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A precise water displacement method for estimating egg volume

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ABSTRACT. Relationships between egg volume and an array of life-history traits have been identified for many bird species. Despite the importance of egg volume and the need for precise and accurate measurements, egg volume is usually estimated using a mathematical model that incorporates length and width measurements along with a shape variable. We developed an instrument that provides precise estimates of egg volume and can be easily used in the field. Using Clapper Rail (*Rallus longirostris*) eggs, we compared egg volumes measured using our instrument with estimates based on linear measurements. We found our instrument to be both precise and accurate. Compared with a method based on linear measurements of eggs, use of our instrument reduced variation in egg volume estimates by 1.6 cm^3, approximately 8% of the volume of a Clapper Rail's egg. Further advantages of our technique include ease of use, increased accuracy of field-based volume estimates, and increased resolution of variation in egg volume estimates. In addition, our technique does not require postdata collection processing time and did not influence hatching success. Also, for Clapper Rails and similar species, our technique can be combined with other techniques (e.g., egg flotation) so that both egg volume and embryonic stage can be estimated at the same time.

SINOPSIS. **Un método preciso de desplazamiento de agua para estimar el volumen del huevo**

Las relaciones entre el volumen del huevo y una gran cantidad de caracteres en las historias de vida han sido identificadas para muchas especies de aves. A pesar de la importancia del volumen del huevo y la necesidad de medidas más precisas, el volumen del huevo es comúnmente estimado usando modelos matemáticos que incorporan medidas del largo, ancho y forma del huevo. Nosotros creamos un instrumento que proporciona estimativos precisos del volumen del huevo y puede ser fácilmente usado en el campo. Usando huevos de *Rallus longirostris* comparamos las medidas de los volúmenes de los huevos usando nuestro instrumento con estimados obtenidos mediante mediadas lineales. Encontramos que nuestro instrumento fue preciso. Comparado con métodos que se basan en medidas lineales de los huevos, el uso de nuestro instrumento reduce la variación de los estimativos del volumen de los huevos en 1.6 cm^3, aproximadamente 8% del volumen de los huevos de *Rallus longirostris*. Ventajas adicionales de nuestra técnica incluye facilidades de uso, incremento en la precisión en los estimativos de volumen realizados en el campo y un incremento en la disminución de la variación de los estimativos del volumen del huevo. Adicionalmente, nuestra técnica no requiere tiempo de manejo después de la colección de los datos, y no afecta el éxito de eclosión. También, para *Rallus longirostris* y especies similares, nuestra técnica puede ser combinada con otras técnicas (e. g., flotación de los huevos) de tal forma que simultáneamente se puedan estimar el volumen del huevo y el estadio embrionario.

Key words: Clapper Rail, egg length, egg width, Hoyt's formula, *Rallus longirostris*

The size and volume of bird eggs are known to be related to many important life-history traits, including female mass (Nol et al. 1997), egg composition (Ricklefs 1984, Arnold 1989, Reynolds et al. 2003), hatching success (Williams 1994, Potti 2008), nest success (Roper 1992), hatchling size (Blomqvist et al. 1997), and nestling survival (Galbraith 1988, Grant 1991). Egg volume has been estimated in several different ways, including mathematical models that use mass and various morphological measurements (Anderson et al. 1970, Hoyt 1979, Bridge et al. 2007). However, each of these methods has limitations. For example,

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measurements of length and width are prone to intra- and interobserver measurement error and fail to address variation in egg shape (Christians 2002). Although photographic techniques have been developed to account for intraspecific variation in egg shape (Mänd et al. 1986, Bridge et al. 2007), the most widely used method for estimating egg size uses measurements of length and width along with a shape variable generalized among species (Hoyt 1979, Amundsen et al. 1996, Catry and Furness 1997, Bridge et al. 2007).

Here we describe a method for measuring egg volume using water displacement. Although other investigators have used water displacement to measure egg volume (Loftin and Bowman 1978, Morris and Chardine 1986, Székely et al. 1994, Flint and Grand 1996), we believe that our instrument is more accurate, easier to construct, and simpler to use than any described previously. We tested our instrument using Clapper Rail (*Rallus longirostris*) eggs and compared our results to those obtained using an alternative method (Hoyt 1979).

METHODS

Our instrument consisted of a main cylinder (a 15-cm-long Pyrex cylinder) with an enclosed spout on the side, about 5 cm from the top, to accommodate a standard filtration flask-type nipple that was fused in place (Fig. 1). We used a modified Pyrex graduated cylinder for the main body of the instrument because this material is shock and stress resistant and can be produced in most glass shops. The size of our device was based on the dimensions of Clapper Rail eggs, but dimensions can be altered to accommodate different-sized eggs.

Before use in the field, we cut approximately 2 cm from the bulb-end of a 1.5 cm^3 disposable transfer pipette (VWR Labshop, Batavia, Illinois) and approximately 3 cm from the transfer tip, leaving an internal diameter of 4 mm at the transfer tip. We then used Parafilm (Structure Probe, West Chester, Pennsylvania) to create an air-tight bond between the nipple and pipette tip.

To use our instrument, we placed it on a flat, level surface and filled the cylinder with fresh water. We allowed the water in the cylinder to equilibrate to air pressure and create surface tension at the end of the pipette tip. Once equilibrated, a small scale and catch basin was placed under the pipette tip (Fig. 1). An egg placed into the cylinder forced a volume of water equal to the egg's volume through the pipette tip and into the catch pan. The mass of the displaced water was equal to the volume of the egg because 1 cm^3 of water weighs 1 g.

To measure precision and accuracy, we used our instrument to measure the water displaced

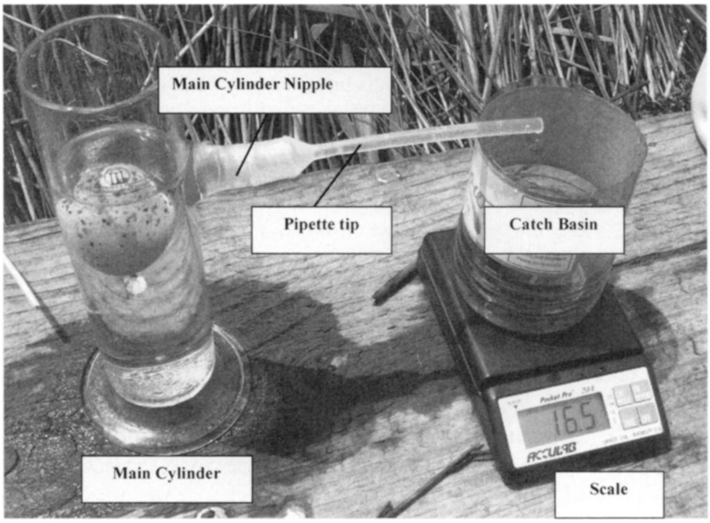

Fig. 1. The instrument we designed to measure the volume of Clapper Rail eggs along with a catch basin and scale.

by a steel cylinder of known volume (10.37 cm^3). The mass of water displaced by the cylinder was measured using a balance (±0.01 g; TS400 Precision Standard, Ohaus, Pine Brook, New Jersey), and this process was repeated 10 times. We defined precision as the statistical variability present in the sample, attributed to the sampling device. We defined accuracy as the overall distance between the observed volume measurements and their true value (Walther and Moore 2005). We estimated the precision of our instrument as the variance of the 10 volume measurements. The accuracy of our instrument was calculated as the mean departure of the 10 repeated measurements from the known volume of the cylinder.

Field testing. From April 2007 to August 2007, we located 24 active Clapper Rail nests in the Pascagoula River Marsh Coastal Preserve (30° 25′N, 88° 34′W) and the Grand Bay National Estuarine Research Reserve (30° 20′N, 88° 24′W), both located in Jackson County, Mississippi. Nests contained clutches of eggs with embryos at all stages of development, as identified using methods described by Rush et al. (2007). We used 18 nests to test our instrument and six nests served as controls to assess possible negative effects of our technique. In the field, we measured the mass of water displaced by eggs with a smaller, more compact, battery-operated scale (±0.1 g; Acculab Pocket Pro 250-B, Acculab, Edgewood, New York).

The volume of eggs ($N = 150$) from the 18 nests (mean clutch size = 7.7 ± 1.2 [SE] eggs) was measured (mean volume = 19.25 ± 0.18 cm^3). Eggs with more developed embryos broke the surface of the water (Rush et al. 2007). In such cases, we gently pushed the egg below the surface using a small wire or piece of grass. The volume of the portion of the wire or grass below the water's surface was then measured and subtracted from the estimated volume of the egg.

In addition to egg volume, one author (SAR) measured egg length (L) and breadth (B) at the widest point of each egg using dial calipers (±0.1 mm). These measurements were used to estimate egg volume based on Hoyt's formula (Volume = $K_v * LB^2$), using 0.51 as the volume coefficient K_v. We used 0.51 as the volume coefficient because Hoyt (1979) indicated that this value could be used to obtain relatively precise estimates of egg volume for most species of birds (within 2% of actual).

We removed eggs from the instrument by slowly inverting the instrument, allowing eggs to slide into our hands. Measuring an egg and removing it from the device took about 30–45 s. After measurement, eggs were dried and returned to nests. Nests were monitored every 2 to 5 d until all eggs hatched or the nest failed.

To compare volume estimates obtained using Hoyt's (1979) method with estimates derived using our technique, we developed a linear mixed-effects model, based on a normal distribution, and analyzed in the statistical package R (Bates and Sarkar 2007, R Development Core Team 2008). Application of linear mixed effects models allowed us to simultaneously control for a lack of independence among samples (i.e., eggs within clutches). In addition, we used measurements obtained with our instrument to calibrate the volume coefficient (K_v) from Hoyt's formula to obtain an estimate specific for the Clapper Rail eggs in our study. We report all values as means ± SE.

RESULTS

Repeated measurements of the steel cylinder indicated that our instrument was precise (precision = 0.004) and accurate (accuracy = 0.05 ± 0.01 cm^3). The accuracy of our instrument was equivalent to less than 0.3% of the mean volume of a Clapper Rail's egg (19.72 cm^3). In addition, measurements of the volume of Clapper Rail eggs determined using Hoyt's (1979) equation were greater (mean differences = 1.6 ± 0.28 cm^3; $t = 6.2$, $P < 0.001$), a difference of approximately 8 ± 1.4% from measurements obtained using our method.

Using the measurements of Clapper Rail eggs obtained using our instrument and calibrated against Hoyt's formula (1979), our data indicated that volume could be more precisely predicted when a volume coefficient of K_v = 0.49 was used. However, application of this refined coefficient still resulted in a measurement error of 1.31 ± 0.26 cm^3 or 7 ± 1.3% of the average volume of a Clapper Rail egg as measured in our study. Hatching success was 100% for both experimental and control nests, indicating that our technique did not influence hatching success.

DISCUSSION

The accuracy and precision of our instrument exceeded those of other water displacement techniques (Hanson 1954, Loftin and Bowman 1978, Thomas and Lumsden 1981, Székely et al. 1994). Error associated with the accuracy of our instrument was equivalent to less than 0.3% of the volume of a Clapper Rail's egg. Our results also indicate that, for Clapper Rails, the precision of egg volume estimates derived using Hoyt's (1979) technique can be increased using a volume coefficient of 0.49 (rather than 0.51). Therefore, we believe that the accuracy of the results obtained using Hoyt's formula can be improved when a species-specific volume index is used. However, for our study, application of this new volume coefficient still resulted in a measurement error of 7% of the mean volume of Clapper Rail eggs. We attribute this difference to the considerable variation in egg shape (roundness of the eggs) within and between clutches of Clapper Rail eggs (S.A.R., unpubl. data). This variation may not be accounted for when estimating egg volume based on linear measurements. However, we believe that by incorporating water displacement, our method accounts for this variation and will prove useful for estimating the volume of the eggs of most other species of bird. In particular, we believe investigators will find our method useful for estimating the volume of eggs of most waterbirds and other species of birds where embryos can survive short periods of submersion (Kozicky and Schmidt 1949, Oney 1954, van Paassen et al. 1984, Alberico 1995).

Despite the improved accuracy of our technique over methods based on linear measurements, our technique does have several limitations. For example, immersion in water may be impractical under certain field settings, such as in a boat, and is not possible for eggs that are cracked or near hatching. In addition, our water displacement method likely requires a longer handling time than the method where the length and width of eggs are measured with calipers. Further, there may be accuracy or precision problems associated with using water displacement methods to measure the volume of small eggs. For example, applying our technique to measure the volume of an egg similar to those of most warblers (e.g., about 2 cm^3), the resolution of our balance ($\pm$0.1 g) results in a 5% measurement error. If a more accurate balance was used, precision would still be limited by the surface tension properties of water and the minimum expected measurement error would remain restricted to the volume of the last drop of water displaced by the egg. The precision of water displacement methods is, therefore, inversely related to the volume of the egg measured. This is an issue that may limit the practical application of our technique to larger eggs. However, investigators looking to measure the volume of an egg must consider the limitations and benefits of each of the techniques available and select the method best suited to their particular goals.

Finally, we believe our technique has several advantages over those based on linear measurements (Hoyt 1979) and photography (Mänd et al. 1986, Bridge et al. 2007). First, measurements using our technique are more accurate than the linear measurement approach of Hoyt (1979). Second, our instrument can also be used to estimate the stage of embryonic development (Rush et al. 2007). Finally, our approach does not require specialized software or postprocessing efforts of any kind (Bridge et al. 2007).

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