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EVALUATION OF A PHOTOGRAPHIC TECHNIQUE FOR ESTIMATING BODY SIZE IN LIZARDS FROM A DISTANCE

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***Abstract.*—Body size is often an important factor in understanding an animal's ecology. In squamates, snout-to-vent length (SVL) is a primary metric for quantifying body size. Measuring SVL typically requires capture and handling of animals, which may be difficult due to a species' behavior or its presence in complex habitat. Here, we evaluated an alternative to the capture, handling, and direct measurement of lizards via the use of paired photographs of animals *in situ* and with a ruler positioned in place of the lizard. Paired digital images were processed using the freely available image analysis software ImageJ. We found that snout-to-hindlimb length (SHL) measured directly on an animal was highly correlated with its SVL. In turn, SHL estimated from images was highly correlated with SHL measured directly on the animals and had an average error of 19.4 ± 16.2% (1 SD) compared with measurements made on the animals themselves. Estimates of total length from images were also highly correlated with total length measurements made directly on the animals and had an average error of 14.8 ± 12.9% (1 SD). There was no correlation between image measurement error and the distance from which photographs were made. Our results identify that the use of digital photographs and image analyses can in some cases eliminate the need to capture and handle lizards to quantify body size.**

Key Words.—ImageJ; image analysis*; Sceloporus occidentalis*; snout-to-vent length; SVL; Western Fence Lizards.

INTRODUCTION

Body size is one important part of understanding an organism's physiology, locomotion, reproduction, growth, and life history, and for making comparisons among taxa and individuals (Calder 1984; Meiri 2010). In many reptiles and amphibians, an animal's length is used as the primary metric for body size (Meiri 2010). Methods for quantifying length vary depending on the species in question, but snout-to-vent length (SVL) is one of the most common methods of assessing body size in lizards, as it is in many reptiles.

Determining SVL over time generally requires capture of an animal, a factor that may pose many challenges in species that are particularly wary or swift, or that occur in habitats where capture is difficult (e.g., arboreal species). Additionally, excessive or repeated handling may expose animals to elevated stress or loss of body parts (e.g., tail autonomy), a factor that can decrease survival in some circumstances (reviewed in Bateman and Fleming 2009), though some have found that routine handling of lizards did not affect corticosterone levels (Langkilde and Shine 2006). For these reasons, techniques for measuring body size remotely may facilitate greater opportunities for the study of additional species.

Magnusson (2003) discussed the value of visual size estimation in crocodilians when conducted by an experienced observer. However, visually estimating size can introduce bias when done by inexperienced researchers and is limited by the observer's experience with the taxa at hand. An alternative to visual estimation and the capture and direct measurement of species is the use of digital photographs and image analysis, a technique that has been used to determine size of amphibians in a controlled setting (Davis and Grayson 2007; Davis et al. 2008). Photographic size estimation has also been used *in situ* for some other taxa. For example, video cameras and mirrors have been used to estimate body size in coral reef fishes (Pfister and Goulet 1999). However, this technique is unlikely to be as useful in a more expansive, terrestrial setting. Van Rooij and Videler (1996) used stereo-photography to measure length in wild fish, but this method required costly and cumbersome equipment that would also make photographing terrestrial vertebrates difficult. Chang et al. (2009) used photographs of a calibrated board and Tuna (*Thunnus obesus*) to estimate fish size, however, this was done on fishing vessels as animals were being harvested. To our knowledge, the use of similar techniques has yet to be evaluated in terrestrial herpetofauna. The use of image analysis should facilitate the collection of morphological data when handling is not recommended. Here, we used digital photographs and image analysis software to estimate lengths of the Western Fence Lizard (*Sceloporus occidentalis*). We tested whether there was a direct correlation between snout-to-hindlimb length (SHL) and

FIGURE 1. Photograph of (left) an Western Fence Lizard (*Sceloporus occidentalis*) *in situ* before being captured and measured in hand and (right) a second photograph of a ruler in place of the lizard. Both photographs are shown here at 33% of their original size and were cropped but were not otherwise altered. (Photographed by Catherine Yusuda)

SVL in captured lizards and whether SHL and total length (TL) can be estimated using image analysis software.

MATERIALS AND METHODS

Study site and species—We conducted our study at the Angelo Coast Range Reserve, Mendocino County, California. We sampled three different habitat types: riparian cobblestone substrate, grassland, and areas around residences and dwellings. We used the Western Fence Lizard as our model species for this study because of its ease of capture (see below) and because two different size classes, juveniles and adults, can be found in close proximity, therefore allowing us to evaluate a greater range of sizes.

Data collection.—We conducted our study from 14–17 September 2010. When a lizard was first seen, one observer marked his or her own position and immediately took a digital photograph of the animal using a Canon EOS Digital SLR camera with a Canon lens with focal length of 55–255 mm. We kept the lens at 255 mm focal length and used auto-focus for all pictures. All camera settings were unchanged before the next image was taken. After the first image was taken, a second observer captured the lizard either by hand or with a noose and placed a ruler at the location where the animal was previously located. We placed the ruler lengthwise along the same axis in which the lizard was positioned and arranged the ruler so that measurement increments would be visible in the second photograph (Fig. 1). The photographer then took a second picture from the same location and position in which the first image was taken. On the ventral side of the lizard we measured SVL and TL in a straight line from the snout tip to the end of the tail. We also measured SHL of each animal to compare with measurements made from digital photographs because the cloaca is not typically visible from the dorsal side of the animal in photographs. We measured SHL from the snout tip to the anterior side of the hindlimbs as seen from the dorsal side of the lizard. In a few cases the lizard would flee before an initial photograph could be taken. In these instances we would capture the lizard, take the necessary measurements, and the photographer would then take photographs as soon as the lizard stopped fleeing after release. The same observer took all pictures to standardize the height at which photographs were taken and another observer recorded all direct measurements. After the lizard was released, we measured the distance from the photographer to the ruler. We marked each lizard on its ventral side using indelible ink to avoid repeating procedures on previously measured animals.

ImageJ analyses.—We used ImageJ, version 1.44 (National Institute of Health, Available from http://rsb.info.nih.gov/ij/), a free image analysis software, to analyze photographs. Using the "Straight Line" tool, we calibrated a 1 cm increment for each pair of photos from the photograph of the ruler arranged in place of the lizard. We used 1 cm increments for all measurements because mm increments are often difficult to read on rulers in photographs (Werner 2011). We used "Set Scale" and set the known 1 cm increment as the "Global" scale to analyze the second image of the

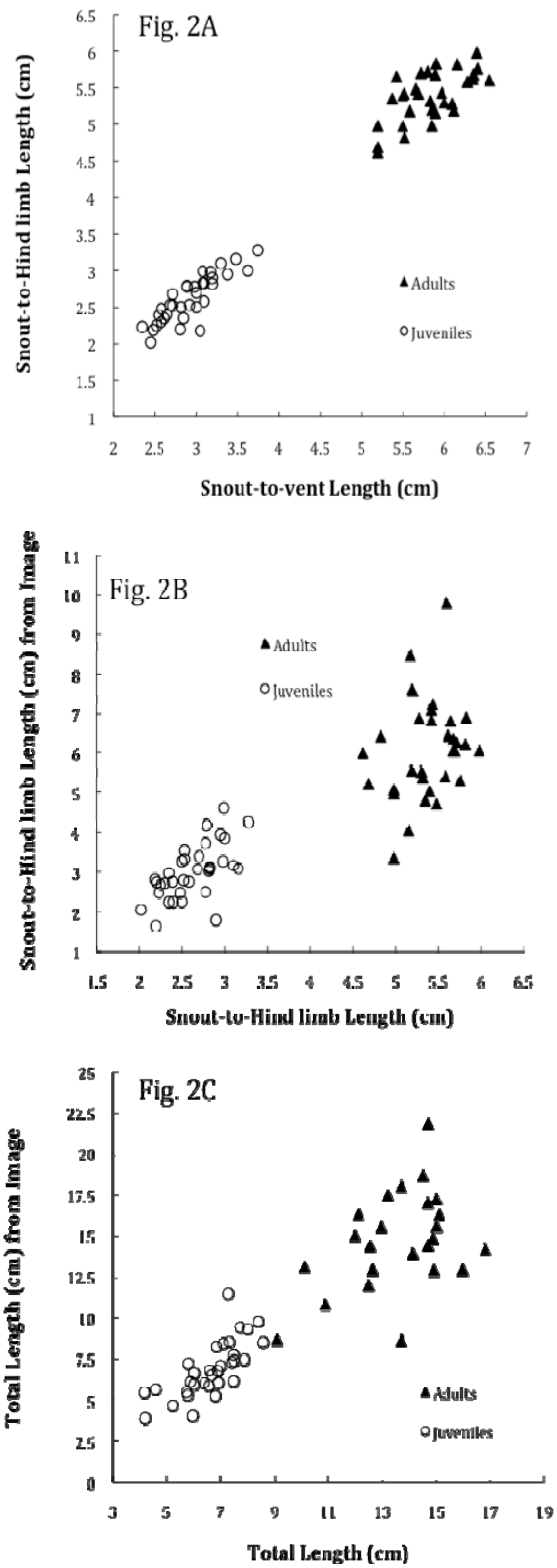

FIGURE 2. Plots showing degree of correlation between (A) snout-to-vent length and snout-to-hindlimb lengths measured directly on each animal, (B) snout-to-hindlimb length measured directly on animals or estimated from images, and (C) total length measured either directly on animals or estimated from images.

lizard. We then opened the paired picture of the lizard and used the “Freehand Line” tool to measure SHL and TL of each animal from the digital photographs. All image measurements were made by an observer blind to the size measurements made directly on each lizard in hand. We did not measure TL in images in which the lizard’s tail was not visible and these images were excluded from analyses of TL.

Statistical analyses.—Owing to a lack of individuals of intermediate size, most data were not normally distributed and thus violated assumptions necessary for parametric analyses. Therefore, we used nonparametric Spearman’s rank correlations ($\alpha = 0.05$) to determine the degree to which variables were correlated. We tested for correlations between SHL and SVL measured directly on the animals, between the estimated SHL from the images and directly measured SHL, between the estimated TL from the images and directly measured TL, between the directly measured SHL and relative error of SHL estimates, between the directly measured TL and the relative error of TL estimates, between the distance from the animal to the photographer and relative error of TL estimates, and between the distance from animal to the photographer and relative error of SHL estimates.

RESULTS

We captured 64 lizards (30 adults and 34 juveniles) over four days of sampling. Animals were bimodally distributed in size with no individuals of intermediate length (Fig. 2). The average measured SVL was 4.31 cm (range: 2.35–6.55 cm). We photographed all measured animals with their entire body in the frame.

Measurements of SHL were highly correlated with SVL measurements when both measurements were taken directly on animals in hand ($\rho = 0.93$, $P < 0.001$; Fig. 2A). Estimates of SHL using the digital image analyses were also highly correlated with SHL measured directly on the animals ($\rho = 0.87$, $P < 0.001$; Fig. 2B). However, SHL measurements made using images had an average error of 19.4 ± 16.2% (1 SD) relative to measurements made on the animals themselves. Estimates of TL using the digital image analyses were also highly correlated with TL measured directly on the animals ($\rho = 0.90$, $P < 0.001$; Fig. 2C). However, TL measurements made using images had an average error of 14.8 ± 12.9% (1 SD) relative to measurements made on the animals themselves.

Photographs of the lizards were taken an average of 3.1 ± 1.6 m (1 SD) from the animals but ranged as far away as 9.6 m from an animal. The photograph taken at 9.6 m resulted in SHL and TL image measurements that were within approximately 26% of the respective measurements taken on the animal. We found no correlation between the distance a photograph was taken from an animal and the relative error associated with estimated SHL (ρ = -0.10, P = 0.42) or estimated TL (ρ = 0.08, P = 0.56) from digital images. Additionally, we found no correlation between the size of animals and the relative errors of SHL estimates (ρ = -0.12, P = 0.34) or TL estimates (ρ = 0.04, P = 0.78). However, we found a significant correlation between the size of the relative errors in SHL and the size of the relative errors in TL measured using the digital images (ρ = 0.61, P < 0.001). In other words, either a pair of pictures had low errors for estimates of both measurements (SHL and TL) or both estimates had high associated errors.

We photographed five of the 64 animals with their bodies not parallel to the plane of the image. Average error in estimates of SHL for these five non-parallel photos was 20.3 ± 20.1% compared with an average error of 18.7 ± 15.3% in photos where animals were parallel to the plane of the photograph. Average error in TL from the five non-parallel images was 21.7 ± 13.1% compared with 13.6 ± 12.9% for images where the animal was parallel to the plane of the image. The similarity and comparable ranges for these errors suggest that measurements of SHL and TL from images wherein animals were not parallel to the image plane were not more error prone than the same measurements from normal images.

Discussion

Our data indicate that SHL in *Sceloporus* lizards, and potentially other species with similar morphology and behavior, can serve as an adequate proxy for SVL. However, caution may need to be taken for species with ornate (e.g., spines or crests) or convex dorsa (as in many Chameleons) as these may distort the digital measurement of SHL from images. *Sceloporus occidentalis* has a relatively flat and smooth dorsum and so SHL was easily measured from either a dorsal or lateral perspective. Measuring SHL is useful because it in turn allows the calculation of an animal's size from digital images. The use of digital photographs and image analysis to estimate SHL and TL in *S. occidentalis* appears reliable given the high degree of correlation between estimates from image analyses and true field measurements. Additionally, our study incorporated a wide range of distances from the observer to the animal yet there was no correlation between this distance and the relative error of SHL or TL. This technique should therefore be valuable in a field setting where researchers are presented with animals at varying distances. Also important is the lack of correlation between a lizard's size and the relative error of the size estimate. This suggests that the application of this technique should apply equally well to the range of sizes that we saw here.

Size estimation from digital photographs may be hindered by the fact that three-dimensional animals are compressed into two-dimensional space in digital images. This likely results in some loss of measurement precision, especially when the lizard is positioned at odd angles to the photographer (i.e., is not parallel to the plane of the photograph), although our results suggest that normal and non-parallel images did not differ appreciably in error. It may be that a lizard's sinusoidal alignment or vertical angle (e.g., positioned flat versus in the top of a push-up) or its tail being hidden may affect measurement accuracy, even if the animal is in the same plane as the photograph. Werner (2011) noted the value of using a scale object when photographing reptiles but did not address how the curvature or angle of the animal might impact the effectiveness of the scale object. It would therefore be useful to test this technique on species with different morphologies and behaviors that may affect the accuracy of the technique outlined here.

The feasibility of estimating body sizes from digital photographs will presumably be affected by the habitat of the lizard. Lizards in this study were photographed when they were within 2 m from the ground and were typically perched on an object. However, many lizard species use different habitats. Placing a ruler high in a tree or on a tree trunk may be difficult when working with arboreal species. Many fossorial species, like many scincid lizards, or aquatic species, like certain varanids, may also be challenging to photograph. Such species are often seen in leaf litter or water so obtaining a useful photograph of the animal's entire body may not be possible. It is also possible that narrow endemic species may be specialized to a particular habitat that would make this technique challenging to use (e.g., camouflage or refugia use). Future experimentation may determine the efficacy of this technique with a wider array of species and habitats.

It is important to note that we marked all animals in this study to avoid pseudoreplication. If the goal is to repeatedly measure an animal's size over time, it may be necessary to capture observed individuals initially and use a visual mark like a glass bead or other method to allow later identification of the animal from a distance. This would permit the researcher to handle an animal only once and still collect data for the same individual over time for use in estimating growth. Without previously marking an animal, care would need to be taken to avoid double counting individuals in the population where inferences about population structure or demography are desired.

The photographic method described here is a useful way of estimating SVL in lizards. Image analysis can allow field biologists to collect measurements from multiple animals without investing considerable time trapping, pursuing, or capturing animals. Furthermore, this method reduces handling time of an animal and minimizes the likelihood of additional stress, though the importance of this decreased stress is likely to vary from case to case and quantification of stress hormones in one species found no effect of capture and handling (Langkilde and Shine 2006). An additional caveat is that photography itself may impact the animal. Huang et al. (2011) found that shutter sounds decreased anole display behavior in the same way that predator calls did. Thus, caution may be needed before applying the method outlined here under some circumstances, such as in behavioral research.

Although the estimation technique that we outline allows for efficiency in certain respects, there are at least a few additional burdens imposed by this method. Field data collection should be done in pairs to ensure that photographs are taken similarly of the lizard and ruler, additional time must be spent on the analysis of the photographs, and the field measurements may lack information on sex, as well as body mass and gravidity, among others. Whether the reduction in precision in calculating body size is a significant drawback will depend on the purpose for which the size measurements are made. For example, our estimates of 14–19% error may be acceptable for gauging size classes of many lizards in wild populations, but may be inadequate for predicting the outcome of agonistic interactions in territorial displays or sexual selection. The technique outlined here is nevertheless likely to still be valuable in many cases.

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