ORNIS SCANDINAVICA 11: 223–227. Copenhagen 1980

Trans-illumination of passerine bird eggs in field studies on clutch-size and incubation

Anders Enemar and Ola Arheimer

Enemar, A. and Arheimer, O. 1980. Trans-illumination of passerine bird eggs in field studies on clutch-size and incubation. – Ornis Scand. 11: 223–227.

A handy device for trans-illuminating eggs of passerine birds in the field is described. Recommendations are given for the interpretation of developmental stages of trans-illuminated eggs in order to decide if a clutch is complete. Special attention is paid to the first four days of the incubation period when the swelling of the yolk mass is easily registered providing information on the beginning of the incubation behaviour during the laying period, on the frequency of non-developing eggs, and on the date of laying onset.

A. Enemar, Dept of Zoology, Univ. of Gothenburg, P.O. Box, S-400 33 Gothenburg, Sweden. O. Arheimer, Magasinsvägen 58, S-681 00 Kristinehamn, Sweden.

Introduction

When a bird's nest with eggs is found and the intention is to establish the clutch-size, it is necessary to check the nest again at least once after an interval of one or more days to ascertain that laying is over and, accordingly, that the clutch is complete. Such return visits are not always possible. Nests are sometimes found by accident during general field excursions or during field work not related to clutch-size studies. Much information is therefore lost. Warm eggs or an apparently incubating bird do not necessarily indicate that laying is over. Due to the transparency of the fresh egg and with the aid of a simple device for trans-illumination it is often possible to establish, on the spot, whether a found clutch is complete or not. The same device has also proven useful for dating the beginning of laying, for investigating the beginning of incubation in relation to the completion of the clutch, as well as for recording the frequency of non-developing eggs.

In this communication the method of trans-illumination and its utility will be described and views on the interpretation of the observations will be given, based on egg dissections and experience during the long-term ornithological field work in the subalpine birch forests at Ammarnäs, Swedish Lapland (the LUVRE project). During the course of time the application of the method has greatly improved the quality and the usefulness of the nest-card files of this project.

The trans-illumination device

A common torch equipped with two batteries of 1.5 volt each is used as light source. In front of the bulb a shallow cup is mounted. It is made of rubber or other opaque material and has a small hole in the centre at the bottom. The egg under inspection is placed on its "side" over the hole through which the light passes into the egg. To keep out disturbing day-light the egg is inspected through a tube made of opaque material and attached to the rim of the cup (Fig. 1).

The selected developmental stages

The amount of information obtained by trans-illumination depends upon the egg-size, egg-shell thickness and colouration, and the strength of the light beam. In the present context the description will be restricted to those structures in the egg which can be observed in the field with the aid of the rather weak light beam of the torch irrespective of the egg-size (thrush egg and smal-

Accepted 17 January 1980

© ORNIS SCANDINAVICA 0030-5693/80/030223-05 $02-50/0

Fig. 1. The trans-illumination device with an egg of a Bluethroat in position to be examined. (Photo: Mats Forsberg and Urban Wikenborg/LUVRE).

ler) and pigmentation. Five stages, each separated by about 24 h of efficient incubation, are selected and can be identified rather objectively. These stages are shown in Fig. 2 and are based on the development of the lastlaid egg of seven species (*Turdus iliacus, T. pilaris, T. philomelos, Luscinia svecica, Prunella modularis, Phylloscopus trochilus, Emberiza schoeniclus*). They can be characterized and interpreted as follows (Fig. 2).

Stage 1. The egg appears as a more or less translucent body with a dark circular field in its centre. This field is the yolk mass (the egg cell). The boundary between the yolk and the surrounding translucent material (the albumen or egg white) is very distinct. This is the picture of a fresh egg, and it lasts for most of the first 24 h of incubation.

Stage 2. The picture is similar to that of Stage 1, the only difference being that the contour of the yolk mass is less distinct and the size of the yolk field has increased somewhat. This indicates that the process of yolk swelling has started. The stage occurs after approximately 24 h of incubation.

Stage 3. The dark yolk field which is now somewhat lighter has expanded considerably but is still surrounded by a translucent margin which is between 1 and 2 mm wide. This enlargement is caused by the swelling of the yolk mass due to uptake of water from the surrounding albumen (Romanoff 1960). At the same time the rather regular circular shape of the yolk mass is lost. The yolk mass has moved from the centre of the egg to the upper side where it spreads under the shell membranes. This stage indicates that the egg has been incubated approximately two days (48 h).

Stage 4. The dark field is of the same moderate opacity as in Stage 3 and covers the whole egg apart from the air chamber at the blunt end and most often also a small area at the pointed end. This means that the process described for Stage 3 has progressed. The transformation of the shape of the yolk mass is now manifested by the rupture of the thin membrane surrounding the yolk (the vitelline membrane) which is successively replaced by the growing extra-embryonic membranes of which one will later completely envelop the yolk (formation of the yolk sac). Stage 4 is ready when incubation has lasted about three days.

Stage 5. This stage includes the rest of the incubation period and is characterized by an increasing and finally complete opacity of the egg caused by the growing extra-embryonic membranes and their blood vessels and also by the embryo itself. During the first few days of this stage a trace of red emanating from the blood can be discerned, especially in the light-coloured eggs.

The five stages are easily recognized with the exception of Stage 2 which might be less clear to an untrained observer. Stage 4 can also provide problems due to inconsistency of its main characteristic, the translucent pointed end of the egg. Its duration may vary within and between species and depends in part on the shape of the egg.

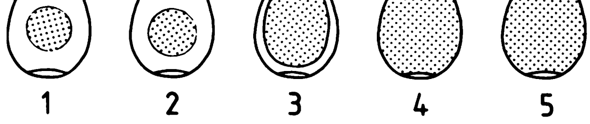

Fig. 2. Stage 1 to 5 of the incubated egg, showing the swelling of the yolk mass during the first four days of incubation (Stage 1 is the fresh egg). The intervals between the stages are approximately 24 h.

It should be noticed that the criteria of the first four stages are based on the change of size and form of the yolk. The development of the vascular plexus around the embryo, the area vasculosa, is not easily visible in these stages of most species when using the field technique recommended here. The area vasculosa is a circular field about half the diameter of the yolk mass in Stage 3 eggs, and it does not yet cover the whole yolk mass in Stage 4. The size and blood volume of the area vasculosa substantially contributes to the opacity of the egg in early Stage 5.

Applications of the method

Determination of clutch-size

The problem is to establish that the number of eggs counted are in complete clutches only. Trans-illumination can help in most cases. If the eggs are more or less opaque throughout (Stage 5), incubation has lasted for four days or more. This means that laying has ceased. It is most accurate to accept only Stage 5 as the criterion of a complete clutch. With experience one can evaluate clutches also from earlier phases of incubation. This is based on the fact that the passerine birds are known to normally lay their eggs at intervals of about 24 h. Therefore, if the eggs are found to belong to Stage 3 then no egg has been laid during the last two days indicating that laying is over. The only disadvantage in accepting Stage 3 is that irregularities in the laying sequence due to severe weather and the like are more likely to cause misinterpretations than when advanced stages are considered.

A complication is that incubation often starts before the laying of the last egg or eggs. Therefore the eggs may show different stages when the clutch is examined during the first few days of incubation. Consequently all eggs must be investigated in search of the least incubated egg which is crucial for the evaluation of the laying status of the clutch. This egg should have reached at least Stage 3 in a clutch which is accepted as complete. As the incubation behaviour at the end of the laying period is not adequately known for most species it is adviceable to adopt as a general rule to check all eggs of the clutch under examination.

Another complication is the occurrence of eggs which due to fertilization failure or to early death of the embryo remain in Stage 1 or 2. They can be considered abnormal eggs provided that the healthy eggs of the clutch have attained Stage 5 indicating that the eggs of Stage 1 or 2 are lagging further behind than can be explained by normal laying intervals. This means that no conclusion can be made on the basis of the information from only the first examined egg of a clutch if this belongs to Stage 1 or 2, not even for species which are known not to start incubation until laying is over. This strengthens the general recommendation that all eggs should be investigated in order to secure a reliable evaluation of the clutch.

Frequency of non-developing eggs

The number of eggs which fail to develop can be established in clutches where the normal eggs have attained Stage 5. This evaluation is mostly restricted to eggs which have not been successfully fertilized (appearing as eggs of Stage 1) and to eggs where development ceases not later than Stage 3. It should be stressed that all Stage 1 eggs which fail to develop are not unequivocally "sterile." They may contain dead embryos of a very early developmental stage (blastodiscs).

Beginning of incubation

As indicated above, information about the beginning and intensity of incubation in relation to the progress of laying can be obtained provided that the clutch is examined before all eggs entered Stage 5. When all eggs of a complete clutch show the same stage one can conclude that incubation did not start until after the laying of the last egg. Insignificant differences between eggs are almost always found and they may be mainly explained by the fact that the first eggs are repeatedly heated by the female bird when she is sitting in the nest to lay the subsequent eggs. Moreover, there may be differences between eggs already at laying because the ready eggs may remain for different lengths of time in the lower oviduct before being deposited in the nest.

In order to carefully investigate when the initiation of incubation occurs during the laying period the clutch should be examined daily from the early phase of laying. It is not necessary to have the eggs marked according to laying order but it may facilitate interpretation. Fig. 3 shows a summary of the results from three clutches in such an investigation of the Fieldfare *Turdus pilaris*. Each clutch consisted of five eggs. Trans-illumination started the day the third egg was laid. It can be concluded from Fig. 3 that a gradually increasing incubation occurs during the time of laying. This is clearly seen when the developmental stages of the different eggs are compared at the age of two days (i.e. 1A, 2B, 3C, 4D and 5E of Fig. 3). All eggs have an enlarged yolk mass, but the swelling of the yolk increases with the laying order of the egg. The increase in amount of incubation during laying is also demonstrated by the fact that the first egg enters Stage 5 about five days after laying, the other eggs after about four days.

An early start of incubation in advance of the laying of the last eggs, could also be discovered by only one examination of the clutch when the results are similar to those of day C, D, and E of Fig. 3.

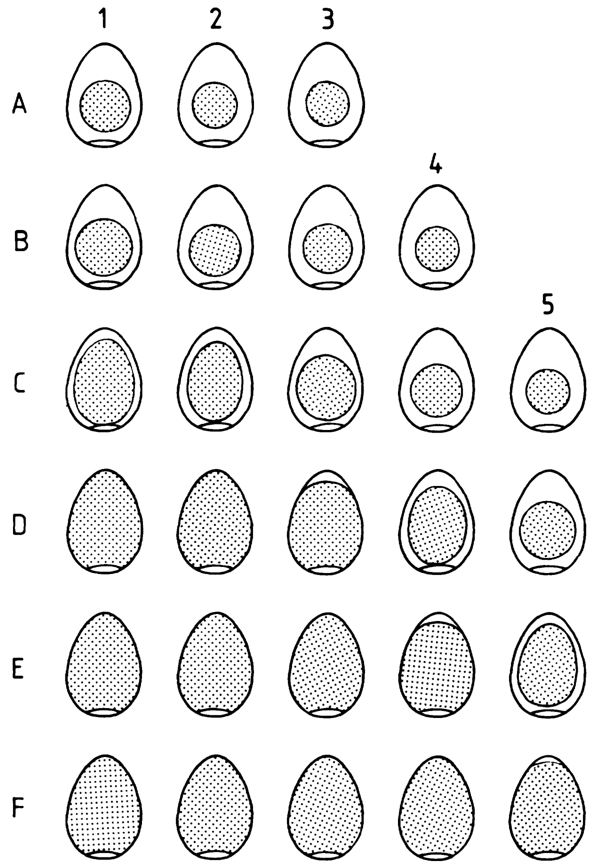

Fig. 3. Trans-illumination pictures of the eggs in a 5-egg clutch of the Fieldfare *Turdus pilaris*. Trans-illumination started on day A when the third egg was laid.

Date of laying onset

The laying date of the first egg in a clutch can be estimated provided that the clutch is examined during the laying period or during the first few days after completion of the clutch when all eggs have not yet entered Stage 5. The laying of the least incubated (= last laid) egg (or all eggs if they are of the same stage) is dated by determining its developmental stage. The date of arrival of the first egg is then easily calculated from the number of eggs in the clutch. The precision of this method is reduced for the following reasons:

1. When dating the last laid egg it is presumed that full incubation has occurred from its laying. This is often the case when incubation starts during the laying period.
2. It is not possible to decide whether an egg of Stage 1 was laid before or after midnight (the change of date).
3. Even if the laying interval most often is approximately 24 h there are species differences. Even moderate, unknown deviations can bias the calculations considerably when clutches are large. (For example, the interval between eggs is about 20 h in the Redwing (Arheimer 1978a), which means that the period of time between the laying of the first and sixth egg is only four days).
4. Difficulties in interpreting intermediate stages contribute to the uncertainty.

Taking the examination results on day D of the 5-egg clutch of Fig. 3 as an example, the interpretation is as follows. The least incubated egg seems to be Stage 2, but could be Stage 1, or even approaching Stage 3, although the staging of the older eggs makes the last assumption less probable. Since four eggs were laid before the last one the conclusion is that laying probably started five days before day D, although four and six days cannot be excluded.

To summarize it is not usually possible to date the start of laying within less than two to three days on basis of a single examination. The precision is, however, at least as good as the one where the laying date is derived from the date of hatching; an often used method. The precision may be improved if trans-illumination is carried out on two or more consecutive days.

Comments

Methods and criteria for determining the incubation stage of eggs in the field have been worked out for game birds and terns, including both floatation methods (Westerskov 1950, Hays and LeCroy 1971) and trans-illumination techniques (Hanson 1954a, b, Weller 1956). Enemar (1958) trans-illuminated eggs of Blackbird when studying the beginning of incubation, and so did Arheimer (1973, 1978b) in the Redwing, although both investigators overlooked the swelling of the yolk mass and erroneously interpreted the early expansion of the dark area in the egg to indicate growth of the extra-embryonic membranes including area vasculosa. Rydén (1978) was not always able to determine the laying sequence when examining the vascularized area around the embryo in trans-illuminated Blackbird eggs.

The swelling of the yolk mass is a well-known phenomenon among developmental physiologists, but is unknown to or ignored by ornithologists. It is important that the yolk remains unaffected in the unfertilized egg even after weeks of effective incubation. The swelling of the yolk can thus be taken as a reliable indication that incubation has started, a conclusion which is possible to make already the first few days of incubation before the embryos or the extra-embryonic membranes are visible.

The advantage of considering the change of the yolk in incubation studies is that the differences between the developmental stages in the eggs are easily recognized in the field with a rather simple trans-illumination device. Corresponding developmental differences between eggs with more advanced embryos are more difficult to ascertain, even with the aid of more efficient equipment, due to the increasing opacity of the egg content. Moreover, the initial differences caused by the onset of incubation during the laying period may be successively masked by individual differences in developmental rates between embryos.

The method presented in this paper is applicable irrespective of species for checking if the eggs have attained Stage 5 indicating whether or not a clutch is complete. Some training is recommended in order to take advantage of the "yolk-swelling period" to study the beginning of incubation behaviour or to determine on what data laying started. Even if the swelling of the yolk according to our experience seems to be a regular process, identically timed in different species, the change in the properties of the yolk mass cannot be determined with the same accuracy in all species due to differences in egg size and form, shell colour and thickness. It is adviceable, therefore, to establish a chart of the yolk swelling progress for the investigated species corresponding to those shown in Fig. 2 and Fig. 3. This will serve as a guide during the trans-illumination work in the field.

Acknowledgements – We are grateful to Aino Falck-Wahlström for drawing the figures, to Mona Möllerstedt for typing the manuscript, to Britt Betz for revising the English, and to the Swedish natural science research council for financial support (B 2180–103).

References

Arheimer, O. 1973. The breeding biology of the Redwing *Turdus iliacus* in subalpine birch forest, Ammarnäs, Swedish Lapland. – Vår Fågelvärld 32: 1–10. (Swedish with English summary).

– 1978a. Laying, incubation, and hatching in the Redwing *Turdus iliacus* in subalpine birch forest at Ammarnäs, Swedish Lapland. – Vår Fågelvärld 37: 297–312. (Swedish with English summary).

– 1978b. Number of clutches and clutch-size of the Redwing *Turdus iliacus* in subalpine birch forest at Ammarnäs in Swedish Lapland. – Anser, Supplement 3: 15–30. (Swedish with English summary).

Enemar, A. 1958. On the initiation of incubation in the Blackbird (*Turdus merula*). – Vår Fågelvärld 17: 81–103. (Swedish with English summary).

Hanson, H. 1954a. Criteria of age of incubated Mallard, Wood Duck, and Bobwhite Quail eggs. – Auk 71: 267–272.

– 1954b. Apparatus for the study of incubated bird eggs. – J. Wildl. Mgmt 18: 191–198.

Hays, H. and LeCroy, M. 1971. Field criteria for determining incubation stage in eggs of the Common Tern. – Wilson Bull. 83: 425–429.

Romanoff, A. L. 1960. The avian embryo. – Macmillan, New York.

Rydén, O. 1978. Egg weight in relation to laying sequence in a South Swedish urban population of the Blackbird *Turdus merula*. – Ornis Scand. 9: 172–177.

Weller, M. W. 1956. A simple field candler for waterfowl eggs. – J. Wildl. Mgmt 20: 111–113.

Westerskov, K. 1950. Methods for determining the age of bird eggs. – J. Wildl. Mgmt 14: 56–67.