

EGG DISSECTION SOP: PHOTOGRAPHIC GUIDES

APPENDIX 1: Avocet embryo aging guide developed by Joe Skorupa (U.S. Fish and Wildlife Service) and modified by Josh Ackerman (U.S. Geological Survey).

Figure 1:

Fresh American Avocet egg.

In a fertile egg, a broad white ring surrounds a clear central region (area pellucida). In an infertile egg, there will only be a small white dot (about the size of the area pellucida in a fertile egg). The egg pictured here (three different camera exposures of same egg) was fertile, but the area pellucida does not show very well, so it (as well as the ring around it) has been highlighted on the central photo to illustrate scale and location.

(J.P. Skorupa, U.S. Fish & Wildlife Service)

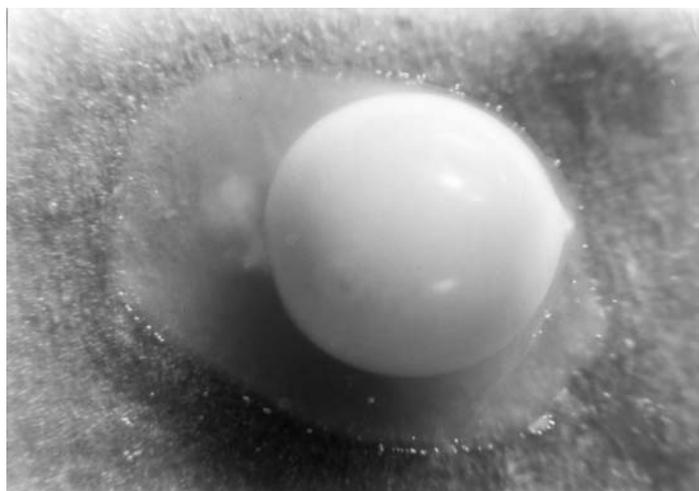
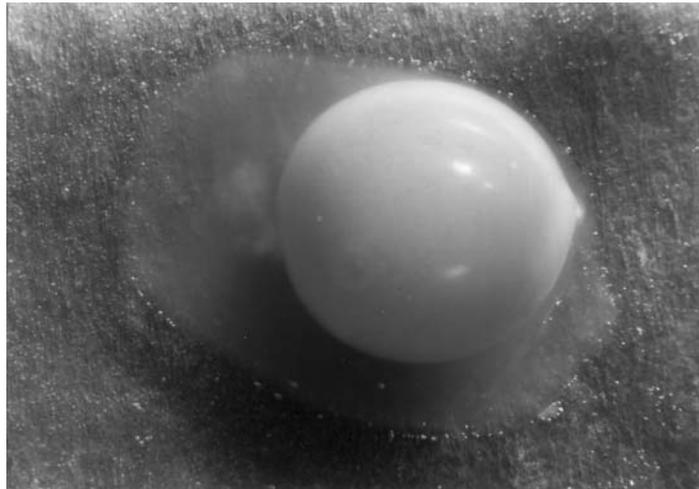


Figure 2:

American Avocet egg after 3 days incubation.

The most prominent characteristics at this stage (known as the “streak” stage) are the heart and the major blood vessels of the yolk sac. In these photos the heart is the dark spot in the upper central area of the yolk sac. If the embryo is alive, you will be able to notice that the heart is beating even after you have placed the sample in a sample jar. (Top two photos are different camera exposures of the same egg.)

(J.P. Skorupa, U.S. Fish & Wildlife Service)

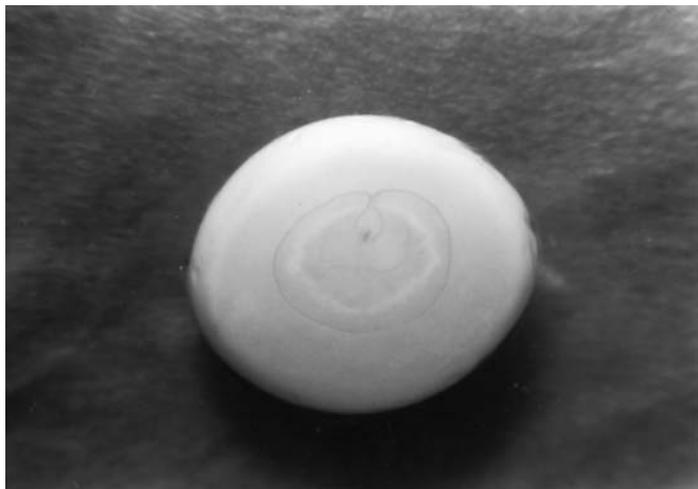


Figure 3:

American Avocet egg after 6 days incubation.

The most prominent characteristics at this stage are the mid-brain bubble at the posterior end of the head (above the eyes), the eyes (which should be very distinctly visible and developed) and the limb buds. The limbs are still paddle-shaped with only slight definition (if any) of digits. The entire embryo is still naked and only about 2 cm in length.

(J.P. Skorupa, U.S. Fish & Wildlife Service)

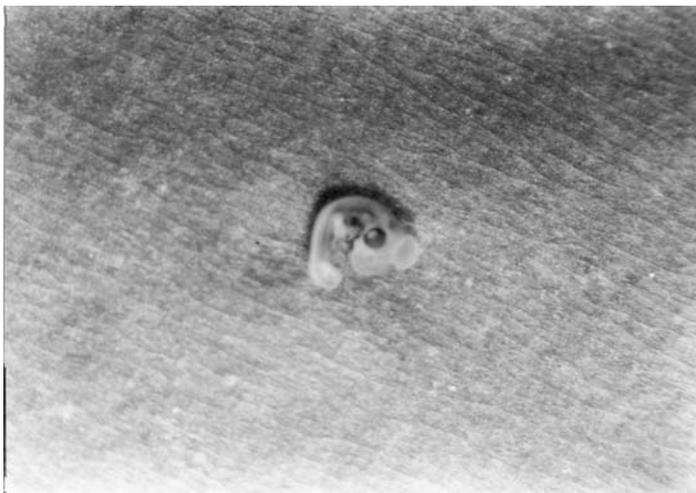


Figure 4:

American Avocet egg after 9 days incubation.

The most prominent characteristics at this stage include a more typically shaped head with the mid-brain bubble absent (upper picture) or only slightly visible (lower picture), a distinctly formed bill that is still shorter than the width of the head, extremely prominent eyes, and more fully developed limbs making the transition to distinctly visible digits. At this stage the embryo is still completely naked, although the skin is beginning to show distinct “pimpling” in the regions of the major feather tracts.

(J.P. Skorupa, U.S. Fish & Wildlife Service)



Figure 5:

American Avocet egg after 12 days incubation.

The most prominent characteristics at this stage include a bill that is longer than one width of the head, large eyes with distinctly visible eyelids that are just beginning to close down, fully developed limbs and the first signs of feathers in the caudal region (a peppered rump, at late day 12, early day 13). The mid and upper back, neck, and head are always still naked at day 12. As illustrated in the upper third of this figure, the yolk sac is still larger than the embryo (lying in the shadows to the left of the yolk sac) at this stage.

(J.P. Skorupa, U.S. Fish & Wildlife Service)

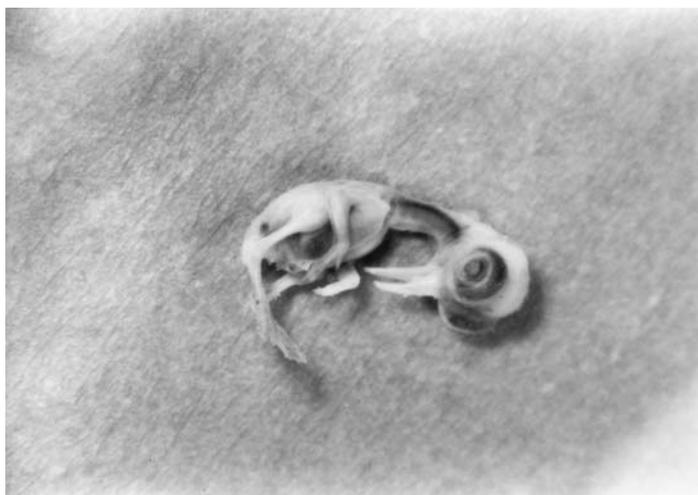


Figure 6:

American Avocet egg after 15 days incubation.

The most prominent characteristics at this stage include feather development that results in a slightly to well “peppered” head, eyelids closing down to more of a horizontal slit than a circular opening, and obviously visible egg tooth & toenails. Overall, the body is still rather sparsely feathered such that the jugular vein is still clearly visible.

(J.P. Skorupa, U.S. Fish & Wildlife Service)

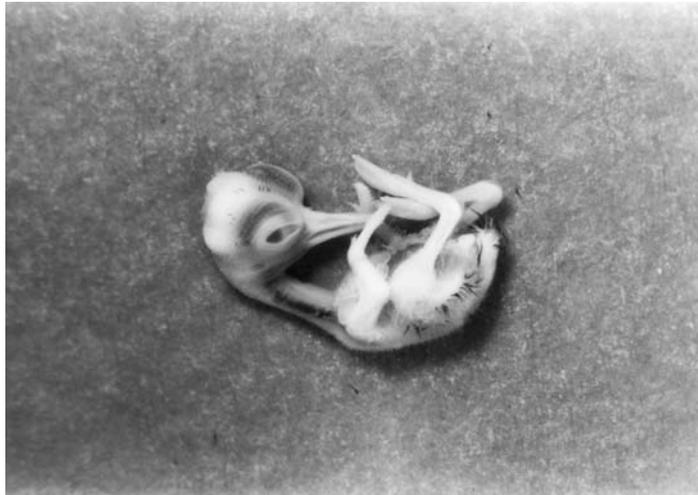


Figure 7:

American Avocet egg after 18 days incubation.

The most prominent characteristics at this stage include more fully feathered facial, head, and neck region, closed eyes, and absence of nare caps. Jugular vein no longer clearly visible, but major femoral blood vessels still clearly visible. The eyelids are still obviously bald. The legs and feet still have a thin “bony” appearance.



(J.P. Skorupa, U.S. Fish & Wildlife Service)

Figure 8:

American Avocet egg after 20 days incubation.

The most prominent characteristics at this stage include very fleshy and bulky looking legs and feet (without clearly visible blood vessels), pigmented/feathered eyelids, and thick feathering of the head. Between this stage and hatching (at 22-25 days), the primary external changes in appearance are the continuing “bulking-up” of the legs and feet and the progressive reduction and absorption of the yolk sac.



(J.P. Skorupa, U.S. Fish & Wildlife Service)

NOTES ABOUT ARTIFICIAL INCUBATION OF EGGS

Sept 18, 2009

Josh,

Below are the Hamburger and Hamilton (1951)** chicken stage equivalents of various avocet incubation ages as per determinations by Paul Martin (UCD Dept. of Avian Science):

<u>Incubation Days</u>	<u>H&H Stage</u>
3	16
6	23+ - 25
9	33
12	36
15	37 - 38
16	39

Paul also noted that: “*Embryos that are 18 days old or older will be hard to distinguish from each other. It looks as though the 20-day embryos have lighter colored feet and have definite scales compared to the 18-day embryos.*”

[My experience has been that there is substantive bulking-up of the mass (fleshiness) of the legs and feet between 18 and 20 that is fairly obvious.]

** Hamburger, V., and H.L. Hamilton. 1951. A series of normal stages in the development of the chick embryo. J. Morph., 88:49-92.

The avocet and stilt eggs producing the embryos in the photographs were incubated @ 37.5 degrees C in a Jamesway 252 incubator @ 56% humidity. The eggs were candled daily and for eggs that were held long enough to pip into the air cell, they were transferred to another Jamesway 252 incubator that had been set up as a hatcher @ 37.5 degrees and 76% humidity.

Time to hatch for eggs that were delivered to the hatchery as fresh eggs were as follows:

Avocet = 23.4 days; range = 22-25 days; based on n = 20 eggs

Stilt = 23.1 days; range = 21 – 24 days; based on n = 20 eggs

[My experience in the Tulare Basin of the southern San Joaquin Valley, where day time temperatures during much of the nesting season are very hot (desert-like environment), was that average hatching time was closer to a 21+ to 22+ day time frame, although there was some variability and the ranges shown above that Paul Martin documented in his incubators covered the full range of variability we were seeing in the field (no hatch earlier than 21 days or later than 25 days); I think Grant (AOU Monograph) also found hatch-out to be a little shorter than 23+ days in the hot environment of the Salton Sea, but I haven't gone back and checked his monograph to be sure I'm remembering that correctly.]

Cheers, **Joe Skorupa**

APPENDIX 2: Avocet and Stilt embryo age equivalents to the Mallard embryos pictured below. After Caldwell, P. J., and A. E. Snart. 1974. A photographic index for aging Mallard embryos. Journal of Wildlife Management 38:298-301.

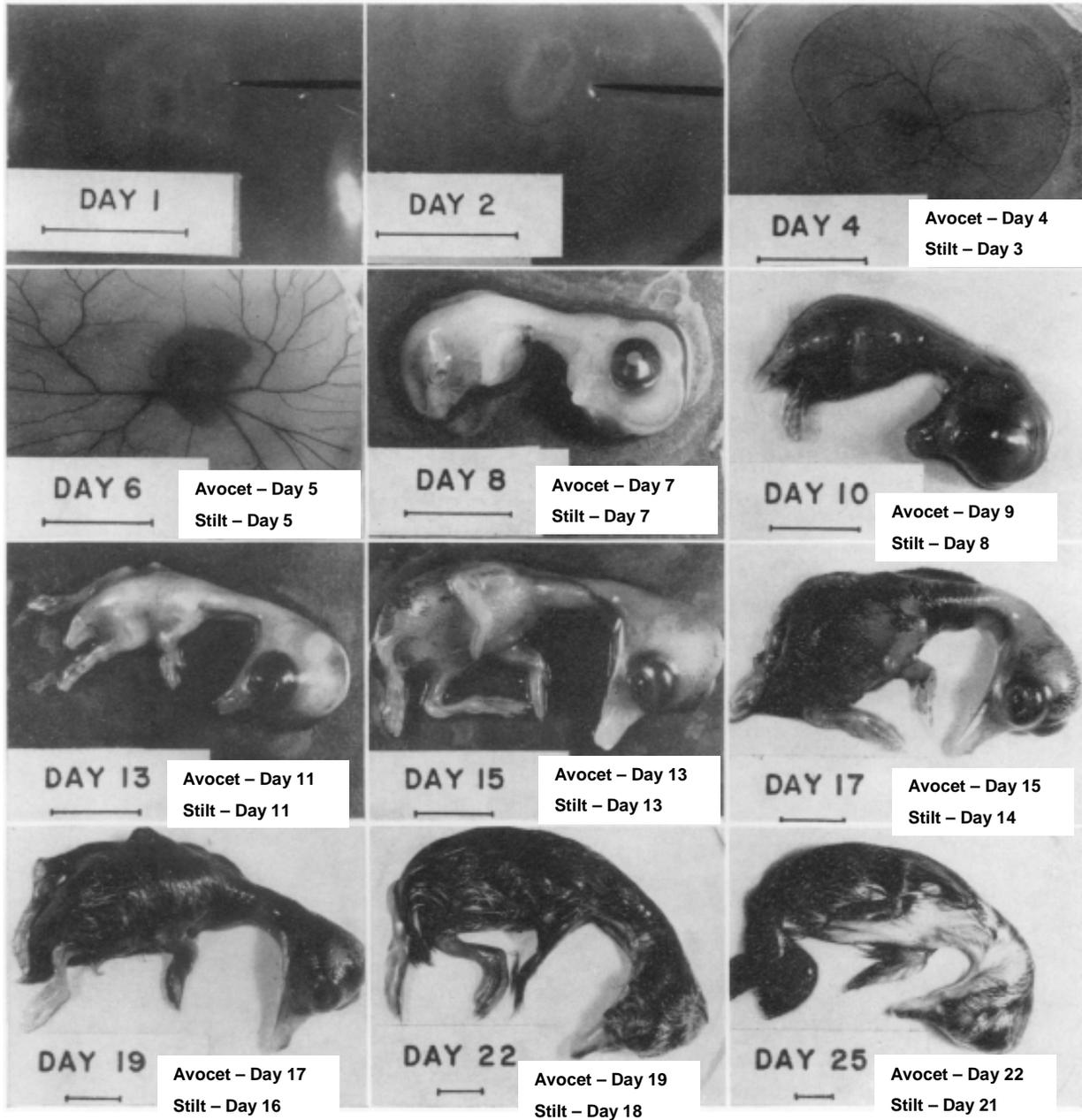


Fig. 1. Photographic index to age of mallard embryos. Line below age designation represents 1 cm.

Doug Roster, Bill Hohman, And Joe Skorupa modified for avocets and stilts.

APPENDIX 3: Normal and Deformed 9 day old Stilt embryos.

Normal Stilt embryo after 9 days incubation, stage 34.

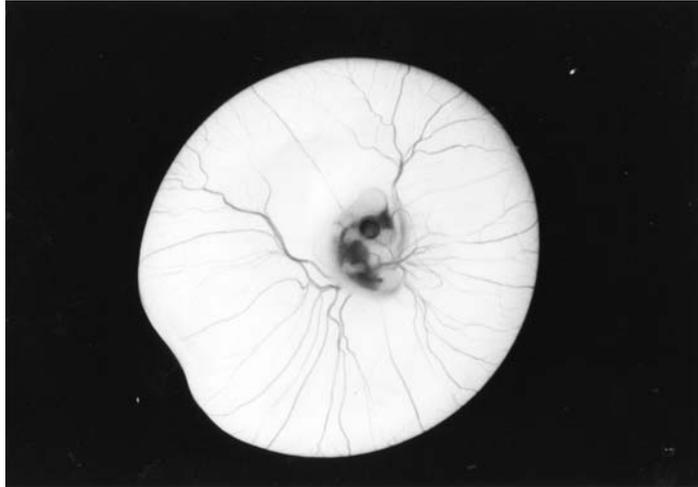


Deformed Stilt embryo after 9 days incubation, ca. stage 33 (stage 38 by eyelids). No legs, reduced eyes and brain hernia (not visible). Reduced head.

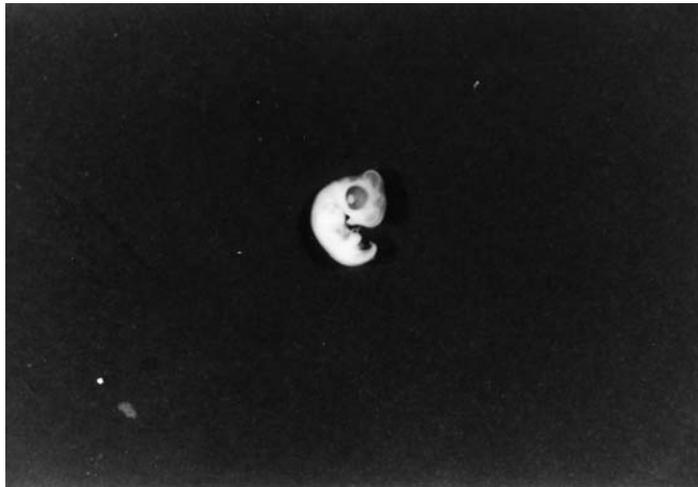


APPENDIX 4: Additional photographic series of artificially incubated Stilt (code=02) and Avocet (code=03) eggs that covers ca. 5 days to 14 days.

Avocet embryo after 5 days incubation, stage 26.



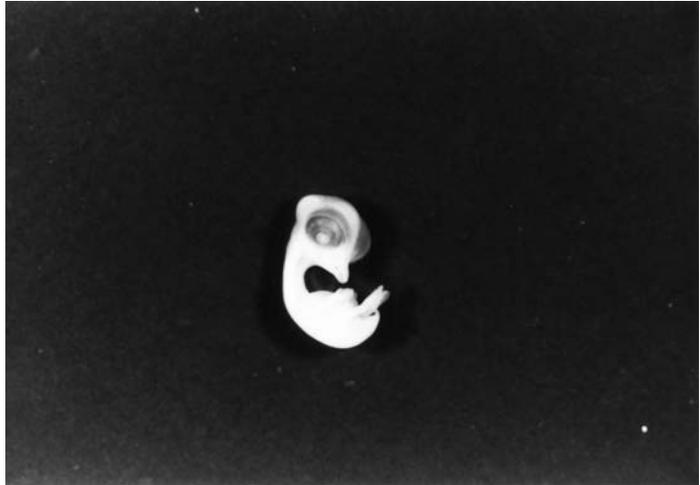
Avocet embryo after 7 days (+?) incubation, stage 27-28.



Stilt embryo after ca. 8 days incubation, stage 30.



Stilt embryo after 10 days incubation, stage 35.



Stilt embryo after ca. 10-11 days incubation, stage 35-36 (eyelid).



Stilt embryo after 11 days incubation, stage 36 (eye).



**Stilt embryo after 12 days
incubation, stage 36+.**



**Avocet embryo after ca. 12 days
incubation, stage 37.**



**Stilt embryo after ca. 12-13 days
incubation, stage 37 (eye).**



Stilt embryo after ca. 13-14 days incubation, stage 38- (eye).



American Avocet, 24 hours post-hatch.

