Validation of the post-hoc method to estimate snout-vent length in the order Caudata

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15 **Abstract.**

16 Amphibians are the most endangered class of vertebrates, with a high rate of decline recorded

17 since the 20th century. Even activities related to the study of these animals, for instance, handling

18 them to collect individual biometric parameters, can have negative effects on amphibian health.

19 A post hoc method for estimating snout-vent length from dorsal photographs has been developed

20 to reduce handling time and stress for individuals, thereby improving precision and repeatability

21 of measurements. However, to date, this methodology has been tested on only approximately 1%

22 of known salamanders, thereby limiting its broad applicability. Here, we tested this method on a

23 diverse sample of Caudata comprising 25 species across 5 families, characterized by diverse

24 morphologies. The correlation between predicted SVL (estimated from dorsal photographs) and

25 observed SVL (measured directly from ventral photographs) values was assessed using Linear

26 Mixed Models. The results showed a significant correlation between observed and predicted

27 SVL, with an average and constant discrepancy of approximately 1.6 mm. When considering the

28 increase of SVL, there was a slight tendency to underestimate SVLe in newts, plethodontids, and

29 proteids. Estimation errors slightly increased with the SVL. The error increased in larger newts,

30 while decreased in larger plethodontids. Our study highlighted the reliability and applicability of

31 adopting this methodology for data collection in all Caudata species.

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Amphibians are the most endangered class of vertebrates, with a dramatically increased rate of

decline recorded over recent decades (Blaustein et al., 1994; Houlahan et al., 2000; Stuart et al.,

2004). Due to their physical and physiological sensitivity, even a well-intentioned human activity

like handling them for research purposes can have negative effects on amphibians' health (Huber

et al., 2019). Handling individuals is often essential in field data collection, allowing researchers

to gather biometric parameters from captured individuals. However, if precautions are not taken,

handling amphibians can facilitate the direct transfer of pathogens (Mendez et al., 2008; Phillott

et al., 2010). A less appreciated, yet concerning effect of handling is the induction of high stress

in wildlife (Huber et al., 2019). For example, individual handling can cause high stress that

might contribute to altering their behaviour (Bliley and Woodley, 2012; Woodley and Porter,

2015) and physiology (Caipang et al., 2014; Möstl and Palme, 2002), having important

cascading negative effects on their biology and consistently increasing their susceptibility to

environmental threats (Bliley and Woodley, 2012; Karaer et al., 2023; Woodley and Porter,

2015). Even brief manipulations can significantly alter their internal temperature (Lunghi et al.,

2016) and compromise their immune defences, which increases their exposure to highly virulent

pathogens (Gabor et al., 2015; Raffel et al., 2006).

Measuring amphibians in the field is not always trivial: their wet, slippery skin significantly

reduces grip during handling, and this effect is further enhanced when individuals struggle

(Lunghi et al., 2022). This can prolong the handling time and reduce measurement precision.

Some approaches have been developed to potentially address these issues (Barzaghi et al., 2025).

Tools like the "Mander Masher", the "Salamander stick" and the "Modified Salamander stick"

helped researchers to increase the precision of recorded data (Margenau et al., 2018; Walston and

Mullin, 2005; Wise and Buchanan, 1992); however, no concerns were raised about the possibility

of also reducing individual stress. In recent years, the use of digital images has significantly

improved data quality and animal safety (Lunghi et al., 2020a; Mott et al., 2010; Speybroeck and

Steenhoudt, 2017). An example is the post-hoc estimation of the length, that involves using a

digital camera to take photographs of individuals in the field, which are then processed in the lab

to extrapolate multiple measurements using a standard reference (Cialente et al., 2025; Lowe and

McPeek, 2012; Lunghi et al., 2021). With this technique, animal handling is limited to

positioning the individual on a specific surface, with no further contact (Lunghi et al., 2021).

This not only reduces handling time and the stress placed on individuals (Lunghi et al., 2016) but

also lowers measurement error rate, enabling the collection of endless measurements for each

individual (Lunghi et al., 2020a). The reduction of these issues is achieved because high-quality

photos, even when taken dorsally, allow estimation of an important parameter hidden from view,

for example the snout-vent length (SVL) (Lunghi et al., 2022; Lunghi et al., 2020a; Mott et al.,

2010). The reliability of the post-hoc method to estimate SVL has been tested only on

approximately 1% of the salamander species known to science (Lowe and McPeek, 2012;

Lunghi et al., 2022; Lunghi et al., 2020a; Mott et al., 2010), and it requires further validation to

be widely employed in studies on Caudata.

The aim of this study was to evaluate the reliability of the SVL post-hoc estimation on different

species of Caudata, giving particular emphasis on testing the methodology on morphologically

distinct groups of species. We constructed a dataset of high-quality photographs including 569

individuals belonging to 25 different species (on average, 22.76 ± 13.58 individuals per species).

We identified five functional groups based on the general body shape of individuals:

Amphiumids/Sirenids (including the genus *Amphiuma* and *Siren*), Newts (genus *Ichthyosaura*,

Lissotriton, *Notophthalmus*, *Triturus*), Plethodontids (genus *Aneides*, *Desmognathus*, *Eurycea*,

Gyrinophilus, *Plethodon*, *Pseudotriton*, *Stereochilus*), Salamanders (genus *Ambystoma* and

Salamandrina), Proteids (genus *Necturus*). The photographs were obtained with the procedure

described in (Lunghi et al., 2020b); captured individuals were placed next to a reference scale on

a white flat horizontal surface and dorsal photographs were taken perpendicularly (Fig. 1A).

Ventral photographs of the same individuals were also taken following the same procedure (Fig.

1B). Measurements were taken to the nearest mm using the program ImageJ (Troscianko and

Stevens, 2015). First, SVL was measured by a single operator for each individual using a

photograph of the ventral aspect. Then, for the same individuals, four operators estimated the

SVL from the dorsal photographs (hereafter, SVLe), obtaining a repetition of four measurements

for each individual. The landmark used to measure SVL is the cloaca, located between the

hindlimbs and the tail. This area is demarcated in almost all salamander species by a conical

frustum shape from a dorsal view, that narrows from the hind legs to the tail base (Fig. 1C). The

posterior opening of the cloaca is placed among an imaginary line that delimits the end of the

frustum from the base of the tail (Fig. 1C). In the genus *Salamandrina* additional landmarks can

help to locate the cloaca. Vertebrae and ribs are well visible from the dorsal view, and the

posterior opening of the cloaca corresponds perfectly to the intervertebral sulcus after the third

caudosacral vertebra and ribs (Fig. 1D). In some species belonging to the Plethodontidae family

(e.g., *Pseudotriton spp.*, *Gyrinophilus spp.*) the shape of the frustum is not always visible, due to

the width of their robust tail. In these cases, the third fold behind the hindlimbs can be used as

landmark to establish the correct position of the posterior end of the cloaca (Lunghi et al., 2020a)

(Fig. 1E). Dorsal photographs were provided to the operators without any additional information

to ensure unbiased measurements (MacCoun and Perlmutter, 2015).

103   We used Linear Mixed Models (LMMs) (package nlme; Pinheiro et al., 2016; R Development

104   Core Team, 2023) to assess the potential relationship between SVL and SVLe. The SVLe was

105   used as the dependent variable, while the mean-centered SVL was used as an independent

106   variable. We added the morpho-group of the analysed species (hereafter: group) as a further

107   independent variable to evaluate whether this trait may affect the precision of SVL estimation.

108   The interaction between SVL and groups was added as an additional factor. The identity of

109   operators and the species were assigned as random variables. Considering the possibility of non-

110   constant variance of the error associated with both predictors, we fitted an extended model using

111   a combination of a power variance function (SVL, continuous variable) and a variance identity

112   structure (group, categorical variable). The comparison between the four models was performed

113   using a likelihood ratio test (Lewis et al., 2011) and the Akaike information criteria (AIC).

114   Likelihood ratio tests were also used to evaluate the significance of the best model terms.

115   Additionally, marginal (R^2_m) and conditional (R^2_c) coefficients of determination were calculated

116   (Bartoń, 2016). We then calculated the standardized squared residuals to explore the potential

117   effects of observed SVL and groups. We performed an additional LMM to assess the model's

118   goodness of fit and to examine the variability of squared residuals in relation to the fixed

119   variables (SVL and groups). In this case, we used the squared residuals as the dependent

120   variable, while the other fixed and random variables remained constant (see above: full model).

121   The results of the model comparison are shown in Table 1. The full model, including the power

122   variance function and the variance identity structure, showed the lowest AIC (8144.837),

123   suggesting that including heteroscedasticity significantly improved the model fit. We identified a

124   significant correlation between the estimated (SVLe) and the centered SVL ($F_{1, 2171} = 272520.50$,

125    $P < 0.001$), while no effect was observed for the group ($F_{4, 20} = 1.76$, $P = 0.177$) (Table 2). A

126   significant effect was observed for the interaction between the SVL and the group ($F_{4,2171}$ =

127   11.25, $P < 0.001$). The slope of the regression line was approximately 1.00 ($\beta = 1.003$, $P <$

128   0.001), indicating that operators' estimates closely resembled the real SVL. However, we

129   observed significant variability in the estimation accuracy between the studied groups of

130   amphibians. In three of them, newts ($\beta = -0.038$, $P = 0.018$), plethodontids ($\beta = -0.029$, $P <$

131   0.001), and proteids ($\beta = -0.038$, $P < 0.001$), there was a slight tendency to underestimate body

132   length in larger individuals (Fig. 2A). This pattern was not significant in Salamanders ($\beta = -$

133   0.016, $P = 0.171$). Residual variability slightly increases with increasing body size (0.82).

134   Considering the residual variability among groups, there was high heterogeneity in the

135   estimation of SVL for newts (1.32), while for plethodontids the estimations were more consistent

136   (0.68). The model explained the overall variance in the dependent variables accounting for

137   99.83% of the variance ($R^2_m = 0.998$). The inclusion of random effects further increased the

138   overall model explanation to 99.99% ($R^2_c = 0.999$). The coefficient for SVL (0.978) indicates

139   that the estimation of SVL was highly reliable, with a slight underestimation of the real values.

140   The discrepancy between observed and predicted SVL (RMSE) was ~ 1.6 mm. The squared

141   residuals were significantly correlated to SVL ($F_{1,\ 2171} = 58.78$, $P < 0.001$) and to the interaction

142   between SVL and group ($F_{4,21791} = 11.35$, $P < 0.001$); no effect of the single variable group was

143   observed ($F_{4,\ 20} = 2.47$, $P = 0.078$). The estimation error generally increased in larger individuals

144   ($\beta = 0.087$, $P < 0.001$). However, we observed specific patterns in two groups: when SVL

145   increased, the error increased more quickly in newts compared to other groups ($\beta = 0.124$, $P =$

146   0.021), while the opposite occurred in plethodontids ($\beta = -0.07$, $P = 0.004$) (Fig. 2B). Residual

147   error increases markedly with body size (1.6) and, among groups, newts showed the highest

148   estimation error heterogeneity (2.30). The model explained a large proportion of the overall

149 variance in the dependent variables (88.96%, $R^2m = 0.889$), and this explained variance

150 increased to 99.99% ($R^2_c = 0.999$) after the inclusion of random effects.

151 Our study demonstrated the high reliability of this method for measuring SVL from dorsal

152 photographs in Caudata species. Ventral SVL values were generally well retrieved by our dorsal

153 measurements, regardless of the species analysed or the operator performing the measurements.

154 The inclusion of morphologically distinct amphibian groups allowed us to test the robustness of

155 this method across species and families characterized by different body shapes and sizes.

156 Notably, the average estimated error remained remarkably low (1.6 mm), indicating a high level

157 of measurement precision of this method as previously reported (Lunghi et al., 2020a). Some

158 differences in accuracy were detected between groups. A slight increase in error estimation was

159 observed in large newts, whereas higher precision was observed in larger plethodontids.

160 However, these variations were minor and did not compromise the overall reliability of the

161 approach.

162 Our study demonstrates that this method can be widely adopted to estimate SVL across all

163 Caudata species. This can be particularly important in long-term monitoring, where populations

164 are sampled repeatedly over extended periods, and where excessive, repeated handling could

165 impose significant stress and adverse effects on population health. Another important feature of

166 this method is its ease of application, especially in field data collection. It enables enhancing the

167 contribution of citizen science by providing useful data using only a camera and a scale reference

168 (e.g., a ruler). However, photos must be taken using appropriate precautions and methods (e.g.,

169 perpendicular framing, sufficient lighting) to obtain material of sufficient quality. Furthermore,

170 attention must be paid to photographing only animals that are still and relaxed to obtain the best

171 subjects for post-hoc measurements. Data obtained from species not included in our dataset (e.g.,

172 Asiatic species) can be used to further test its reliability and broad applicability. By using this

173 approach, we have not only provided researchers with a tool to enhance data collection quality

174 but also identified a method that likely reduces stress on salamanders collected during field

175 studies.

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270 **Table 1.** Results of the likelihood ratio test comparing different models. The best model with the

271 lowest AIC is in bold. Basic: predicted SVLe (dependent variable), observed SVL and groups

272 (independent variables), species and operator identity (random variables). Full: addition of the

273 power variance function (var_SVL) and the variance identity structure (var_Group). The last two

274 models only include one of these functions that considers heteroscedasticity in the error

275 distribution.

| Model	df	AIC	BIC	logLik	Test	L.Ratio	p-value
basic	13	8993.433	9067.869	-4483.717			
full	**18**	**8144.837**	**8247.901**	**-4054.418**	**1 vs 2**	**858.597**	**<0.001**
var_SVL	14	8456.582	8536.743	-4214.291	2 vs 3	319.745	<0.001
var_Group	17	8493.228	8590567	-4229.614	3 vs 4	30.646	<0.001

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278 **Table 2**. Results of LMMs analysis performed on the best AIC model related to A) estimated

279 SVLe, and B) distribution of error estimations. Significant factors are in bold.

	Value	Std.Error	DF	t-value	p-value
A) Model related to SVLe					
(Intercept)	56.710	0.828	2171	68.510	< 0.001
centered_SVL	1.003	0.004	2171	228.280	**< 0.001**
Group_Newts	1.274	1.038	20	1.230	0.234
Group_Plethodontids	-0.165	0.912	20	-0.181	0.858
Group_Proteids	-0.206	1.134	20	-0.181	0.858
Group_Salamanders	0.642	1.006	20	0.638	0.530
centered_SVL*Group_Newts	-0.038	0.016	2171	-2.360	**0.018**
centered_SVL*Group_Plethodontids	-0.029	0.005	2171	-5.663	**< 0.001**
centered_SVL*Group_Proteids	-0.038	0.006	2171	-6.188	**< 0.001**
centered_SVL*Group_Salamanders	-0.016	0.011	2171	-1.367	0.171
B) Model related to error estimations					
(Intercept)	0.208	1.392	2171	0.149	0.881
centered_SVL	0.087	0.024	2171	3.592	**< 0.001**
Group_Newts	6.581	1.738	20	3.787	**0.001**
Group_Plethodontids	1.295	1.432	20	0.904	0.377
Group_Proteids	1.869	1.591	20	1.174	0.254
Group_Salamanders	1.962	1.570	20	1.250	0.226
centered_SVL*Group_Newts	0.124	0.054	2171	2.310	**0.021**
centered_SVL*Group_Plethodontids	-0.070	0.024	2171	-2.880	**0.004**
centered_SVL*Group_Proteids	-0.020	0.026	2171	-0.778	0.437
centered_SVL*Group_Salamanders	-0.049	0.038	2171	-1.299	0.194

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282   **Figure 1.** An example of dorsal (A) and ventral (B) photographs used in this study to correlate

283   predicted and observed snout-vent lengths (SVLe and SVL). In the photographs, the top and

284   bottom ends are indicated to estimate (A) and measure (B) the SVL in a male of *Lissotriton*

285   *vulgaris*.

286   Methods of identification of the posterior cloacal edge in different Caudata groups. C) Landmark

287   based on the frustum shape placed on *Lissotriton vulgaris*; 1. smaller base of the frustum /

288   posterior end of the vent. D) Landmarks based on intervertebral sulci applied on *Salamandrina*

289   *terdigitata*; 1. ilium and sacral rib; 2. caudosacral ribs (I, II, II); 3. Intervertebral sulci; 4. III

290   sulcus / posterior end of the vent. E) Landmarks based on skin folds applied on *Pseudotriton*

291   *ruber*; 1. Hindlimbs; 2. folds (I, II, III); 3. III fold / posterior end of the vent.
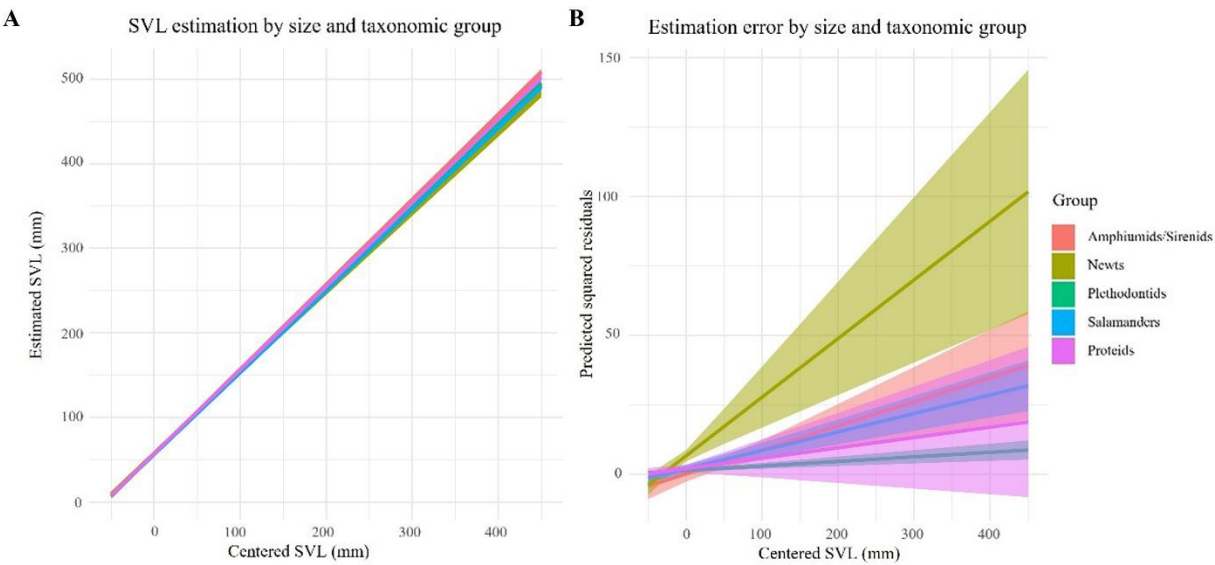
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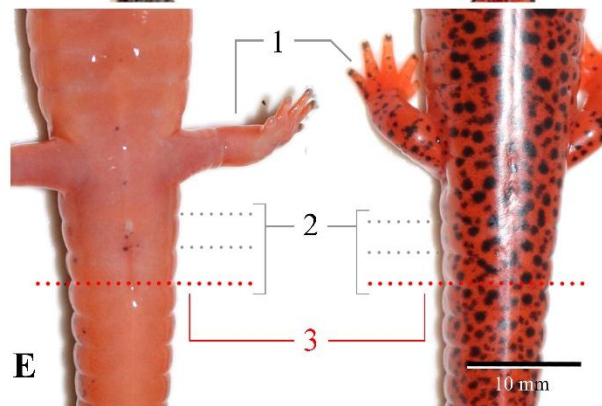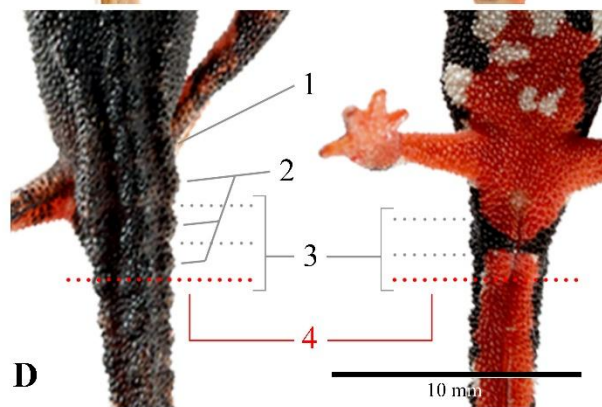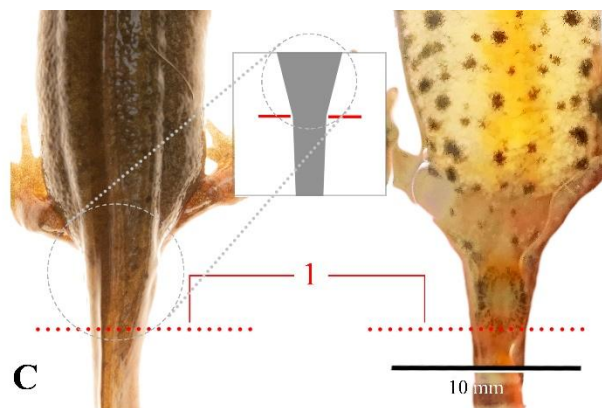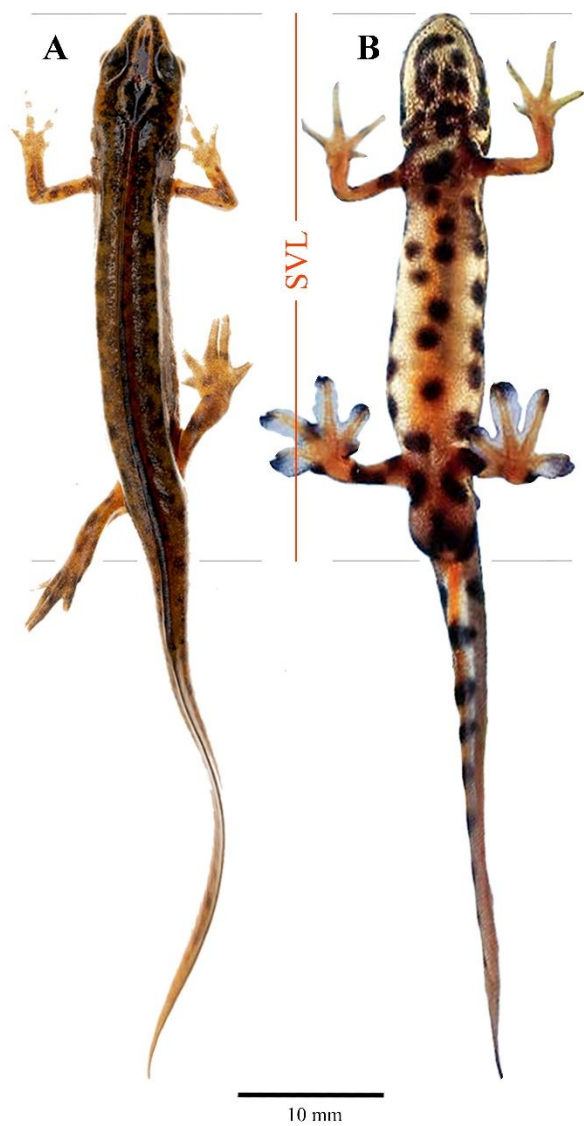
296 **Figure 2.** Plots showing the LMM results for the correlation of A) estimated snout-vent length

297 (SVLe) and B) Estimated error with the mean-centered SVL.

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