Bull. Environ. Contam. Toxicol. (1985) 35:173-182

Bulletin of Environmental Contamination and Toxicology

Changes in Eggshell Thickness during Incubation: Implications for Evaluating the Impact of Organochlorine Contaminants on Productivity

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The impact of organochlorine contaminants on the productivity of avian populations and individuals often is assessed by evaluating the relationship between eggshell thickness and contaminant levels in eggs (Blus et al. 1974; Ohlendorf et al. 1979; Laporte 1982). A decrease in eggshell thickness of about 20% is considered to indicate low reproductive success for a population or individual (Anderson and Hickey 1972). Eggshell thinning of this magnitude can be induced by organochlorine contaminants (see Mendenhall et al. 1983 for discussion). Thus, when the presence of organochlorine contaminants and the occurrence of shell thinning is observed in a sample of eggs, the interpretation that organochlorines are adversely impacting the reproductive performance of the birds is made. This approach is reasonable as long as other factors do not substantially reduce eggshell thickness. Since eggs collected by field researchers contain embryos at various stages of development, the amount of eggshell thinning which occurs as the embryo develops is of particular interest in this context.

The eggshell is an important source of calcium for the developing embryo (Romanoff and Romanoff 1949). In domestic fowl, the weight of the eggshell decreases during incubation by about 5% (Simkiss 1967). Vanderstoep and Richards (1970) noted that changes in shell thickness and weight of eggs began during the last quarter of the incubation period. Eggshell thinning during incubation is not well documented for nondomestic species. In a laboratory study with Coturnix quail (Coturnix coturnix), the shells of fully incubated eggs were 7.3% thinner than those of unincubated eggs (Kreitzer 1972). Field studies have shown that the shell thickness of naturally incubated eggs of cedar waxwings (Bombycilla cedrorum) decreased throughout incubation (Rothstein 1972), but such changes were not significant for brown pelicans (Pelecanus occidentalis; Anderson and Hickey 1970) or for several heron species (Ohlendorf et al. 1979; Laporte 1982).

The relationship between eggshell thickness and egg residue levels can be evaluated without reference to developmental stage of the embryo if the amount of eggshell thinning during incubation is small. If it is large, then adjustments are required before attributing eggshell thinning to the adverse effects of environmental contaminants. This study was conducted to determine the amount of eggshell thinning that takes place during the incubation period of several species of birds. If significant eggshell thinning did occur, the time at which these changes are detectable was estimated.

MATERIALS AND METHODS

Eggs from five species were used to evaluate the change in eggshell thickness during incubation: mallard (*Anas platyrhynchos*), black-crowned night heron (*Nycticorax nycticorax*), American kestrel (*Falco sparverius*), barn owl (*Tyto alba*), and screech owl (*Otus asio*). The mallard represented species with precocial young, whereas the others represented species with altricial young. Eggshell thickness of all five species is known to decline when the birds are exposed to DDE (Ohlendorf et al. 1979; Mendenhall et al. 1983).

Mallard eggs were obtained from a commercial source (Whistling Wings, Hanover, IL)[1]. These eggs were randomly assigned to one of 11 incubation times (Table 1). For all other species, eggs were taken from captive pairs maintained at the Patuxent Wildlife Research Center, Laurel, MD. Captive birds were fed Nebraska brand bird-of-prey diet. Diets of raptors were supplemented with rats or day-old chicks. Eggs were removed as they were laid and were numbered sequentially. Since the laying sequence and the pair from which the egg was obtained were known for each egg, a

Table 1. Sampling times during incubation for each species.

Species	Sampling times (days)									
Mallard	0	3	6	9	12	15	18	20	22	24	P[a]
Black-crowned Night Heron	0	7	11	14	16	18	P				
American Kestrel	0	9	14	18	20	22	24	P			
Barn Owl	0	10	15	20	23	26	29	P			
Screech Owl	0	14	18	22	P						

[a]Eggs left to pipping. Pipping normally occurred at 25, 20, 25, 30, and 26 days, respectively, for the species listed.

[1]Use of trade names or names of suppliers is for identification purposes only and does not constitute endorsement by the Federal government.

latin square design was used to assign each egg to one of several sampling times during incubation (Table 1). This design allowed the variation due to laying sequence and pair differences to be eliminated from the experimental error but required that the number of sampling times equal the number of eggs laid.

Eggs from all species were artificially incubated in a Petersime incubator (Model No. 5) at 37.6^{o} C and 55% relative humidity. Eggs were removed from the incubator on the designated sampling day and candled to determine viability. Length and breadth of each egg were measured to the nearest 0.01 cm. Eggs were opened at the equator, the embryo discarded, and the inside of the eggshell rinsed with running water. Eggshells were air-dried a minimum of 30 days before weighing to the nearest 0.01 g. Eggshell thickness was measured to the nearest 0.001 mm by interpolating from the scale of a dial indicator with rounded contact points. Several measurements of each egg were taken at equidistant points around the equator and averaged to obtain one measure of eggshell thickness. The thickness index which is a ratio of eggshell weight to egg size (Ratcliffe 1967) was calculated as a second measure of eggshell thickness. When membranes were separated from the eggshell, only this index could be used to measure eggshell thickness.

Changes in shell thickness (mm) and in the thickness index were evaluated by analysis of variance (ANOVA). If significant differences were found, Bonferroni multiple comparison procedures (Neter and Wasserman 1974) were used to determine at which stages of incubation the shell thickness or thickness index differed from that of unincubated eggs. The significance level for each group of comparisons was 0.05.

When data for the latin square design were insufficient to evaluate changes in eggshell thickness, additional eggs were obtained from pairs in the breeding colonies. These eggs were either left unincubated or incubated to pipping and were handled and measured in the same manner as study eggs. Data from these eggs were combined with data from the eggs in the original study. An ANOVA blocked on pair was used to compare the shell thickness and thickness index of these unincubated and pipped eggs.

The amount of variation associated with design factors was estimated for both measures of eggshell thickness by assuming a random effects model and equating mean squares with their expected values (Neter and Wasserman 1974). The latin square design used for heron and raptor species allowed the variation associated with two types of factors to be estimated. One source of variation related to characteristics which differed between pairs. The second source related to characteristics which differed between individual eggs of pairs, such as incubation stage and laying order. Only the variation associated with incubation stage could be estimated for the measurements from mallard eggs.

RESULTS AND DISCUSSION

Shell thickness and thickness index of mallard eggs changed little until the last few days of the incubation period (Fig. 1). The mean thickness index of eggs incubated 24 days and of pipped eggs was significantly ($P<0.001$) less than that of unincubated eggs. No significant changes in shell thickness or thickness index of black-crowned night heron eggs were found during incubation (Fig. 2). The shell thickness of American kestrel eggs differed significantly ($P<0.01$) among incubation times, but the thickness index of these eggs did not differ (Fig. 3). The apparent increase in shell thickness at day 9 and 20 was a result of somewhat larger eggs being assigned to these sampling times. The thickness index did not reflect these increases because this measure of eggshell thickness adjusted for the size of the egg.

Less than 50% of the latin square was completed for barn owls and screech owls. Some pairs laid few or no eggs and those eggs that were laid experienced high mortality in the incubator. Therefore, the analysis for these two species was based only on unincubated and pipped eggs. The mean thickness index of pipped barn owl eggs was significantly ($P<0.05$) less than that of unincubated eggs (Table 2), but the mean shell thickness of the two groups did not differ. The shell thickness and thickness index did not differ between unincubated and pipped screech owl eggs.

Incubation stage accounted for only 2.3% and 11.5% of the variation in shell thickness (mm) and in the thickness index, respectively, of mallard eggs. Likewise, the percent of the total variation associated with incubation stage for heron and raptor species generally was small for both measures of eggshell thickness (Table 3). The laying order of heron eggs was associated with a moderate amount of variation, but measurements from kestrel eggs did not show such a relationship. Although the differences between pairs were typically a significant ($P<0.01$) source of variability, the percent of variation due to all between-egg factors was as large or larger than that due to differences between pairs. This result may not hold for free-ranging birds since captivity may reduce the differences between pairs in factors, such as diets, which influence eggshell thickness.

Generally, the amount of eggshell thinning observed for the species in this study was well below the amount of eggshell thinning which has been related to adverse reproductive effects. Because the amount of eggshell thinning which occurred during incubation was small, the relationship between eggshell thickness and egg residue levels can be used to evaluate the impact of organochlorine contaminants on the reproductive status of birds without reference to the development stage of the embryo. Eggs from precocial species with pipping embryos should be used cautiously in this assessment.

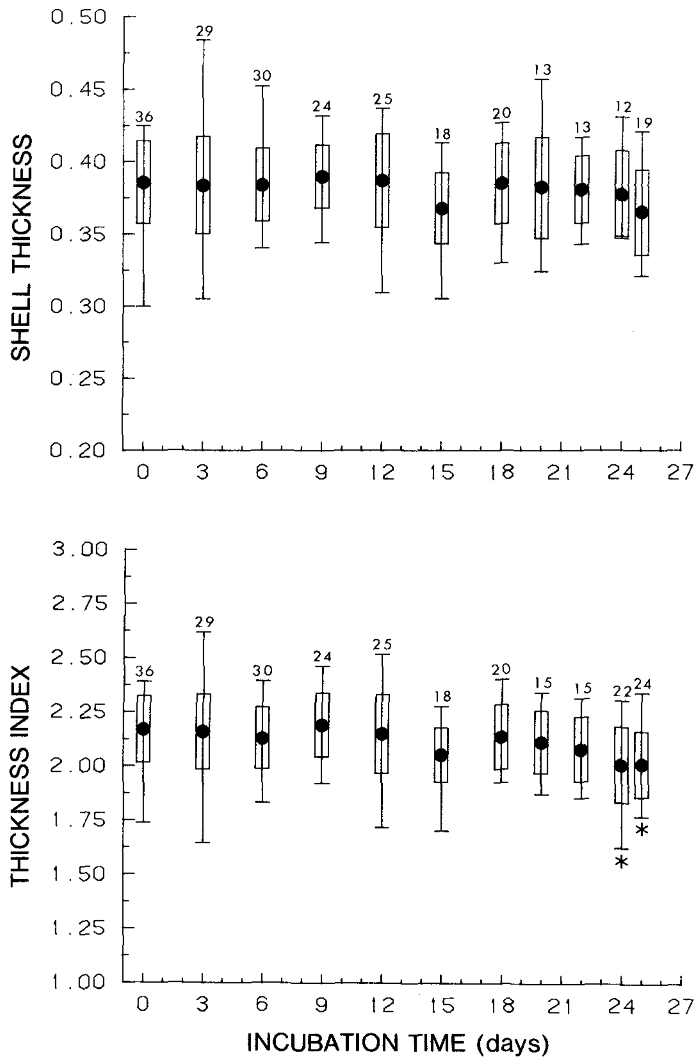

Figure 1. Shell thickness (mm) and thickness index of mallard eggs during incubation. Means are indicated by circles. The mean $\pm$ 1 standard deviation is represented by the box. Ranges are indicated by the lines. Numbers above each mean indicate sample size. Means which are significantly ($P<0.05$) different from the mean of unincubated eggs are identified with an asterisk.

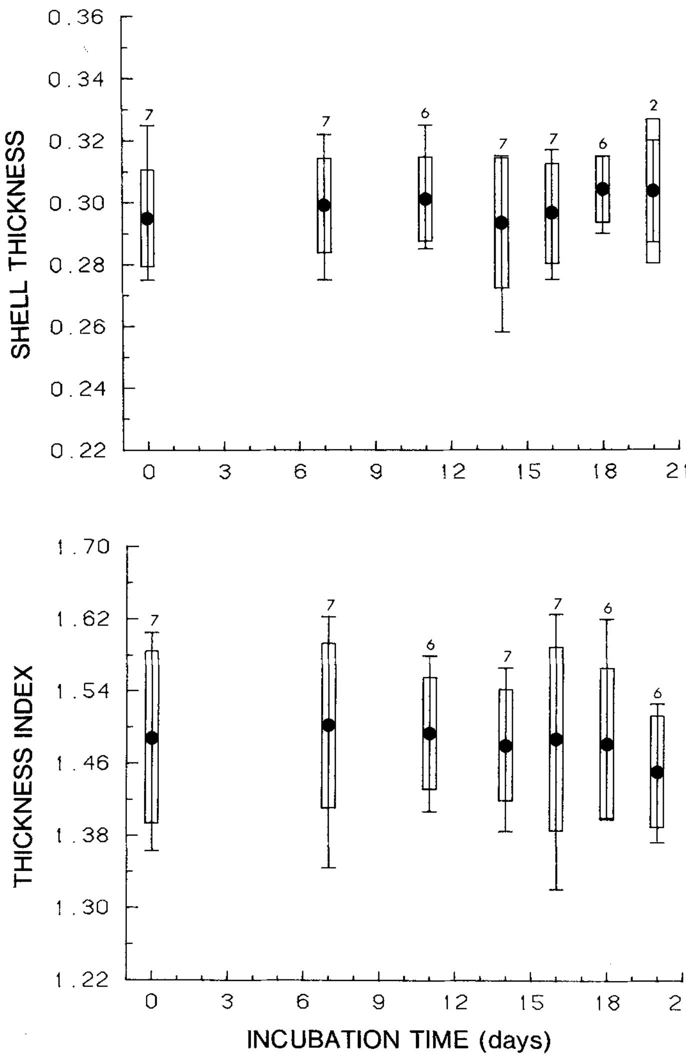

Figure 2. Shell thickness (mm) and thickness index of black-crowned night heron eggs during incubation. Means are indicated by circles. The mean ± 1 standard deviation is represented by the box. Ranges are indicated by the lines. Numbers above each mean indicate sample size.

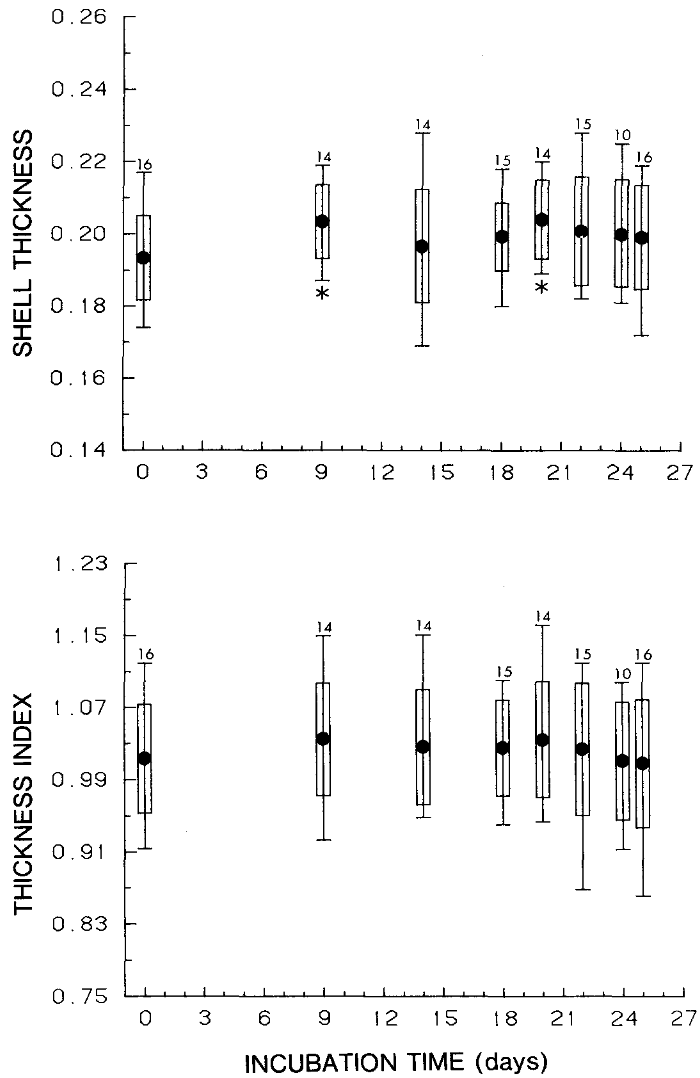

Figure 3. Shell thickness (mm) and thickness index of American kestrel eggs during incubation. Means are indicated by circles. The mean ± 1 standard deviation is represented by the box. Ranges are indicated by the lines. Numbers above each mean indicate sample size. Means which are significantly ($P<0.05$) different from the mean of unincubated eggs are identified with an asterisk.

Table 2. Mean shell thickness and thickness index of unincubated and pipped eggs from 5 species of birds.

Species	Shell Thickness (mm)				Thickness Index			
	No. pairs	Unincubated	Pipped	% Change	No. pairs	Unincubated	Pipped	% Change
Mallard		0.386[a]	0.365[b]	-5.6 (-11.5, +0.4)[c]		2.17[a]	2.01[d]	-7.2[e] (-12.1, -2.3)
Black-crowned Night Heron	7	0.295	0.304	+2.9 (-4.0, +9.9)	7	1.49	1.45	-2.5 (-7.8, +2.8)
American Kestrel	16	0.193	0.199	+3.0 (-0.9, +6.9)	16	1.01	1.01	-0.5 (-4.6, +3.6)
Barn Owl[f]	11	0.310	0.303	-2.4 (-5.9, +1.0)	12	1.62	1.56	-3.8[e] (-7.3, -0.4)
Screech Owl[f]	5	0.231	0.233	+0.8 (-4.0, +5.3)	4	1.24	1.18	-4.7 (-10.9, +2.0)

[a] Mean based on 36 eggs.
[b] Mean based on 19 eggs.
[c] 95% confidence interval based on analysis of variance results.
[d] Mean based on 34 eggs.
[e] Mean of pipped eggs significantly ($P<0.05$) different from mean of unincubated eggs.
[f] Additional unincubated and pipped eggs were included with eggs from the original study design.

Table 3. Percent of variation associated with design factors in the analysis of eggshell thickness measurements for 4 species of birds.

Species	Shell Thickness				Thickness Index			
	Between pairs	Between eggs			Between pairs	Between eggs		
		Incubation stage	Laying order	Error[a]		Incubation stage	Laying order	Error[a]
Black-crowned Night Heron	54.0[b]	2.4	14.8[b]	28.8	36.1[b]	0.0	28.4[b]	35.5
American Kestrel	60.3[b]	5.5[b]	0.9	33.3	55.6[b]	0.6	1.5	42.3
Barn Owl	26.4[b]	4.2		69.4	27.2[b]	9.8[b]		63.0
Screech Owl	56.9[b]	0.0		43.1	10.0	18.4		71.6

[a]Variation not explained by factors in the study.
[b]Significant ($P<0.01$) sources of variation relative to amount associated with error. Tests based on appropiate mean square error ratios from the analysis of variance results.

Acknowledgments. We thank Paula Decker and Jeanne Grillo for preparation of the figures; Kathy Klimkiewicz and Stan Wiemeyer for reviewing the manuscript.

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Received August 4, 1984; accepted October 5, 1984.