

## AVIAN EGGSHELL THICKNESS: VARIABILITY AND SAMPLING

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Studies comparing recently collected eggs with those in archival collections have shown that shell thickness in certain predatory (including fish-eating) birds has decreased significantly since the introduction and widespread use of persistent chemical insecticides, particularly DDT (Ratcliffe, 1967, 1970; Hickey and Anderson, 1968; Anderson and Hickey, 1970, 1972; Peakall, 1970; Blus, 1970; Blus et al., 1972; Koeman et al., 1972). This decrease in shell thickness is correlated with reproductive failures in some of these species, with populations declining when thinning persists for a period of years. Baseline data on eggshell thickness depend on studies of archival egg collections, which have had few additions since the 1930's—when severe restrictions were placed on egg collecting as a conservation measure. For more recent data, interested researchers must often collect eggs themselves, and they are faced with many problems. These include optimal allocation of time and resources to obtain adequate samples, while minimizing the impact of egg collecting on the reproduction of a species.

We feel that a knowledge of the variability of shell thickness in species is essential to determination of the proper size and composition of samples. To illustrate this, we have measured and analyzed eggs in museum collections of the following: Black-crowned Night Heron (*Nycticorax nycticorax*), White Ibis (*Eudocimus albus*), Clapper Rail (*Rallus longirostris*), Mockingbird (*Mimus polyglottus*), and Loggerhead Shrike (*Lanius ludovicianus*). Our analyses of these data are intended (1) to probe the variability in eggshell thickness and to discuss some of the factors which contribute to this variability; (2) to show how a knowledge of variation can enable one to estimate sample sizes needed for detecting specified differences in shell thickness; and (3) to discuss the advantages and disadvantages of collecting complete clutches of eggs.

### METHODS

Techniques for measuring eggshell thickness have been described by Anderson and Hickey (1970) and Ratcliffe (1970). We measured thickness using a modified Starrett Model 1010M thickness gauge, accurate to 0.01 mm. Three measurements were taken at different places around the blow-hole of each egg of the three nonpasserine species. Only those eggs with the blow-hole located near the "equator" were used. Eggs of the Mockingbird and Loggerhead Shrike are too small to measure thickness directly with the instrument described above. To estimate their thickness, the length and breadth of these eggshells were measured to the nearest 0.05 mm, using a vernier dial caliper

graduated in 0.05-mm intervals, and the shells were weighed to the nearest 0.01 g on a 100-g-capacity, top-loading, pan balance. These measurements were then used to calculate Ratcliffe's (1970) thickness index, a parameter closely correlated with actual thickness. We have endeavored to use only eggs that appeared clean inside and those with small blow-holes, in order to control weight variability.

Significant variation in eggshell thickness over broad geographic areas has been demonstrated for some species (Anderson and Hickey, 1970, 1972). Thus, for each species we have restricted our analysis to eggs collected in a relatively small region on the assumption that geographic variation is reduced to a trivial level. Black-crowned Night Heron eggs were from Massachusetts, Rhode Island, Connecticut, and extreme eastern New York. White Ibis and Loggerhead Shrike eggs were from Florida, Clapper Rail eggs from Virginia, and Mockingbird eggs from South Carolina and nearby Georgia. All of these eggs were collected between 1876 and 1943.

We assume that eggs in museum collections represent random samples from the given localities. We found no indication in the collections or in the field notes of the collectors that certain eggs were selected in preference to others, except that they usually tried to take fresh *first* clutches. All collectors probably attempted to obtain complete clutches. Their original field notes verify that complete clutches were selected after repeated visits to the nest or upon flushing a bird off the nest in species where incubation generally does not begin until the last egg is laid (Lloyd Kiff, pers. comm.). Collectors also accumulated or communicated knowledge of what a full clutch of eggs should be for the species. Thus, we believe that incomplete clutches were rarely taken, and that the inadvertent inclusion of a few of these clutches in our large samples does not significantly bias our data.

We assume that shell thickness remains essentially constant after an egg is collected and dried, that is, that the materials do not sublimate or otherwise decrease.

Statistical procedures follow those presented in Sokal and Rohlf (1969).

#### RESULTS AND DISCUSSION

*Variability*—Variation in shell thickness for each of the five species has been analyzed as a Model II (random) nested analysis of variance (anova), with unequal sample sizes (Table 1), using the clutch of eggs as the primary sampling unit. For the nonpasserine species, the procedure separates variation in thickness into three hierarchical levels: among clutches, among eggshells within clutches, and among measurements (error).

Variation in thickness of shell among clutches probably depends on: differences in the stages of incubation, differences related to clutch size, genetic and physiological differences between females, differences in diet among females within and between local populations, differences in gene pools between local populations, differences in environmental conditions between years, and other unknown factors (Kreitzer, 1972; Romanoff and Romanoff, 1949; Rothstein, 1972). Variation among eggs within clutches is probably due to day-to-day differences in calcium metabolism and eggshell deposition in individual females. The main factors which contribute to the among-measurements (error) mean square are: differences in eggshell thickness at the three

TABLE 1  
NESTED ANALYSIS OF VARIANCE FOR EGGSHELL THICKNESS FOR FIVE SPECIES

Species and Source of variation	Degrees of freedom (df)	Mean squares (MS)
Black-crowned Night Heron <sup>a</sup>		
Among clutches	81	.00423583*
Among eggs	254	.00048310*
Among measurements (error)	672	.00003343
Total	1007	
White Ibis <sup>a</sup>		
Among clutches	193	.00344592*
Among eggs	407	.00081443*
Among measurements (error)	1202	.00005435
Total	1802	
Clapper Rail <sup>a</sup>		
Among clutches	59	.00513168*
Among eggs	466	.00033180*
Among measurements (error)	1052	.00005133
Total	1577	
Mockingbird <sup>b</sup>		
Among clutches	80	.00489155*
Among eggs (error)	231	.00067398
Total	311	
Loggerhead Shrike <sup>b</sup>		
Among clutches	72	.00383473*
Among eggs (error)	255	.0043458
Total	327	

<sup>a</sup> Based on three thickness measurements (mm) per egg.

<sup>b</sup> Based on one thickness-index measurement per egg.

\*  $P < .001$

points of measurement, imperfections in the micrometer within the limits specified by the manufacturer, and human error in applying and reading the instrument.

An analysis of variance was performed on White Ibis data to test differences among nine localities in Florida. These differences were found to be non-significant ( $P < .01$ ). We conclude that most of the variation among clutches is due to individual differences in females and possibly to yearly differences in environmental conditions.

In the Mockingbird and Loggerhead Shrike, we made only one determination of the thickness index for each egg. For this reason, variation due to the intrinsic inaccuracies in measuring and to other within-egg variables could

TABLE 2  
STATISTICS OF EGGSHELL THICKNESS FOR FIVE SPECIES

Items analyzed	Species Studied				
	Black-crowned Night Heron <sup>a</sup>	White Ibis <sup>a</sup>	Clapper Rail <sup>a</sup>	Mocking- bird <sup>b</sup>	Loggerhead Shrike <sup>b</sup>
Variance					
Among clutches ( $s^2c$ )	0.0003055	0.0002832	0.0001829	0.0010949	0.0007567
Among eggs ( $s^2ec$ )	0.0001499	0.0002533	0.0000935	0.0006740	0.0004346
Among measurements ( $s^2$ )	0.0000334	0.0000544	0.0000513	—	—
Percent of the variability					
Among clutches	62.50	47.93	55.81	61.90	63.52
Among eggs	30.66	42.87	28.52	38.10	36.48
Among measurements	6.84	9.20	15.66	—	—
Overall mean thickness					
or index	0.28348	0.34752	0.25792	0.54332	0.51506
Number of clutches	82	194	60	81	73
Number of eggs	336	601	526	312	328
Clutch size					
Mean	4.10	3.10	8.77	3.85	4.50
Range	2-7	1-5	4-12	2-5	2-6

<sup>a</sup> Based on direct thickness measurements (mm).

<sup>b</sup> Based on thickness-index measurements.

not be separated from variation among eggs within clutches. Thus, differences among eggs within clutches cannot be tested and the number of levels in the anova is reduced to two. The coefficients of variation for thickness index among eggs within clutches for these species are similar to those for shell thickness for the other three species.

Statistics derived from the anova are presented in Table 2. Variances have been calculated from expected mean squares. Variation at each level is also expressed as a percentage of the total variance. Most of the variation occurs among clutches, but a substantial proportion is found among eggs within clutches. The percentage of the variation occurring among measurements of the same egg is small.

Percentages of variation for groups and subgroups are fairly consistent from species to species. A notable exception is found in the White Ibis, in which the percentage of the total variation occurring among eggs within clutches was greater than for the other species. This finding is related, in part, to obvious differences in size and shape of eggs within clutches. Generally, eggs in clutches of other species were more uniform in these characteristics. Furthermore, because eggs of White Ibis are larger and have

thicker shells than the other four species considered here, there is greater opportunity for variability.

*Complete clutches vs. one egg per clutch*—To get at the question of whether to collect whole clutches or some fraction of each, we computed the *relative efficiency* (RE) of one design with respect to the other. RE is a ratio, usually expressed as a percentage, of the variances resulting from the two designs being compared. The appropriate formula as given in Sokal and Rohlf (1969:289) is

$$RE = \frac{s_{\bar{y}}^2 \text{ of design B}}{s_{\bar{y}}^2 \text{ of design A}} \times 100$$

where  $\bar{y}$  here represents the mean of a group of clutches. The expected variance among clutch means ( $s_{\bar{y}}^2$ ) for each design can be derived from estimated variance components for each level in the nested analysis of variance (Table 2) according to the formula:

$$s_{\bar{y}}^2 = \frac{s^2}{mec} + \frac{s_{ECC}^2}{ec} + \frac{s_C^2}{c},$$

where  $s^2$ ,  $s_{ECC}^2$ , and  $s_C^2$  are the respective estimates of the variance among measurements within eggs, among eggs within clutches, and among clutches; and  $m$  is the number of measurements per egg,  $e$  the number of eggs sampled per clutch, and  $c$  the number of clutches sampled.

We computed estimated variances of  $\bar{y}$  for a theoretical design A in which the entire clutch is utilized and for a second design, B, in which the sampling unit is one egg selected randomly from each clutch. In design A, the number of eggs measured per clutch ( $e$ ) has been determined as the whole number nearest to the mean clutch size for the respective species (see Table 2). In design B,  $e = 1$ . Values of  $m = 3$  and  $c = 10$  were constant for all calculations of  $s_{\bar{y}}^2$  for Black-crowned Night Heron, White Ibis, and Clapper Rail. These values were selected because our empirical estimates of variances were based on three measurements per egg, and ten clutches approximate the required sample sizes estimated in the next section of this paper. The value  $c = 10$  was also used for Mockingbird and Loggerhead Shrike, but  $m = 1$ . Thus, there is no estimate of  $s^2$  within measurements and the formula is reduced to two components,

$$s_{\bar{y}}^2 = \frac{s_{ECC}^2}{ec} + \frac{s_C^2}{c}.$$

Table 3 shows the gain in efficiency of design A compared to design B. The relatively large differences in thickness observed among eggs within

TABLE 3  
RELATIVE EFFICIENCY (RE) OF COLLECTING WHOLE CLUTCHES (DESIGN A) VERSUS  
COLLECTING ONE EGG FROM A CLUTCH (DESIGN B)<sup>1</sup>

Species	Mean clutch size	$s_y^2 A$	$s_y^2 B$	$\frac{s_y^2 B}{s_y^2 A} \times 100$	Increase in RE (%) of Design A over Design B
Black-crowned Night Heron	4	.3457283	.4665033	134.9	35
White Ibis	3	.3736778	.5546333	148.4	48
Clapper Rail	9	.1951889	.2935000	150.4	50
Mockingbird	4	.0001263	.0001769	140.0	40
Loggerhead Shrike	5	.0000844	.0001191	141.2	41

<sup>1</sup> The number of measurements per egg and the number of clutches are constant in each design.

clutches (expressed as  $s_{ECC}^2$ , see Table 2) contribute substantially to the sample variance ( $s_y^2$ ).

In utilizing whole clutches, the investigator should be aware that inadvertent or intentional collection of a high proportion of incomplete clutches may bias the data, especially if sample sizes are small. Such conditions would occur if only the first one or two eggs of a clutch are collected and the shell thicknesses of successive eggs tend to differ from those of the first eggs. Thus, care should be taken to collect complete clutches. If partial clutches are collected the investigator should randomly choose the eggs.

Other designs could be compared in which the number of measurements per egg ( $m$ ) or the number of clutches ( $c$ ) would vary. However, variability between measurements ( $s^2$ ) is small, and relative efficiency would be changed little by increasing the number of measurements per egg. If one wished to use one egg per clutch and still achieve a variance equivalent to that of design A, the percentage increase in the number of clutches to be sampled for each species would be approximately that shown in the last column in Table 2.

The relative efficiency of one design with respect to another is not very meaningful unless one considers the relative effort and time (or cost) involved in obtaining the data. Cost will vary greatly, depending on whether the species is abundant, whether colonial or solitary nester, how well it conceals its nest, and other characteristics. For most studies, the cost of removing the egg contents and measuring shell thickness is much less than that of finding sufficient nests and collecting the eggs. The investigator may also be confronted with a limited season in which eggs are available, extended travel cost and time, and other factors. Under such limitations, design A, which minimizes the number of clutches needed, will usually require the

least amount of time in the field. However, the total number of eggs to be measured is greater, as entire clutches are utilized.

The investigator must weigh the efficient utilization of time and resources against the impact on a species that collecting might have. Most species, except large birds of prey and albatrosses (which have long incubation periods), will lay replacement eggs when the entire clutch is taken or destroyed (Welty, 1962:295; Thompson, 1964:242). This trait was sometimes used by early oologists to get fresh eggs and/or increase their total take. Today, it might be used to minimize the impact of egg collecting. For instance, when the nests of a small proportion of breeding pairs are actually subject to the removal of the entire clutches, less disturbance occurs than when some fraction of the clutch is taken from a greater number of nests. Such disturbance, even though only one egg is taken, may also result in nest desertion by the birds.

*Sample size estimates*—After the sampling design has been selected, the investigator will want to know the number of clutches needed from each of “*a*” groups of nests in order to detect a minimum true difference,  $\delta$ , in mean eggshell thickness. This assumes a probability,  $P$  (power), that the difference will be detected if it exists, and a probability,  $\alpha$ , of incorrectly claiming a difference when none exists. Sokol and Rohlf (1969:247) present a relatively simple formula for estimating the sample size if one has a priori information of the sample variance. The formula (with  $c$  substituted for Sokol’s  $n$ ) is as follows:

$$c \geq 2 \left( \frac{\sigma}{\delta} \right)^2 \{ t_{\alpha[\nu]} + t_{2(1-P)[\nu]} \}^2,$$

where  $c$  = number of clutches,  $\sigma$  = true standard deviation for clutches,  $\delta$  = the smallest true difference between means that one desires to detect,  $\nu$  = degrees of freedom of the sample standard deviation with  $a$  groups and  $c$  replications (clutches) per group,  $\alpha$  = significance level,  $P$  = desired probability (power) that a difference will be found to be significant. Values of  $t_{\alpha[\nu]}$  and  $t_{2(1-P)[\nu]}$  are from a two-tailed  $t$ -table with  $\nu$  degrees of freedom, and probability levels of  $\alpha$  and  $2(1-P)$ .

The sample estimate of the coefficient of variation (C.V.) and the percent difference between means that one wishes to detect can be substituted for  $\sigma$  and  $\delta$ , respectively, because only the ratio of  $\sigma$  and  $\delta$  are necessary, not their actual values. We computed estimates of sample sizes (number of clutches) needed to detect thickness differences ( $\delta$ ) of five and 10 percent (percentages arbitrarily selected) for each of the five species at significance levels of 0.05 and powers of 0.9 (Table 4). These estimates are based on a collecting

TABLE 4  
ESTIMATES OF THE NUMBER OF CLUTCHES NEEDED FROM A LOCALITY TO SHOW  
DIFFERENCES ( $\delta$ ) IN THICKNESS OF EGGS<sup>1</sup>

Species	Coefficient of variation	Collecting Design			
		$\delta = 10$ percent		$\delta = 5$ percent	
		Whole clutches	One-egg clutches	Whole clutches	One-egg clutches
Black-crowned Night Heron	6.55	11	15	38	51
White Ibis	5.54	8	12	27	40
Clapper Rail	5.42	8	12	26	39
Mockingbird	6.56	11	15	38	53
Loggerhead Shrike	5.67	8	11	28	40

<sup>1</sup> Significance level ( $\alpha$ ) of 0.05 and power ( $P$ ) of 0.9.

design in which complete clutches are utilized and each egg is measured three times. The formula is very sensitive to changes in the ratio of ( $\sigma/\delta$ ). This accounts for the marked dissimilarity in numbers of sets required for detecting five and 10 percent differences.

The estimates of required sample sizes are based on estimates of the variance from relatively large samples of archival eggs. We believe these estimates are relatively precise, but they may also be conservative. For instance, we have assumed that variation has not increased in the post-DDT era. This assumption may not be valid, but we do not have sufficient samples of these five species at this time to analyze variation in such eggs. If recent eggs are sufficiently more variable in thickness, the sample sizes required would be somewhat larger.

Table 4 also presents estimated sample sizes for a one-egg-per-clutch collecting design. The number of clutches is increased by the difference in relative efficiency between the two designs.

SUMMARY

Measurements of shell thickness of the eggs of five species were subjected to nested analyses of variance. The analyses separated variation into two or three levels for which variances and percentages of the total variation were derived. The results show that differences among measurements of the same egg contribute little to the sample variance, whereas differences among eggs within clutches contribute nearly as much as differences among clutches. It is more efficient and less costly to collect entire clutches of eggs in most studies of shell thickness. Using entire clutches, sample sizes needed to detect differences of 10 percent in shell thickness (at given significance levels and power) were estimated to be eight to 11 clutches for the species studied. For differences of five percent, 26 to 38 clutches are required. Guidelines are presented which may assist other



workers in evaluating the efficiency of their sampling designs, and in estimating sample sizes for detecting differences in eggshell thickness in wild birds.

## ACKNOWLEDGMENTS

We thank L. J. Blus, D. R. Clark, Jr., and L. F. Stickel for their advice and critical reading of the manuscript. Data presented in this paper could not have been obtained without the generous cooperation of curators and their assistants at the following museums and universities: American Museum of Natural History, Carnegie Museum, Clemson University—Department of Zoology, Florida State Museum, Harvard University—Museum of Comparative Zoology, University of Massachusetts—Department of Zoology, Ohio State University—Museum of Zoology, Philadelphia Academy of Sciences, Smithsonian Institution—U.S. National Museum of Natural History, and Yale University—Peabody Museum of Natural History.

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31 JANUARY 1974.