RELATION OF "CLUTCH-SIZE" TO NUMBER OF OVA OVULATED BY STARLINGS

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THE CLUTCH SIZE of birds has been widely used as a measure of biological processes. The data can be obtained without killing the bird or performing any technical manipulation. However, the relation of the number of eggs actually ovulated to the number in the nest has not been examined except incidentally. The purpose of this contribution is to compare the number of eggs ovulated by Starlings (*Sturnus vulgaris*) with the number in the nest box. Problems of interpretation of clutch size will be discussed.

"Clutch" to an ornithologist means the number of eggs in a nest that are being incubated. It is generally assumed that the loss of eggs from accidents or predation is negligible and that the number in a clutch is essentially the same as the number ovulated. Yet, it has been known for a long time that eggs are occasionally found on the ground. Some species such as Rheas (*Rhea americana*) commonly scatter eggs for a week or so before starting to lay in a nest. Others such as ducks lay eggs in nests of other ducks or even pheasants. Pheasants (Kabat et al. 1948) have been shown to lay only part of their eggs in a nest. Among passerine birds the number of eggs lost has been considered to be negligible and the clutch size has been assumed to be a good measure of the egg production, although Davis (1955a:274) raised some doubts.

Interest in this problem arose from an attempt to determine whether Starlings are determinate or indeterminate layers. Davis (1955b) had shown that the clutch size for Barn Swallows and Magpies was no more or no less than normal when eggs were removed from or when eggs were added to the nests. Experiments of this type were begun on Starlings in 1953 and it was soon suspected that the number of eggs in the nest might not be a good measure of the effect of removing or adding eggs. Comparison was then made with the number of eggs ovulated; as determined by counting the number of post-ovulatory follicles.

METHODS

The work was conducted on the forested grounds of a hospital near Baltimore. Some 60 nest boxes (18 x 6 x 6 inches) were nailed to trees high enough to be accessible only by a ladder. The contents of the boxes were observed sufficiently frequently (daily during laying) to determine the date on which eggs appeared in the box. Some nests were not disturbed, other nests had 4 eggs added when the first egg appeared and

others had eggs removed as laid (see Davis 1955b for more details of procedure).

After the laying stopped, the female was captured on the nest. Birds were obtained at various stages of incubation and even a few days after the young hatched. The birds were caught during the day if possible, but some wary individuals had to be caught at night. The bird was killed in the laboratory and the entire ovary fixed in Bouin's solution. The ovaries from 1953-56 were all prepared histologically in 1956. The ovary was stained *in toto* in alumcochineal, sectioned serially, placed on large slides, and covered. The number of post-ovulatory follicles (POF) in each ovary was counted.

Recognition of post-ovulatory follicles: Some years ago in determing the number of eggs laid by certain cuckoos, through sectioning the ovary and counting the post-ovulatory follicles (Davis 1942a, 1942b), the confusingly similar appearance of burst atretic follicles was discovered (Davis 1942c). The bursting of atretic follicles presumably serves to remove large quantities of yolk material in large ova that undergo atresia. The granulosal and thecal layers of the follicle break, permitting the yolk to flow out of the follicle. However, the mesothelium of the ovary does not break so that the yolk is kept within the ovary. The burst atretic follicle (BAF) superficially resembles a post-ovulatory follicle (POF), but can be distinguished by lack of a break in the mesothelium and (usually) presence of yolk or yolk residue either in the follicle or around the outside. Also the BAF is circular rather than elliptical. These differences remain clear for many days and confusion could result only in the case of greatly regressed follicles. No confusion is likely for birds that are incubating or feeding young. It is possible to distinguish the age of various groups of follicles and Davis (1942d) showed that cowbirds ovulated several groups ("clutches") of eggs.

Definition of clutch: "Clutch" to a poultry worker (unlike the usage among ornithologists) is the number of eggs laid consecutively in a daily series. The "clutch" for the Domestic Fowl thus may be as small as 2. The term "cycle" is apparently used to refer to the length (in days) of the interval between days without laying, and does not refer to the number of eggs. Thus Heywang (1938) listed the eggs per cycle (clutch) and the number of such cycles; he reported that in his flock about half the hens had cycles of two days. For the rest of this paper, clutch will mean the number of eggs found in a nest. This pragmatic definition conforms to current ornithological usage and has the merit of being an item of data that can readily be obtained.

A problem arises in the assignment of the date of laying. The boxes were examined after 9 A.M. to give the birds time to lay before checking. However, in some cases an egg was surely laid after examination of the box and thus erroneously credited to the succeeding day. Actually, the true date of laying is not essential.

RESULTS

The number of post-ovulatory follicles and the number of eggs in the nest-box are given in table 1, as well as other pertinent data. It is

TABLE 1

DETAILED RECORDS OF CLUTCHES AND POST-OVULATORY FOLLICLES

Bird	*Year*	*Dates eggs laid*	*Clutch*	*Follicles*		*Date collected*	*Treatment*
				I	*II*		
986	1954	April 23, 26	2	5	2	April 29	Add
988	1954	Before April 20	5	6	—	April 24	Normal
989	1954	Before April 22	6	4	—	May 4	Normal
990	1954	Before April 20	5	4	—	May 4	Normal
992	1954	Before April 21	4	0	1	May 4	Normal
996	1954	May, 7, 8, 9, 10, 11	5	7	—	May 18	Subtract
1000	1954	Before May 24	3	4	—	June 9	—
1003	1955	April 15, 16, 17, 19	4	4	—	April 25	Subtract
1004	1955	April 17, 18, 19, 20	4	5	—	April 25	Add
1005	1955	April 12, 13, 14, 15, 16	5	5	—	April 25	Normal
1007	1955	April 18, 19, 20, 21, 22	5	2	5*	April 25	Subtract
1008	1955	April 15, 16, 17, 18, 19	5	5	—	April 25	Subtract
1009	1955	April 16, 17, 18, 19, 20	5	7	—	April 25	Normal
1012	1955	April 16	1	4	—	April 26	Add
1020	1955	April 15, 17, 18, 19	4	4	—	April 26	Subtract
1021	1955	April 17, 18, 19, 20	4	5	—	April 26	Add
1023	1955	April 15, 16, 17, 18, 19	5	3	—	April 26	Subtract
1024	1955	April 17, 18, 19, 20	4	6	—	April 26	Subtract
1025	1955	April 17, 20	2	4	—	April 26	Add
1026	1955	April 14, 15, 16, 17, 18, 19	6	6	—	April 26	Subtract
1027	1955	April 19, 21, 22, 23	4	5	3	April 26	Add
1037	1955	April 22, 23, 24, 25	4	5	4	April 29	Add
1038	1955	Before May 11	4	5	5	May 11	—
1040	1955	Before April 29	6	4	—	May 11	Normal
1052	1955	May 28	1	4	6	May 30	—
1053	1956	Before May 9	3	5	—	May 9	—
1054	1956	Not known	6	6	—	April 27	—
1055	1956	Not known	7	6	—	May 25	—
1056	1956	May 2, 3, 4, 5	4	4	—	June 3	—
6421	1954	Before May 24	4	0	5	June 10	—
4053	1954	Before June 9	3	1	5*	June 9	—
4486	1954	Before April 26	4	6	—	April 18	—

I Refers to recent follicles.

II Refers to an older group.

*These 2 birds also had 7 very old follicles.

apparent that some serious discrepancies occur between the clutch size and the number of post-ovulatory follicles. Among the 32 birds, clutch size and the number of POF are the same in only 7 cases. In 9 cases the clutch is larger and in 16 cases the clutch is smaller than the number of POF.

Consider some specific cases. Bird 996 had 7 POF but had only 5 eggs

in the box. These 5 eggs were laid in a daily sequence that would not arouse suspicion concerning interruptions. Based on the extent of regression of the POF, it seems likely that the missing eggs were dropped before the date (May 7) the first egg was laid in the box. Bird 1012 had only 1 egg in the nest but ovulated 4. Bird 1023 had 5 eggs in the nest but ovulated only 3. Bird 992 was feeding 4 young when caught in the box but had no POF in the ovary.

Actually in table 1 the eggs ovulated averages 4.38 which is almost the same as the clutch size of 4.22 because the gains from several birds laying in a nest cancel the losses of eggs.

The differences between clutch size and number of POF may result from several causes. Unfortunately, it seems difficult to assign a cause

TABLE 2

COMPARISON OF EGGS OVULATED AND CLUTCH SIZE (FIRST CLUTCHES)

POF or eggs	*Normal nests*		*Addition nests*		*Subtraction nests*		*Total nests*	
	Ovulated	*Clutch*	*Ovulated*	*Clutch*	*Ovulated*	*Clutch*	*Ovulated*	*Clutch*
1	0	0	0	1	0	0	0	1
2	0	1	0	1	1	0	1	2
3	0	0	0	0	1	0	1	0
4	3	5	2	6	2	6	7	16
5	1	3	5	2	1	9	7	15
6	1	1	0	0	2	1	3	2
7	1	0	0	0	0	0	1	0
	6	10	7	10	7	16	20	36
Mean	5.0	4.3	4.7	3.7	4.3	4.7	4.8	4.3

in a particular case and thus indicate the relative importance of the several possible causes. A clutch larger than the number of POF can obviously occur when several females lay in a single nest. For example, bird 1055 was collected specifically because two females were using the same box. A lower clutch size can occur in several ways. The bird may drop eggs on the ground. Eggs may ovulate into the body cavity. A bird (male or female) may remove eggs (e.g. males are known to throw eggs out when the female is killed). A predator, such as a Blue Jay, may remove single eggs. It is apparent that clutch size is a dubious measure for certain aspects of reproduction.

It seems unlikely that predation or disturbance of nests was entirely responsible for the discrepancies here observed. Starlings vigorously defend their nests against squirrels and Blue Jays. Although many hours were spent in the area both checking the clutch-size and observing behavior, no squirrel or jay was seen molesting a nest. When disturbance by

boys or male Starlings did occur, the eggs were generally seen on the ground below the nest.

The problem of determinate laying was examined from the viewpoint of the number of POF and the clutch size. The last column of table 1 (Treatment) shows whether the nest was unmolested (normal) or eggs were added (add) or eggs removed (subtract). Table 2 shows the number of eggs in normal nests, in nests to which eggs were added, and in nests from which eggs were subtracted. "Ovulated" refers to the number of POF seen in the ovary. "Clutch" refers to the number of eggs in the nest. For example, in nests to which eggs had been added there were 6 cases of a clutch of 4 and 2 cases of a bird that ovulated 4 eggs. Since there is no statistical difference among the "normals", "addition" and "subtraction" groups, all have been totaled in the last column. To restrict the data to first clutches, only birds that completed laying in April were used. (More data are available for clutch size than for eggs ovulated because not all females that laid were captured and their ovaries successfully sectioned.) While differences in the averages exist, the lack of consistency prevents one from concluding that the addition of eggs causes a reduction in the number laid and the removal of eggs causes an increase. It is, therefore, concluded that starlings are determinate layers.

DISCUSSION

The validity of conclusions about a contrast between clutch size and the number of ova ovulated depends upon the correct counting of the POF. In birds collected during the incubation period, the POF are large and readily identifiable under low power. Also BAF are easily distinguished. Some direct comparisons have been made. The number of eggs laid by captive pheasants was compared (Meyer *et al*, 1947) with the number of POF and a very close agreement obtained, even though the hens may have eaten some eggs. Kabat *et al.* (1948) found a very close correlation even for birds killed several months after laying. From studies of wild pheasants, Buss *et al.* (1951) concluded that many eggs are dropped on the ground and that desertion of a partial clutch is common. From these results it is concluded that the number of POF counted in birds killed during incubation is very close to the true number ovulated.

The evaluation of the extent of difference of clutch size and number of eggs ovulated requires a reconsideration of conclusions derived from a study of clutch size. For some purposes the difference is immaterial. For example, the productivity of a species depends upon how many eggs survive and the fact that some eggs never reach the nest does not affect

the use of clutch size to describe actual productivity. However, potential productivity may be affected and, if some factor affects the number of eggs wasted, then some management procedure may increase the clutch size by increasing the number actually reaching the nest.

If one is assessing the effect of some factor on clutch size then it may be essential to know the number of eggs ovulated. The alternative is to assume that the factor has no effect on the proportion of ovulated eggs that are placed in the nest. The generalities developed by Lack (1948) and Davis (1955a) need reexamination. For example, seasonal changes in clutch size might be due to changes in proportion of eggs put into the nest. The increase in clutch size in the early part of the laying period (see Davis 1955a for examples) may merely mean that a higher proportion of eggs is wasted at the beginning. The common finding that yearling birds lay fewer eggs than do adults may merely mean that yearlings have to learn to get the eggs into the nest.

While many additional examples could be cited, it seems only necessary to note that problems arise in interpretation. Unfortunately, for a variety of obvious reasons it is unlikely that many birds will be collected for examination of ovaries by the tedious procedure of serial sections. In some cases (gulls) the POF are macroscopically visible, but still the bird must be killed. Thus, there remains for most work simply an evaluation of the possibility of error due to the assumption that, in the groups whose clutch sizes are being compared, the number of eggs that exist in the nest is the same proportion of the total ovulated.

SUMMARY

The number of eggs ovulated by Starlings (*Sturnus vulgaris*) was determined by serial sections of the ovary and compared with the number (clutch) of eggs in the nest box of each bird. For 32 birds the number of ovulations was the same as the clutch-size in only 7 cases. The ovulations were greater in 16 cases and smaller in 9. A variety of causes can result in loss of eggs. An excess can result when 2 females lay in the same box.

The fact that clutch-size is not necessarily an indication of number of eggs ovulated means that interpretations of the biological implications of changes or differences in clutch-sizes need to be examined carefully.

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