J. Field Ornithol., 66(3):418–423

AIR CELL DEVELOPMENT AND MASS CHANGES IN EASTERN BLUEBIRD EGGS DURING INCUBATION

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Abstract.—When Eastern Bluebird (*Sialia sialis*) eggs were laid no air cells were present; 12 h later air cells had an average diameter of 5.3 mm. Following this rapid initial development, air cells increased in diameter an average of 0.59 mm/d and 14 d after laying had an average diameter of 13.0 mm. Initial development of air cells was enhanced by cool air temperatures while subsequent development was dependent on heat. Air cells developed faster after the onset of incubation. Air cell size can be used to determine the sequence of laying and to estimate the age of eggs, but such applications are limited by variations in the rate of air cell formation. Eggs lost an average of 0.38 g, or 12.2% of their initial mass, during incubation. Air cell diameter on the day before hatching was correlated with mass loss during incubation.

DESARROLLO DE CELDAS DE AIRE Y CAMBIOS EN PESO EN HUEVOS DE *SIALIS SIALIS* DURANTE LA INCUBACIÓN

Sinopsis.—Cuando se depositaron huevos de *Sialis sialis* no había celdas de aire presentes; 12 h después las celdas de aire tenían un diámetro promedio de 5.3 mm. Tras este rápido desarrollo inicial las celdas de aire crecieron en diámetro a razón de 0.59 mm día^{-1} y 14 días tras la eclosión tenían un diámetro promedio de 13.0 mm. El desarrollo inicial de celdas de aire fue apoyado por bajas temperaturas del aire mientras que el desarrollo subsiguiente dependió del calor. Las celdas de aire se desarrollaron más rápidamente después del comienzo de la incubación. El tamaño de la celda de aire puede utilizarse para estimar la secuencia y la edad de los huevos, pero éstos usos están limitados por las variaciones en la razón de formación de la celda de aire. Los huevos perdieron un promedio de 0.38 g, o un 12.2% de su peso inicial, durante la incubación. Se correlacionó el diámetro de la celda de aire el día antes de la eclosión con la pérdida en peso durante la incubación.

As embryonic development occurs in an egg, the size of the air cell at the blunt end of the egg increases. The relative size of the air cell has been used for many years as an indicator of the sequence of laying or the stage of incubation (Westerkov 1950). During incubation eggs lose, as water vapor, between 10 and 23% (mean = 15%) of their initial mass (Ar and Rahn 1980). The size of the air cell is proportional to the amount of water vapor lost (Carey 1983). Air cell development and egg mass loss during incubation have been described for many species (Ar and Rahn 1980); however, for Eastern Bluebirds (*Sialia sialis*) the only documentation of egg mass loss is from 24 eggs weighed by Hamilton (1943) and one clutch of five weighed by Pinkowski (1974). I am not aware of any reports describing air cell development in Eastern Bluebirds.

Eastern Bluebirds lay 2–6 eggs per clutch at the rate of one per day, typically begin incubation on the day the penultimate egg is laid, and have a 13–14 d incubation period during which the eggs are incubated only by the female, who periodically leaves the eggs unattended during the day (Pitts 1976).

METHODS

During 1981–1987 and 1993 I studied eggs of Eastern Bluebirds using nest boxes in Obion and Weakley Counties, Tennessee. Nests were checked daily during egg laying and every 1–3 d during incubation. I assumed that each egg was laid at 0700 hours CST (Hamilton 1943; Pitts, unpubl. data). Each egg was numbered with a soft lead pencil on the day it was laid. A dial caliper, which could be read to 0.01 mm, was used to measure air cell diameter, maximum egg length, and maximum egg width; all measurements were rounded to the nearest 0.1 mm for calculations. Eggs were weighed with either an Ohaus 1010 Reloading Balance (sensitivity = 0.013 g) or an Acculab Model 221 electronic digital scale (sensitivity = 0.01 g). The mass and air cell diameter of each egg were measured 1–15 times during incubation. SAS (SAS Institute, Inc. 1988) was used for statistical analysis and graphics.

RESULTS

I made a total of 630 air cell measurements on 262 eggs in 145 clutches, including multiple measurements, separated by varying intervals, on some, but not all, eggs. At the time of laying an Eastern Bluebird egg does not have an air cell. On several occasions I flushed the female from a nest containing a recently laid egg without an air cell; as I held the egg or as I measured other eggs in the clutch, an air cell formed in the new egg. Air cells initially consisted of several small bubbles which coalesced into one; usually the air cell was clearly developed and had smooth, distinct margins within 2 h of laying. Rarely (less than 0.5% of eggs), the air cell formed in the pointed end of an egg. In one case I observed air cell formation in a recently laid egg, but an hour later the air cell had disappeared; at my next inspection, 24 h later, the egg had a normal air cell.

The average air cell diameter 24 h after laying was 5.3 mm (n = 79, SD = 1.24). Following this rapid initial stage of development, air cells increased in diameter an average of 0.59 mm per day; 14 d after laying, the average diameter of air cells was 13.0 mm (n = 32, SD = 1.00). The largest air cell I observed, 15.3 mm or 90.5% of the egg diameter, was measured 15 d after laying. Figure 1 illustrates air cell development. Westerkov (1950) noted that within a species large eggs will have larger air cells than small eggs at the same stage of incubation. Egg width varied from 15.1 to 18.4 mm (n = 262, mean = 16.48, SD = 0.53). To compensate for this variation I plotted air cell size as a percentage of the egg width (Fig. 2).

Air cell development was slower prior to the onset of incubation than during incubation. This was determined by comparing air cells of first eggs (which are not incubated until the fourth or fifth egg is laid) and the fifth eggs (which are incubated immediately and regularly following laying) in clutches of five. After each egg had been in the nest 3 d, the average air cell diameter of the first egg in 24 clutches was 7.98 mm whereas the average air cell diameter of the fifth egg in 15 clutches was

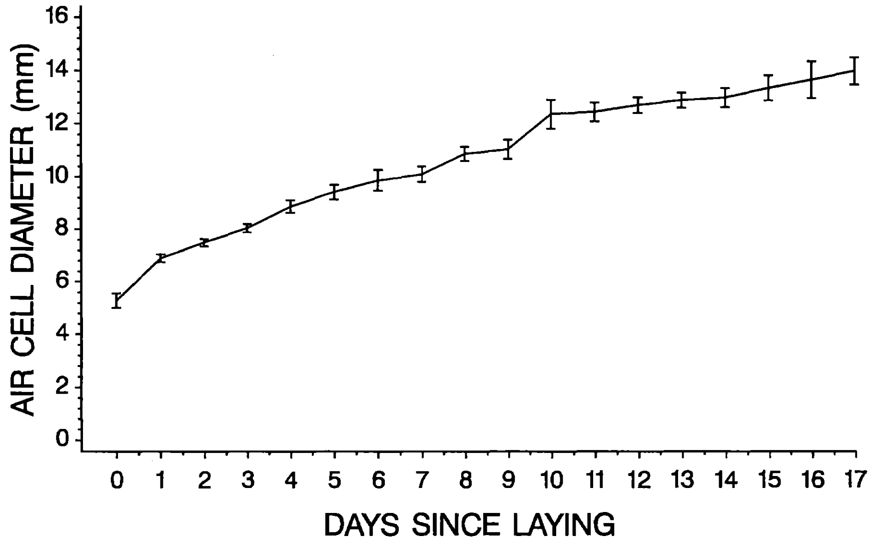

FIGURE 1. The relationship between air cell diameter and the number of days since laying. Means are connected and each bar represents ± 1 SE. Regression equation for air cell diameter on days since laying: y = 6.26 + 0.52x; n = 630; r^2 = 0.87.

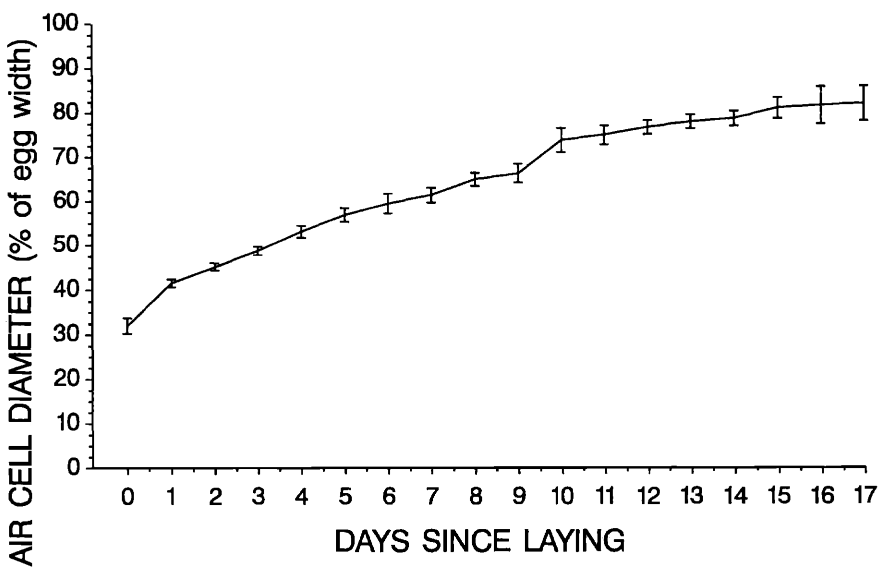

FIGURE 2. The relationship between air cell size (=air cell diameter × egg width^{-1} × 100) and the number of days since laying. Means are connected and each bar represents ± 1 SE. Regression equation for air cell size on days since laying: y = 37.84 + 3.16x; n = 630; r^2 = 0.88.

TABLE 1. Air cell formation in individual eggs in a clutch of four during laying and the first 3 d of incubation.

Egg #	Air cell diameter (mm)[a]					
	Day 1[b]	Day 2	Day 3	Day 4	Day 5	Day 6
1	6.1	7.7	8.0	8.5	8.3	8.9
2	—	5.0	7.8	7.8	8.3	8.9
3	—	—	5.0	7.7	8.2	8.8
4	—	—	—	6.7	7.7	8.8

[a] Measured at approximately 1300 hours each day.
[b] Day the first egg was laid; incubation began on Day 4.

8.95 mm (t = 6.15, $P <$ 0.0001). As a result of the possibility that egg size might vary enough to cause differences large enough to affect this conclusion, I calculated air cell/egg diameter ratios; these also differed significantly (first eggs mean = 0.49, fifth eggs mean = 0.54; t = 5.51, P $<$ 0.0001).

Prior to incubation, air cells increased in size faster in early nests than in later nests. I measured air cells of 71 eggs that had been in the nest 1.2–1.6 d and were either first, second or third in the laying sequence. These eggs had undergone the initial stages of air cell formation but had not been incubated. The eggs were divided into three groups; group 1 was laid before 1 May, group 2 was laid 1 May–29 June, and group 3 was laid 30 June–28 August. Air cells in group 1 (n = 25) averaged 44.2% of the egg diameter, air cells in group 2 (n = 32) averaged 40.1% of the egg diameter, and air cells in group 3 (n = 14) also averaged 40.1% of the egg diameter. The air cells in group 1 were significantly larger than the air cells in groups 2 (t = 5.87, $P <$ 0.00001) or 3 (t = 4.64, $P <$ 0.00001).

Within 1–3 d after laying each egg usually had an air cell similar in size to the air cell of the preceding egg. For example, in Table 1, eggs 1 and 2 had similar air cells 24 h after egg 2 was laid. Two days after the last egg was laid, all eggs in the clutch had air cells of similar size.

The average mass of 101 eggs from 26 clutches that had not been incubated was 3.12 g (range = 2.27–3.58, SD = 0.29). During incubation each egg lost an average of 0.38 g (range = 0.19–0.63, SD = 0.09); the percentage mass loss was 5.5–23.7 with a mean of 12.2. The average mass loss (assuming an incubation period of 14 d) was 0.027 g per day. In 70 eggs whose mass and air cell size I measured on the day of laying and again on the day before hatching, mass loss and air cell size were significantly correlated (r = 0.84, $P <$ 0.0001).

DISCUSSION

Air cell diameter of Eastern Bluebird eggs increased throughout incubation. Due to the rapid initial stage of air cell formation the rate of increase was highest during the first day; following the onset of incubation, the increase was approximately linear. A similar pattern of development occurs in Red-winged Blackbird (*Agelaius phoeniceus*) and American Robin (*Turdus migratorius*) eggs (Carey 1979). Use of the air cell/egg width ratio (Fig. 2) is slightly more accurate for determining the age of an egg but has the disadvantage of requiring two measurements.

The initial stage of air cell formation was apparently enhanced by cool temperatures; unincubated eggs in the later, warmer part of the nesting season developed air cells more slowly than did eggs laid in the earlier, cooler part of the nesting season. This difference is consistent with the statement of Romanoff and Romanoff (1949:657) that air cells initially form as a result of contraction of the egg contents when the egg is cooled below the temperature of the bird's body; presumably, the greater the difference between egg temperature and air temperature at the time of laying, the faster an air cell would form. Following the initial stage of formation, air cell size was related to the amount of embryonic development, which is stimulated by warming the egg.

The rate of air cell growth was affected by several factors. As noted above, the initial stage of air cell formation occurred more rapidly in early clutches than in later clutches. The sequence of an egg in a clutch may also affect the rate of development as air cells formed faster during incubation than prior to incubation. This is in contrast to the conclusion of Dunn et al. (1979) who found that the onset of incubation does not affect the rate of air cell formation. Air cells in first laid eggs in a clutch may develop more slowly than in the last laid egg because the first eggs will not be incubated for 2–4 d (depending on clutch size), whereas the last egg will be incubated immediately after laying. Air temperature also affected the rate of air cell development.

No information is available on the temperature variations that normally occur in Eastern Bluebird eggs during incubation. Incubation results in the maintenance of a fairly constant egg temperature in most passerines (White and Kinney 1974), but I suspect that individual Eastern Bluebirds differ in their attentive-inattentive patterns sufficiently to cause some variation in egg temperature which in turn affects air cell development.

Relative sizes of air cells can be used to determine the sequence of laying in some clutches. Eggs that have been together in the same nest for 48–72 h will usually have air cells of similar size (Table 1). As a result of potential measuring errors, I consider a difference of 0.5 mm between two air cells to be the minimum for determining egg sequences; if two air cells differ in size by less than 0.5 mm, I think it is not safe to distinguish between the ages of the eggs. If an investigator is attempting to document the sequence of laying with the minimum number of nest inspections, I recommend that the visits be no more than 48 h apart during the egg laying stage. For example, if the nest for Table 1 had been inspected on days 1, 3 and 5 or on days 2 and 4, each of the eggs could have been assigned its correct position in the sequence. If inspections had been made on days 1 and 4, or on days 1 and 5, however, at least two eggs could have been incorrectly assigned because differences in their air cells were less than 0.5 mm.

The average mass loss I recorded during incubation (12.2%) is similar to the loss of 12.6% reported by Hamilton (1943) and 13.2% reported by Pinkowski (1974) for Eastern Bluebirds. The range in mass loss I observed in successfully hatching eggs (minimum mass loss = 5.5%, maximum mass loss = 23.7%; range = 18.2%) is much greater than the range found by Hamilton (minimum mass loss = 11.8%, maximum mass loss = 13.4%; range = 1.6%). Carey (1983) pointed out that by emphasizing average values of water loss for species, the importance of variability may have been overlooked. She noted that water loss can vary as much as 3.5-fold in successfully hatching eggs, a conclusion that my observations support.

ACKNOWLEDGMENTS

This manuscript was prepared while I was on an Alma and Hal Reagan Faculty Development Leave from the University of Tennessee at Martin. I thank Ken Yasukawa, Donald F. Hoyt, and an anonymous reviewer for comments on the manuscript.

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Received 8 Apr. 1991; accepted 29 Dec. 1994.