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# Relative Sensitivity of Several Measures of Eggshell Quality to the Stage of Embryonic Development

R. S. Bennett

USEPA Environmental Research Laboratory, 200 SW 35th Street, Corvallis, Oregon 97333, USA

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Eggshell quality is often used as an indicator of the effects of environmental contaminants on avian reproduction. Although eggshell thickness has been used most commonly to measure quality, eggshell breaking strength has been shown to be a more sensitive indicator of contaminant effects in some situations (Carlisle et al. 1986, Bennett et al. 1988). As embryos develop, calcium is mobilized from the shell to the embryo for skeletal formation. Approximately 80% of the calcium in a chicken hatchling is derived from the shell (Romanoff 1967).

Bunck et al. (1985) found little change in the eggshell thickness during incubation of eggs from five avian species and concluded that "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." Vanderstoep and Richards (1970) found that embryonic development in chickens resulted in a gradual reduction in eggshell strength without affecting thickness. This study was designed to determine if embryonic development affected eggshell strength and weight per unit surface area in mallards (*Anas platyrhynchos*) and northern bobwhite (*Colinus virginianus*). Changes in these parameters during development could confound efforts to detect contaminant-related changes in eggshell strength if the number of days of embryonic development was not taken into consideration.

## MATERIALS AND METHODS

Eggs from 33 pairs of bobwhite producing at least five intact, fertile eggs within a week were incubated for 0, 5, 10, 15 or 20 days using a randomized block design. One hundred eighty intact, fertile mallard eggs were incubated for 0, 5, 10, 15, 20 or 25 days using a completely randomized design. Bobwhite eggs were collected from a colony at the U.S. Environmental Protection Agency Environmental Research Laboratory, Corvallis, OR. Mallards eggs were purchased from Whistling Wings, Hanover, IL. All eggs were incubated in Humidare® Incubators at 37.5°C and 60 to 80% relative humidity rotating 90° every 2 hours.

At the assigned stage of incubation, intact eggs with live embryos were measured for length and width to the nearest 0.1 mm and eggshell strength according to methods in Bennett et al. (1988) using a Tinius Olsen® Series 1000 Universal Testing Machine with platen surfaces advancing at 4 mm/min. Strength was measured as the load (kg) put on the equator of an egg positioned between two flat surfaces at the time of fracture. Eggs were cut at the equator using a high-speed rotary saw and the contents removed, except the shell membranes. Shells were air dried at room temperature for 2 days. Mean eggshell thickness was measured to the nearest 0.005 mm at four locations around the equator using a micrometer. All instruments were calibrated prior to each use. Eggshell weight was measured to the nearest 1 mg and standardized for egg size as the weight per unit of egg surface area ($mg/mm^2$). Because eggs approximate a prolate spheroid, surface area was estimated as:

$$\text{Surface area} = \frac{\pi\ \text{width}^2}{2} + \frac{\pi\ \text{length} \times \text{width}}{2}.$$

Changes in egg weight and eggshell quality parameters were evaluated using one-way analysis of variance (ANOVA) for mallards and ANOVA blocked on pairs for bobwhite. A one-sided Dunnett's test (SAS Institute Inc. 1989) was used to determine which, if any, developmental stages produced lower mean values than unincubated eggs, using a significance level of $\alpha$=0.05.

## RESULTS AND DISCUSSION

The treatment (i.e., embryonic development) did not have detectable effects on eggshell thickness in either species (Table 1). This is similar to the results of Bunck et al. (1985). The sample size of eggshell thickness measurements was reduced during the last sample period for both species because the shell membrane can easily separate from the crystalline structure. A significant reduction in eggshell strength was observed in bobwhite eggs at 20 days of incubation (17% less than controls) and mallard eggs at 20 days (15%) and 25 days (22%) of incubation (Table 1). Eggshell weight per unit surface area ($mg/mm^2$) was significantly reduced in bobwhite eggs at 20 days of incubation (8% lower than controls), but a significant change was not detected in mallard eggs.

Although there was no evidence of change in eggshell thickness during embryonic development in bobwhite and mallards, eggshell strength and weight was significantly reduced during development, especially during the latter stages when calcium mobilization from the shell was greatest. The relationship between eggshell thickness and egg residue levels may be useful to evaluate contaminant effects without reference to the stage of embryonic development, but the relationship between eggshell strength and egg residue levels should be qualified by developmental stage. Because the interaction of contaminant-related changes on eggshell quality and changes related to embryonic development is unknown, eggs collected from the field for measurement of eggshell strength should be unincubated or early in embryonic development.

Table 1. Mean (± SE) egg weight and eggshell strength, thickness, and weight of bobwhite (n=33) and mallard (n=30) eggs at several stages of incubation. Values with asterisks are significantly different ($P < 0.05$) from unincubated eggs.

| Day of incubation | Egg weight (g) | Eggshell strength (kg) | Eggshell thickness (mm) | Eggshell weight (mg/mm$^2$) |
|---|---|---|---|---|
| | | Bobwhite | | |
| 0 | 9.80±0.13 | 1.211±0.032 | 0.225±0.000 | 0.396±0.005 |
| 5 | 9.48±0.13* | 1.231±0.033 | 0.230±0.003 | 0.394±0.004 |
| 10 | 9.25±0.13* | 1.236±0.043 | 0.226±0.003 | 0.394±0.004 |
| 15 | 9.09±0.13* | 1.207±0.040 | 0.224±0.003 | 0.393±0.004 |
| 20 | 8.78±0.13* | 1.006±0.033* | 0.229±0.008 | 0.363±0.005* |
| | | Mallard | | |
| 0 | 63.78±0.76 | 3.402±0.111 | 0.406±0.005 | 0.811±0.011 |
| 5 | 63.35±0.93 | 3.246±0.120 | 0.410±0.005 | 0.823±0.010 |
| 10 | 61.16±1.21 | 3.139±0.130 | 0.393±0.006 | 0.795±0.011 |
| 15 | 61.28±1.45 | 3.224±0.139 | 0.411±0.009 | 0.825±0.021 |
| 20 | 59.08±0.74* | 2.890±0.085* | 0.391±0.005 | 0.778±0.026 |
| 25 | 56.96±0.80* | 2.642±0.102* | 0.397±0.012[1] | 0.773±0.014 |

[1] Sample size was 13 due to degradation of eggshell.

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