Estimating Egg Mass in Lizards Using X-radiography

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Data on age-specific reproduction are critical to testing life history theory, and frequently yield greater insight regarding the evolution of life histories (Stearns 1992). Such data can be difficult to acquire for reptiles in natural populations, because determination of egg masses and clutch mass may require destructive sampling or that individuals be maintained in captivity until oviposition. X-radiography is a non-invasive means of determining egg number and egg size in reptiles (Gibbons and Greene 1979; Hinton et al. 1997; but see Kuchling 1998), and has been widely used on individuals to determine population demographics (e.g., Congdon and van Loben Sels 1991, 1993). Another potential application of X-radiography is estimation of egg mass (Congdon et al. 1983; Girardin 1993). Here, I demonstrate that: 1) X-radiography produces adequate resolution of eggs in small lizards, and 2) egg dimensions can be used to accurately predict egg mass in the lizard *Sceloporus undulatus*. These data suggest that X-radiography is a valuable tool for measuring age-specific reproductive traits in lizards.

In the springs of 1997 and 1998, I collected gravid females of *S. undulatus* on the Savannah River Site, Aiken, South Carolina, USA. X-radiographs of these females were made with a table-top unit (Emerald 130, Eureka Xray Tube Co., Chicago, Illinois, USA). Lizards were photographed while lying on their dorsal surface, at a distance of 100 cm from the X-ray tube, using Cronex 10 film and a fast detail screen (STERLING Diagnostic Imaging, Newark, Delaware, USA). X-radiographs were taken over a wide range of output settings to determine settings that maximized clarity of eggs.

To determine the relationship between egg size and egg mass, I used eggs laid by gravid *S. undulatus* in the laboratory. Gravid females were collected from a population in Burlington County, New Jersey, USA, during the summers of 1997 and 1998. Females were transported to the laboratory, where they were maintained in 6 L terraria on a layer of damp sand or sphagnum moss. Terraria were checked twice daily for newly laid eggs. When eggs were discovered they were removed and weighed to the nearest 0.01 mg using an electronic balance. Egg width and egg length were measured to the nearest 0.01 mm with digital calipers. I used multiple linear regression to produce a model to predict egg mass based on egg length and egg width.

A sensitivity analysis of the model was performed to determine the accuracy with which egg length and egg width must be measured to satisfy the requirement of estimating egg mass within 5% of actual mass. Average length and average width were used as values for the regression model parameters to calculate a reference value for predicted egg mass. Length or width was then varied systematically in 1% intervals and predicted egg mass was recalculated. Percent deviation of the new prediction from the reference value was used as an indication of percent error in estimation of egg mass associated with a given percent error in measurement of egg length or egg width.

A total of 175 eggs from 22 females were measured. Both egg length and egg width were significantly correlated with egg mass (Fig. 1). Multiple linear regression produced the following model, relating egg length (L), egg width (W), and egg mass (M),

$$M = 0.0378 \cdot L + 0.081 \cdot W - 0.682$$

The model explained 92% of the variation in egg mass ($F_{2,172}$ = 965.51, $p < 0.01$, adj. $r^2 = 0.92$). The average residual was $3 \cdot 10^{-10} \pm 0.002$ indicating that the data were distributed evenly around the regression line. When the regression equation was used to predict egg mass for the 175 eggs, average error in predicted egg mass was 0.1 ± 0.6%. The absolute deviation of predicted egg mass from actual egg mass averaged 3.1 ± 0.4%. Thus, the regression equation was capable of predicting egg mass with high accuracy.

Using X-radiography procedures described above, I obtained clear images of lizard eggs. An output of 5 mAs and 44 kV provided the sharpest detail for measurement of egg dimensions (Fig. 2). Similar output can be achieved using a less expensive, portable X-ray unit (Model 903, MinXray Inc., Northbrook, Illinois, USA). Therefore, X-radiographs can be taken at a remote field station or, if necessary, in the field. Such a design would permit determination of egg mass and clutch mass with little disturbance, and would be ideal for use with animals included in demographic studies.

Measurements of egg dimensions from X-radiographs should produce highly accurate estimates of egg mass. Sensitivity analysis

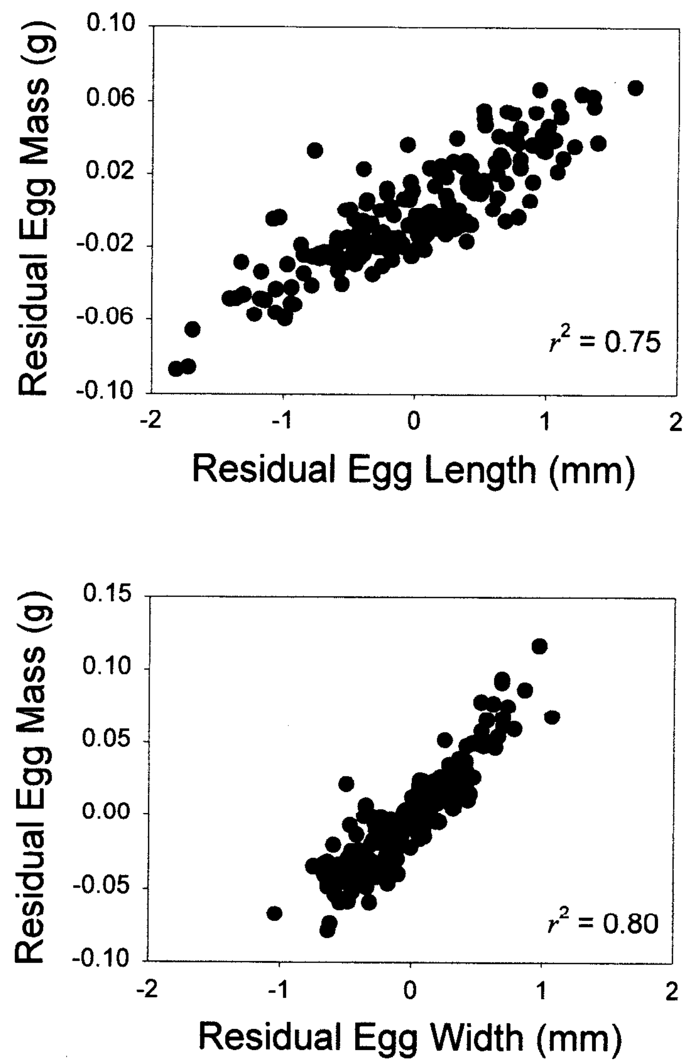

FIG. 1. Relationships between residual egg dimensions and residual egg mass in the lizard *Sceloporus undulatus*. Partial correlations for both egg length (N = 172, $t = 22.6$, $p < 0.001$) and egg width (N = 172, $t = 26.2$, $p < 0.001$) were highly significant.

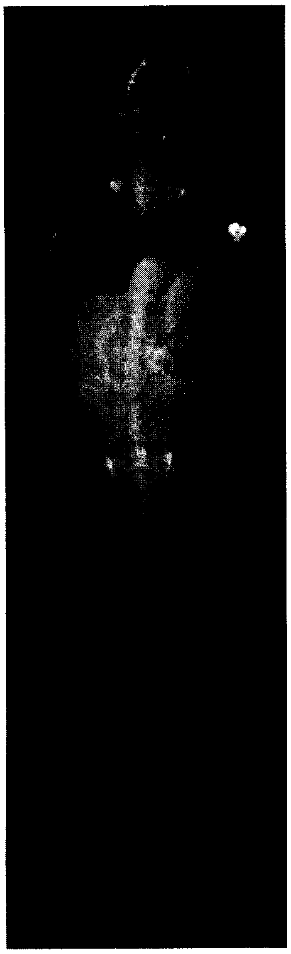

FIG. 2. X-radiograph of a gravid female obtained with an output of 5 mAs and 44 kV (see text for details). This individual was carrying eight eggs, all of which were clearly visible and could be measured directly from the X-radiograph.

indicates that a deviation of less than 5% from actual egg mass requires that egg length and egg width each be measured within 0.17 mm of actual values. This is a very reasonable margin of error, considering the accuracy of digital calipers (± 0.01 mm). The most likely source of error in egg dimensions is magnification of the image, caused by the X-ray technique (≈6.4%, Congdon and van Loben Sels 1991). The magnitude of error caused by image distortion can be reduced by including objects of standard length in each X-radiograph, which can be used to determine a correction factor for egg dimensions (Graham and Petokas 1989). To assess the accuracy of egg dimensions determined from X-radiographs, I measured egg dimensions of a clutch of eggs laid one day after being X-rayed. On average, egg length and egg width measured from X-radiographs deviated 0.15 mm and 0.13 mm, respectively, from measures of actual eggs (N = 5), which would result in an average error in egg mass estimation of 4%. At least one source of error would be eliminated when egg dimensions are obtained from X-radiographs. Because lizard eggs have a flexible shell, measures of egg dimensions are prone to error resulting from compression of the egg. X-radiographs provide a fixed image for measurement, eliminating errors resulting from compression by hands or calipers.

I conclude that egg mass, and thus reproductive output and relative clutch mass, in *S. undulatus* can be inferred from dimensional measurements of eggs in X-radiographs. This technique provides a valuable tool for following reproductive output of individuals in a demographic setting, and will add to our existing knowledge of reproductive patterns in reptiles. Undoubtedly, predictive equations for estimating egg mass from X-radiographs can be generated for other species (e.g., Girardin et al. 1993). Future studies of reproductive allocation should exploit the advantages of X-radiography to determine aspects of offspring size, as well as offspring number.

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