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Novel, non-invasive method for distinguishing the individuals of the fire salamander (*Salamandra salamandra*) in capture-mark-recapture studies

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Abstract. Recently we started implementing a highly efficient, non-invasive method of direct individual marking (i.e., typifying) in a population study of the fire salamander, *Salamandra salamandra*. Our technique is based on the unique alphanumeric code for every individual, generated upon the numbers of openings of repellent/toxic skin glands in the yellow areas of the selected regions of the body. This code was proved reliable in the sample of 159 individuals from two separate populations and enabled easy and quick recognition of recaptured animals. The proposed method is inexpensive, easily applicable in the field, involves minimum stress for the animals and does not affect their behaviour and the possibility of repeated captures of "marked" (i.e., coded) individuals. It is particularly suitable for dense populations.

Keywords. *Salamandra salamandra*, individual recognition, non-invasive, natural markings.

The capability to accurately identify individual animals within a population is fundamental for Capture–Mark–Recapture (CMR) studies, which provide basic information regarding population ecology. Individual identification is indispensable for gaining information on individual growth rates, attainment of sexual maturity, rates of reproduction, survival and other life-history parameters and for the studies of various aspects of behaviour (Davis and Ovaska, 2001). The accurate assessment of population size and density is vital for efficient management and protection of species (Bradfield, 2004). Numerous techniques are used for individual marking of amphibians: toe clipping (Donnelly et al., 1994; McCarthy and Parris, 2004; Liner et al., 2007), pattern mapping (Donnelly et al., 1994; Arntzen and Teunis, 1993; Gamble et al., 2008), branding, polymers and pigments (Donnely et al., 1994), photographic identification (Kurashina et al., 2003; Plăiaşu et al., 2005; Gamble et al., 2008; Clemas et al., 2009), Passive Integrated Transported (PIT) tags (Jehle and Hödl, 1998), Visible Implant Elastomer (VIE) tags (Campbell et al., 2009), and Visible Implant Alphanumeric (VIA) tags (Clemas et al., 2009; Osbourn et al., 2011). Being cheap and simple, the most frequently used invasive marking method is toe clipping (Donnelly et al., 1994). However, this method involves stress and tissue damaging, which can increase, for example, the risk of infection (McCarthy and Parris, 2004). Also, the toes can regenerate; therefore these markings cannot be considered permanent (Donnelly et al. 1994; Davis and Ovaska, 2001; Ursprung et al., 2011). In addition, recent studies demonstrated decreased rates of recapture following the marking by toe clipping (Davis and Ovaska, 2001; McCarthy and Parris, 2004; McCarthy et al., 2009).

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Currently, photo-identification is the most frequently used non-invasive method in population studies of amphibians. Photo-identification was previously used in population studies of several amphibian species; however, it was often proved reliable only in short-time studies because the patterns of spots, i.e. markings on the skin change over time (Arntzen and Teunis, 1993; Kurushina et al., 2003; Plăiaşu et al., 2005). Another frequently stated drawback of individual identification based on colouration pattern was the time-consuming analyses of the photographs (Plăiaşu et al., 2005).

Previous "marking" schemes considered solely the colouration pattern; we propose the inclusion of glands openings, supposing they vary less than the colour pattern. This modification considerably reduces the time necessary to analyse the photographs.

To simplify the recognition of recaptured individuals in large populations, the addition of toe clipping to non-invasive approaches was suggested (Plăiaşu et al., 2005). However, it should be kept in mind that amphibians' toes (or complete limbs) can be deformed and/or lost due to various naturally causes, e.g. sublethal predation or infection (Gray et al., 2002; Stopper et al., 2002; Bowerman et al., 2010; Sessions and Ballengée, 2010); also, amphibians can regenerate clipped toes (Ursprung et al., 2011). Besides, Davis and Ovaska (2001) showed that the number of recaptured animals with clipped toes was only 40%, compared to 60% of animals with fluorescent markings; that result suggested higher mortality or altered behaviour among the animals with clipped toes.

Here we propose a highly efficient, non-invasive method for recognizing individual animals, particularly suited for the fire salamander (*Salamandra salamandra*). The method is based on the combination of the numbers of openings of the excretory ducts of skin glands in certain body regions. It causes minimum stress and there is no need to firmly restrain or anesthetize the animals. To our knowledge, our study is the first capture-mark-recapture assessment of *Salamandra salamandra* populations ever performed in the Balkan Peninsula.

The present study is based on the sample of 159 fire salamander individuals (157 adults, 2 juveniles), which were captured and photographed in the field. The study was conducted from March to November 2012, in two localities near the city of Banja Luka (Republic of Srpska, Bosnia and Herzegovina). The samples from the two populations consist of 91 and 68 individuals, respectively. We made three photographs of every individual: its left parotoid gland, right parotoid gland, and the entire dorsal side of the body. We used the digital camera SONY DSC-S980. Also, we clipped the fourth toe from the right hind limb. Immediately after processing the individuals were released at the exact places of capture.

The analysis of the number of toxic glands openings (black dots) on yellow surfaces in the selected regions of the body was conducted in the laboratory, based on the obtained photographs. A database of photographs was formed, necessary for subsequent identification of potential recaptures. We assigned an identification code to every individual, which consisted of three combinations of numbers and letters. The first combination depicts the number of gland openings on the yellow surface on the left parotoid gland, the second shows the number of gland openings on the yellow surface on the right parotoid gland, and the third set presents the number of gland openings on the yellow surfaces on the mid-dorsal side of the body, along the vertebral column (see Fig. 1). The codes were entered into the Excel database, along with individual descriptions, which enabled easy and quick search.

The identification code for the individual shown in Fig. 1 is: 14L/17R/14V, and it represents the following: 14 glands openings on the yellow surface covering the left parotoid gland (A), 17 openings on the yellow surface on the right parotoid (B), and 14 glands openings on the yellow surfaces along the trunk and tail in the two parallel rows following the vertebral column (C). Note that we considered only the fully circular glands openings: five incomplete ones on the right side of the head were not counted.

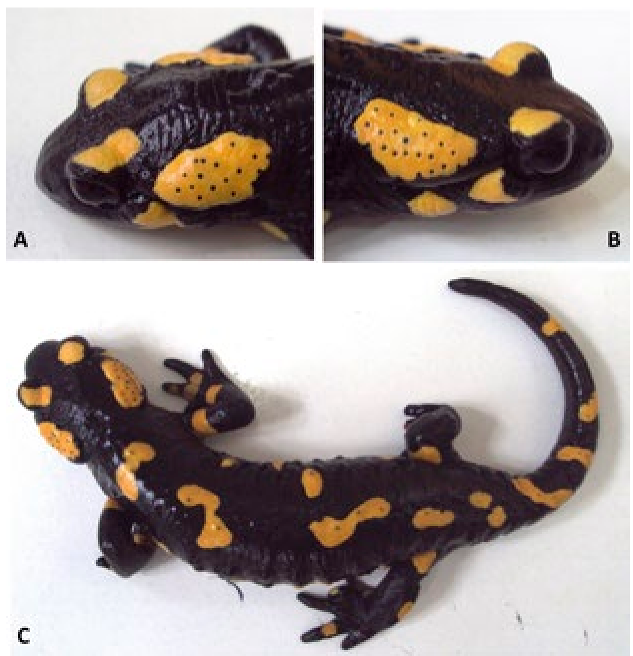

Fig. 1. Fire salamander's body regions with the skin glands openings important for generating the unique identification code

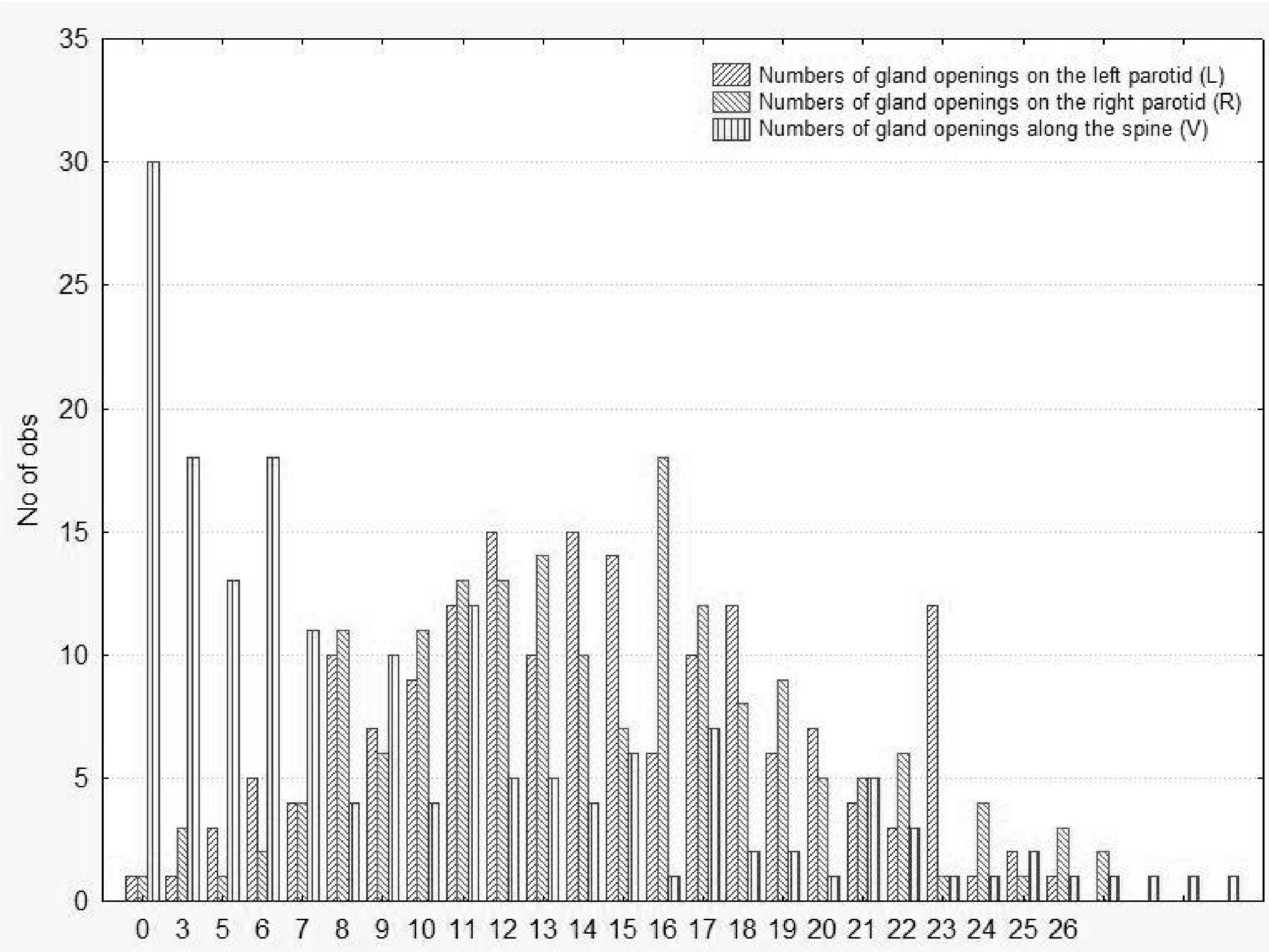

Fig. 2. Histogram of glands opening numbers in the selected body regions.

The number of the glands openings on the yellow surface on the left parotoid gland ranged from 0 to 26, and on the right parotoid it was between 1 and 29; on the yellow areas following the vertebral column along trunk and tail there were 0 to 36 glands openings (pooled sample, 159 individuals; Fig. 2). This range of variation allows huge numbers of possible combinations within populations, i.e. there is only a minute probability of finding several individuals with the identical code and pattern.

We assumed that the numbers of glands openings (i.e., yellow surfaces they are encircled by) do not considerably change over time, at least not in adult animals. However, in this initial phase of the capture-mark-recapture study, we performed the standard toe-clipping procedure in order to have an independent validation of photo-identification. Among the 159 processed animals, we recaptured eleven individuals; all were recognized by the means of individual codes and spot patterns. Time lag between the initial capture and subsequent recapture ranged from one to six months. Using only the part of the alphanumeric code representing the numbers of poison glands openings on the yellow areas covering the parotoid glands, we successfully distinguished 72.5% and 89.7% of the samples from the two populations. With the inclusion of an additional trait, i.e. the number of glands openings on the yellow patches along the midline of the trunk and tail (two parallel rows of openings directly above the vertebral column, see Fig. 1), the confidence of identification reached 100%, i.e. we precisely distinguished all the individuals from both populations.

Our alphanumeric code was proved unique in almost all individuals from the two sampled populations. Comparison of the codes of all 159 individuals (pooled sample, both localities) showed that only two individuals (1.26%) had the identical codes, i.e. the matching combination of glands openings on the head and body (8L/12R/0V). However, checking the photographs in the database enabled quick and successful distinguishing of these two animals, based on the differences in the pattern of yellow patches on the dorsal side of the body.

The technique we propose satisfies the demands for the ideal marker (Beausoleil et al., 2004; Gibbons and Andrews, 2004): an animal is subjected to no pain and to minimum stress, it is identifiable, the technique is easily applicable in the field (no software is necessary), it's economical, does not lead to increased mortality and/or reduced growth or reproduction, it has no effect on the behaviour of marked animals (or of the other animals towards the marked ones), and does not affect the probability of future capturing of marked compared to unmarked animals. If the cameras with high resolution and zooming performances are available, the animals can even be photographed directly in their natural environment, without the need of disturbance and capturing (compare, for example, Lambert et al., 2012).

Considering all the above mentioned, the technique we propose is highly suitable for *Salamandra salamandra* populations, such as the two sampled in this study. Toe clipping, which we also applied in this preliminary phase of the CMR study, facilitated the recognition of recaptured individuals. This invasive procedure shall be abandoned if we prove satisfying accuracy of our newly proposed methodology. Although the colour patches pattern is liable to changes during ontogeny (e.g. Beukema, 2011), we can assume that in adults it does not change dramatically. On the other hand, the arrangement of salamanders' poisonous glands does not seem to change during lifetime (McManus, 1935). In the years to come it is necessary that we continue monitoring this trait in our study populations, in order to check the possible pattern changes in various life stages.

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