IRCF REPTILES & AMPHIBIANS
CONSERVATION AND NATURAL HISTORY

METHODS

Are Squash Boxes Necessary? The Use of Dorsal Photographs to Determine Snout-vent Lengths of Slow Worms (*Anguis fragilis*)

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In previous studies, squash boxes (also known as squeeze boxes) have been used to measure the lengths of snakes (e.g., Beartram and Larsen 2004; Cross 2000). In this study, we used this technique to record biometric data as part of an ongoing monitoring project of a translocated population of Slow Worms (*Anguis fragilis*), a semi-fossorial legless lizard usually found in relatively moist and densely vegetated habitats, such as meadows and open woodlands (Speybroeck et al. 2016).

At the survey site in Wandlebury Country Park, Cambridge, UK (OSGB Grid Reference TL500533), Slow Worms are located through the use of 0.5-m^2 pieces of bitumen roofing felt placed at even intervals (Sewell et al. 2013). Each individual is weighed and sexed before being photographed (Plattenberg and Griffiths 1999). This procedure includes taking dorsal pictures to record head patterns (for individual recognition) and lateral images to calculate body measurements using ImageJ1 (IJ1) software (Schneider et al. 2012). The latter involves holding the lizards in place on the base of a clear container with the aid of soft sponge (the "squash box") to allow its underside to be recorded.

In order to make processing individuals more effective and to reduce stress on the animals, we proposed that snout-vent length (SVL) calculations could be taken using dorsal photographs without the need for the squash box. This technique has been used in other studies for limbed lizards, for which SVL measurements were made using the hindlimb position to estimate the location of the vent (e.g., Lambert et al. 2011; Mott et al. 2010). We were uncertain if this would be effective for Slow Worms (or similar closely related species); however, on close examination a noticeable constriction point between the pelvic region and tail base corresponds with the location of the vent (Stokely 1947; Fig. 1).

To determine if this would be sufficiently accurate, we conducted a rapid comparative study. Dorsal and ventral photographs of all individuals caught over three surveys in May and June 2018 (n = 24) were analysed using IJ1 software. This included calculating SVL as well as total length

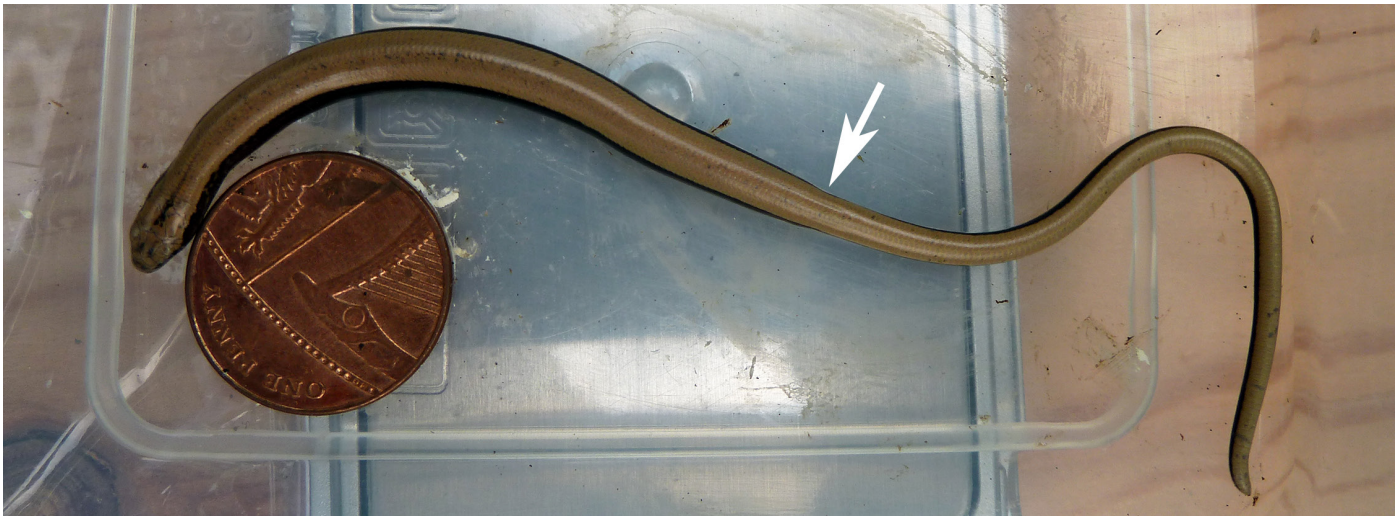

Fig. 1. Dorsal photograph showing the estimated location of the vent of the Slow Worm (*Anguis fragilis*). Photograph by Iain Bray.

IRCF Reptiles & Amphibians ISSN 1098-6324

(TL), which would serve as a control since the latter measurement should not vary between the two methods. We also calculated the percentage difference between the dorsal and ventral TL and applied that to the dorsal SVL data to generate a corrected result. We used paired t-tests to compare the TL, SVL, and corrected SVL datasets.

We found no significant difference between the TL results ($p > 0.11$); although, this was not as strong as expected and was noticeable when examining variation in the data for some of the results. This may be the outcome of natural differences in a Slow Worm's body, such as those caused by contractions at different times; however, more likely is surveyor error due to such factors as the subject not being flush with the base of the squash box (both for dorsal and ventral photographs), variability in the angle of the picture, and/or errors in the use of the measurement software.

We also found no significant differences in SVL between the dorsal and ventral calculations ($p > 0.16$) or for the corrected SVL data ($p > 0.86$).

Our results suggest that accurately estimating vent location from dorsal photographs of Slow Worms is possible and that SVL calculations can be made without using the squash box. However, those same results drew attention to the importance of minimizing errors when collecting data in the field. We suggest that the use of squash boxes be minimized to reduce stress, especially for species such as *A. fragilis* in which tail autotomy is prevalent.

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