EGGSHELL THICKNESS VARIABILITY IN THE WHITE-FACED IBIS

DAVID E. CAPEN

Many recent papers have reported the occurrence of persistent chemicals, such as DDT, and the associated thinning of eggshells of birds (Cooke 1973). Eggshell thinning is commonly documented by comparing recently-collected eggs with eggs found in museum collections. There are a number of factors, other than persistent chemicals, which may contribute to the variation in the thickness of eggshells. Some of these factors are geographic location (Anderson and Hickey 1970, 1972); the genetics, physiology, and diet among females (Romanoff and Romanoff 1949); the stage of incubation (Vanderstoep and Richards 1970, Kreitzer 1972); the order in which the eggs are deposited (Romanoff and Romanoff 1949, Berg 1945); and the size of the clutch (Rothstein 1972). Hence, when collecting eggs to study shell thickness, one must be aware of these factors to determine the proper composition of the sample.

I considered it important to conduct a study of eggshell variability of a species nesting in the wild, since most related investigations have been conducted in the laboratory, usually with the domestic chicken (*Gallus gallus*). The purpose of this study was to evaluate variability in the shell thickness of eggs of a population of the White-faced Ibis (*Plegadis chihi*). The parameters studied were (1) the length of incubation, (2) the order in which the eggs were deposited, and (3) the clutch size.

METHODS

During May 1974, White-faced Ibis eggs were collected from active nests in the Bear River delta, Box Elder Co., in northern Utah. More than 1000 pairs of Ibises comprised the study colony. Eggs were taken from 112 of 220 nests where egg deposition was synchronous. The nests were visited each afternoon during the egg-laying period, and all eggs were marked to indicate the order of deposition. Complete clutches most commonly contained either 3 or 4 eggs.

I selected a random sample of 56 nests when egg-laying ceased. From each nest I took 1 egg, also randomly, so that the completed sample contained 8 replicates of the first egg deposited in a nest, 8 replicates of the second egg deposited, etc. Corresponding samples (8 replicates of the first egg, second egg, . . .) were collected from both 3-egg and 4-egg clutches. Thus, the 56-egg sample included 24 eggs from 3-egg clutches and 32 eggs from 4-egg clutches.

A second sample of 56 eggs was collected after incubation had progressed to an average of 17 days, or about 4 days prior to hatching. All eggs contained embryos. This sample was selected in the same manner as before.

After the eggs were removed from the nests, they were refrigerated for 4–6 days, then

TABLE 1

THE COLLECTION DESIGN AND MEAN EGGSHELL THICKNESS (MM) FOR SAMPLES OF WHITE-FACED IBIS EGGS

	Order of Egg Deposition				
	O_1	O_2	O_3	O_4	Sample Means
6-days Incubation (I_1)[1]					.324
3-egg Clutches (C_1)	.309[2]	.331	.325	—	.322
4-egg Clutches (C_2)	.321	.314	.341	.325	.325
17-days Incubation (I_2)					.310
3-egg Clutches (C_1)	.308	.324	.321	—	.318
4-egg Clutches (C_2)	.302	.307	.306	.300	.304
Sample Means	.310	.319	.323	.312	.317

[1] Mean incubation for early eggs = 5.8 days; for late eggs = 16.6 days.
[2] Each figure represents a mean for 8 eggs.

cut around the equator, the contents removed, and the eggshells rinsed with warm tapwater. Eggshells were dried on absorbent paper for 24 h in a low-humidity laboratory.

Eggshell thickness measurements were made with a Starrett No. 1010M dial micrometer, read to the nearest .01 mm. Six measurements were taken, equally spaced around the equator of each eggshell.

To obtain a desired precision of 95% for the eggshell thickness measurements, I calculated the variance for the 6 measurements of each eggshell, then computed the number of measurements necessary to be 95% confident of remaining within a 5% limit of error (Steel and Torrie 1960:86). These calculations showed that, on the average, 5.3 measurements from each eggshell were necessary for the desired precision. Since I had taken a sufficient number of measurements, only the mean thicknesses for the eggshells, expressed to the nearest .001 mm, were used in the analyses.

A 2 × 2 × 4 (Incubation [I] × Clutch size [C] × Order of deposition [O]) factorial design (Steel and Torrie 1960:194) was the basis for the collection of eggs (Table 1). A frequency distribution of the data exhibited no kurtosis. For the statistical analyses, the collection design was partitioned into 3 separate factorial designs: a 2 × 2 × 3, a 2 × 4, and a 2 × 3. This was necessary because 2 cells ($I_1C_1O_4$ and $I_2C_1O_4$) do not exist, i.e. there are no fourth eggs deposited in nests with 3-egg clutches. The data were tested by standard analysis of variance techniques.

RESULTS

Sources of variation.—The analysis of variance results are presented in Table 2. The effects of 3 variables on eggshell thickness were investigated in the 2 × 2 × 3 analysis: the length of incubation, the order of egg deposition, and clutch size. The effect of incubation was significant ($P < .01$). Eggs which were incubated for an average of only 5.8 days had shells that were 4.3% thicker than shells from eggs which had undergone 16.6 days of incuba-

TABLE 2

ANALYSIS OF VARIANCE FOR SHELL THICKNESS OF WHITE-FACED IBIS EGGS

Designs and Sources of Variation	Degrees of freedom (df)	Mean squares (MS)	F
$2 \times 2 \times 3$			
Incubation (I)	1	.00373	8.88***
Order (O)	2	.00149	3.55**
Clutch Size (C)	1	.00048	1.14
I × O	2	.00034	< 1
I × C	1	.00159	3.79*
O × C	2	.00094	2.24
I × O × C	2	.00048	1.14
Error	84	.00042	
Total	95		
2×4			
Incubation	1	.00753	18.82***
Order	3	.00056	1.40
I × O	3	.00055	1.38
Error	56	.00040	
Total	63		
2×3			
Incubation	1	.00023	< 1
Order	2	.00163	3.70**
I × O	2	.00002	< 1
Error	42	.00044	
Total	47		
Nested factorial			
Incubation	1	.004643	13.66***
Order	5	.000987	2.37**
Clutch Size	1	.000738	< 1
I × O	5	.000340	< 1
I × C	1	.002065	6.08**
Error	98	.000416	
Total	111		

* $P < .10$; ** $P < .05$; *** $P < .01$.

tion. The order of deposition also significantly ($P < .05$) affected the eggshell thickness. In general, the first and last eggs had thinner shells than those in between. The 2 different clutch sizes showed a non-significant ($P > .10$) effect.

Three 2-way interactions and one 3-way interaction were tested. All were non-significant ($P > .10$) except one. A significant ($P < .10$) effect was obtained with the incubation × clutch size interaction. The nature of the implied relationship is illustrated in Fig. 1. Apparently, there was a greater decrease in shell thickness during incubation for eggs in 4-egg clutches than for eggs in 3-egg clutches.

Only the effects of incubation and order of deposition were tested in the 2-factor designs. Data from 4-egg clutches were used in the 2 × 4 analysis. The only significant ($P < .01$) factor was the length of incubation. In the 2 × 3 analysis, however, where only data from 3-egg clutches were used, the significant factor was the order of deposition ($P < .05$). The length of incubation did not significantly affect the shell thickness of eggs from 3-egg clutches. This, to some degree, confirms the interpretation of the significant interaction discussed above.

The data were also analyzed using a nested, factorial design (Sokal and Rohlf 1969:256). Sums of squares for the analysis of variance were obtained using regression techniques for an unbalanced design. Though not as straightforward, this is actually a more powerful analysis since the design used all available data. The results were essentially the same as those of the previous analyses, though more convincing (Table 2). Again, the effect of incubation length was significant ($P < .01$), as was the order of deposition ($P < .05$). The incubation × clutch size interaction was significant ($P < .05$) and more noticeable than before.

DISCUSSION

There was no apparent interference from pecticides in the eggs collected in this study. Eggshells did not differ in thickness from shells of eggs collected prior to 1940 and preserved in museums. The mean thickness for the pre-1940 museum eggs collected in Utah was 0.324 mm ($N = 29$) (unpublished data, Denver Wildlife Research Center, U.S. Fish and Wildlife Service). For a comparable sample of eggs in this study, shell thickness also averaged 0.324 mm ($N = 56$).

Incubation.—I was not surprised to observe the significant decrease in shell thickness between the eggs collected soon after incubation had begun and those taken just prior to hatching. A developing embryo obtains calcium from the eggshell. Simkiss (1967) estimated that 5% of the shell calcium may be used by the chicken embryo. Kreitzer (1972) reported a 7.3% decrease in shell thickness between incubated and unincubated eggs of the Coturnix Quail (*Coturnix japonica*). Rothstein (1972), who studied a species nesting in the wild, the Cedar Waxwing (*Bombycilla cedrorum*), demonstrated a similar association between incubation and eggshell thickness.

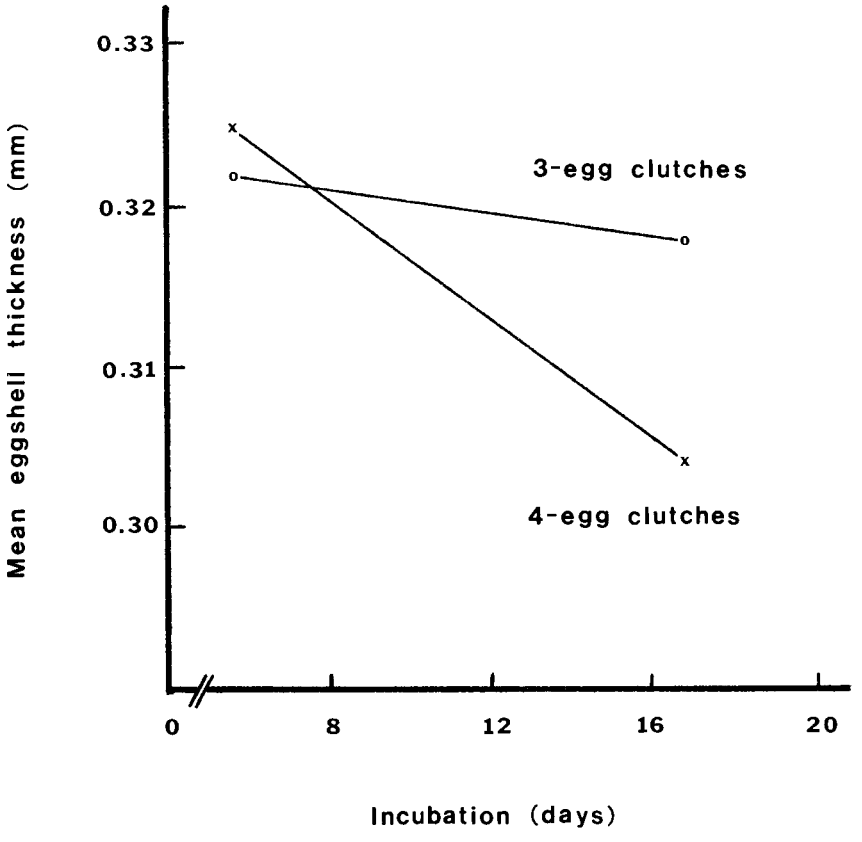

FIG. 1. The effect of incubation on shell thickness in White-faced Ibis eggs from 3- and 4-egg clutches.

The data in this study, showing a 4.3% decrease in shell thickness over approximately 11 days incubation, correspond with other published information. However, most of the decrease is a function of eggs from 4-egg clutches, which showed a 6.5% decrease in shell thickness, while eggs from the 3-egg clutches dropped only 1.2% (Fig. 1). On the average, eggs from 4-egg clutches were incubated only 0.7 days longer than those from 3-egg clutches. There is only a slight probability ($P < .02$) that the relationship is due to sampling error, hence there may be cause for additional study.

Order of deposition.—The relationship between eggshell thickness and the order in which the eggs are laid has apparently not been investigated in wild birds. In the chicken, shell thickness usually changes throughout the laying

cycle. Generally, the first and last eggs have the thickest shells (Wilhelm 1940, Berg 1945). My data with the eggs of the White-faced Ibis suggest the opposite relationship, i.e. the first and last eggs are usually the thinnest. Nevertheless, there is noticeable variation (Table 2), and the order of egg deposition should not be overlooked when collecting eggs for determination of shell thickness.

Clutch size.—Complete clutches of both 3 and 4 eggs are common in the White-faced Ibis, and many other species (Lack 1968:330). Thus, I believed that clutch size should be evaluated as a variable which might affect shell thickness. The analyses indicated that clutch size did not have a significant effect on thickness, despite the different responses to incubation discussed above. Rothstein (1972) reported that shell thicknesses were different in eggs of the Cedar Waxwing collected from 3-egg clutches and those collected from 4- and 5-egg clutches. However, Rothstein could not account for variation in the sequence of egg deposition which, as he pointed out, may have confounded the variation attributed to different clutch sizes. The shell thickness–clutch size relationship is worthy of investigation in other species.

Sampling.—There are some practical implications from these results. When collecting eggs to measure shell thickness, one should attempt to take as few eggs as possible, yet sample enough to detect a desired difference, with confidence. Hence, one should select a sampling scheme which provides as little variation as possible among eggs. Klaas et al. (1974) analyzed variation in eggshell thickness in 5 species and concluded that the most efficient sampling procedure was to collect entire clutches. These authors emphasized that this scheme allows for the most efficient use of time and resources, while minimizing the impact on the reproductive success of the species. Their recommendations are valid for many species.

For the White-faced Ibis, I favor a different sampling design. Intuitively, from the data presented in this paper, the most efficient design would be one where the two significant sources of variation in shell thickness are eliminated. Thus, I recommend: (1) collecting eggs before incubation has progressed, and (2) collecting one egg per nest, sampling only the first eggs or only the second eggs laid. Of course, this plan is practical only when the egg laying sequence can be determined.

The following comparison illustrates the efficiency of my recommendations. Klaas et al. (1974) presented eggshell thickness data for the Black-crowned Night Heron (*Nycticorax nycticorax*) and reported the sample estimate of the coefficient of variation (C.V.) as 6.55. For White-faced Ibis eggs (this paper) taken during early incubation, the C.V. was 6.54, thus our samples are clearly comparable. Klaas et al. (1974:162) used a formula (Sokal and Rohlf 1969:247) for estimating the sample size necessary to detect a minimum

difference in mean eggshell thickness. These authors estimated that it would be necessary to collect 38 clutches (112 eggs) to show a 5% diffrence in shell thickness with eggs of the Black-crowned Night Heron. Using a different design, collecting one egg at random from a clutch, the authors calculated that 51 eggs would be required. I used the same formula, significance level, and power as Klaas et al. (1974) and calculated that only 34 eggs from the White-faced Ibis would be needed, providing the eggs collected were the first laid in each clutch. My sample for this calculation consisted of 16 eggs. With a similar sample, but taking the second eggs laid in each nest, it was estimated that 41 eggs would be needed.

I must qualify my recommendations where shell thickness comparisons involve museum eggs. The sequence of egg deposition in clutches preserved in museums is rarely, if ever, known, thus eggs in museums are samples which represent all orders of deposition and clutch sizes. The most comparable field sample then, would be either one egg at random from many clutches or the collection of entire clutches. However, for a comparison of shell thickness differences between years or geographical areas, it may be more efficient to eliminate the variability due to the sequence of egg deposition.

My suggested sampling scheme may be the most practical for colony-nesting species. For birds such as the White-faced Ibis, proper synchronization of nesting activities may influence reproductive success; therefore renesting, if it occurs at all, may have much poorer success (unpublished data). Also, there may be more nestlings than adults can feed, and many do not survive. Thus, taking one egg from a nest may not hinder reproductive output. In species possessing these characteristics, the impact of egg collecting may be minimized by selecting one egg per clutch rather than entire clutches. In other species, it may be desirable to collect entire clutches, rather than single eggs. Hence, when collecting wild bird eggs to detect differences in eggshell thickness, one should consider the biology and behavior of the species as well as the factors which contribute to eggshell variability.

SUMMARY

Eggs of the White-faced Ibis were examined for natural variability in shell thickness. Eggs collected soon after they were laid had thicker shells than those collected just prior to hatching. Eggshell thickness also varied with the order in which eggs were laid. Different clutch sizes (3 or 4 eggs) did not contribute to the variability in shell thickness. A design for collecting eggs to determine shell thickness is suggested. The most efficient sampling scheme for eggs of the White-faced Ibis, and perhaps other species, involved collecting only one egg per nest rather than entire clutches.

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UTAH COOPERATIVE WILDLIFE RESEARCH UNIT, UTAH STATE UNIV., LOGAN 84322 (PRESENT ADDRESS: SCHOOL OF NATURAL RESOURCES, UNIV. OF VERMONT, BURLINGTON 05401). ACCEPTED 29 OCT. 1975.