults. We photographed juveniles in a drop of water through a plastic dish, whereas Elgue et al. (2014) photographed adults in the field, dry, and upside down. Allometric variations (because juveniles were growing) and image quality differences were both influencing the similarity scores produced from our database. In spite of these difficulties, we consider the comparison of similarity scores a useful quantitative approach because the allometric variations are unavoidable in early stages, and the algorithm of the software still works at low scores (Bendik et al. 2013); thus, we strongly recommend this method. The manipulation of small animals without harming them is difficult and time consuming. If the SVL of the animal is < 10 mm, a drop of water reduces its movement and the animal can be photographed in a natural position from below with no handling.

We determined the minimum size at which researchers can reliably use the color pattern in *M. montevidensis* to be 8.8 mm SVL. However, because the age at which an individual attained pattern stability was highly variable and our sample size was small, we take caution in interpreting our results. The last individual developed a stable color pattern nearly one year after front limb emergence, which suggests that older animals would be at the point of stabilizing their pattern in the field. Therefore, we recommend the body size of this animal, 13.2 mm SVL, as the minimum size at which the color pattern is reliable for photo-identification.

Diet of the juveniles during captivity may have affected their growth and ontogeny of the color patterns. We expect that animals in nature might be larger at the same age as our study subjects and may therefore develop the color pattern earlier. Diet is also important because of its relationship with skin toxins (Darst et al. 2005; Mebs et al. 2005; Hantak et al. 2013). Some species sequester skin toxins from their food, so it is possible that a shift in coloration depends on the acquisition of these toxins (Caorsi et al. 2014). We found that the appearance of the unken reflex and the development of the red coloration in hands, pelvic patch, and axillar spots were synchronous, and occurred earlier than the stabilization of the color patterns. The body parts that developed a red coloration are most visible during the unken reflex behavior of *M. montevidensis*. The belly pattern in some *Melanophryniscus* species is exhibited as part of the unken reflex (Toledo and Haddad 2009; Caorsi et al. 2014), but it does not seem to be involved in the defensive behavior of *M. montevidensis*. The belly pattern would not be subjected to the same selective pressures as the coloration, pelvic patch, or axillar spots and there may be no advantage to their early appearance. Further studies of early stages of the *Melanophryniscus* genus are needed to determine the role of diet in the development of coloration and the unken reflex behavior.

Finally, there have been no reports of juvenile recaptures in *M. montevidensis* (Pereira and Maneyro 2016b). Therefore, information about the early juvenile stages of *Melanophryniscus* species is scarce (Caorsi et al. 2012; Caorsi et al. 2014). Young juveniles usually have high mortality rates, which means few are in the environment, and because young use different environments than the adults, they are intrinsically difficult to find (Semlitsch 2008; Walston and Mullin 2008; Pereira and Maneyro 2016b), but it is also plausible that photo-identification methods are not effective when applied to early stages. The determination of the minimum size at which the color pattern stabilizes improves the accuracy of this marking method for juveniles. We consider visual implant elastomers, already tested in 6 mm juvenile Blue Crabs (*Callinectes sapidus*; Davis et al. 2004), as a complementary method to the photo-identification to identify younger and smaller individuals in population and life-history studies of *Melanophryniscus* species.

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Literature Cited

Abadie, M. 2012. Fotoidentificação como alternativa de marcação não invasiva para o sapinho microendêmico *Melanophryniscus admirabilis* (Anura: Bufonidae). Graduate Thesis, Universidade Federal Do Rio Grande Do Sul, Porto Alegre, Brasil. 26 p.

Bendik, N.F., T.A. Morrison, A.G. Gluesenkamp, M.S. Sanders, and L.J. O'Donnell. 2013. Computer-assisted photo identification outperforms visible implant elastomers in an endangered salamander, *Eurycea tonkawae*. PLoS ONE 8: e59424. doi:10.1371/journal.pone.0059424

Caorsi, V.Z., R.R. Santos, and T. Grant. 2012. Clip or snap? An evaluation of toe-clipping and photo-identification methods for identifying individual Southern Red-Bellied Toads, *Melanophryniscus cambaraensis*. South American Journal of Herpetology 7:79–84.

Caorsi, V.Z., P. Colombo, M.D. Freire, I.B. Amaral, C. Zank, M. Borges-Martins, and T. Grant. 2014. Natural history, coloration pattern and conservation status of the threatened South Brazilian Red Bellied Toad, *Melanophryniscus macrogranulosus* Braun, 1973 (Anura, Bufonidae). Herpetology Notes 7:585–598.

Carreira, S., and R. Maneyro. 2015. Lista roja de los anfibios y reptiles del Uruguay. Una evaluación del estado de conservación de la herpetofauna de Uruguay sobre la base de los criterios de la Unión Internacional para la Conservación de la Naturaleza. Dirección Nacional de Medio Ambiente, Montevideo, Uruguay.

Darst, C.R., P.A. Menéndez-Guerrero, L.A. Coloma, and D.C. Cannatella. 2005. Evolution of dietary specialization and chemical defense in poison frogs (Dendrobatidae): a comparative analysis. American Naturalist 165:56–69.

Davis, J.L.D., A.C. Young-Williams, A.H. Hinesa, and O. Zmora. 2004. Comparing two types of internal tags in juvenile Blue Crabs. Fisheries Research 67:265–274.

Dirección Nacional de Medio Ambiente (DINAMA). 2010. Plan de mediano plazo 2010/2014. Hemisferio Sur, Montevideo, Uruguay.

Elgue, E., G. Pereira, F. Achaval-Coppes, and R. Maneyro. 2014. Validity of photo-identification technique to analyze natural markings in *Melanophryniscus montevidensis* (Anura: Bufonidae). Phyllomedusa 13:59–66.

Ferner, J.W. 2007. A review of marking and individual recognition techniques for amphibians and reptiles. Herpetological Circular No. 35. Society for the Study of Amphibians and Reptiles, Salt Lake City, Utah, USA.

Gollmann, G., and B. Gollmann. 2011. Ontogenetic change of colour pattern in *Bombina variegata*: implications for individual identification. Herpetology Notes 4:333–335.

Gosner, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16:183–190.

Hantak, M.M., T. Grant, S. Reinsch, D. Mcginnity, M. Loring, N. Toyooka, and R.A. Saporito. 2013. Dietary alkaloid sequestration in a poison frog: an experimental test of alkaloid uptake in *Melanophryniscus stelzneri* (Bufonidae). Journal of Chemical Ecology 39:1400–1406.

Langone, J.A., M.V. Segalla, M. Bornschein, and R.O. de Sá. 2008. A new reproductive mode in the genus *Melanophryniscus* Gallardo, 1961 (Anura: Bufonidae) with description of a new species from the state of Paraná, Brazil. South American Journal of Herpetology 3:1–9.

Löhner, L. 1919. Über einen eigentümlichen Reflex der Feuerunken nebst Bemerkungen über die "tierische Hypnose". Pflügers Archiv für die Gesamte Physiologie des Menschen und der Tiere 174:324–351.

Lowe, D.G. 2004. Distinctive image features from scale-invariant keypoints. International Journal of Computer Vision 60:91–110.

Maneyro, R., and S. Carreira. 2012. Guía de Anfibios de Uruguay. Ediciones de la Fuga, Montevideo, Uruguay.

Mebs, D., W. Pogoda, R. Maneyro, and A. Kwet. 2005. Studies on the poisonous skin secretion of individual Red Bellied Toads, *Melanophryniscus montevidensis* (Anura, Bufonidae), from Uruguay. Toxicon 46:641–50.

Mebs, D., M.G. Wagner, W. Pogoda, R. Maneyro, A. Kwet, and G. Kauert. 2007. Lack of bufadienolides in the skin secretion of red bellied toads, *Melanophryniscus* spp. (Anura, Bufonidae), from Uruguay. Comparative biochemistry and physiology. Toxicology & Pharmacology 144:398–402.

Morrison, T.A., D. Keinath, W. Estes-Zumpf, J.P. Crall, and C.V. Stewart. 2016. Individual identification of the endangered Wyoming Toad *Anaxyrus baxteri* and implications for monitoring species recovery. Journal of Herpetology 50:44–49.

Pereira, G., and R. Maneyro. 2016a. Use of reproductive microhabitat by *Melanophryniscus montevidensis* (Anura: Bufonidae) from Uruguay. Zoological Science 33:337–344.

Pereira, G., and R. Maneyro. 2016b. Movement patterns in a Uruguayan population of *Melanophryniscus montevidensis* (Philippi, 1902)(Anura: Bufonidae) using photo-identification for individual recognition. South American Journal of Herpetology 11:119–126.

Semlitsch, R.D. 2008. Differentiating migration and dispersal processes for pond-breeding amphibians. Journal of Wildlife Management 72:260–267.

Toledo, L.F., and C.F.B Haddad. 2009. Colors and some morphological traits as defensive mechanisms in anurans. International Journal of Zoology 2009:1–12.

Walston, L.J., and S.J. Mullin. 2008. Variation in amount of surrounding forest habitat influences the initial orientation of juvenile amphibians emigrating from breeding ponds. Canadian Journal of Zoology 86:141–146.

Zank, C., F.G. Becker, M. Abadie, D. Baldo, R. Maneyro, and M. Borges-Martins. 2014. Climate change and the distribution of neotropical red-bellied toads (*Melanophryniscus*, Anura, Amphibia): how to prioritize species and populations? PLoS ONE 9: e94625. doi: 10.1371/journal.pone.0094625

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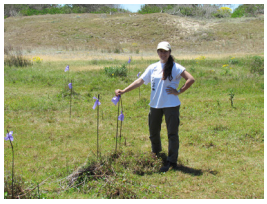

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