CRITERIA OF AGE OF INCUBATED MALLARD, WOOD DUCK, AND BOB-WHITE QUAIL EGGS

BY HAROLD C. HANSON

IF field studies of birds are to continue to yield new findings that are qualitatively and quantitatively commensurate with the effort involved, techniques that yield new and more precise data must be constantly evolved. From more refined data, basic relationships that might otherwise be missed become apparent. This is particularly true in the case of the study of nesting birds, the eggs of which offer the possibility of more dynamic interpretation than is generally realized. With this in mind the writer developed a portable egg candler and a method of photographing incubated eggs with transmitted light.

The primary purpose of candling eggs is, of course, to ascertain the age of the embryos, and through the examination of many nests, to accurately and efficiently determine the chronology of the nesting season. Candling also enables the investigator to anticipate the date of hatching so that return visits, if desired, can be made on the appropriate dates and the final outcome of the nest more readily determined. In the case of altricial species such as the Mourning Dove (*Zenaidura macroura*), the visit can be so timed that the young can be banded just before they leave the nest.

Descriptions of a portable egg candler, an egg volumeter, and the technique of photographing incubated eggs with transmitted light have been presented elsewhere (Hanson, 1954, Journ. Wildl. Mgt., 18: in press). This report, primarily a continuation of the above report, presents photographs and notes on the developmental stages of three species: the Mallard (*Anas platyrhynchos*), Wood Duck (*Aix sponsa*), and Bob-white Quail (*Colinus virginianus*). These photographs should also be useful in determining the stage of development of eggs of other species whose incubation periods are similar. Photographs and notes on the incubation of Mourning Dove eggs will be presented later in conjunction with other studies of this species' nesting cycle.

The writer is indebted to William E. Clark, Natural History Survey photographer, for his patient assistance in preparing the plates showing eggs—which were made from admittedly difficult negatives; and to Francis J. Kruidenier of the University of Illinois Department of Zoology for a critical and helpful reading of the manuscript.

CRITERIA OF AGE IN INCUBATED EGGS

The number of days an egg has been under incubation can be accurately determined with the aid of an egg candler for about the first half of incubation; during the second half of incubation, when the egg becomes more opaque, aging becomes progressively less accurate as there are fewer observable characters on which to base an estimate. This is particularly true of large eggs but may also be true of small eggs such as quail eggs, which have an incubation period of about three weeks. The eggs of Mourning Doves usually can be accurately aged throughout most of their incubation since their development is so swift that day-to-day changes are quite readily recognized.

The following features and successive stages may be used as criteria in estimating the stage of incubation. They are presented in the approximate order of their appearance.

1. Color of the yolk and the distinctness of its outline. The yolk area becomes darker and more orange a day or two after incubation begins, the time element depending on the species involved.

2. Blastoderm becomes apparent.

3. Embryo first becomes visible.

4. Flexed embryo with beating heart plainly seen. Vitelline veins become visible giving the embryo spider-like aspect.

5. Embryo thickens, is obviously within an embryonic membrane, the amnion. Branching of vitelline veins becomes more obvious or visible.

6. Greatest distance between anterior vitelline veins.

7. Greatest diameter of area vasculosa as demarcated by the sinus terminalis.

8. Appearance of the amnion and length of flexed embryo.

9. Increasing diameter of amnion (the embryo not always seen as it moves about within the amnion).

10. Nature of embryonic movements, how rapidly the embryo flexes, whether it sways about within the amnion.

11. The extent to which the over-riding allantois obscures the amnion and enclosed embryo and the *area vasculosa* (see mallard, stages 8-11).

12. Position of embryo within egg.

13. Proportion of egg area occupied by embryo when the egg becomes too opaque for other structures to be seen.

14. Size of air sac.

For the sake of convenience, the structures listed above are reviewed in plate 15.

How readily these characters can be seen and/or their reliability depends upon the following factors:

1. Strength of light source.

2. Size and shape of egg.

3. Thickness of shell and amount of pigmentation.

4. Position of egg in the nest just prior to candling. If the egg has been in a tilted position in the nest prior to examination, the embryo and associated structures will be crowded at one end of the egg. Because curvature of the egg is greater at the ends, the diameter of the yolk sac, for example, will appear less than it would if the embryo is situated under the mid-portions of the shell. Usually up-ending the opposite end of the egg will cause an embryo in the early stages of development to float upwards and assume a more easily studied position near the center of the egg. Older embryos require more time to adjust their position.

The notes which follow on the appearance of the eggs of the Mallard Duck, Wood Duck, and Bob-white Quail at various stages of incubation are intended to supplement the photographs of the eggs (plates 16-21). Often they may offer only an approximation of the true situation within the egg, and would doubtless need considerable correction if revised on the basis of exposed and dissected material. The photographs of some of the stages admittedly are not completely satisfactory, but in the case of large eggs, such as the Mallard, they show more detail than is seen in a portable egg candler during the last half of incubation. Most of the details shown in the photographs of late stages of incubation can, however, be seen in a laboratory candler using a 75 watt miniature enlarging bulb. The reverse situation is also true. Detailed structures seen in a candler in the early stages of incubation could not always be photographed clearly despite repeated efforts. The heart, which was quite readily seen in nearly all eggs examined, did not register on film.

MALLARD (plates 16 and 17)

Fresh egg.—Egg, especially if shell is light colored, appears fairly translucent. No sharply demarcated zones; egg varies in color from whitish at the tips to light yellow and light orange at the center.

1st day.—Differs from fresh egg by darker hue of yolk area. The blastodisc, a small dark round spot about 4 mm. in diameter, can be detected in light colored eggs in the center of the yolk area when the egg is rotated.

2nd day.—Yolk area darker and better defined. Area vasculosa 7-12 mm., averaging 10 mm. in diameter.

3rd day.—Yolk area now a dark orange in color and its limits rather sharply defined. Area vasculosa spreading rapidly, now averages 20 mm. in diameter, its periphery demarcated by the sinus terminalis. Embryo now visible for first time, averaging about 4.5 mm. in length, and forms, in conjunction with initial lateral outgrowths of the vitelline veins, a small cross.

4*lh* day.—"Heart-beat stage." Embryo flexed, 6-8 mm. in length, beating heart clearly visible. Area vasculosa averages about 30 mm. in diameter (25-32 mm.).

Anterior and posterior vitelline veins form in *area vasculosa* making the embryo appear spider-like. Greatest spread of anterior vitelline veins averages 19 mm. (15-24).

5th day.—Amnion becomes visible, varying from 6-9 mm. in diameter; the amnion, as well as the embryo within, sways about freely when egg is quickly rotated. Length of flexed embryo 6-8 mm. Area vasculosa averages 43 mm. (34-48). Greatest width between vitelline veins averages 22 mm. (19-32 mm.).

6th day.—Embryo now moves slowly to and fro (as a result of rhythmic contraction of muscle fibers in the amnion) and flexes. Eye is now very prominent.

7th day.—Embryo now, and for several subsequent days, is in stages of development that are not readily seen, since it moves about within the amnion considerably below the inner surface of the egg shell. The embryo also becomes less visible in successive days by the over-growth of the allantois, which spreads gradually out between the amnion and the chorion and the *area vasculosa*. Thus, the relatively simple radiating patterns of the veins of the *area vasculosa* become obscured by the vessels of the highly vascular allantois (days 8–11). These vessels, continuous over the flattened sac-like allantois, do not terminate in a peripheral vessel like the *sinus terminalis*, but turn under at the advancing lip of the allantois. Eventually the allantois surrounds most of the egg contents; its gradual advancement beneath the egg membranes and chorion can be seen in stages 8 through 11, the advancing edge being demarcated by the looped blood vessels. In relaxed flexed position, the embryo measures about 11 mm. in length. Amnion 22–29 mm. in diameter; *area vasculosa* now covers most of egg surface "below" air sac.

8th day.—Embryo 11–13 mm. long in flexed position; amnion 26–28 mm. in diameter. Air sac now increases more rapidly in size.

9th day.—Embryo 16–22 mm. long in relaxed yet flexed position; amnion 25–29 mm. in diameter. Embryo now very active.

10th day.—Embryo 25 mm. (22–27 mm.); amnion 35 mm. in diameter; eye 3–4 mm. in diameter. Kicking action of legs indicated by the spasmodic movements of the amnion and adjoining membranes.

11th day.—No significant change in embryo and amnion size measured. Embryo only moderately active; head and body about equal in size.

12th day.—Embryo about 33 mm. long; amnion about 40 mm. in diameter. Embryo movement now slow and gently pulsating. Embryo lies across shell in flexed position.

13th day.—Embryo in relaxed position occupies two-thirds of distance across egg.

14th day.—Embryo occupies four-fifths of distance across egg; embryo mass fills about two-thirds of dark area of the egg.

15th day.—Embryo moves slowly; lies in fairly tightly flexed position. Extraembryonic blood vessels appear enlarged as compared with earlier stages.

16th day.—Little detail of egg contents now visible. Embryo occupies threequarters of darkened portion of the egg.

17th day.--Thick blood vessels at the small end of the egg only detail yet visible.

21st day.—Entire egg is opaque except for air sac and a small area at the small end of the egg.

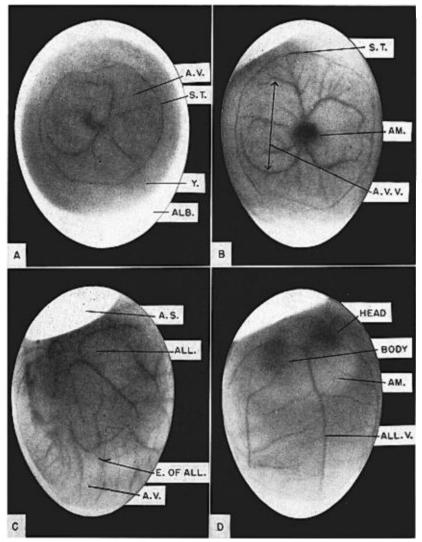
22nd and 23rd days.—Continuing increase in the size of the air sac is the only character that can be seen in these stages. If candling light is of low intensity, the air sac will be the only character easily seen after 12-14 days of incubation.

24th day.-Embryo active; can be heard moving about within shell.

25th day.-Shell pipped and embryo has broken into air sac space.

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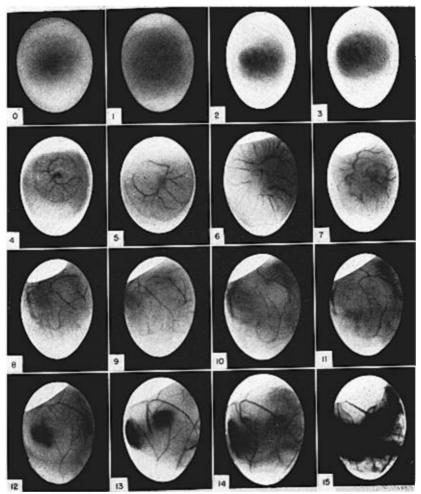




SELECTED STAGES OF WOOD DUCK AND MALLARD EGGS TO SHOW EMBRYOLOGICAL FEATURES USEFUL IN DETERMINING STAGES OF INCUBATION. A. Mallard at 4 days. B. Wood duck at 6 days. C. Mallard at 8 days. D. Wood duck at 11 days. Symbols: A.V.—area vasculosa; S.T.—sinus terminalis; Y.—yolk; Alb. albumen; A.M.—amnion; A.V.V.—anterior vitelline veins; A.S.—air sac; ALL.— Allantois; E. of All.—edge of allantois; All.V.—allantoic vein.

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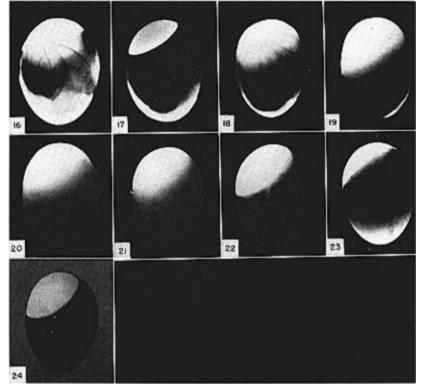


MALLARD EGGS. Days of incubation designated by numerals on plate.

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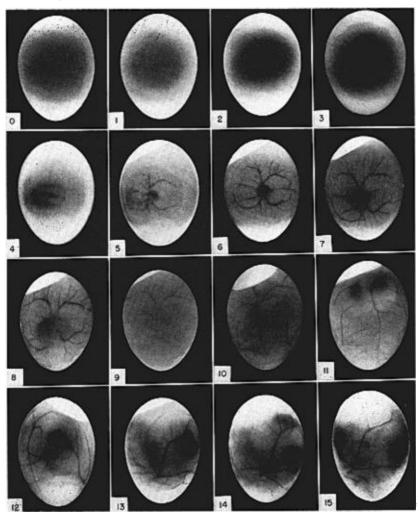
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PLATE 17



MALLARD EGGS (cont'd). Sixteen through 24 days of incubation.

PLATE 18



WOOD DUCK EGGS. Days of incubation are indicated by numerals on plate.

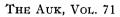
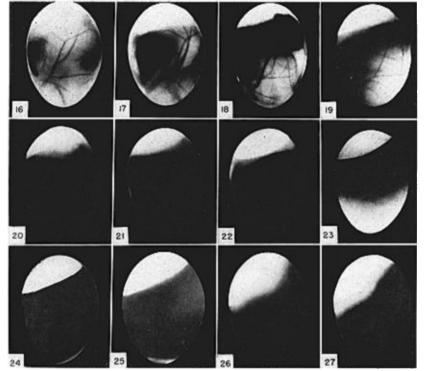


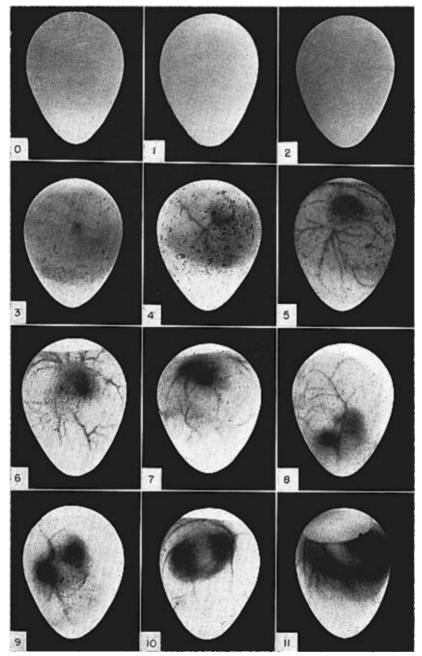
Plate 19



WOOD DUCK EGGS (cont'd). Sixteen through 27 days of incubation.

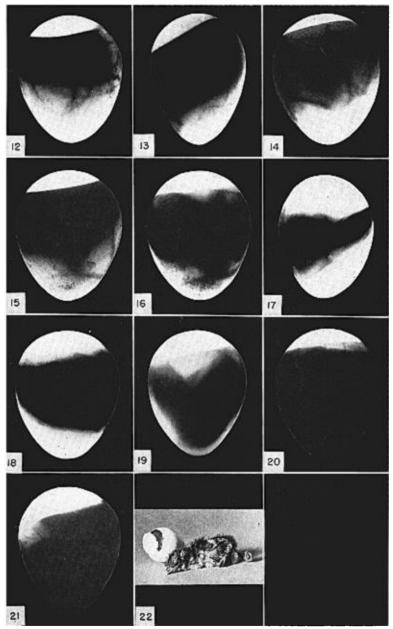
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PLATE 20



BOB-WHITE QUAIL EGGS. Days of incubation are indicated by numerals on plate.

PLATE 21



BOB-WHITE QUAIL EGGS (cont'd.). Twelve through 21 days of incubation.

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WOOD DUCK (plates 18 and 19)

Fresh egg.—Differentiation or contrast between yolk and albumen not prominent. Ist day.—Volk area becomes somewhat darker and better defined. Primitive streak 1/16 to 1/8 in. in length—can be seen best when egg is slightly rotated, lies at mid point of yolk area.

2nd day.—Yolk area darker and well defined.

3rd day.—Blastodisc about 9 mm., lighter area (area pellucida) about embryo 4-5 mm., width of vitelline veins 4.5 mm.

4th day.—Embryo not strongly flexed, area vasculosa 16 mm. (Embryo is more easily seen with the aid of a green filter.)

5th day.—"Heart-beat stage," i.e. heart as a functioning organ readily seen for the first time. Area vasculosa 27 mm. in diameter; cervical flexure observable, embryo and vitelline veins appear spider-like in outline. Distance between anterior veins about 15 mm.

6th day.—Embryo 6-7 mm. in length, its outlines obscured by the rapid increase in size of the amnion; area vasculosa 41 mm. in diameter, greatest spread of anterior vitelline veins 15 mm. Vitelline veins now have many easily observed branches.

7th day.—Outline of the embryo as it lies enclosed in the amnion not readily apparent.

8th day.-Air sac shows sudden increase in size.

9th day.—Allantois spread about one-half way around air sac; embryo very active, about 12 mm. long, flexed.

10th day.—Allantois now surrounds all but about one-sixth of air sac; blood vessels of allantois less apparent due to lack of contrast. Embryo about 17 mm., flexed; eye spot 4-5 mm.

12th day.-Legs clearly seen and frequently moving.

13th day.---Embryo about 12 mm. long as normally flexed.

15th day.—Embryo lies openly flexed in a semicircle; its "diameter-length" is about 33 mm.; allantois now surrounds entire egg.

16th day.—Body of embryo about 19 mm.

17th day.—Embryo flexed to form a coil or ring for the first time; the wings and legs are now seen to move appreciably, the head and neck move occasionally, body more quiescent.

18th day.-Embryo now extends across shell and curves half-way around it.

19th day.—Embryo a compact mass; most of egg now black.

21st day.—Egg is all dark except for air sac and a small area at the extreme opposite end of shell.

BOB-WHITE QUAIL (plates 20 and 21)

1st day.—Yolk area shows some darkening, but is not sharply delimited.

2nd day.—No appreciable changes noted.

3rd day.—Embryo 4 mm.; blastodisc 8–9 mm. in diameter. These characters barely discernible.

4th day.—"Heart beat stage." Embryo about 7 mm. long, distance between anterior vitelline veins 12-14 mm. Embryo with vitelline veins has a spider-like appearance.

5th day.—Embryo 8 mm., and is now mobile. Eye spot visible; amnion 9-11 mm. diameter. Original vitelline veins now a vein complex.

7th day.—Embryo now moves about freely in amnion, but is not seen when egg cools, as it sinks in the amnion sac to inner part of egg. Amnion 16 mm. in diameter.

8th day.—Flexed embryo about 10 mm.; flexes and sways in amnion. Eye spot large. Air sac prominent for first time.

91h day.—Embryo 13 mm. in length, eye spot 3.5 mm.; movements within amnion slower. Egg is noticeably darker.

10th day.—Embryo about 15 mm., eye spot 4 mm., moves and flexes more slowly. 15th day.—Embryo now lies across width of egg near air sac.

16th day.—Only air sac visible in candler.

17th day.-Egg opaque; only air sac seen.

Illinois Natural History Survey, Urbana, Illinois, August 1, 1953.