

Validity of photo-identification technique to analyze natural markings in *Melanophryniscus montevidensis* (Anura: Bufonidae)

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Abstract

Validity of photo-identification technique to analyze natural markings in *Melanophryniscus montevidensis* (Anura: Bufonidae). Individual identification is useful for answering a variety of biological questions about animal life histories. Most of the techniques used to mark amphibians are invasive and can cause negative effects, compromising individual survivorship and biasing studies. Photo-identification consists in the identification of specimens based on photographic records of unique color-design patterns. This technique has been used with success in several amphibian species. *Melanophryniscus montevidensis* is an endangered anuran species inhabiting the Uruguayan Atlantic coast. The general pattern of coloration is black with red and yellow blotches on the belly. In this study, we validated the technique of photo-identification assisted by software for individual recognition in *M. montevidensis* using natural markings. Field trips were performed over 16 months during which, the ventral color pattern of specimens was photographed. The photos were edited and analyzed with the Wild-ID 1.0 software for photographic reconnaissance. An efficiency of 100% was obtained in the visual recognition and 90% in the detection of recaptures using the software. The use of photo-identification using natural marks is an effective technique in this species, because the color pattern of the belly was highly variable among individuals and remained unchanged in individuals over the 16 month period. In this evaluation the use of software for photo-identification was necessary for the treatment of large databases.

Keywords: amphibians, individual identification, non-invasive technique, Uruguay.

Received 4 April 2014.
Accepted 22 July 2014.
Distributed August 2014.

Resumen

Validez de la técnica de fotoidentificación con marcas naturales en *Melanophryniscus montevidensis* (Anura: Bufonidae). La identificación individual de animales es muy útil para responder diversas preguntas biológicas relacionadas con su historia de vida. La mayoría de las técnicas utilizadas para marcar anfibios son invasivas y pueden causar efectos negativos sobre los individuos, comprometiendo su sobrevivencia y los resultados de las investigaciones. La fotoidentificación consiste en identificar especímenes en base a registros fotográficos, usando patrones de diseño-coloración únicos para cada animal. Esta técnica ha mostrado resultados exitosos en varias especies de anfibios. *Melanophryniscus montevidensis* es un anuro presente en la costa atlántica uruguaya cuya conservación se encuentra comprometida. El patrón general de coloración es negro con manchas rojas y amarillas en el vientre. En este estudio se valida la técnica de fotoidentificación asistida por software para el reconocimiento individual en *M. montevidensis*, utilizando marcas naturales. Se realizaron salidas durante 16 meses en las que se registró fotográficamente el patrón ventral de coloración de los ejemplares. Las fotografías fueron editadas y se utilizó el software de reconocimiento fotográfico Wild-ID 1.0. Se obtuvo una eficacia del 100% en el reconocimiento visual y 90% en la detección de recapturas empleando el software. El uso de la fotoidentificación a partir de marcas naturales es una técnica efectiva en esta especie, ya que el patrón de coloración analizado resultó altamente variable entre individuos e invariable en un mismo individuo a través del tiempo. Es importante utilizar un software para asistir la tarea de fotoidentificación, ya que hace viable el tratamiento de grandes base de datos.

Palabras claves: anfibios, identificación individual, técnica no invasiva, Uruguay.

Validade da técnica de foto-identificação para analisar marcas naturais em *Melanophryniscus montevidensis* (Anura: Bufonidae). A identificação individual é útil para responder uma variedade de questões biológicas sobre a história de vida dos animais. A maioria das técnicas usadas na marcação de anfibios é invasiva e pode provocar efeitos negativos, comprometendo a sobrevivência e introduzindo vieses nos estudos. A foto-identificação consiste na identificação dos indivíduos baseada em registros fotográficos de padrões individuais de coloração ou de desenho. Essa técnica tem sido usada com sucesso em diversas espécies de anfibios. *Melanophryniscus montevidensis* é um anuro ameaçado de extinção que habita a costa atlântica uruguia. O padrão geral de coloração é preto com manchas vermelhas e amarelas no ventre. Neste estudo, validamos a técnica de foto-identificação auxiliada por software para o reconhecimento individual *M. montevidensis* usando marcas naturais. Viagens de campo foram feitas ao longo de 16 meses, durante as quais foi fotografado o padrão de coloração ventral dos indivíduos. As fotografias foram editadas e analisadas com o auxílio do software Wild-ID 1.0 para reconhecimento fotográfico. Obtivemos uma eficiência de 100% no reconhecimento visual e de 90% na detecção de recapturas usando o software. O uso de foto-identificação usando marcas naturais mostrou-se uma técnica eficaz para essa espécie, pois o padrão de coloração ventral é altamente variável entre os indivíduos e permaneceu inalterado ao longo do período de 16 meses. Em nossa avaliação, o uso de software é necessário para o tratamento de grandes bases de dados.

Palavras-chave: anfibios, identificação individual, técnica não-invasiva, Uruguai.

Introduction

Individual identification of animals is fundamental for answering several biological questions related to demographic, reproductive or ethological aspects, as for evaluation of survival rate, migrations, and body growth, among others (Williams *et al.* 2002, Cove and Spínola 2013). Several methods for marking amphibians have been developed and used in the field: toe-clipping, marks for injection of elastomers, cold, heat, silver nitrate or tattooing, and application of chips or transponders (Donnelly *et al.* 2001, Ferner 2007). Natural markings as well as molecular markers like microsatellites are alternative techniques (Jehle and Amtzen 2002). An ideal marking technique should have no effect on the health and behaviour of the animal, it should last for the duration of the study, and should be easy to apply with minimal cost (Ferner 2007). It is very difficult to meet all of these criteria simultaneously, and hence the choice of an adequate technique will depend on the objectives and limitations of the study and the species under study.

The most used technique for individual identification of amphibians has been toe-clipping due to its easy application and low cost (Donnelly *et al.* 2001). However, occasionally, and similar to other invasive techniques, toe-clipping has been proven to have negative consequences on individuals, affecting both the survival of animals and the results of the study (e.g., biased mortality rates) (Davies and Ovaska 2001, Bloch and Irschick 2004).

Photo-identification methods (PIMs) using natural markings is a non-invasive technique, which requires permanent external phenotypic differences. Although this technique has been used in amphibians (Donnelly *et al.* 2001), difficulties for its application or the high costs made it a delicate choice (e.g., production of analog drawings or photos). Nowadays, such difficulties have been resolved with the use of digital photography. The efficiency of this technique depends upon the speed and training

of the researcher in identifying individuals. However, it becomes more complex when the photographic database is large (Gamble *et al.* 2008, Hastings *et al.* 2008, Hiby *et al.* 2009, Sherley *et al.* 2010). Software for rapid photo-identification has recently been developed, using an algorithm that chooses from a database the photos that are more similar for visual confirmation (Bolger *et al.* 2011).

Methods of photo-identification have been used and tested successfully in anurans of the genus *Melanophryniscus* (Abadie 2012, Caorsi *et al.* 2013), which show great individual variation in coloration (Maneyro and Carreira 2012). *Melanophryniscus montevidensis* is distributed throughout the Uruguayan Atlantic coast and southern Rio Grande do Sul, Brazil. The general coloration of the body is black with red and yellow marks on palms and soles, including very variable markings in the lower abdomen. The distribution of this species has been notably reduced during the last decades, and hence is considered an endangered species according to IUCN (IUCN red list, 2013). The decline of the species has been attributed to the increase of urbanization (Maneyro *et al.* 1995). Recently, climate change has been added as a potential threat for the conservation of this species (Toranza and Maneyro 2013). The biological information of this species generated from the use of non-invasive techniques is essential in order to establish conservation actions without compromising the populations in the wild. The objective of the present work is to validate the technique of photo-identification based on software using natural markings in *M. montevidensis*.

Materials and Methods

Capture and Photography of Specimens

Specimens of *Melanophryniscus montevidensis* were captured in the Barra de la Laguna de Rocha (Rocha, Uruguay). We collected the specimens from five temporary ponds in 16 field

trips each lasting three days, between September 2011 and February 2013. The individuals were captured manually, kept in individual containers (maintained in suitable humidity and temperature), and identified with a number. When taking photos, the animals were placed upside down on a white colored plastic area. Care was taken to get the toads clean and dry at the moment of photography, in order to be able to capture all the ventral markings. The cameras used were NIKON, L810 (16.0 MP) and P500 (12.1 MP) models. Two to four photos of each individual were taken. Then, after 24 hours maximum, the animals were released at the place of capture.

Test of the Program Effectiveness

In the laboratory, the most suitable photo was selected (in terms of sharpness and position of the animal) and edited. When necessary, brightness and contrast were modified, and each photo was cut in order to obtain only an image of the abdomen (Figure 1). The objective of this cut was to minimize the sources of error due to the photo background and the invariant regions of individual coloration (head and extremities) (Bolger *et al.* 2011). Wild-ID 1.0 was the free software used for the analysis of recaptures of photographs (Bolger *et al.* 2011). It returns the 20 photos that were more similar to the analyzed one for visual confirmation of recapture. The software is based on an algorithm that recognizes and compares “key points independent from the scale” (Lowe 2004). Finally, a score of coincidence among pairs of photos is elaborated.

To test the effectiveness of the program, 410 photos from database were selected for examination, corresponding to 20 recaptures (40 photographs) and 370 individuals that were captured only once (370 photographs). Visual matching (VM) was performed by comparing every possible combination of these photos in pairs by a single person (this task resulted in 83845 comparisons). Then, this selection was processed by the Wild-ID software in computer-assisted matching (CAM). Data of position in

the ranking of comparison and the similarity coefficient among pairs were taken from the software. For VM, as well as CAM, the time employed to obtain the results was measured.



Figure 1. Variation of ventral coloration of *Melanophryniscus montevidensis*. Photos taken from the analyzed database.

Analysis of the Full Database

The complete database of 16 months was also analyzed to obtain results with all recaptures (total number of recaptures, percentages of monthly recaptures, and multiple recaptures of the same individual). The software Wild-ID was run in a computer with a processor Intel Core i5 1.8GHz, 4,0GB RAM and Windows 8 (64-bit). The regressions and statistical tests were performed in Statistica 8.0 and XLSTAT 2014.

Results

Capture and Photography of Specimens

During the study 7,436 photographs were taken. A total of 1,878 photos were selected based upon the criteria stated in Materials and

Methods. These photos corresponded to one picture of each individual in each capture event.

Test of the Program Effectiveness

Photograph editing (adjusting brightness, contrast and cropping) required 30–60 s per photograph. The time required for VM of recaptures to prove the efficiency of the software lasted around 10 hours, meanwhile the time required for CAM lasted no more than three hours and 40 minutes: 10 minutes for the software analysis and about three hours and 30 minutes for visual confirmation.

In the proof of software all recaptures were recognized via VM, while CAM recognized 18 representing a 90% efficacy. From the detected recaptures, 14 were the first option in the list of individuals returned by the software, and the other four were listed in a second, fourth, fifth, and thirteenth place. The coincidence scores varied from 10^{-6} to 0.23. No false positives (pair of photos taken wrongly as recaptures) were obtained with either method.

Analysis of the Full Database

A total of 268 recaptures were recognized from the database, including 29 individuals that were recaptured more than once. The percentage of recaptures increased with the number of sampling events ($r^2 = 0.83$; $p < 0.0001$) and the size of database ($r^2 = 0.76$; $p < 0.0001$). Also, the relationship between the number of captures and the number of recaptures was positive and significant ($r^2 = 0.84$; $p < 0.0001$) (Figure 2).

Taking into account the recaptures recognized by the software, the one with greatest temporal distance had 14 months difference (Figure 3). To verify whether there was temporal variation in the coloration of all the recaptured specimens, a further examination of photos was carried out and no changes in the pattern of marks were found. It is important to notice that 66 individuals showed deformities in the limbs: ectrodactyly, brachydactyly, or syndactyly.

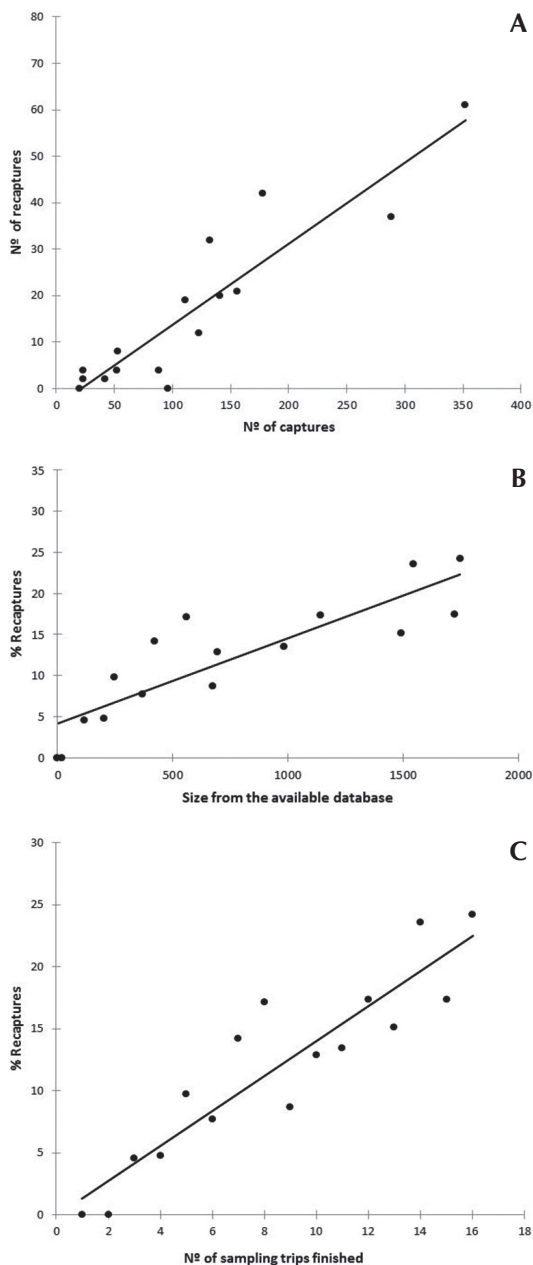


Figure 2. Linear regressions among the number of recaptures and the number of captures ($r^2 = 0.838$; $p < 0.0001$) (A); % of recaptures and size from the available database ($r^2 = 0.755$; $p < 0.0001$) (B); and % of recaptures and number of sampling trips finished ($r^2 = 0.834$; $p < 0.0001$) (C).

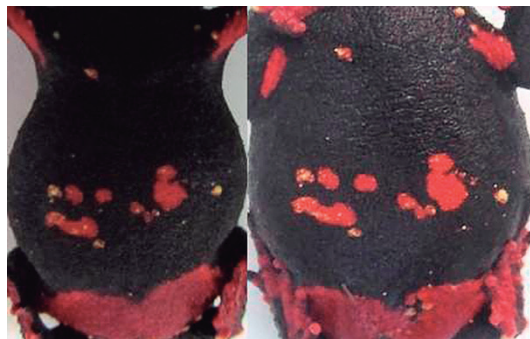


Figure 3. Photographs of an individual of *Melanophryniscus montevidensis* recaptured with a 14 months difference.

Discussion

PIM showed good results for *M. montevidensis*, with a 100% effectiveness in the case of VM. CAM seems to be an effective technique, although it was not free of error; a main strength of this technique is the short time required, considering the large database. The software contributes mostly to such strength, since it decreases over half the time used when compared to the manual analysis of photos. Another positive aspect of the technique is the absence of false positives, which is due to the way the software is managed, since recaptures require the operator to have a visual confirmation. The characteristics of the natural markings analyzed in this species, with a high degree of variation among individuals (Maneyro and Carreira 2012) could also have contributed to the good performance of the CAM. The absence of false positives is very important since in many analysis (e.g. estimations of body growth, identification of migrants), commission errors can give more biased results than omission errors do (Krebs 1999).

Taking into account the conservation status of *M. montevidensis* (IUCN red list, 2013) and the characteristics of its coloration (Maneyro and Carreira 2012), photo-identification through

natural markings seems to be one of the best alternatives for individual identification of this species. Additionally, it has low economic cost, the procedure is simple, fast, and shows good results. Invasive marking techniques would be risky for the viability of the population owing to the scale of the study and because of the possible health impact of these techniques. Toe-clipping may be less efficient on these animals, because a high percentage of abnormalities in the limbs was observed (4.1 %), which could generate an additional error in the analysis of recaptures. On the other hand, although the ablation of phalanges has been considered a viable technique for amphibians (Phillot *et al.* 2007), several studies demonstrate its inconvenience for a good health of the animal as well as for the results of the research (McCarthy and Parris 2004, Parris and McCarthy 2008).


Like other studies in the genus, our results show the feasibility of working with a big database for photo-identification in *Melanophryniscus* (Abadie 2012, Caorsi *et al.* 2012). Additionally, the association of number of recaptures to variables like sample size in each event, number of events or size of database can be taken in mind in order to better plan future field work (e.g. evaluate if it is more convenient make larger samples or more sampling events).

Working on *Litoria genimaculata*, Kenyon *et al.* (2009) found a low PIM efficiency (61.15%), contrary to the present results. This difference is probably a consequence of the characteristics of the natural markings analyzed, since the coloration of the specimens of *L. genimaculata* is not as variable among conspecific individuals, as it is in *M. montevidensis*. Also, these authors reported changes in natural markings during the 10 month period of the research. Other similar studies with species of the genus *Melanophryniscus* (*M. admirabilis* and *M. cambaraensis*), show that the efficiency of the PIM, although similar to the reported data in the present paper, never reached the 100% as in VM (Abadie 2012, Caorsi *et al.* 2012). This is probably because *M. admirabilis* and *M. cambaraensis* has less variable

coloration than *M. montevidensis*. However, this does not explain why when photos were analyzed by the software, the efficacy was lower in *M. montevidensis*. This difference could be because of the factors that affect the software, like the uniform quality of photos or the background of the image (Bolger *et al.* 2011, Abadie 2012). Improving these aspects would result in a more accurate CAM.

The utilization of PIM is an effective technique in this species and could also be efficient for other species of the same genus with similar coloration patterns (as in *M. sanmartini*, *M. atroluteus* or *M. dorsalis*). Such efficiency may be based on the pattern of analyzed coloration in the present study, resulting highly variable among individuals and invariable in a unique individual, through a period of time. The non-invasive character and the low cost of this technique should be emphasized, being the best option for individual identification. We encourage field biologists to rely on the possibility of using software to help with photo-identification of individuals in those species with variable color markings and under conservation threat

Acknowledgments

We acknowledge to Ross Alford and two anonymous reviewers whose comments significantly improved the manuscript. We are very grateful to Zulema Coppes for translating this study to english version, as to María Pía Aristimuño and Arley Camargo for their important suggestions. To Michelle Abadie and Valentina Caorsi, for assisting in the use of Software Wild-ID. We would like to thank the colleagues of “Laboratorio de Sistemática e Historia Natural de Vertebrados”, and the park rangers of Laguna de Rocha for supporting our field work. The authors acknowledge the financial support of Agencia Nacional de Investigación e Innovación (ANII) in the FCE_2009_1_2514 project. 

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Editor: Ross Alford