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THE USE OF DIGITAL IMAGES FOR THE INDIVIDUAL IDENTIFICATION OF AMPHIBIANS

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***Key words**: marking technique, Bombina variegata, ventral pattern, digital identification, identification code*

INTRODUCTION

Mark-recapture techniques are widely used in amphibian demographical studies (Beausoleil et al., 2004; Donnely et al., 1994; Schmidt, 2003). A variety of marking techniques are available for adult amphibians, with permanent or temporary marks, either date-specific or individual specific. Long-term studies, including monitoring, require permanent marks, that ideally should be individual-specific. Among the marking techniques used for adult amphibians are: toe clipping, pattern mapping (Donnely et al., 1994), branding (Donnely et al., 1994; Ehmann, 2002), passive integrated transponders and microtags (Sinsch, 1992; Jehle & Hödl, 1998), skin grafting (Rafinski, 1977; Plytycz & Bigaj, 1993), the use of polymers and pigments (Donnely et al., 1994; Cogălniceanu, 1997).

The ideal mark should be as free of pain and/or stress as possible, allow the individual identification of the animal, be easy to apply in the laboratory and the field, cost-effective and able to utilize materials that are easy to obtain, should not cause death or have sub-lethal effects on fitness, or influence the behavior and detection probability of the marked individuals (Beausoleil et al., 2004).

The most frequently used marking technique for amphibians is toe clipping. This marking technique involves some level of stress and tissue damage that might increase the risk of infection (Parris & McCarthy, 2001; McCarthy & Parris, 2004; May, 2004; Funk et al., 2005).

Pattern mapping was until recently a rather costly and time-consuming technique. The decrease in cost of digital cameras and their improved quality has made this technique more attractive, since it allows the rapid marking of large number of individuals in the field. It is similar to the finger prints technique used in individual human identification for over a century.

Pattern mapping was used in a variety of amphibian species for the identification of both adults (Loafman, 1991) and larvae (Eitam & Blaustein, 2002). This marking technique is limited only to those species of amphibians that have highly variable dorsal (among Romanian taxa: *Salamandra salamandra*, *Rana temporaria, Pelobates fuscus, P. syriacu*s, *Bufo viridis)* or ventral (e.g. *Triturus vulgaris, T. dobrogicus, T. cristatus, Bombina* sp.) color patterns. Most attention was given to *Bombina* sp., for belly pattern identification of hybrids (Covaciu-Marcov et al., 2004; Voros et al., 2002), but also for individual identification (Delarze et al., 2000; Seidel et al., 2001). In this paper we present a simple method for individual recognition of the yellow bellied toad (*Bombina variegata*) based on belly pattern.

MATERIAL AND METHOD

This study is based on 162 adult and subadult yellow bellied toads captured and photographed in the field during 2004 and 2005, in the Haţeg Geopark and Retezat National Park (Department of Hunedoara, Romania). Each individual was immobilized between the two sides of a Petri dish with foam on one side. The time required for handling and photographing an individual was about one minute. The method does not require prior anaesthesia of the animals and involves minimal stress. The animals were released imediately after. The images were downloaded in the laboratory, stored in a database and analyzed using *Image Tool* ver. 3.00 software (http://ddsdx.uthscsa.edu). Of the 162 individual photos, 28 could not be used due to low quality of the digital photo caused by a twisted position of the animal, improper light, or to the presence of plant debris or mud stains.

A large number of photographs makes recaptured individual recognition time consuming. We decided to develop an identification code based on belly pattern characteristics, assuming that it did not change in time. Since the photographed individuals were collected from several populations, it was possible to analize a high variety of ventral model patterns. In populations with large number of individuals it is recommended that animals photographed for the first time are toe-clipped, for rapid identification in later recaptures.

RESULTS AND DISCUSSIONS

The code developed for individual identification of the belly pattern of *Bombina variegata* consists of eight numbers, according to the different characters observed. The first refers to the sex or life stage, while

the rest to the presence or pattern of ventral yellow spots. Each characteristic coded acts as a filter, separating the images in two or more categories (Table 1, Fig. 1).

The criteria used are based on previous studies used for belly pattern discrimination of hybrids (Stugren & Vancea, 1968; Voros et al., 2002; Covaciu-Marcov et al., 2004) or other amphibian species (Novitsky, 1996).

Tabel 1. Characters of belly patterns considered for the individual identification code

No.	Character	State	Score
1	Sex or life stage	- Male - Female - Subadult	0 1 2
2	Spots on the throat	- Separated - Combined	0 1
3	Spots on arm	- Combined - Separated - Combined on just one side (left/right)	1 0 2 / 3
4	Spots on the chest	- Combined - Separated	1 0
5	Spots on upper body	- Combined - Separated	1 0
6	Spots on the middle part of the belly	- Combined - Separated	1 0
7	Spots on the lower part of the belly	- Combined - Separated	1 0
8	Spots on the pelvis	- Separated - Combined, above/below the hind legs insertion point	0 1/2

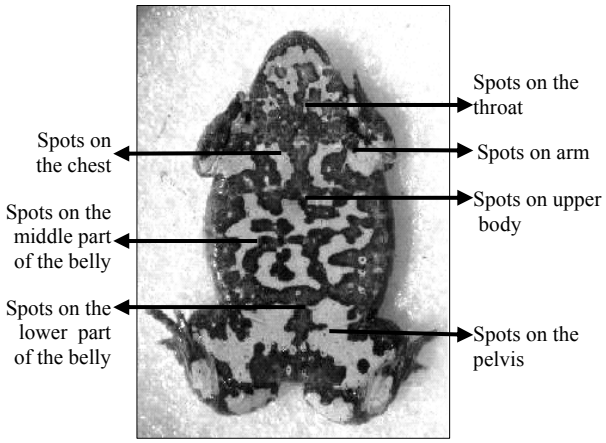


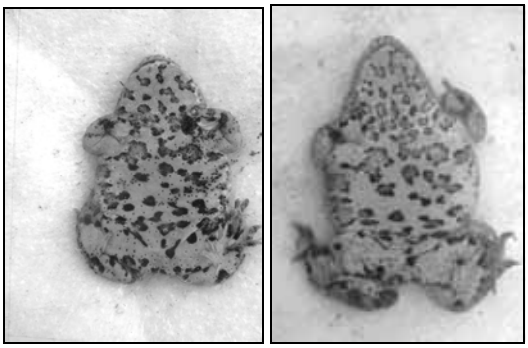
Fig. 1. The characteristic belly patterns used in individual identification.
The code for this individual is 1 0 0 0 0 0 0 0.

The state of a character used in individual identification based on belly pattern should be highly variable, allowing for discriminating between individuals. Ideally, a two-state character should be present close to 50-50 in the population. The percentage of the different states of the characters used in identification shows that, except for character 7 (i.e. spots on the lower part of the belly), all proposed characters have a good discriminating power (Table 2).

Table 2. The usefullness of the code proposed tested with 162 individuals. The percentage of each character state is given (n = 162).

Character	State	Character state (%)
Sex or life stage	- Male - Female - Subadult	43.54 37.10 19.35
Spots on the throat	- Separated - Combined	71 29
Spots on arm	- Combined - Separated - Combined on just one side (left/right)	54.78 22.29 10.19/ 12.74
Spots on the chest	- Combined - Separated	23.46 76.54
Spots on upper body	- Combined - Separated	45.06 54.04
Spots on the middle part of the belly	- Combined - Separated	40.74 59.26
Spots on the lower part of the belly	- Combined - Separated	91.98 8.02
Spots on the pelvis	- Separated - Combined, above/below the hind legs insertion point	6.79 69.75 / 23.46

Arntzen & Teunis (1993) reported of belly pattern changes in *Triturus cristatus* in time, but our preliminary results do not confirm this for *Bombina variegata*. We have tested this method on individuals repeatedly captured in Ciopeia (Hațeg Geopark), but managed to correctly identify the previously photographed individuals (Fig. 2).



29 mm 2.2 g 47 mm 7.5 g

Fig. 2. The same individual photographed in May (left) as subadult (Code: 2 1 1 0 1 1 1 1), and September (right) of 2005 as an adult (Code: 1 1 1 0 1 1 1 1). Snout-vent length and wet weight are also given.

CONCLUSION

In a comparative analysis of the costs of two marking techniques (PIT-tags versus pattern maps), Arntzen et al. (2004) showed that individual identification based on pattern analysis is more efficient for short-term studies or for small populations. One of the disadvantages of the method is the long time required for image analysis. Apart from this aspect, we came across images taken in the field that could not be properly analysed due to the low quality of the image. Despite these drawbacks, the

pattern mapping method used for *B. variegata* has several major advantages: (1) it is a non-invasive method, with no health risks associated and little stress involved, (2) digital images after coding can be easily retrieved, (3) it can be done in the field and requires little time, (4) it is becoming more cost-effective, and (5) each animal can have a recapture history in time, allowing for complex population size models to be used.

REZUMAT

Marcarea individuală a animalelor este o tehnică frecvent utilizată în studiile populaționale şi în programele de monitoring. Tehnicile de marcare tradiționale ale amfibienilor sunt fie traumatizante, fie nu sunt permanente. O alternativă la aceste metode este identificarea individuală a adulților pe baza fotografiilor digitale. Metoda se poate aplica speciilor ai căror indivizi au un model dorsal sau ventral unic. Analiza modelului dorsal sau ventral pe baza unor caractere specifice permite codificarea acestora. În urma codificării imaginile pot fi stocate într-o bază de date iar identificarea în momentul recapturării să se poată realiza rapid, chiar în condițiile unor populații cu sute de indivizi. Metoda nu este traumatizanta si poate fi utilizată repetat, fără a afecta probabilitatea de recapturare a animalelor marcate.

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