The Condor 89:663–665

AN IMPROVED PROCEDURE FOR COUNTING PORES IN AVIAN EGGSHELLS[1]

GILBERT W. BLANKESPOOR
Department of Biology, Augustana College, Sioux Falls, SD 57197

Key words: Avian eggshell; counting pores; staining technique.

Efforts to count pores in avian eggshells have most often made use of procedures first described by Tyler (1953). In general, eggshells are boiled in a solution of NaOH to remove shell membranes and free organic matter from pore mouths and pore channels, etched with acid (usually nitric acid) to enlarge the pore channels, and then treated with a dye (usually methylene blue but also other dyes as in Ar and Rahn 1985) to make pores visible and countable. Typically, the dyes are applied to the inner surface of a shell fragment in water solution. After application, capillary action moves the dye solution to the outside surface of the shell where its appearance in pore mouths makes counting of pores possible.

A number of other procedures has been used to count pores in avian eggshells. Tyler and Fowler (1979) painted the outside surface of eggshells with acid fuchsin to highlight grooves and pore mouths. Hoyt et al. (1979) projected light through previously etched pore channels and then counted screen images. Tullet (1975) applied procedures of scanning electron microscopy (SEM) to the inner surfaces of eggshells. Silyn-Roberts (1983) used SEM techniques in conjunction with uranium-stained pores to specifically identify nonobstructed pore channels.

In this note I describe still another procedure for counting pores. The procedure has the following attributes: (1) it is simple to apply; (2) it makes actual counting easy; (3) it provides some information about pore size.

METHODS

The procedure described below applies specifically to eggshells of the Red-winged Blackbird, *Agelaius phoeniceus*. It would differ for other species only in detail.

A fine-pointed forceps is used to separate a fresh, nonincubated egg into two longitudinal halves. Yolk and albumin are flushed away and the clean hemispheric eggshells dried in a desiccator. The eggshells can be stored in the desiccator indefinitely.

In the next step, an entire eggshell half is transferred to 40 ml of a hot (approximately 85°C) 5% (wt./vol.) NaOH solution for 8 min. The use of hot rather than boiling NaOH avoids bumping damage to the fragile eggshell. While still hot, the eggshell is transferred convex surface up to a flat surface of absorbent material. As soon as possible, the eggshell is flushed with a 2% (wt./vol.) solution of acid fuchsin. Before application, the dye solution must be vigorously swirled. The dyed eggshell is then transferred to a desiccator.

RESULTS AND DISCUSSION

What presumably happens during the procedure is as follows. When the eggshell is removed from the hot NaOH, the pore channels are filled with NaOH solution and both surfaces are covered by a film of the same solution. When the eggshell is placed on a flat surface, water evaporation begins, principally from the exposed convex surface. The combined effect of capillary action and evaporation from the outer surface causes NaOH solution to move through the pore channel towards the outer surface. Now the outer surface is painted with acid fuchsin which becomes colorless at pH 12 to 14. After painting, NaOH solution continues to move out onto the outer surface of the egg and where it contacts acid fuchsin, the dye becomes colorless.

Two Red-winged Blackbird eggshell halves to which the procedure has been applied are shown in Figure 1. The eggshell on the left had a conductance (Ar et al. 1974) of 1.36 mg $H_2O \cdot day^{-1} \cdot torr^{-1}$ while the eggshell on the right had a conductance of 0.72 mg $H_2O \cdot day^{-1} \cdot$

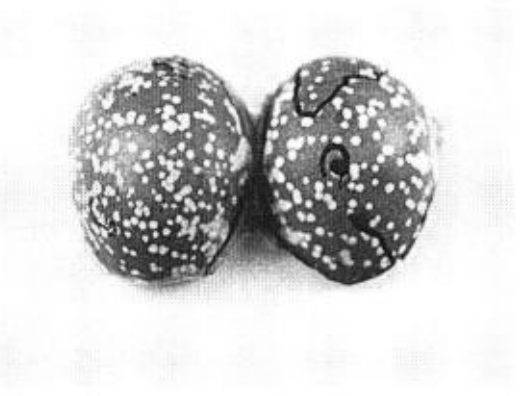

FIGURE 1. Two Red-winged Blackbird eggshells that have undergone the staining procedure described in the text. A pore mouth occurs at the center of each colorless circle.

[1] Received 8 September 1986. Final acceptance 2 March 1987.

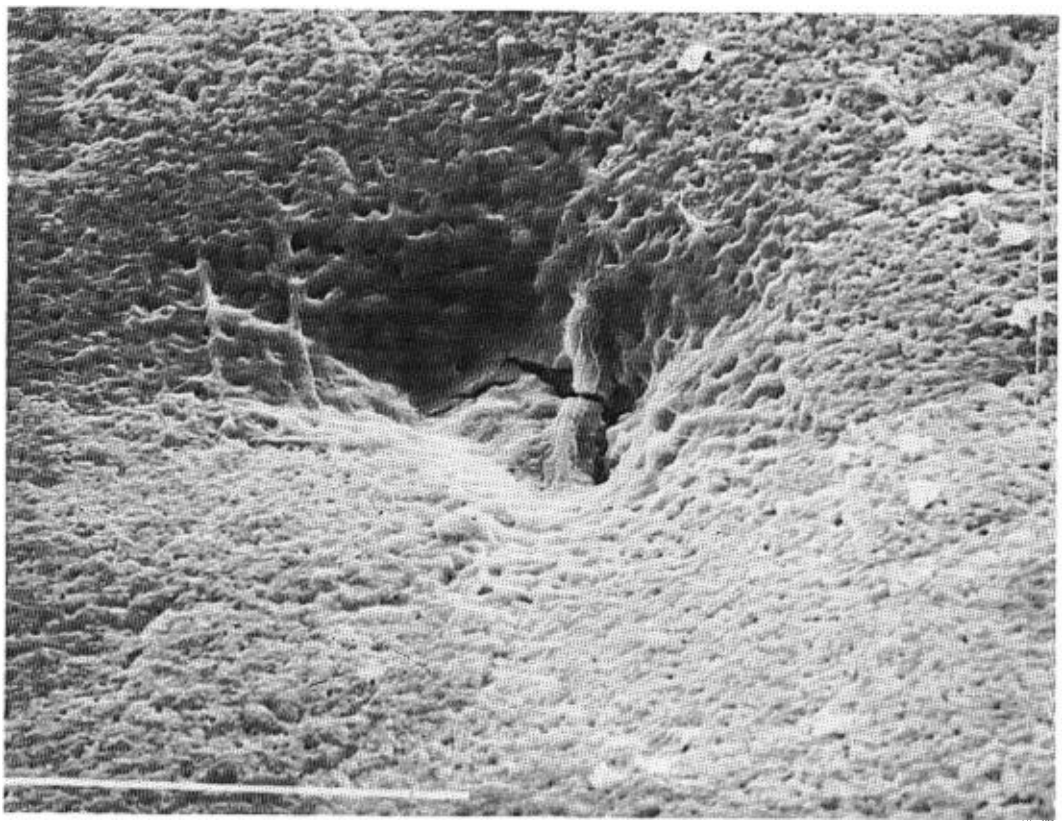

FIGURE 2. Scanning electron micrograph of a single eggshell pore of the Red-winged Blackbird. Note that the pore mouth is mostly occluded. Short scale bar = 5 μm.

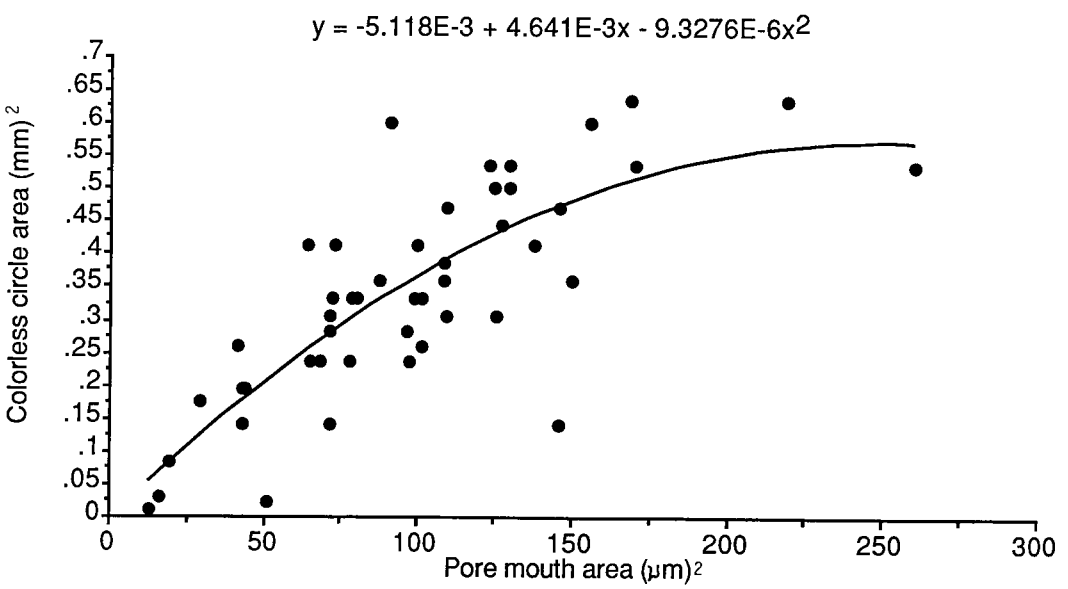

FIGURE 3. Relationship between actual pore mouth area and the area of corresponding colorless circles on the stained eggshell.

torr^{-1}. Note that pores on the outer surface of the eggshell are represented by colorless circles of varying sizes. As can be concluded from Figure 1, counting of pores is a very simple matter. One of the dyed pores was selected for examination with SEM (Fig. 2). As can be seen from the micrograph, the procedure worked well even though the pore channel was mostly occluded.

The staining technique also provides information about pore size. Forty-seven dyed pores were selected for measurements of actual pore mouth area and the area of the corresponding colorless circle on the stained eggshell. SEM images (scanning angle = 45°) were used to measure pore mouth area and a micrometer-equipped light microscope was used to measure the area of the colorless circles. Pore mouths were assumed to be either circular or elliptical. The relationship between these two areas is given in Figure 3 where $R^2 = 0.629$. When the two outlying data points are deleted from the data set, R^2 increases to 0.758. Probably contributing to unexplained variation are difficulty in accurately measuring actual pore area (note irregular shape of pore mouth in Fig. 2) and influence of shell thickness on movement of dye solution through pore channels. Although all of the pores included in Figure 3 were from one eggshell, it is possible to use the procedure to make comparisons among eggshells from different eggs. However, such comparisons are useful only when the procedure is applied in exactly the same way (timing, concentration of solutions, etc.) to each egg.

LITERATURE CITED

AR, A., AND H. RAHN. 1985. Pores in avian eggshells: gas conductance, gas exchange and embryonic growth rate. Respir. Physiol. 61:1–20.

AR, A., C. V. PAGANELLI, R. B. REEVES, D. G. GREENE, AND H. RAHN. 1974. The avian egg: water vapor conductance, shell thickness and functional pore area. Condor 76:153–158.

HOYT, D. F., R. G. BOARD, H. RAHN, AND C. V. PAGANELLI. 1979. The eggs of the Anatidae: conductance, pore structure, and metabolism. Physiol. Zool. 52:438–450.

SILYN-ROBERTS, H. 1983. BSE Z contrast imaging of uranium stained pores in the avian eggshell. J. Microsc. (Oxf.) 130:111–114.

TULLETT, S. G. 1975. Regulation of avian eggshell porosity. J. Zool. (Lond.) 177:339–348.

TYLER, C. 1953. Studies on eggshells. II. A method for marking and counting pores. J. Sci. Food Agric. 4:266–272.

TYLER, C., AND S. FOWLER. 1979. The size, shape and orientation of pore grooves in the egg shells of *Rhea* sp. J. Zool. (Lond.) 187:283–290.

The Condor 89:665–667

NESTING OF THE ROSS' GOOSE AND BLUE-PHASE SNOW GOOSE IN THE SAGAVANIRKTOK RIVER DELTA, ALASKA[1]

STEPHEN R. JOHNSON
LGL Ltd., 9768 Second Street, Sidney, British Columbia V8L 3Y8, Canada

DECLAN M. TROY
LGL Alaska Research Associates, Inc., 505 W. Northern Lights Blvd., #201, Anchorage, AK 99503

Key words: Ross' Goose; blue goose; Snow Goose; nesting; populations; arctic Alaska.

Since 1980 we have been studying the small population (100 to 200 pairs) of Snow Geese (*Chen caerulescens*) nesting on Howe Island in the Sagavanirktok River delta, Alaska (Fig. 1). Our study has included (1) an annual (1980 to 1987) round-up and banding of geese during the brood-rearing/molt period, (2) behavioral observations of molting geese during 1981, and (3) behavioral observations of incubating geese during 1984. During these investigations we documented nesting by Ross' Geese (*Chen rossii*) and blue-phase Snow Geese (hereafter blue geese).

ROSS' GEESE

Nearly all Ross' Geese nest in the central arctic of Canada (Ryder 1972, McLandress 1983). The breeding range is predominately adjacent to the Queen Maud Gulf with some breeding records from Southhampton Island and the Hudson Bay coast (see summary in Bellrose 1976). Ross' Geese typically nest in mixed colonies with Snow Geese (Ryder 1972, Kerbes et al. 1983). In recent years the breeding population has increased (Kerbes et al. 1983) and their range has expanded, mostly eastward to Snow Goose colonies along the west coast of Hudson Bay (Frederick and Johnson 1983). Ross' Geese have been found nesting on Banks

[1] Received 24 September 1986. Final Acceptance 10 February 1987.