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Digital Analysis of Photographs for Snake Length Measurement

For all its apparent simplicity, accurately determining the length of a snake is a surprisingly difficult task, particularly if the snake is alive or conscious at the time; venomous or very large specimens present additional challenges. A variety of methods have been proposed over the years, each with its own unique advantages and drawbacks, as detailed below. In this paper, we present a validation of the use of digital photography for the measurement of conscious, unrestrained snakes. Digital imagery to measure snakes has been used and presented by others (Measey et al. 2003; McMartin 2013; Penning et al. 2013), but we hope to bring additional attention to the potential of this method, as well as to quantify both its precision and accuracy.

There are a variety of obstacles to accurately determining lengths of snakes, not the least of which being their tendency to struggle and actively resist straightening. Even small snakes are difficult to straighten by force, especially if appropriate efforts are made not to injure or handle them in an inhumane fashion. Resolving this problem through euthanization or anaesthetization of the snake (Setser 2007; Cundall et al. 2016) poses its own problems. For example, many studies require collecting repeated data on an individual, and euthanasia may require equipment or chemicals that are inaccessible or non-permissible. Furthermore, it is possible, though uninvestigated, that active muscle tension could influence intervertebral distance in snakes and, if so, the muscle relaxation after death, paralysis, anaesthesia, or sedation may allow the snake to "stretch" beyond resting length. Conversely, long-term preservation may lead to shrinkage (Klauber 1943; Reed 2001; Simmons 2014) and measurement of preserved snakes is not necessarily any more accurate than measurement of live ones (Natusch and Shine 2012; Cundall et al. 2016). An alternative is mechanical restraint via "squeeze boxes" (Bertram and Larsen 2004). In this method, the snake is placed in a rigid box and pinned between a transparent lid and a soft foam interior, but this method is potentially injurious, may distort the snake's shape, and requires the transport of bulky boxes, which may be infeasible for fieldwork. Furthermore, squeeze boxes merely are a method of restraint, and thus snake must still be measured by one of the methods discussed (Fitch 1987; Frye 1991).

If a snake is sufficiently placid to be measured without restraint, such as by a flexible ruler (Blouin-Demers 2003) or a string along the back (Rivas et al. 2008), significant obstacles are posed by the snake's skin, which is both elastic and connected with variable looseness to the neural spines of the vertebral column. As a scientist works their way down the length of the snake with a string or tape, the skin may shift relative to the underlying vertebral column and stretch between or outside of points where the investigator places their fingers to anchor the string, potentially resulting in significantly distorted lengths. This suggestion is supported by comparing data from two prior studies in which the target species differ in how easily the skin moves relative to the underlying body (HCA, pers. obs.); the species with more mobile skin (*Eunectes murinus*, Rivas et al. 2008) shows a higher coefficient of variation than similar measurements on a species with less mobile skin (*Pantherophis obsoletus*, Blouin-Demers 2003).

Digital photographic analysis using computer-based tools offers a powerful alternative to these methods. Snakes can be measured while awake, unrestrained, in natural or minimally disturbed postures, without risk of injury to the snake or scientist, all while achieving high accuracy and precision. We wish to emphatically note that this method is not novel to this paper, and various programs that implement it in some manner have been available for free since at least 2001:

<http://serpwidgets.com/main/measure>

<https://sourceforge.net/projects/snakemt/>

<https://play.google.com/store/apps/details?id=com.theultimatelabs.snake&hl=en)>

Previous studies of these programs have focused on camera optical effects (McMartin 2013) and comparisons of precision

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(variability of measurements of the same subject) compared to tape-measure methods with only one or two experimenters performing measurements (Measey et al. 2003; Penning et al. 2013). However, no previous efforts have specifically examined consistency across large numbers of digitizers nor, most crucially, the accuracy (difference between the results and reality) of this method. In this paper, we quantify both the accuracy and precision of this method across a large number of testers (N = 37), and identify potential sources of error, as well as how to avoid or minimize them.

Materials and Methods

Digital equipment and programs.—Photographs of snakes were taken on a Fujifilm Finepix HS50EXR 16-megapixel digital camera, strings were photographed using an iPhone 4S (8 megapixel), and analyses were performed using ImageJ (v1.49 to v1.51, National Institute of Health). ImageJ was selected as the software because it is actively maintained by the NIH and can be expanded upon by the user with customized scripts and plug-ins. However, no modifications beyond the core program were used in these trials.

Photographic subjects.—To test the accuracy and precision of this method, we used seven photographic subjects, consisting of four snakes (*Crotalus willardi*, *Lichanura trivirgata*, *Senticolis triaspis*, and *Epicrates subflavus*) and three pieces of string. Use of the snakes as study subjects allowed assessment of the precision of this method on the target taxa, while the strings provided a measure of accuracy, because the true lengths of the strings were known. All subjects were placed in a cylindrical container (a 5-gallon bucket or 20-gallon trash container, typical of hardware stores and zoos in the USA) of measured diameter, which served to both contain them and function as a calibration object. Snakes were from the collection of Zoo Atlanta and were alert, unrestrained, apparently healthy and behaving normally. A snake hook was used to gently arrange each snake so that no portions of the body overlapped and the entire belly was in contact with the bottom of the container with the head held at or near horizontal; no snakes were restrained in any manner. Multiple photographs were only taken when behavioral issues (vertical body movements, crossing over itself) or focus problems rendered prior photographs unusable; only a single photograph per snake was used for all digitizing and analyses. Strings were manually arranged similarly. Photographs were taken with the camera held horizontally directly overhead by hand at an approximate distance of between 0.7 and 1.3 m from the subject. Pictures were not cropped or otherwise altered prior to analysis, and cameras were set to automatically adjust various parameters as per their internal default programming. All photographs and instructions used are included in the online archive (http://gozips.uakron.edu/~hastley/SnakeLengthSSM.zip).

Analyses of Photographs.—Testers imported photographic images singly into ImageJ and a line segment was drawn across the diameter of the cylindrical container, which was used to set the scale (i.e., calculate the conversion from pixels to centimeters) based on manual measurements of the containers. Testers then selected the “segmented line” tool and clicked points along the midline of the snakes or strings until reaching the tail or end of the string, with an unspecified number of points used (see below) (Fig. 1A). The “fit spline” procedure was then used, which converted the straight segments into a curved spline (Fig. 1B), which was then measured and recorded. Each image was calibrated individually.

Testing procedure.—These photographs and a set of instructions were given to a total of 37 testers, with two alternate instruction formats for single testers and those as part of a class (see below). All testers were unaware of the values of any data previously gathered and unaware of the true lengths of the strings. Five testers were individuals with significant computer skills and some with significant experience with snakes; these subjects received no further instruction beyond the standardized instruction sheet (Supplementary Materials). One of the authors (VEA) used this experiment as a teaching tool for an introductory physics lab (PHY 2048L, 32 students) at Florida Polytechnic University to teach the students about basic statistics and the concepts of accuracy, precision, bias, and blinding. A subset of the students (9 individuals) also recorded (in pixels) the diameter of the bucket or trashcan (used for calibration), as well as the length in pixels and number of points used to construct the segmented line along each snake or string; this method was used in order to assess whether number of points used influenced the resulting snake or string lengths. The testers and author (VEA) were blind to any data previously collected, as well as the known string lengths, until after the data had been anonymized and transferred to the author in charge of objective analyses (HCA).

Outlier policy.—To account for a variety of possible user errors, we examined outliers in detail, defined as data outside of 1.5 times the interquartile range (Tukey 1977). Outliers were excluded in several specific cases: 1) failure to calibrate at all (diagnosed as lengths more than 100-fold greater than the median due to failure to convert pixels to cm); 2) user failure (observed in the lab or diagnosed as a single student producing all outliers); 3) string mis-calibration (all string lengths differ from true values by the same ratio, suggesting the user applied the wrong calibration length); 4) snake mis-calibration (outlier can be converted to a non-outlier by multiplying by the ratio of the correct calibration length to an incorrect one, suggesting the used applied the wrong calibration length); and 5) extreme error (outlier differs from median by more than 40%, suggesting major failure of either calibration or digitizing). All outliers meeting one of these criteria were deleted, while any other outliers remained as part of the dataset. The point-number dataset was screened on the basis of length in pixels, with values differing from the median by more than 300 pixels (suggesting significant digitizing error) being removed as user error, leaving a total of 51 points across all images.

Data analyses.—Summary statistics were computed for each image, including mean, standard deviation, standard error, 95% confidence interval, median, and interquartile range. To account for different means, standard deviation was converted to the coefficient of variation by dividing by the mean for snakes and the true value for strings, 95% confidence ranges were normalized similarly, and quartile coefficients-of-dispersion were calculated from quartile data. To assess accuracy, two-tailed t-tests were conducted on string data, compared to the true lengths of the strings. To determine the influence of point number, the mean values from the full dataset were subtracted from the observed mean values (for snakes) or known values (for strings) to determine a residual, and a least-squares regression was performed between number of points and the pooled residuals.

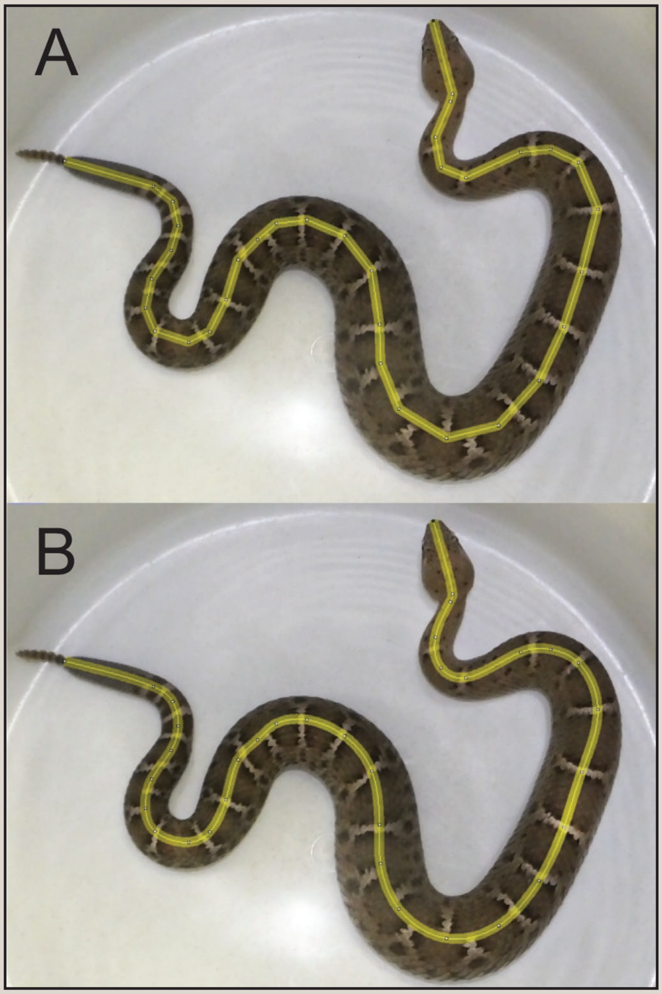

Fig. 1. Digitized backbone line on *Crotalus willardi* as a segmented line (A) and after spline fitting (B).

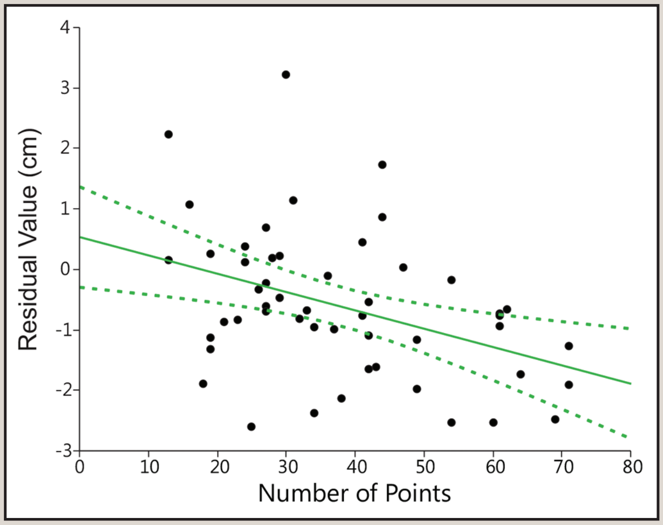

Fig. 2. Influence of number of points used on Residual Value. Increasing the number of points causes a slight but significant decrease in measured size.

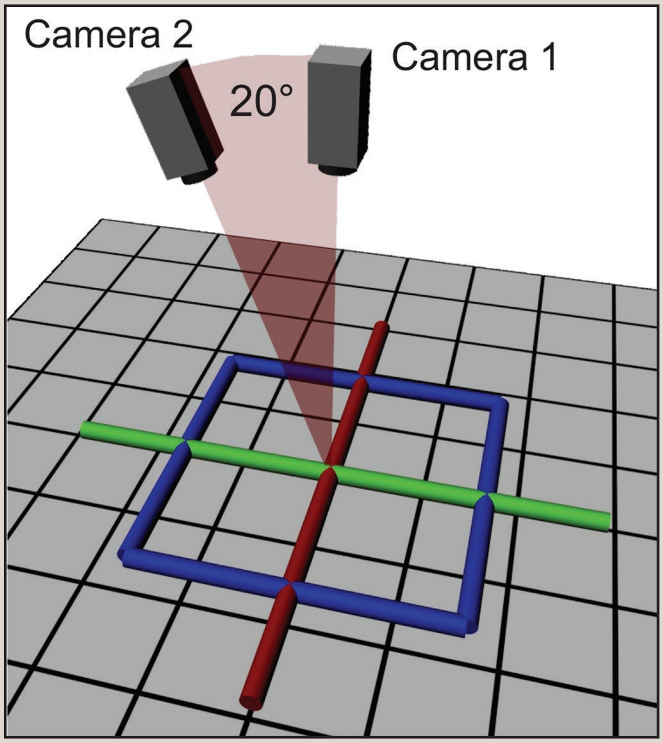

Fig. 3. Effect of camera orientation on estimated length. Camera 1 is positioned directly above the viewing plane (grey & black grid), while Camera 2 has been rotated by 20° about the axis of the red cylinder. Images taken from the perspective of Camera 2 will show the length of the green cylinder as 94% of the true length, while the red cylinder's length will be accurate, and the blue square's perimeter will be 97% of the true perimeter.

Results

Lengths measured from digital photographs showed both high accuracy and precision, albeit with a very slight bias towards underestimation (Tables 1–3). Examination of summary statistics showed measures of dispersal (standard deviation, 95% confidence interval width, and inter-quartile range [IQR]) of 4 cm or less (Table 1). Coefficients of variation, normalized 95% confidence intervals, and quartile coefficients of dispersion, normalized for length by various methods (see above), typically showed values of less than 3.2% (Table 2). It is noteworthy that the most broad-bodied species, *Crotalus willardi*, had higher values of these metrics, while strings showed lower values than snakes (Tables 1, 2).

Analyses of the string data showed high accuracy, with means differing from actual string lengths by at most 1.6 cm, or 1.4% (Table 3). However, two-tailed t-tests revealed that these differences were significant, indicating a small, but real, tendency to slightly underestimate length (Table 3). The number of points used in the spline had a small but significant effect on accuracy, with increasing point number resulting in greater underestimates (R^2 = 0.156, F = 9 .0820, p = 0 .0041, Intercept = non-significant, slope = -0.030317, slope standard error = 0.01). However, this slope was so shallow that the addition of an extra 33 points would only lower the length measurement by 1 cm (Fig. 2).

Discussion

Our results show that digital analyses of photographic images can yield both accurate and precise lengths for conscious snakes in naturalistic body postures with minimal disturbance and repositioning. Comparison with previous studies (Blouin-

TABLE 1. Summary Statistics (all units in cm except N): Mean, standard deviation (S.D), standard error (S.E.), 95% confidence interval of the mean (95%+, 95%-, 95% Range), percentiles (75%, Median, 25%), and interquartile range (IQR).

Image	N	Mean	S.D.	S.E.	95%+	95%-	95% Range	75th Pct.	Median	25th Pct.	IQR
String 1 - 119.4 cm	31	117.8	0.8	0.1	118.1	117.5	0.6	118.1	117.7	117.1	1.0
String 2 - 90.2 cm	32	89.9	0.8	0.1	90.2	89.7	0.6	90.5	89.9	89.3	1.2
String 3 - 61.0 cm	32	60.8	0.6	0.1	61.0	60.6	0.4	61.3	60.7	60.4	0.9
Crotalus willardi	32	48.5	2.6	0.5	49.4	47.5	1.9	50.6	47.3	46.7	4.0
Senticolis triaspis	31	103.1	2.5	0.4	104.0	102.2	1.8	104.8	102.8	102.1	2.7
Lichanura trivirgata	32	72.6	2.3	0.4	73.5	71.8	1.7	73.3	72.0	71.3	2.0
Epicrates subflavus	34	154.2	3.1	0.5	155.3	153.1	2.2	154.7	153.6	152.6	2.1

TABLE 2. Relative Summary Statistics (all units in lengths). Coefficient of Variation = S.D./Mean, 95% Range = 95% CI/Mean, Quartile coefficient of dispersion = IQR/(75th Percentile + 25th Percentile). Means were from measurements of snakes and known lengths of strings.

Image	C.V.	95% Range	Quartile coefficient of dispersion
String 1 - 119.4 cm	0.0069	0.0051	0.0042
String 2 - 90.2 cm	0.0092	0.0066	0.0066
String 3 - 61.0 cm	0.0092	0.0066	0.0072
Crotalus willardi	0.0540	0.0389	0.0408
Senticolis triaspis	0.0240	0.0176	0.0132
Lichanura trivirgata	0.0318	0.0229	0.0139
Epicrates subflavus	0.0203	0.0142	0.0069

TABLE 3. Accuracy Measurements, including mean values, difference between the mean and actual values (in cm and lengths), and the results of a two-tailed t-test comparing the measured lengths to the known string length.

Image	N	Mean	Mean Difference (cm)	(lengths)	Two-tailed T-test t	p
String 1 - 119.4 cm	31	117.8	-1.6	-0.01373	-11.008	<0.0001
String 2 - 90.2 cm	32	89.9	-0.3	-0.00278	-1.7161	0.0961
String 3 - 61.0 cm	32	60.8	-0.2	-0.00342	-2.1075	0.0433

Demers 2003; Measey et al. 2003; Setser 2007; Rivas et al. 2008; Penning et al. 2013) shows that digital analysis of photographs results in comparable or superior precision to other methods of manually measuring conscious snakes. We note that these methods should also be applicable for or other elongate creatures, such as caecilians, some salamanders and lizards, and perhaps fishes or worm-shaped invertebrates. Use of digital information also clearly is gentler and safer for the study animals and the investigators. This method is portable, requiring only a camera positioned over a flat surface, a calibration object, and freely-available software from a trusted source (the US NIH), and it allows rapid data collection and processing, even by untrained personnel. It is also compatible with squeeze boxes to restrain animals, should they be necessary or preferred, and can be applied to radiographic images to remove any doubts regarding the location or orientation of the vertebral column. If the camera is mounted securely in a fixed position, post-hoc calibration may also be possible, though if the camera is disturbed it becomes impossible to recover data from the prior photographs.

Further developments of this method could allow considerably easier access to reliable length data in otherwise impossible situations. Determining the length of large crocodiles, for instance, has previously required capture (a difficult and dangerous process), but given their affinity for basking on flat or minimally sloped surfaces, large animals could be accurately measured using a camera mounted to a fixed position over a favorite basking site followed by post-hoc calibration of the enclosure using a pre- or post-photo measurement of a nearby object, such as a rock or log.

To achieve the best possible results from this method, there are certain best practices that should be adopted. The core assumption of this method is that the snake lies approximately in a plane, so it is necessary to have the calibration object as close to the same plane as possible. If the calibration object is beneath the snake, as in our case, the effect of perspective will cause the snake to be measured as longer than it actually is. However, in this case, the ratio between the actual and measured snake lengths will be equal to the ratio between the distance from the camera to the snake and the distance from the camera to the calibration object. Thus by maximizing the camera distance, this distortion can be minimized. Additionally, the camera must be oriented as close to horizontal (i.e., perpendicular with respect to the horizontal plane of the snake) as possible, but a small angle of tilt will have a minimal effect for two reasons (Fig. 3). In the worst-case scenario of a snake which is absolutely straight along a line perpendicular to the axis of rotation of the camera (Fig. 3, green cylinder), the ratio of the snake's apparent length to real length is the cosine of the camera angle. This ratio will be nearly 1 for a wide range of angles; a camera off by 20° will underestimate this linear snake by only 6%. However, this effect disappears if the snake's vertebral axis is parallel to the axis of rotation of the camera, and the ratio in this position will always be 1 (Fig. 3, red cylinder). The interaction of these effects can be seen in a hypothetical 4-m snake laying in a perfect square, photographed by a camera tilted 20° along an axis parallel to one of the sides of the square (Fig. 3, blue square). The lengths of the two sides parallel to the rotation axis will appear accurately, while the two sides perpendicular to the rotation axis will appear to be shorter, and the resultant total estimated snake length will be 3.88 m long, a 3% error comparable to what we observed in our trials simply from calibration and digitizing noise (Fig. 3, blue square). Even this can be eliminated, however, by using a calibration square, which allows detection of and correction for such distortions, as shifted camera angles will cause sides of the square to shorten unevenly and their angles to depart from 90°; a plug-in to correct for this already exists for ImageJ. One of the authors (HCA) has previously been able to use this method to accurately record distances of 2+ m frog jumps to within 1.6 cm using perspective distortion correction in spite of the camera being located more than 60° off vertical (less than 30° from pure lateral view) (Astley et al. 2013). Finally, it is important to maximize both image resolution and calibration object size in the image, as a few pixels of digitizing error will result in much larger calibration or measurement error on a subject that is 100 pixels long versus 2000 pixels long.

This method is not without potential issues or limitations. As tested, we quantified only the total length of the snakes, and cannot separately quantify snout–vent length (SVL), which is especially desirable for wild specimens which have often lost portions of their tail to predators; this could be remedied by manually locating the vent and applying a paint mark to the dorsum at that point. This method also shows a slight tendency to underestimate length and sensitivity to the number of points used to create the spline. We suggest that this may be partially due to users being instructed to follow the animal's midline, not the vertebral column, while only one snake in the test images had longitudinal stripes which could provide a visual aid. Because snakes have highly mobile ribs, and can shift the ribs independently, the vertebral column may not be directly under the optical midline of the body when observed from above. Furthermore, visual estimation of the midline may become more difficult for wider snakes; we note that the highest relative errors, by far, were seen in the stoutest snake, *Crotalus willardi*, followed by the second-stoutest, *Lichanura trivirgata*, while the significantly thinner strings had far lower errors. We also relied solely on ImageJ's native "fit spline" function and did not attempt to manually correct areas where it may have produced insufficient curvature, leading to lower lengths; this may be exacerbated by excessive points. To remedy this, we suggest that future investigators attempt to mark the mid-dorsal line or illuminate the snakes so as to make the scales more visible (though even this may not accurately capture the underlying vertebral column, see above). However, based on our analyses of data provided by inexperienced and experienced volunteers, we feel confident that this method of measurement, properly implemented, will provide a powerful new tool for assessing snake length in a consistent, reliable, and accurate manner both in the lab and in the field. Especially useful situations for implementation of this method would seem to be rapid measurement of large numbers of animals at a field site, non-intrusive tracking of individual animals in zoo or conservation collections, and safe measurement of venomous animals in any situation.

We also emphasize the potential utility of this method for teaching students about basic concepts of precisions and accuracy in scientific measurements, as reflected in the success of one of the authors (VEA) in using the data, software, and instructions (see supplementary material) as a laboratory exercise.

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