NEW WAYS OF MEASURING THE INCUBATION PERIOD OF BIRDS

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As pointed out by Swanberg (1950) and Nice (1954) there has in the past been considerable confusion concerning the proper way to measure the length of the incubation period of birds. Of concern here is not the entire length of time that the adult sits on a clutch of eggs, but the minimum length of time required to bring an average, newly laid egg to hatching under normal nest conditions. For practical purposes it is now generally agreed that the incubation period should be considered the time from the laying of the last egg to the hatching of the last young when all eggs in the clutch hatch.

The confusion lies in the fact that the first eggs of a clutch may receive some heat and undergo a certain amount of development before the clutch is completed or full incubation begins. The eggs tend as a consequence to hatch in the order laid, but the intervals between hatching of the eggs is shorter than the intervals between their laying so that the time that elapses between laying of an egg and its hatching progressively decreases with each additional egg in the clutch. In those species, relatively few in number, where full incubation begins with the first egg laid, the intervals between hatching are more nearly equivalent to the intervals between their laying.

Even when the adult bird is observed sitting on the eggs frequently, or for long periods of time, in the egg-laying period, this does not necessarily mean that the eggs are being subjected to full incubation heat (Swanberg, 1950). This depends on the stage of development of the brood patch which is being formed at this time (Bailey, 1952). Activity records on the adult bird and thermocouples placed in the nest show that in the House Wren, *Troglodytes aedon*, the species here under consideration, full incubation behavior and temperature are not fully established until about the time that the last egg of the clutch is laid (Kendeigh, 1952).

The present study is concerned with the total heat applied to the eggs, as a measure of incubation time, and with the proportional amount of heat applied during the egg-laying, incubating, and hatching periods. The original data were secured between 1934 and 1936 at the Baldwin Bird Research Laboratory, near Cleveland, Ohio. The present analysis was supported by a grant from the National Science Foundation.

Procedure

Thermocouples were inserted under the eggs in six nests of the House Wren; in four nests on the day the first egg was laid, in one nest on the day the second egg was laid, and in one nest on the day the third egg was laid. Each egg was numbered soon after it was laid. All except one nest contained six-egg clutches; the one exception was a four-egg clutch. Continuous nest temperatures were recorded on a Leeds and Northrup recording potentiometer until all the eggs hatched. From these records, it was possible to determine not only the exact time required for the incubation of each egg, but also the egg temperature throughout the egg-laying, incubating, and hatching periods. The way this was done is fully explained elsewhere (Kendeigh, 1963) and need not be repeated here.

LENGTH OF THE INCUBATION PERIOD

The time of laying of the eggs could be determined within a few minutes from the temperature record. It appeared in all cases that the eggs were laid daily during the first attentive period or visit to the nest in the morning, even though the female ordinarily spent the entire night in the box. The average time of egg-laying was 0530 hours, with extremes of 0510 and 0600. The time of hatching was only occasionally discernible from the nest temperature record, so that dependence had to be placed on frequent nest observations. Hatching time was taken as midway in the period to the last time the unhatched egg was observed except when night intervened. Hatching in the House Wren appears never to occur at night. The time of hatching was ascertained within a few minutes in several instances, but may have been as much as three hours off in other cases. Probably the average accuracy of determining hatching time was plus or minus one and one-half hours.

Since each egg was numbered after being laid, the order of hatching could be determined. Of the 34 eggs in the six nests, 30 hatched. Only one of these 30 eggs is known to have hatched out of order; egg number three in nest 10 hatched four hours before egg number two. There are a few instances, however, when more than one egg was found hatched at the same inspection of the nest.

The incubation period, calculated from the time of laying to the time of hatching of the last egg in the clutch for five nests (the last egg did not hatch in one nest, no. 43A) is almost exactly 14 days, or more precisely 336 ± 3.5 hours (mean plus standard deviation). In another sample, of 35 clutches, the length of the incubation period also averages 14 days.

HATCHING PERIOD

In six-egg clutches, egg two hatches on the average about 6 hours after egg one; egg three, 15 hours; egg four, 24 hours; egg five, 32 hours; and egg six, 47 hours. The intervals between hatching of successive eggs are thus 6, 9, 9, 8, and 15 hours. The total time from laying to hatching is: for egg one, 409 hours; egg two, 391 hours; egg three, 376 hours; egg four, 361 hours; egg five, 345 hours; and egg six, 336 hours.

The shorter intervals between hatching of the first eggs in the clutch and the longer time that elapses between their laying and hatching compared with the later eggs in the clutch are best explained by the lower temperatures to which they are subjected during the egg-laying period (Kendeigh, 1963).

INCUBATION TEMPERATURE

The mean temperature of the eggs during the period of full incubation averages 34.9°C in the House Wren, but varies slightly with temperature ($Y = 33.9 + 0.045X$, where X is air temperature). In the six nests (numbers 25, 49, 75, 43, 10, 43A) studied, the calculated egg temperatures were 34.7°, 34.7°, 34.7°, 34.9°, 35.0°, and 35.2°C respectively (Kendeigh, 1963).

After the first eggs hatch, the behavior of the adult on the nest (now combining incubation of the remaining eggs and brooding of the new young) much resembles that when only eggs are present. Egg temperatures remain about the same. During the egg-laying period, the temperature of the eggs is lower, but varies greatly, since it depends on the air temperature, the amount of insolation the nest box receives from the sun, and the often irregular attention the eggs receive from the adult bird. Before one can evaluate the significance of different temperatures on the length of the incubation period, one must know how the rate of development of the embryo varies with temperature.

RATE OF DEVELOPMENT AND TEMPERATURE THRESHOLD

The rate of embryonic development is stated to be proportional to the rate of energy metabolism of the embryo (Barott, 1937), and this can be determined from the rate of oxygen absorption. The rate of oxygen uptake of House Wren eggs was measured at six stages of incubation (0, 2, 4, 7, 10, 12 days) and at six temperatures (21.1°, 26.7°, 32.2°, 35.0°, 37.8°, 40.6°C) by means of a constant-pressure manometric respirometer (Kendeigh, 1940). Omitting the less reliable data for freshly laid eggs (0 days), the rate of oxygen absorption at the other stages of incubation varied linearly with temperature according to the following equations, where Y is the cubic cm of oxygen absorbed per hour and X is the air temperature: 2 days, $Y = 0.00225X - 0.0370$; 4 days, $Y = 0.0086X - 0.1489$; 7 days, $Y = 0.028X - 0.537$; 10 days, $Y = 0.05X - 0.85$; 12 days, $Y = 0.07X - 1.14$.

The temperature thresholds for development at the five ages, calculated by solving the above equations when $Y = 0$, are 16.4°, 17.3°, 19.2°, 17.0°, 16.3°C respectively. The over-all average is 17.2°C. The embryo is inactive at temperatures below the threshold but is not necessarily killed.

Edwards (1902) measured the early growth of the embryo of the domestic fowl at various temperatures and placed the temperature threshold for development of the embryo at 20° to 21°C. Funk and Biellier (1944) criticized Edwards' work and indicated the threshold to be much higher, 26.7°C. One could not expect full development to hatching at any of these low temperatures even though growth proceeds slowly. Since the egg temperature during incubation in this domesticated tropical species is 38°C or above, compared with 35°C in the House Wren, it is not surprising that the temperature threshold is also higher. Another indication that the domestic fowl is more sensitive to a drop in temperature than the House Wren is that the rate of embryonic development decreases 8.2 per cent/°C from 39° to 28°C (Edwards, 1902), compared to 4.8 per cent/°C in the House Wren.

TOTAL HEAT REQUIREMENT

When one knows the temperature threshold for development and that the speed or rate of development is proportional to temperature above this threshold over the entire range of temperatures to which the eggs are normally exposed in the nest, then one can calculate the total heat applied to the eggs. Effective temperature (egg temperature minus temperature threshold) multiplied by total hours for development gives number of degree-hours, a measurement of applied heat.

Since the last egg in clutches is maintained at full incubation temperature until hatched, the total heat requirement is most accurately calculated for this egg. In five clutches, all but no. 43A where the last egg did not hatch, the total degree-hours for the last egg averaged 5,867 ± 144 (Table 1). If this number of degree-hours of heat is required to hatch the last egg in a clutch, one would expect it also would be required for all other eggs in the clutch.

The number of degree-hours that accumulate for each egg during the egg-laying period has been calculated by subtracting the temperature threshold of 17.2°C from the average temperature of the egg during each three-hour period day and night as measured by the nest thermocouple (Kendeigh, 1963) and multiplying by the number of hours involved. These figures, given in Table 1, show clearly that the total degree-hours for hatching is essentially the same for all eggs in the clutch. The average number of degree-hours for 18 eggs, other than the last ones in the six clutches, is 5,989 ± 196. The difference between the mean number of degree-hours for the last egg and for the other eggs of the clutch is not statistically significant by the *t*-test.

There is a reciprocal or intercompensating relation between the number of degree-hours that accumulate between the three portions of the nesting cycle. This is true both between different eggs in the same clutch and in comparisons between different clutches. There is, of course, no accumulation of heat for the last egg during the egg-laying period and the amount

TABLE 1

ACCUMULATION OF DEGREE-HOURS OF HEAT AT VARIOUS STAGES IN THE NESTING CYCLE OF THE HOUSE WREN

Nest	*Egg number*	*Egg-laying period*[1]	*Incubating period*[2]	*Hatching period*[3]	*Total*
No. 25	4	195	5,782	137	6,114
	5	191	5,782	155	6,128
	6	0	5,782	258	6,040
No. 49	3	519	5,363	105	5,987
	4	399	5,363	106	5,868
	5	198	5,363	262	5,823
	6	0	5,363	528	5,891
No. 75	2	632	5,004	97	5,733
	3	566	5,004	141	5,711
	4	411	5,004	449	5,864
	5	248	5,004	461	5,713
	6	0	5,004	703	5,707
No. 43	2	870	4,992	218	6,080
	3	722	4,992	543	6,257
	5	299	4,992	706	5,997
	6	0	4,992	972	5,964
No. 10	2	745	5,079	249	6,073
	3	609	5,079	143[4]	5,831
	4	0	5,079	860	5,939
No. 43A	2	1,266	5,085	107	6,458
	3	1,016	5,085	107	6,208
	4	730	5,085	128	5,943
	5	386	5,085	534	6,005
	6	0	5,085	949[5]	6,034[5]

[1] From hour egg laid to hour last egg in clutch laid.
[2] From hour last egg laid to hour first egg hatched.
[3] From hour first egg hatched to hour particular egg hatched.
[4] Egg number 3 hatched 4 hours before egg number 2.
[5] Assuming this egg had hatched at the end of 336 hours.

that must accumulate during the hatching period is therefore the greatest —for this reason it is the last to hatch. The earliest eggs laid, on the other hand, accumulate the most heat during the egg-laying period and the least during the hatching period. The other eggs fall progressively along a gradient in between.

On account of unusually low air temperatures, egg temperatures during the laying period were lowest at nest 25 (average 23.2°C). This is correlated with the least accumulation of heat during this time and the greatest during the incubating period. The actual incubating period at this nest was 13 days, compared with 12 days at nest 49, 11 days at nests 10, 43, 75, and only 10 days at nest 43A. Nest box 43A was fully exposed to the sun and to high air temperatures, and the average egg temperature during the laying period was 32.0°C. Egg number 2 had accumulated nearly

20 per cent of the necessary degree-hours before egg number 6 was laid.

In another paper (Kendeigh, 1963), I showed that in regard to House Wren eggs a degree-hour of heat is equivalent to 5.6 g-cal. This means that the egg must absorb an additional 5.6 g-cal of heat per hour for each 1°C rise in temperature. The total of 5,867 degree-hours required to hatch an egg is equivalent, therefore, to 32,855 g-cal (32.9 kcal). If the egg temperature drops below the threshold of 17.2°C, a corresponding amount of heat is required to bring it back to the threshold, even though no embryonic growth is involved.

Energy Requirement for Embryonic Development

The energy for embryonic development does not come from the input of heat from the outside but from oxidation of the fats, carbohydrates, and, perhaps, proteins in the yolk and albumen of the egg itself. The rate and amount of energy that is released for maintenance and growth depends on the amount of oxygen absorbed and this is readily measured. The rate of oxygen absorption by the House Wren embryo is, as indicated above, directly proportional to the temperature and the length of time that the egg is maintained at that temperature. The degree-hour, therefore, indicates the mobilization of a definite amount of energy and a definite amount of embryonic growth.

The total oxygen consumption of the House Wren embryo from the time it is laid to hatching is calculated as 184.3 cubic cm. This differs from the 149 cubic cm given by Kendeigh (1940) where the calculation was erroneously made for a 13 instead of a 14 day incubation period. With a respiratory quotient of 0.72, this is equivalent to an energy transformation of 866 g-cal (0.9 kcal).

The total energy utilization for complete development to hatching has been shown for the domestic fowl to be 20 kcal (Barott, 1937) and for the domestic (probably mallard) duck, 39.7 kcal (Kashkin, 1961). These are much larger species, the incubation periods are much longer, and the young hatch in a precocial rather than an altricial state. It is important to note, however, that these energy outputs are constants. This is indicated by Barott when he states: "As the temperature of incubation was lowered the rate of metabolism decreased, but the time of incubation was increased so that the total, which is the product of these two, remained constant."

If 5,867 degree-hours represents the total heat applied to the egg of the House Wren from laying to hatching and the total energy transformation is 866 g-cal, then a single degree-hour represents an energy transformation by the embryo of about 0.148 g-cal. Similarly if 32,855 g-cal of heat from the outside are required to enable the embryo to transform 866 g-cal of energy for growth, the efficiency ratio is only 2.6 per cent.

DISCUSSION

Requirements for the incubation of House Wren eggs to hatching in respect to time in the nest under normal conditions is 14 days, or more accurately 366 ± 3.5 hours; in respect to heat applied it is 5,867 ± 144 degree-hours or 32.9 kcal above the temperature threshold; and in respect to energy transformed it is 0.9 kcal. These measurements are of increasing biological significance and importance. Before we can fully understand the thermodynamics of incubation, the significance of behavioral differences between species, and the role that the environment plays, it is very desirable that measurements of heat and energy requirements for incubation be obtained for many species. This will require considerable effort and refinement of technique.

We have only approximated correct measurements of heat and energy requirements in the House Wren. We need a better method of recording the egg temperature throughout incubation than by puncturing the shell and inserting a thermocouple, by using artificial eggs, or simply by measuring the nest temperature.

Before effective temperature can be calculated we must know the temperature threshold. We have taken 17.2°C for the House Wren, as indicated by our data on oxygen consumption, but the threshold for actual embryonic growth may be different. The rate of embryonic development is not necessarily related absolutely to the rate of oxygen absorption, since oxygen must be used for maintenance as well as for development and the proportion between the two may vary at different temperatures. There should be direct measurement of embryonic growth at various temperatures. At best, 17.2°C represents the alpha threshold (extrapolation to zero rate of development of a regression line obtained at medium temperatures) rather than the true threshold, which may be lower (Shelford, 1929). There may also be a decrease in rate of development at temperatures above some unknown maximum.

When the percentages of normal development that take place in the domestic fowl at various temperatures (Edwards, 1902) are plotted, it is evident that the alpha threshold is 26.7°C, but that some development takes place down nearly to 20°C. It is of interest that the alpha threshold thus determined agrees exactly with the figure given by Funk and Biellier (1944) for "the minimum temperature for embryonic development." I calculated degree-hours of heat (or actually "developmental units" as used by Shelford, 1929) for the House Wren, using 26.7°C as the temperature threshold, but allowing proportional amounts of development to

20°C, and obtained total units of heat considerably less than given above and of almost the same statistical reliability. However, incubation temperatures in the domestic fowl and the relation of embryonic development to temperature are quite different than in the House Wren, as indicated above, and I prefer not to base my calculations on data available for the fowl.

Finally, measurements are desirable, for each different species, of the total energy requirements for the growth of the embryo to hatching. This may be approximated by measuring the total oxygen absorption of the egg, or may be obtained, as did Tangl (1903), by finding the difference between the caloric value of the fresh egg and of the newly-hatched chick, its membranes, and of the unused yolk. No one as yet has divided the energy transformed during the incubation period into that required for maintenance or existence and that for development. This is desirable.

It is obvious that ornithologists are only at a threshold of another sort in the kinds and amount of data that need to be obtained and in the opportunities for detailed analysis of the forces at work in growth, behavior, and ecological relationships.

SUMMARY

The incubation period of the House Wren, measured in time units, is 14 days or 366 ± 3.5 hours. A definite sum total of heat above a temperature threshold is required for hatching a bird's egg. In the House Wren, this is estimated to be 5,867 ± 144 degree-hours or 32.9 kcal. A definite sum total of energy transformation is required by the embryo to complete development to hatching. In the House Wren, the energy requirements for the existence and development of the embryo is estimated at 0.9 kcal.

These units represent progressively more precise ways of measuring the incubation period in birds.

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