AN EVALUATION OF TECHNIQUES FOR MEASUREMENTS OF SNAKE LENGTH

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Abstract: *The efficacy of two measurement techniques was evaluated in a laboratory setting for user variation and practicality. A total of 59 snakes (15* Lampropeltis getula floridana*, 16* Pantherophis guttatus*, 12* Epicrates cenchria maurus*, and 16* Thamnophis sauritus*) were measured using traditional soft-tape measurements paired with restraining tubes. The second measurement method evaluated snake length using the digital imaging software Snakemeasurer© by taking a photo parallel to the surface the snake was resting on with a known length object in the photo for measurement reference. Each snake was measured by two designated measurers (one experienced and one recently-trained) using both measurement techniques. Each researcher and technique produced similar measurements. Digital measurements were not significantly different between measurers while soft-tape measures varied with species.*

Key Words: digital measure, snake length, Snakemeasurer©, straight line length

INTRODUCTION

Varying types of size measurements are used in studies on snakes and can provide valuable data (Fitch, 1987). The typical length measure reported for snakes is the snout-to-vent length (SVL) as it is seen as the most important length measurement (Fitch, 1987), but this has not always been the standard (Seigel and Ford, 1988). Seigel and Ford (1988) reported the three most common measurements of snakes to be SVL, total length (TL), and mass. While SVL is the standard length measurement used for snakes today, TL is still occasionally used (Penning and Cairns, 2012). Typically SVL measurements are recorded using the methodologies of Fitch (1987) due to its time and cost effectiveness in the field. A soft or hard tape measure is used and the snake is gently stretched along the tape length. Other methods of measurement that have been used include restraining tubes (Fitch, 1987), pressboxes (Quinn and Jones, 1974; Bertram and Larsen, 2004), anesthesia (Blouin-Demers, 2003), and digital imaging software (Measey et. al., 2002). All of the above mentioned measurement techniques are prone to three types of error: single user-single measurement technique variation; multiple user-single measurement technique variation; and multiple measurement technique variation (Setser, 2007).

Highly precise and repeatable measurements are becoming increasingly important, especially with the newly discovered bidirectional growth phenomenon reported in marine iguanas (Wikelski and Thom, 2000). This phenomenon was investigated in snakes using data from a long-term field study on *Liasis fuscus*, and based on recapture data, 6.42% of the recaptures reported shrinking lengths (Madsen and Shine, 2001). The same phenomenon was reported in Blouin-Demers *et al.* (2002) for *Pantherophis obsoletus*. Both of these observations were reported to be measurement error and not actual shrinking. Contrastingly, measurement error has not always been considered to cause false results in experimental evaluations (Merila and Bjorklund, 1995).

Several investigators have evaluated the accuracy and precision of length measures of elongate vertebrates using various measurement techniques and varying levels of support exist for every measurement type. Quinn and

Jones (1974) found the squeeze-box technique to produce reliable measurements with little variation, while Setser (2007) found the same technique to produce less reliable results than anesthetizing snakes prior to straight measurement. Bertram and Larsen (2004) reported significantly larger measurements to be produced by straight-line measurements when compared to squeeze-box measurements and recommended one tracing in a squeeze-box measured three times to best balance handling time and accuracy. Cross (2000) evaluated squeeze-box and anesthetized measurement techniques and found <1 cm variation between measurements. Measey *et al.* (2002) compared fixed-ruler measurements to flexible measurements using digital imaging software (Wilcox *et al.*, 1997) and found both measures to be reliable with fixed-rulers producing significantly longer measurements. Blouin-Demers (2003) found all three of the investigated measurement forms (soft-tape while awake, soft-tape while anesthetized, and solid-tape while anesthetized) to be effective and concluded with the recommendation for measuring snakes under anesthesia using a solid ruler. Measurements using soft-tape while the snake is awake is a reasonable alternative, a conclusion also supported by Setser (2007).

Using different measurement techniques not only come at varying economical costs (Setser, 2007), but also have potential ecological impacts (Fitch, 1987). The process of measuring snakes awake with a soft-tape in the field has been shown to have negative growth impacts for several weeks after capture in *Crotalus viridis* (Fitch, 1949). Using anesthesia to measure snakes brings the greatest risk (Fitch, 1987) and has been cautioned against its use if only measurement data are to be taken (Measey *et al.*, 2002). Hand measurements have the drawback of being a "one off" measurement with no capability of being checked by an alternate authority (Measey et. al., 2002). Errors in snake measurements are an important problem (Fitch, 1987) that needs to be addressed with minimally invasive handling techniques in mind (sensu Fellers *et al.*, 1994). Measurement error of SVLs have been found to be so variable that its use has occasionally been abandoned (Houston and Shine, 1994), an unfortunate outcome that may be prevented with new software. Snakemeasurer© is digital imaging software designed to measure snakes based on single-frame images with a known measure within the image. It is freely available (http://serpwidgets.com/main/measure) and has been used in prior publications (Pen-

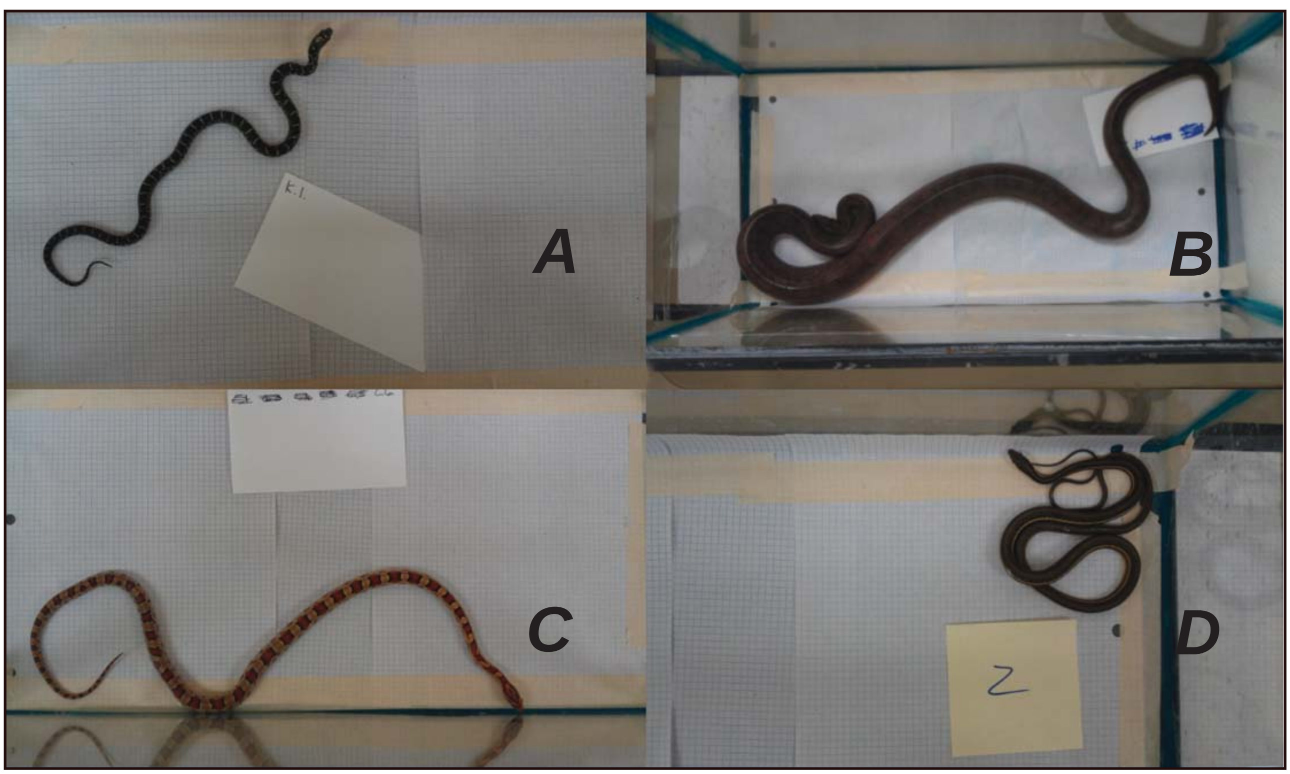

Figure 1. An overhead image of all four snake species [*Lampropeltis getula floridana* (A); *Epicrates cenchria maurus* (B), *Pantherophis guttatus* (C), and *Thamnophis sauritus* (D)] using 0.4 cm graph paper as the measurement reference.

ning and Cairns, 2012). The main objective of our study is to evaluate the effectiveness of Snakemeasurer© for measuring snake length and how it compares to the classic soft-tape measuring technique.

METHODS

Snakes used in this study came from various growth and behavioral studies being performed at the University of Central Missouri during spring 2012. A total of 59 snakes were used for the evaluation of measurement techniques (15 *Lampropeltis getula floridana*, 16 *Pantherophis guttatus*, 12 *Epicrates cenchria maurus*, and 16 *Thamnophis sauritus*). Manual measurements involved the use of a soft-tape measure and various restraining tubes. Snakes were placed in a restraining tube just large enough to accommodate them and were then measured for total length. Total length was used due to the overhead imaging procedures used with the software program. As we are not making any ecological implications based on our length data, total length serves the identical purpose for comparing measures. All snake total lengths were measured twice using soft-tape (once by an experienced researcher and once by a recently-trained researcher) to the nearest 0.1 cm. Snakes were then placed in a 37.8 L aquarium lined with 0.4 cm graph paper where they were free to orient themselves. Snakes were photographed from above with the camera on a parallel plain to the aquarium bottom (Penning and Cairns, 2012). Occasionally snakes were moved gently with a snake hook until an acceptable body position was available for imaging (no overlap or raised body sections). The snake needed to be flat with no portion of the body elevated from the substrate because the imaging software measures only in two dimensions. Both the tip of the snout and tip of the tail needed to be easily viewed within the image. Images were then run through Snakemeasurer© for straight-line length by both researchers using the graph paper as the known measurement length (Fig 1). The experienced researcher remained the same for all measurements but the recently-trained researchers changed between snake species with the exception of *L. g. floridana* and *P. guttatus*.

All statistical analyses were performed on MS EXCEL and MINITAB. Paired t-tests (two-tailed) were performed between measurers of each technique for each species due to the repeated measure design of data collection (McDonald, 2008), as well as its previous use in similar investigations (Measey *et al.*, 2002; Blouin-Demers, 2003). Variables were normally distributed and all tests were considered significant at $p<0.05$.

RESULTS

Implementation

For the experienced user the time to complete the entire procedure was, on average, faster for soft-tape measurements (ca. 2 min). Overall time spent handling snakes was much faster for the digital imaging (<1 min), but required more time for image transfer, downloading, and processing (ca. 3 min).

Reliability

A total of 236 measurements were taken from 59 snakes. For *L. g. floridana*, mean straight line lengths varied only by 1 mm between digital measurers and were not significantly different (paired t-test (15) = -0.453, $p>0.05$), while soft-tape measurements were significantly different with the average varying by 12 mm (paired t-test (15) = -5.467, $p<0.05$). For *P. guttatus*, mean straight line lengths varied by 5 mm between digital measurers and 1 mm between soft-tape measurers and both were not significantly different between measurers (paired t-test (14) = -2.08, $p>0.05$ and paired t-test (14) = -0.4483, $p>0.05$ respectively). For *E. c. maurus*, mean straight line

Table 1. Combined measurement (mean±SD) results from four snake species (a total of 236 measurements). Digital snake measuring using Snakemeasurer© is represented by "Digital Measure", hand measurements using restraining tubes and a soft ruler is represented by "Soft-Tape Measure".

Species	*Digital Measure*	*Soft Tape Measure*	*Paired t-test*
L. g. floridana	511±8.7 mm	478±8.5 mm	t(15) = 15.34, p<0.05
P. guttatus	625±9.14 mm	590±8.32 mm	t(15) = 16.27, p<0.05
E. c. maurus	963±11.08 mm	885±10.23 mm	t(11) = 25.22, p<0.05
T. sauritus	609±4.78 mm	591±5.09 mm	t(15) = 14.44, p<0.05

lengths were not significantly different among digital measurers (paired t-test (11) = 2.128, $p>0.05$) while soft-tape measurers were (paired t-test (11) = -12.749, $p<0.05$). For *T. sauritus*, mean straight line lengths varied by 6 mm and were not significantly different between digital measurers (paired t-test (15) = 1.777, $p>0.05$) while mean straight line length was significantly different between soft-tape measurers (paired t-test (15) = -7.590, $p<0.05$). All combined digital measures were significantly larger than combined soft-tape measures (Table 1).

DISCUSSION

Our results are similar to that of Measey *et al.* (2002) in that one measure consistently produced significantly longer measurements than the other but differ in the longer measure reported. In our study, Snakemeasurer© software reliably produced longer measurements compared to the soft-tape measurements, markedly so in *E. c. maurus*. This species is a notorious resister to manual restraint and the forces at which it can do so have been quantified (Lourdais *et al.*, 2005). All digital measurements were similar when compared between researchers while the soft-tape measures were significantly different between researchers in all but the *P. guttatus* comparison. This supports the notion that measurer variation is higher in hands-on manipulations of snakes and less when using digital imaging software.

Functionally, Snakemeasurer© digital imaging software is similar to anesthetized measures. The snake is not active and time can be taken to measure length carefully with no struggling, resistance, or spinal flexion. Blouin-Demers (2003) found the greatest variation in measurements to be active snakes and the most precise measurements to come from anesthetized snakes. These results support this trend by showing no significant difference between researchers using Snakemeasurer© digital imaging software. When compared between researchers, soft-tape measurements were significantly different in three of the fours species we investigated.

Palmeirim (1998) reported skull measurement variation between different measurers to be 30.7%, almost twice the value of intra-measurer variation. Ecological studies can span several investigators and measurer bias and error has the ability to impact measurement recordings (Madsen and Shine, 2001; Blouin-Demers et al., 2002) even to the extent of rejecting length as a measure in general (Houston and Shine, 1994). With powerful musculature controlling the snake's ability to move and in some cases slightly elongate or compress during handling, gentle stretching procedures used in many measurement investigations could have and have yielded variable results from experimenter manipulation (Houston and Shine, 1994). Coupling measurer and measurement technique variation could have a large impact on the measurements reported. The use of a measuring technique that eliminates as many potential errors balanced with a lessened post-measurement impact on the organism of investigation would be the ideal technique.

In this study, Snakemeasurer© digital imaging software showed the least variation between measurers compared to soft-tape measuring and has added benefits that no other measuring technique can provide. Inter-measurer error can be completely eliminated with this technique even if the length measurer was not the field investigator. Additionally, with the ability to easily store images it is possible to allow additional researchers access to the data allowing for the further elimination of inter-measurer error even in large compiled data sets.

Measey et al. (2002) listed many of the benefits of using digital imaging software, but at the time, warned of its expense and technical complexity. In today's market, digital cameras can be purchased at reasonable prices; the Snakemeasurer© digital imaging software is freely available, and the newly trained researchers in this study had no difficulty using the software. It is a much less expensive alternative to anesthesia and in this study had none of its reported drawbacks (Setser, 2007). Handling time per snake was reduced, although there was a slight increase in total measuring time using the digital software. The expense of added time is a cost to the researcher with the benefit of reduced handling to the snake. The simplest and most cost-effective measuring technique will likely always be soft tape measuring but even this fairly noninvasive technique has been shown to have a negative impact on snake growth (Fitch, 1949). The most precise measurement technique (anesthesia) is the most expensive. Its use requires the most time for the researcher as well as handling time for the snake and can prove to be fatal in some cases (Setser, 2007). When designing future experiments, researchers should design their experiments with all of the above benefits and drawbacks in mind.

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