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ENRICO LUNGI, MARTA BIAGGINI & CLAUDIA CORTI

## RELIABILITY OF THE POST-HOC MEASUREMENT ON *SALAMANDRA SALAMANDRA*

### SUMMARY

The reliability of the post hoc measurement, a non-invasive method for measuring wild animals, was tested on *Salamandra salamandra*. Our results show that SVL measured in the field was lower than SVL estimated from images by three operators and that the estimates were highly consistent. In addition to the potential greater accuracy of this method in estimating SVL compared to the measurements obtained in the field, it allows to significantly reduce the handling times of the animals, thus reducing the stress caused to the salamanders.

*Key words:* Digital images, Fire salamander.

### RIASSUNTO

*L'affidabilità della misurazione post-hoc in Salamandra salamandra.* L'affidabilità della misurazione post-hoc, un metodo non invasivo per misurare gli animali selvatici, è stata testata su *Salamandra salamandra*. I nostri risultati mostrano che la SVL misurata sul campo è inferiore rispetto alla SVL stimata a partire dalle immagini da tre operatori e che le stime sono risultate altamente coerenti. Oltre alla potenziale maggiore accuratezza nella stima della SVL rispetto alle misurazioni ottenute sul campo, questo metodo consente di ridurre notevolmente i tempi di manipolazione degli animali, riducendo così lo stress causato alle salamandre.

*Parole chiave:* Immagini digitali, Salamandra pezzata.

### INTRODUZIONE

Morphometric measurements are crucial data in zoological research. They are needed to assess age classes, individual and population health status (when

used together with individual weight through the calculation of body condition) and morphological characterization (LANZA *et al.*, 2006; BĂNCILĂ *et al.*, 2010). These measurements are critical in studies dealing, for instance, with demography, ecology, phenotypic plasticity, and adaptation. Therefore, obtaining accurate morphological measurements is of primary importance. This can be particularly problematic in field studies, when dealing with species that usually do not maintain linear body orientation. A further goal in studies on wildlife is to reduce handling and decrease both animal stress and the probability of pathogens' transmission, a particularly high risk for amphibians (PHILLOTT *et al.*, 2010; LUNGI *et al.*, 2016). Digital image-based measurements are a useful tool that allows to obtain data on living individuals significantly more accurate than caliper measures (MOTT *et al.*, 2010). This method has been successfully used to measure different salamander species (e.g., MOTT *et al.*, 2010; LUNGI *et al.*, 2020a) but, to our knowledge, it has been never tested on the Fire salamander, *Salamandra salamandra*.

In this study, we test the accuracy of digital image-based measurements in *S. salamandra*, considering SVL (snout–vent length), one of the most common morphometric measures.

## MATERIALS AND METHODS

On 11 April 2021 at night, we surveyed a portion of a population of *Salamandra salamandra* from the northern Apennines (Province of Bologna; Emilia-Romagna, Italy); we focused on an area of about 2,500 m<sup>2</sup>.

Captured salamanders were sexed based on the cloaca swelling; we considered as adults individuals with a total length  $\geq 120$  mm (LANZA *et al.*, 2009). The snout-vent length (SVL) was measured placing and stretching the individuals on a transparent plastic ruler. Prior to release, the salamanders were photographed dorsally to provide high-quality images allowing for estimation of both SVL and total length (TL) with ImageJ software (imagej.nih.gov) (LUNGI *et al.*, 2020a, 2020b) (Fig. 1A).

We used analysis of variance (ANOVA) to assess the potential divergence between SVL measurements in the field and those estimated from images. Using ANOVA we also evaluated the potential divergence in the SVL estimation performed by three operators. We used a linear regression model (GLM) to evaluate whether the divergence between measured and estimated SVL changes with the size of the salamanders; we used the absolute value of the difference between real and estimated SVL as the dependent variable, TL of salamanders and sex (F, M, J) as independent variables and the identity of the operator as the random factor.

RESULTS

We captured and measured 16 Fire salamanders (7 males, 8 females and 1 juvenile). SVL measurements in the field differed significantly from images estimates ( $df = 1, F = 355.1, P < 0.001$ ); the estimated SVL was on average ( $\pm SD$ ) 8.75 ( $\pm 5.30$ ) mm greater (Fig. 1B). No difference in SVL estimation occurred between the three operators ( $df = 2, F = 0.169, P = 0.845$ ) (Fig. 1B). The difference between measured and estimated SVL was significantly affected by the size ( $F_{1, 42} = 5.52, P = 0.024$ ) and sex ( $F_{1, 42} = 8.67, P < 0.001$ ) of the salamanders; the difference increased in larger individuals and in females.

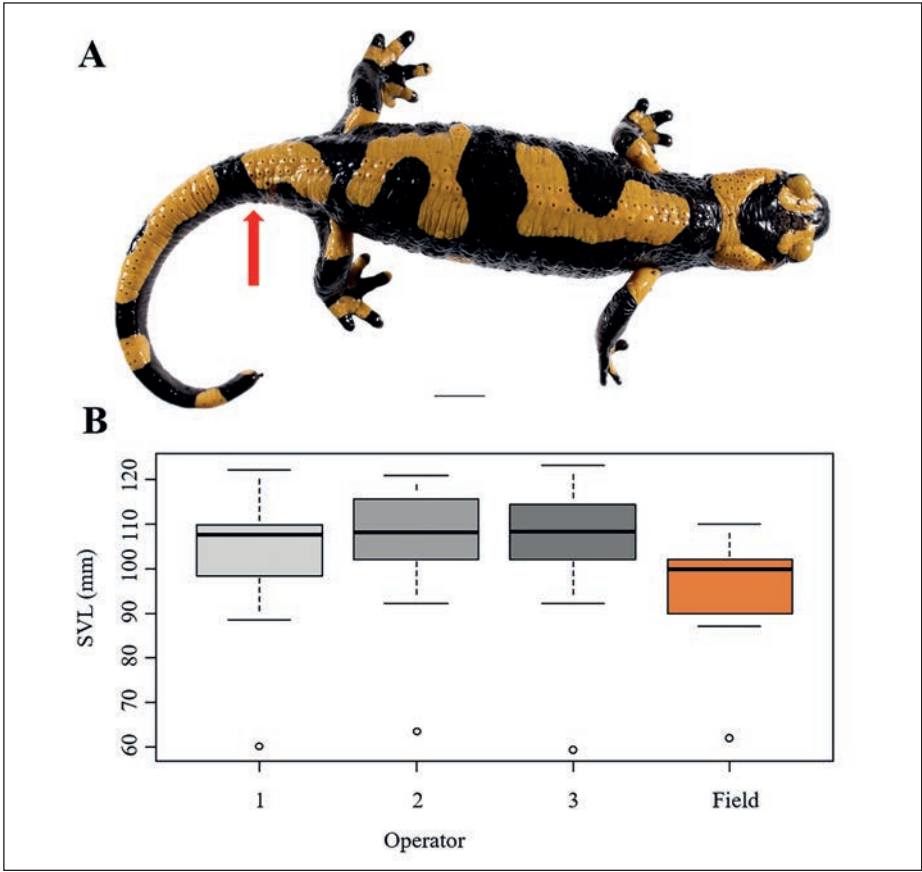


Fig. 1 — A) An example of high-quality image used in this study. The red arrow indicates the area where the opening of the cloaca ends, approximatively corresponding to the point where the tail begins. Barr = 10 mm. B) The boxplots indicate the SVL estimates performed by the three operators and the measurements made in the field.

## DISCUSSION

The estimated measurements of the three operators were highly consistent, highlighting the reliability, also for *Salamandra salamandra*, of the measurements obtained with this method (LUNGI *et al.*, 2020a). SVL measurements performed in the field produced different data, always lower than estimates. Fire salamanders get very agitated when handling, so it is difficult to stretch their body and thus obtain accurate measurements. As expected, the divergence between measured and estimated SVL increased in larger individuals, possibly due to the greater muscle power of larger individuals preventing the operator from adequately stretching their body. This divergence was more pronounced in females; females are usually larger and thus, more difficult to handle. It should be noted that the relatively low number of salamanders sampled (16) is not due to potential difficulties in applying of our method, but represents the total number of individuals observed within the study area. In our study, a single operator measured SVL in the field, but this was sufficient to obtain reliable comparison with the estimated SVL. When this method was tested on European cave salamanders (genus *Speleomantes*) a photo of the ventral side was taken as a reference of the real SVL (LUNGI *et al.*, 2020a). In this study we could not do the same because it was impossible to hold the salamanders still as well as to keep them in supine position. Therefore, further evaluations that include multiple field replicates and museum specimens can strengthen our results and provide more accurate information. The post-hoc method has an additional advantage: it significantly limits handling times of individuals, reducing potential stress (LUNGI *et al.*, 2016) and limiting spread of pathogens.

## LITERATURE

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*Addresses of the authors* — E. LUNGI, Division of Molecular Biology Ruđer Bošković Institute, Zagreb (Croatia); Museo di Storia Naturale dell'Università degli Studi di Firenze, Museo "La Specola", Firenze (I); Natural Oasis, Prato (I); e-mail: enrico.arti@gmail.com; M. BIAGGINI, C. CORTI, Museo di Storia Naturale dell'Università degli Studi di Firenze, Museo "La Specola", Firenze (I).