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Modified Salamander Stick to Facilitate Accurate Measurement of Small Individuals

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Collection of morphometric data is an important component in amphibian research, because age-class, body-condition, and demographics yield important insights into populations (Deichmann et al. 2008; Peterman et al. 2008). Snout–vent length (SVL) is one important morphometric measurement that can help deduce age, sex, and when combined with weight, body condition (Kupfer 2007). Thus, accurate measurement of SVL can be critical for obtaining reliable inferences about populations and individuals. For field studies, optimal devices that facilitate accurate measurements would not require anesthetization of individuals prior to measurements, would minimize handling time, minimize pathogen transmission potential, and be portable and lightweight.

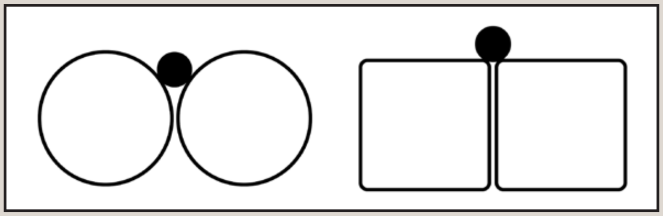

Fig. 1. Profile view of original (round PVC, left) and modified (square PVC, right) Salamander Stick that have equal diameter pipes. While Walston and Mullins (2005) use PVC pipes 2.54 cm in diameter (i.e., larger than our modified Salamander Stick), we depict both versions having equal diameter. Solid black dots represent salamanders of equal diameter, and illustrate their placement when pulled into the crevice.

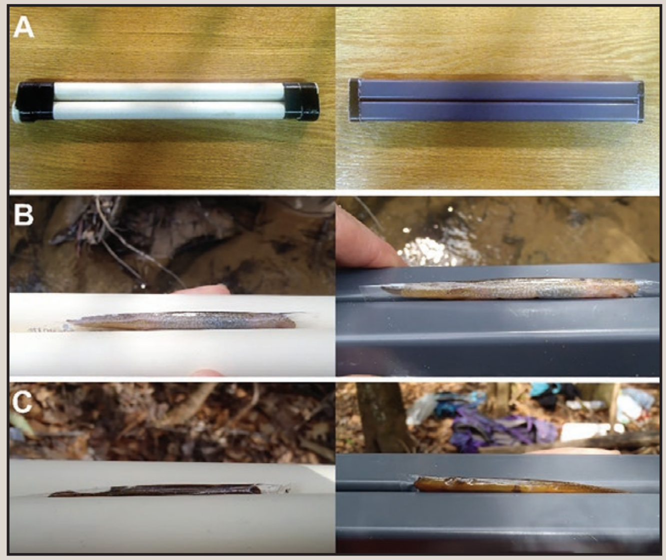

Fig. 2. Comparison of original (round PVC, A-left panel) and modified (square PVC, A-right panel) Salamander Stick for processing salamanders. We found that both versions performed well when processing larger individuals, as individuals remained elevated above the stick with both versions (B; Northern Dusky Salamander [*Desmognathus fuscus*]). We found that processing smaller individuals was easier with the modified Salamander Stick, as smaller individuals were pulled into the crevice of the round PVC (C; Southern Two-lined Salamander [*Eurycea cirrigera*]). Note, both Salamander Sticks shown here measured 1.9-cm in diameter.

Walston and Mullin (2005) developed a tool to measure salamanders called the "Salamander Stick," which improves accuracy compared to measuring individuals that are unrestrained or placed in snake tubes. The original Salamander Stick consisted of two polyvinyl chloride (PVC) pipes cylindrical in shape that measured 40 cm in length and 2.54 cm in diameter. The tubes were wrapped together using duct tape, with a 2-mm gap between them to allow a holding bag to be slipped between the pipes. Anecdotally, we found the original Salamander Stick design performed well when measuring SVL of larger-bodied salamanders, because the individuals remained elevated above the stick and thus the vent was easy to locate (Fig. 1). However, we found that SVL of smaller-bodied salamanders, which are often abundant and compose a substantial portion of the community, was difficult to measure because individuals would slide into the crevice between the pipes (Fig. 2).

Another method for collecting morphometric data of salamanders is digital image analysis, where specimens are photographed alongside reference material (e.g., ruler) and are later measured using computer software. This method has grown in popularity due to the minimal handling time required and potential for high measurement accuracy and precision (Mott et al. 2010). However, to accurately measure SVL the ventral side must be photographed next to the reference material and should be taken at a 90° azimuth. Wise and Buchanan (1992) created a device that satisfies these requirements, called the "Mander Masher," which holds individuals in place, ventral side up, using plexiglass panes and a sponge. However, because the Mander Masher requires that individuals be in contact with a sponge and plexiglass, decontamination is required between measurements to avoid potentially transmitting pathogens. Thus, assuming accuracy is comparable, the Salamander Stick is a better choice for field research because new plastic bags can be used for each individual.

Our objectives for this paper were to: 1) modify the Salamander Stick to facilitate measuring SVL of small-bodied salamanders; and 2) assess the accuracy and precision of our modified Salamander Stick, using digital image analysis and post-euthanization measurements for comparison.

We modified the Salamander Stick design to allow individuals to remain elevated when flush against the pipes. We constructed our modified Salamander Stick using two pieces of square PVC pipe, with each piece measuring 30 cm in length and 1.9 cm × 1.9 cm in diameter. We stacked small strips of Gorilla Tape® at distal ends of one pipe to create a gap when the two pipes were pressed together. Attention should be taken when creating the gap between the two PVC pipes, because too large a gap will allow small salamanders to slide through it, and too small a gap will make it difficult to quickly slide the plastic bag through. Using 5 strips creates a 1.7-mm gap, which allows a press-to-seal plastic bag to be pulled through the gap, but also allows small individuals to squeeze into the gap (e.g., juvenile *Plethodon cinereus* [Eastern Red-backed Salamander]). While working with small plethodontids, we have found that a 3-strip (ca. 1-mm) gap coupled with non-sealable sandwich baggies is optimal. We wrapped 3–4 rows of Gorilla Tape® around both distal ends of the conjoined pipes to secure them together.

To estimate length using the modified Salamander Stick, we placed individuals in plastic holding bags and fed bag openings through the gap of the two PVC pipes. We then pulled the bag through the opening until the salamander was comfortably restrained in a linear position against the crevice. We took length measurements using dial calipers accurate to 0.01 mm. We note that with both Salamander Stick designs, water can be placed in the holding bag to prevent desiccation of aquatic salamanders while handling.

To estimate length using digital image analysis, we placed individuals in a modified Mander Masher and in close proximity to a ruler with the ventral side of the individual being photographed at a 90° azimuth. We used the "Segmented Line" tool in software ImageJ (National Institutes of Health, Bethesda, MD) digital image measurements. Both the salamander and ruler were in focus before images were taken (Fig. 3). Following euthanization, we placed individuals in a linear orientation and re-measured them. Measurements taken post-euthanization were considered to represent approximately true lengths, as specimens could easily be manipulated and carefully measured.

To test whether the modified Salamander Stick provided accurate and precise measurements, we compared total body

Fig. 3. Estimated snout–vent length (left) and tail length (right) using the "Segmented Line" tool in software ImageJ. Solid white lines indicate delineated lengths along the ventral midline. Snout-vent length was measured from the tip of the snout to the posterior end of the cloaca, and tail length was measured from the posterior end of the cloaca to the tip of the tail.

Table 1. Descriptive statistics showing comparison of two length estimation methods (digital image analysis using the program ImageJ, and the modified Salamander Stick) in relation to mean true (i.e., post-euthanization) total body, snout–vent, and tail length of 30 measured *Desmognathus* spp. Mean differences and their standard deviations are reported in millimeters (mm) for each measurement.

Measuring method	Total body length	Snout–vent length	Tail length
Digital Image Analysis (ImageJ)	0.03 ± 4.49	-0.79 ± 3.34	1.21 ± 3.65
Observer 1 + 2 (Salamander Stick)	-0.10 ± 0.66	-0.70 ± 0.52	1.11 ± 0.78
Observer 1 (Salamander Stick)	-0.44 ± 5.06	-0.66 ± 3.39	0.99 ± 5.46
Observer 2 (Salamander Stick)	0.24 ± 3.15	-0.74 ± 2.67	1.23 ± 3.92

length (TBL), SVL, and tail length estimates from modified Salamander Stick measurements and digital image measurements to post-euthanization (i.e., "true") measurements using a paired t-test. We separately tested each observer's modified Salamander Stick measurements and digital image measurements against the true lengths. To quantify and compare the accuracy of the modified Salamander Stick with digital image analysis, we estimated TBL, SVL, and tail length using digital image analysis, and calculated deviations from true measurements (euthanized individuals). To quantify and test the precision of the modified Salamander Stick, two observers independently measured TBL, SVL (i.e., anterior edge of the snout to the posterior end of the cloaca), and tail length (in millimeters [mm]) of 30 salamanders and we compared their respective means using a paired t-test. Means were considered significantly different at $\alpha = 0.05$, and reported as mean ± standard deviation unless otherwise stated. Species used for this study were *Desmognathus fuscus* (Northern Dusky Salamander; N = 10), *D. monticola* (Seal Salamander; N = 17), and *D. ochrophaeus* (Allegheny Mountain Dusky Salamander; N = 3). The salamanders used in this study were part of another study investigating pesticide bioaccumulation, which required euthanization.

Mean estimated TBL, SVL, and tail length measurements using the modified Salamander Stick were not significantly different from true length for observer 1 ($t_{29} = -0.479$, $p = 0.635$; $t_{29} = -1.062$, $p = 0.297$; $t_{29} = 0.995$, $p = 0.328$, respectively) or observer 2 ($t_{29} = 0.411$, $p = 0.684$; $t_{29} = -1.520$, $p = 0.139$; $t_{29} = 1.710$, $p = 0.098$, respectively). Similarly, mean estimated TBL, SVL, and tail length using digital image measurements were not significantly different from true measurements ($t_{29} = 0.033$, $p = 0.974$; $t_{29} = -1.288$, $p = 0.208$; $t_{29} = 1.819$, $p = 0.079$, respectively). Mean TBL, SVL, and tail length estimates deviated from true length by 0.24 to 1.23 mm using the modified Salamander Stick, and 0.03 to 1.21 using digital image analysis, and standard deviations were ≤ 5.46 mm (Table 1). Precision of the modified Salamander Stick was estimated by comparing observer 1 and observer 2 measurements for TBL, SVL, and tail length. All three morphological measurements had mean values < 1 mm when compared (96.28 ± 20.87 and 96.97 ± 22.18 mm for TBL, 51.97 ± 9.48 and 51.89 ± 9.76 mm for SVL, 44.69 ± 14.40 and 44.92 ± 14.32 mm for tail length for observer 1 and observer 2, respectively) and were not significantly different between observer 1 and observer 2 ($t_{29} = -0.849$, $p = 0.403$; $t_{29} = 0.206$, $p = 0.838$; $t_{29} = -0.304$, $p = 0.763$ for TBL, SVL, and tail length, respectively), indicating

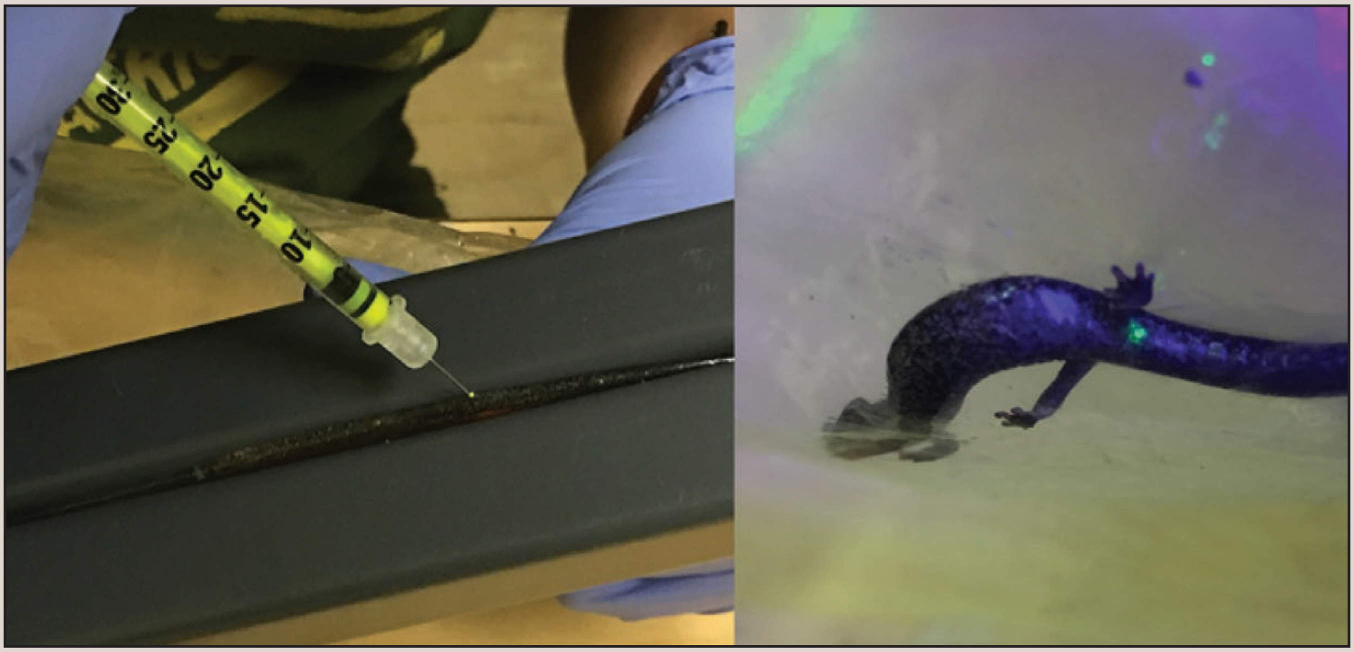

Fig. 4. Visual Implant Elastomer (VIE) injection for *Plethodon cinereus* (Eastern Red-backed Salamander) using the modified Salamander Stick (left) and resulting implant (florescent green dot behind left hind limb) shown under ultraviolet light (right).

high inter-observer precision with the modified Salamander Stick.

We found our modified Salamander Stick worked well for measuring (comparatively) larger-bodied and smaller-bodied salamanders (Fig. 2). In addition, we have found the modified Salamander Stick facilitates measurement of very small individuals such as larval *Desmognathus* spp. and *Eurycea* spp. by providing a flat surface those individuals can be laid on and remain in a linear position while in their holding bag. We feel our modification improves upon a reliable field tool by facilitating accurate measurements of larger-bodied, smaller-bodied, and larval salamanders without sacrificing handling time. Our results show the modified Salamander Stick and digital image measuring methods are similar in accuracy and precision, but the Salamander Stick has the advantage of not requiring decontamination between individuals to minimize risk of pathogen transmission.

Beyond use for morphological measurements, we have also found the modified Salamander Stick to be useful for restraining individuals for Visible Implant Elastomer (VIE) injections. The same reasons that make the modified Salamander Stick beneficial for body measurements (i.e., ability to restrain individuals while reducing handling and processing time), also make it a useful tool for VIE injections. Traditional methods allow for movement of the individual, potentially increasing handling time and stress (Kinkead et al. 2006). Using the above stated Salamander Stick protocol, we placed individuals in a plastic bag and fed each through the gap until individuals were restrained in a linear position, with the target area easily visible (i.e., directly behind the left hind limb; Fig. 4). The VIE was then injected parallel to the skin as recommended by Grant (2008).

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