Use of an Exemplar Versus Use of a Sample for Calculating Summary Metrics of Morphological Traits in Comparative Studies of Anolis Lizards

Comparative morphological analyses of large groups of species are limited by the time needed to perform multiple measurements on several individuals of each studied species. For example, taking complete external morphological data (e.g., Poe 2004) for an individual Anolis lizard for phylogenetic analysis takes us approximately 14 minutes. If even five individuals per species are scored for every species in the Anolis clade, and we estimate 400 species of Anolis, data collection will take over 466 hours. But is it necessary to score lots of specimens to ascertain an informative summary of the population/species? Although more data are nearly always better, researchers must optimize the time spent on data collection relative to the goals of a planned study. If measuring one specimen achieves the same results as measuring (e.g.) five specimens, then measuring one specimen is preferable.

We investigated the efficacy of a time-saving approach for performing comparative morphological analyses of lizard species. In particular, we asked whether use of a single exemplar specimen—the largest male—provides distinguishable results from using means for five conspecific male specimens in some comparative species analyses of traits commonly employed in morphological, ecological, and phylogenetic analyses. We tested for sampling effects related to the number of individuals scored for five commonly used morphological traits and three commonly employed quantitative techniques (e.g., some traits and techniques in Losos et al. 1998; Poe 2004; Latella et al. 2011) in 15 species of Anolis lizards.

Five morphological characters for five adult males each of 15 species of Anolis lizard were collected using specimens from the Poe Lab and the Museum of Southwestern Biology at the University of New Mexico. The five largest adult males available to us for each species were used without concern for geographic origin. The following characters were collected according to procedures established by Williams et al. (1995) and Poe and Yañez-Miranda (2008): body size measured as snout-vent length (SVL) from tip of snout to anterior edge of cloaca; head length (HL) measured from tip of snout to anterior edge of ear opening; femoral length (FL) measured from the midline of the body laterally to the knee; scales across snout (SNSC) counted between second canthals; and lamellae count on phalanges ii and iii of the fourth toe of the hind foot (see above cited papers for more detailed character descriptions). Measurements were taken with digital calipers to the nearest 0.1 mm.

We performed analyses of species using two sets of these data: means for each species using all measured individuals, and values for the single largest male specimen for each species. We compared summary metrics for these two data sets for three techniques: 1) relationship of traits to body size (SVL vs. HL, FL, and lamellae; slope, $R^2$); 2) phylogenetic gap-coding (SNSC, SVL; Thiele 1993), a method that converts measurement data to phylogenetic codes; and 3) principal components (scores for PCI and PCII using all five variables).

Results are summarized in Table 1 and Figs. 1 and 2. We found that use of a single exemplar specimen per species produced

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Fig. 1. Comparison of relationships of traits to body size.
nearly identical results to using summary statistics from five specimens for body size-trait relationships, phylogenetic coding, and principal component scores. Body size-trait relationships differ by less than 0.005 in slope, and by less than 0.008 in $R^2$ (Fig. 1). This similarity among datasets occurred both in traits expected to closely track body size (HL, FL) and in a trait known to correlate more weakly with body size (lamellae). The average difference between phylogenetic codes for each dataset was 0.4 on a scale of 0.0 to 5.0 for SVL and SNSC, and 19 of 30 codes were identical between the datasets (Table 1). Principal component scores were very strongly correlated between datasets (Fig. 2). These results suggest that little would be gained by measuring five specimens rather than one specimen for these analyses (body size-trait relationships; phylogenetic coding; PC scores) for these traits in these Anolis species. A five-fold decrease in data collection time is not trivial in comparative studies of large clades.

In the case of Anolis, the time saved by measuring one specimen rather than five would be on the scale of months, rather than hours. We conclude that some comparative interspecific morphological studies of lizards such as Anolis may proceed more efficiently by measurement of a single exemplar per species rather than multiple individuals. Although results are conclusive for the traits, methods, species, and scale studied here, we do not recommend general extrapolation to other studies. There certainly are studies of morphology—perhaps most studies—wherein measurement of several or even hundreds of individuals per species or population is warranted. Studies of intraspecific variation, of growth within species, and of traits with high variance are obvious examples where many individuals must be measured in order to gain biologically meaningful results. Other cases similar to our study (e.g., interspecific principal component analyses) may produce similar results—i.e., adequacy of use of a single exemplar for some quantitative summary metrics. But these inferences are best made on a case-by-case basis. We believe that the null expectation for a study with unknown variance properties should be that measurement of more than one individual per species/population is warranted. A conservative implication of our results is simply that large sample sizes should not be assumed to be necessary for all studies.

**Literature Cited**


**APPENDIX**

Specimens Examined

Anolis baleatus: Poe 0373–0375, 0395, 0485.

Anolis biporcatus: Poe 1520, 2151, 2155, 2170–2171.

Anolis concolor: Unm 46696, 46703, 46721, 46724, 46746.

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**Table 1. Comparison of phylogenetic codes for snout–vent length (SVL) and scales across snout (SNSC).**

<table>
<thead>
<tr>
<th>Species (Anolis)</th>
<th>SVL (Max)</th>
<th>SVL (Mean)</th>
<th>SNSC (Max)</th>
<th>SNSC (Mean)</th>
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<td>2</td>
<td>3</td>
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<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
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<td>1</td>
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**Fig. 2. Comparison of principal component scores.**
A New System For Marking Hatchling Turtles Using Visible Implant Elastomer

Turtles are a particularly vulnerable taxonomic group, with 40% of all species currently placed on the IUCN Red List (van Dijk et al. 2014). Many recent conservation efforts for turtles focus on the long-term viability of turtle populations, particularly in light of habitat fragmentation, over-colling, road mortality, and other anthropogenic stressors (Doak et al. 1994; Heppell 1998; Gibbs and Shriver 2002; Shoemaker et al. 2013). However, to determine long-term population viability, information must be collected on the demography of the target organism, including survivorship of juveniles (Shaffer 1981; Boyle 1992; Akçakaya and Sjögren-Gulve 2000). Mark-recapture methods are well-suited for studies of hatchling survivorship and ecology (Morafka 1994; Heppell 1998). However, many marking techniques for turtles, such as notching, drilling, and branding, may not be practical for use with hatchling turtles since a) the marks may not remain visible as the organism grows, and b) the marking process itself could harm animals of such small sizes (Plummer 1979; Davy et al. 2010).

Davy et al. (2010) recently proposed the use of Visible Implant Elastomer to mark small or hatchling turtles; however, their suggested marking systems do not allow easy identification of individual turtles and focus primarily on designating cohort marks for groups of hatchlings. Here, we present an intuitive, inexpensive, and easily recognizable individual marking system using Visible Implant Elastomer.

Methods—Visible Implant Elastomer (VIE), available from Northwest Marine Technology, Inc. (Shaw Island, Washington, USA), is a tagging agent that has successfully been used to mark fish and amphibians (Bailey et al. 1998; Bailey 2004; Butt and Lowe 2007; Hutchens et al. 2008). VIE is a bio-compatible material that leaves a well-defined pigment mark that fluoresces under UV light. VIE consists of a two-part system: a colored component and a curing agent. After the two components are mixed, the elastomer is injected into an area of translucent tissue (Fig. 1A), creating a long, distinctive mark (Fig. 1B). If necessary, a small amount of liquid bandage can be applied over the injection site to further ensure that the cured mark is not lost. The Northwest Marine Technology, Inc. (2008) VIE injection manual describes complete VIE application methods.

Results—A total of 101 hatchling Northern Map Turtles were marked using VIE between 9 May 2012 and 29 August 2013. Twenty-one individuals were recaptured at least once, and four individuals were recaptured two or more times (Table 1). No juveniles marked in 2012 were recaptured in 2013. The marking scheme was also successfully applied to five hatchling Bog Turtles in August 2013 to test the utility of this marking scheme for another species.

A single Graptemys geographica hatchling marked in the 2012 field season was recaptured three times between the months of May and July of that year. When captured on 25 July 2012, the plastral VIE marks had faded somewhat but were still visible to the naked eye. The marks were easily recognizable under UV light. This individual increased in mass and straight-line carapace length from 7.4 g and 33.9 mm on 9 May 2012, to 42.0 g and 66.3 mm on 25 July 2012. This equates to a 568% increase in mass and a 95% increase in shell length in 77 days. This is also the only individual in this study recaptured more than two months after marking, as most individuals were either not recaptured at all or were recaptured once within one month of marking.

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