THE DETERMINATION OF INCUBATION STAGE IN STARLING EGGS

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INTRODUCTION

Field workers often wish to determine the stage of incubation of eggs when the dates of laying are not known. Hays and LeCroy (*Wilson Bull.*, **83:** 425–429, 1971) summarized studies on precocial and semiprecocial birds in which eggs were aged by the degree to which they floated in water. In this study on the Starling (*Sturnus vulgaris*), we show that the eggs of an altricial species can be aged in the same way. Furthermore, the sequence in which eggs are laid can be determined by the size of the air space at the blunt end of the egg, which is easily visible in the eggs of certain passerines.

MATERIALS AND METHODS

The study involved measurements of eggs in nearly 100 clutches laid by Starlings in nesting boxes near London Grove, Pennsylvania in 1974. Boxes were checked daily during the laying period of the first clutch, and frequently thereafter. Every egg was marked with dots made by an indelible black felt tip pen, the number of dots indicating its sequence in the clutch. At each visit, the diameter of the air space was measured with dial calipers.

Several nests were selected for periodic measurement of egg flotation. Eggs were placed in a beaker of tepid water, and the angle between a horizontal plane and the long axis of the egg was measured to the nearest 5° with a protractor. If the egg floated, the diameter of the portion above the surface of the water was measured with dial calipers.

Specific gravity was calculated for a series of 27 eggs of known age. Seventeen of these were collected after 3 to 12 days of incubation, the last egg in clutches of 4, or the fourth or fifth egg in clutches of 5; 10 others were collected 0 to 5 days after laying without regard to laying sequence. Normal incubation period is 12 days. Eggs were measured with dial calipers and weighed to the nearest mg on a Mettler balance. An index of the specific gravity was calculated by dividing the weight in g by the product of the egg length and the square of the egg diameter in cm^3. This index, divided by 0.524, (Romanoff and Romanoff, The Avian Egg, New York, Wiley, 1949: 108), provided an estimate of the specific gravity of the egg in g/cm^3. In assigning days of incubation it was assumed that incubation began on the laying of the fourth egg.

RESULTS

Fresh eggs sink in water. The blunt end of older eggs floats higher until the egg rests vertically on its narrow end, and still older eggs float to the surface. The change in the attitude of the egg in water is caused

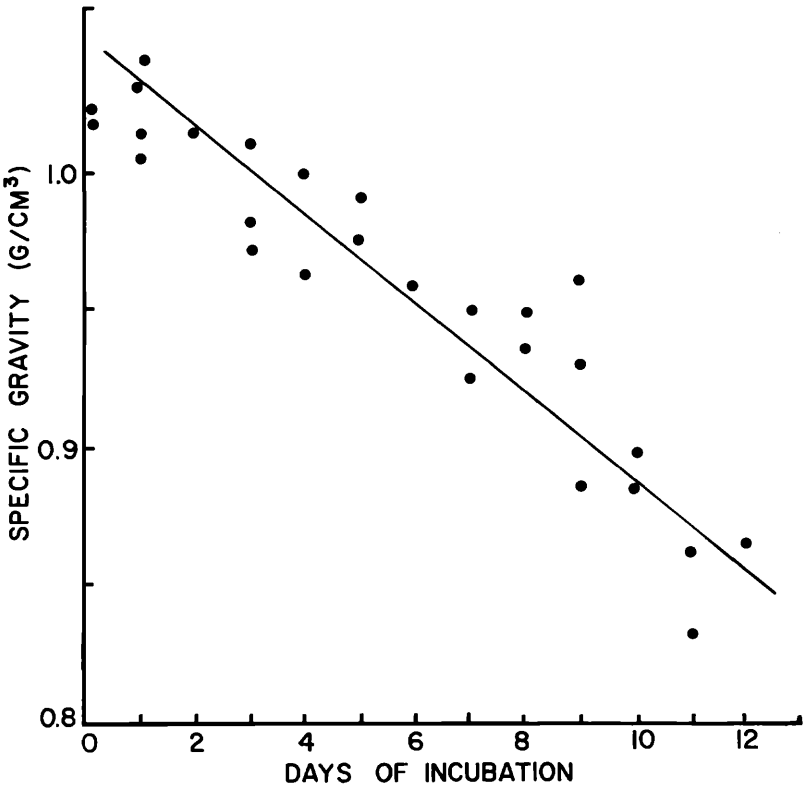

FIGURE 1. Specific gravity of Starling eggs as a function of stage of incubation. The regression equation is: specific gravity = 1.037 − 0.0141 days of incubation, $r = -0.931$, n = 27, standard deviation of specific gravity about regression line = 0.0214.

by a decrease in specific gravity (Fig. 1) resulting both from evaporation and respiration by the embryo. From specific gravity measurements alone, the number of days of incubation of the egg can be predicted with 95% confidence by the following regression equation: days of incubation = 64.64 − 61.60 (specific gravity) ± 2.83 days.

Figure 2 shows the degree of flotation in developing Starling eggs in relation to the age of the first egg laid. In clutches of five eggs, data for first through fourth eggs are combined, as there was no clear difference among them. Fifth eggs were noticeably retarded in development, suggesting that incubation started shortly after the fourth egg was laid (as suggested by Kessell, *Amer. Midl. Nat.*, **58:** 257–331, 1957). Eggs float to the surface 3 to 6 days after incubation commences, after specific gravity has fallen below 1.0 g/cm³ (Fig. 1).

The age of a Starling clutch can be reliably determined by the follow-

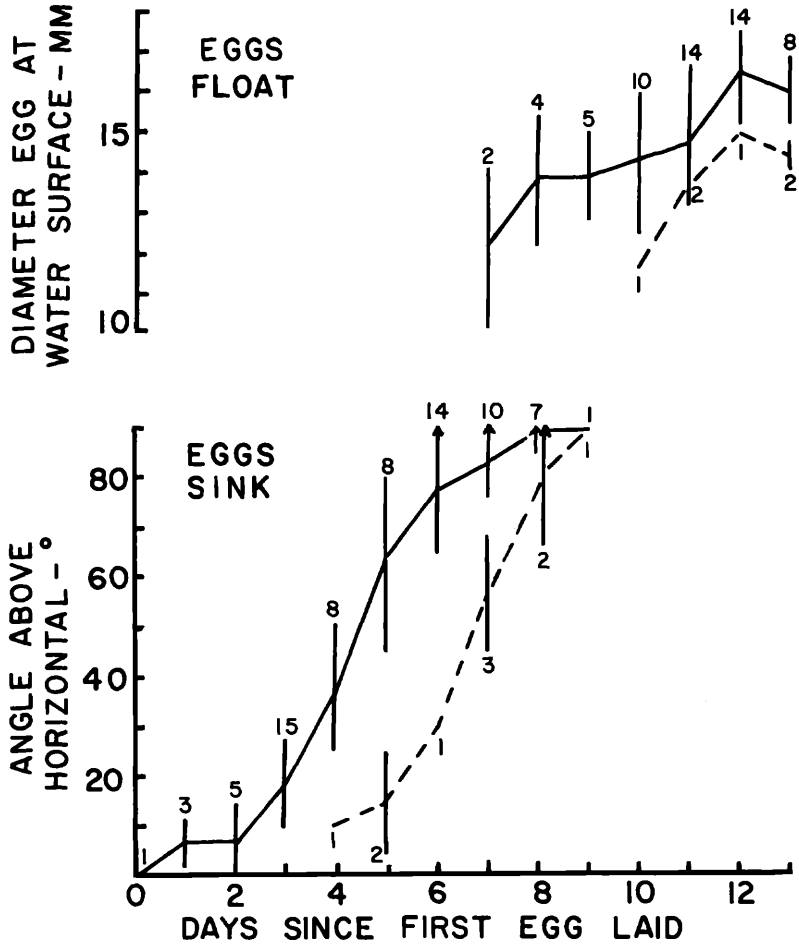

FIGURE 2. Pattern of flotation of Starling eggs as a function of age. Only eggs in clutches of 4 or 5 are included, and only those that hatched. The vertical bar gives SD, whereas the number above each bar is the sample size. Data for the first four eggs of each clutch are combined in the solid line, whereas data for fifth eggs are shown by the broken line.

ing flotation criteria: If no eggs float, the first egg was laid 0 to 5 days previously. If some but not all eggs float, the first egg was laid 6 to 9 days previously, and the clutch is certain to be complete. If all eggs float, the first egg was laid at least 10 days before, and hatching is likely to occur in the next 5 days (12 days after the laying of the fourth egg). (In clutches with more or fewer than 4 or 5 eggs, the commencement of incubation in relation to the laying of the first egg may vary.)

Air spaces begin to form shortly after the egg is laid and increase in

TABLE 1.

Diameter of air spaces in Starling eggs as a function of the number of days since laying.[1]

Days since laying	Mean (mm)	SD (mm)	n
0[2]	6.77	1.05	384
1	9.09	0.93	326
2	10.25	0.98	230
3	11.16	1.12	166
4	12.14	1.19	115
5	13.09	1.17	63
6	14.15	1.68	42
7	15.23	1.46	35
8	15.67	1.09	36
9	16.36	1.07	41
10	16.76	1.11	43
11	17.53	1.46	37
12	17.73	0.83	27
13	18.15	1.29	17
14	19.26	1.11	9
15	18.45	0.64	2

[1] All eggs from season combined, regardless of outcome.
[2] Excluding eggs whose air spaces had not yet formed.

size with the number of days since laying, unaffected by the onset of incubation. Romanoff and Romanoff (1949: 657) state that "Almost immediately after the egg is laid, the air cell is produced by contraction of the egg contents as they cool from the hen's body temperature .... Thereafter, the air cell grows larger as the egg contents evaporate."

Most freshly laid Starling eggs have no air space. When first visible, the air space appears as a faint light-colored patch having an irregular outline. In the first half hour, the air space does not enlarge, but it does become more distinct and its edges become smooth and round. In the next 48 hr, the diameter of the air space increases at a rate of about 0.25 mm/hr (based on 47 cases in which the same egg was measured more than once daily). Although the formation of the air space may begin at different times after laying, air spaces were always clearly visible within 3 hr of laying.

Table 1 gives the average size of air spaces as a function of age. Detailed analysis failed to demonstrate significant differences in rate of air space formation among different eggs in a clutch or between eggs in early and late nests.

The size of the air space increases with age, but variability in size also increases, and after the first 2 or 3 days, the size of the air space no longer provides an index of age accurate to one day. If nests are checked every 2 days during laying and eggs are marked, the sequence of laying can be ascertained with 100% accuracy. At intervals of three days, however, occasional errors would be made.

TABLE 2.

Summary chart for use in determining stage of a clutch on a single visit.

Flotation	Air space[1] (by late morning)	Specific gravity[1] (g/cm^3)	Days since laying of egg 1	No. eggs in clutch (if none lost)	Days of incubation of egg 1, if begun 3 days after laying
	If any air space is <7.23 mm, the egg was laid today. Clutch is incomplete or completed today.	Throughout development, days of incubation = 64.64 − 61.60 (specific gravity) ± 2.83 days.	0	1	
			1	2	
			2	3	
If no eggs float, egg 1 has 2 or fewer days of incubation.	If all air spaces >10.25 mm, clutch is complete.	If >1.03, the egg has 3 or fewer days of incubation.	3	4	0 (Incubation begins)
			4	(5)	1
			5		2
If some, but not all eggs float, egg 1 has 3–6 days incubation.		If >1.0, egg has 5 or fewer days of incubation.	6		3
			7		4
			8		5
			9		6

TABLE 2. Continued.

Flotation	Air space[1] (by late morning)	Specific gravity[1] (g/cm^3)	Days since laying of egg 1	No. eggs in clutch (if none lost)	Days of incubation of egg 1, if begun 3 days after laying
If all eggs float, egg 1 has 7–12 days of incubation.		If <0.9, egg has 7 or more days of incubation.	10		7
			11		8
			12		9
		If <0.86, egg has 10 or more days of incubation.	13		10
			14		11
			15		12
			Hatch		Hatch

[1] Statements have 95% confidence limits.

Table 2 summarizes all the indicators of egg development, and shows how they can be used to determine the status of a clutch with only a single visit to the nest. Measurement of air spaces shows whether the clutch is complete, and flotation indicates the general stage of incubation. If an accurate balance is available, the estimated specific gravity can be used to estimate more closely the stage of incubation. Thus, a field worker can quickly obtain valuable data on the status of Starling clutches with a minimum of effort and disturbance to the nest, and with little equipment or expertise.

Further work on passerine eggs should be conducted to see whether the pattern for Starlings is typical; Table 2 would be far more valuable to field workers if it also applied to other species with a similar incubation period.

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