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Porosity of Eggshells From Wild and Captive, Pen-Reared Alligators (*Alligator mississippiensis*)

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ABSTRACT Artificially incubated fertile eggs from wild alligators have a significantly better hatch rate than those of captive, pen-reared alligators, possibly due to differences in the morphology of the eggshells. We compared the morphology of eggshells of wild alligators to those of captive alligators living in semi-natural environmental pens. Lengths and widths of eggs were measured and volume was determined, assuming an ellipsoid shape. Eggs were also evaluated for the quality of the eggshell (the presence or absence of rough deposits). Pieces of shell were cut from unincubated eggs and from eggs incubated for 55 days (just before hatching) and examined by scanning electron microscopy. Open pores on the outer surface of the shells were counted and thickness of the pieces was measured from micrographs. Results indicated that the number of pores on eggshells was lowest in eggs of captive alligators with early embryonic death. The number of pores was intermediate in eggs with early embryonic death from wild alligators, and the number of pores was highest in eggs with full-term embryos from wild or captive alligators. It is suggested that decreased porosity of eggshells may be associated with early embryonic death, is more prevalent in captive animals, and may, therefore, be related to poor hatch rate among pen-reared alligators.

Since 1964 the Louisiana Department of Wildlife and Fisheries has studied the feasibility of raising alligators in captivity for commercial and conservation purposes. Today, Louisiana has many operating alligator farms and more planned. Some of the problems of breeding and rearing captive alligators have been solved, but others have not. One remaining problem involves the poor hatchability of eggs. Although eggs from the wild and eggs from captive animals in semi-natural environmental pens are collected and artificially incubated under identical conditions, the hatch rate from the former is greater than 90%, while that of the latter ranges from 30% to 60% (Joanen and McNease, '87).

Preliminary studies (Shirley, '82) have indicated that eggs produced by captive, fish-fed alligators have thicker shells than those produced by captive, nutria-fed alligators. He hypothesized that the thick shells of the fish-fed captive group might contribute to early embryonic death. However, eggs from captive, nutria-fed alligators also do not hatch as successfully as those from wild alligators. Therefore, the purpose of the present study was to compare eggshells of wild alligators with those of captive nutria-fed alligators and to determine the relationship between shell morphology and embryonic death.

MATERIALS AND METHODS

Collection and incubation of eggs

Eggs of *Alligator mississippiensis* were collected from nests in the wild and from nests constructed in semi-natural pens by captive, pen-reared alligators. Eggs were collected at or near the Rockefeller Wildlife Refuge, Grand Chenier, Louisiana, within 48 hours of laying during a 2-week period in the summer of 1987. They were marked for upright orientation (the "upper" surface is that at which the embryonic membranes attach), identified by clutch, covered with wiregrass nesting medium (*Spartina patens*), and transported in single layers. The presence of an opaque eggshell band indicating fertility (Ferguson, '82) was checked on each egg. This band usually appears within 24 hrs after laying and expands with the growth of the embryo. Some fertile, unincubated eggs from nests in the wild and from nests in two pens, as well as several infertile eggs, were packed in boxes and transported to New Orleans for laboratory evaluation.

The remaining eggs were incubated in humid environmental chambers as previously described (Joanen and McNease, '76, '77, '81) with temperatures at 29.4–32.7°C. After 55 days of incubation, just before hatching, fertile eggs from each of the nests from the wild and each of the nests from the two captive pens, as well as infertile eggs which had also been incubated, were removed from the incubators and transported to New Orleans for examination.

Volume and status of eggs—quality of shells

Lengths and widths (at the widest circumference) of one egg selected at random from each nest were measured to the nearest 0.01 mm with vernier calipers. The volume of each egg was determined by using the equation for an ellipsoid (Beyer, '81) which closely approximates the shape of an alligator egg. Each egg was also evaluated for the presence of rough, knobby deposits on the shell. A score of 1 was arbitrarily assigned to shells with rough deposits, and a score of 2 was assigned to eggs with smooth shells.

Eggs which had been incubated for 55 days were opened to verify contents. Fertile eggs (ones with opaque bands on the shells) contained either full-term embryos or necrotic tissue, indicative of early embryonic death (usually within two to three weeks after laying). Infertile eggs had no evidence of opaque banding or necrotic embryonic tissue. All fertile incubated eggs from nests in the wild had full-term embryos. To complete the analysis, eggs with early embryonic death from wild alligators were selected and examined in the summer of 1988. Eggs were then divided into the following groups: 1) wild, fertile, unincubated; 2) captive, fertile, unincubated; 3) wild, fertile, incubated with early embryonic death; 4) captive, fertile, incubated with early embryonic death; 5) wild, fertile, incubated with full-term embryo; 6) captive, fertile, incubated with full-term embryo; 7) wild, unincubated, infertile; 8) captive, unincubated, infertile; 9) wild, incubated, infertile; 10) captive, incubated, infertile.

Scanning electron microscopy

Three pieces of shell (approximately 1 cm^2 each) were cut, including samples from the upper surface of the middle and both ends of each egg. The eggshell membrane was removed, and pieces of shell were rinsed in distilled water and dehydrated in a graded series of alcohols. The outer surfaces of the pieces of shell were then sputter-coated with gold palladium and viewed at 20 kV in a JEOL T-300 SEM. Starting at an edge of each piece of shell at a magnification of ×35, open pores were counted on four consecutive SEM display screens along the middle of the piece of shell. Then the pieces were oriented end-on and micrographs (×100) were taken. The thickness of each piece of shell was measured to the nearest 0.01 mm on the micrograph. There were no differences in thickness or number of pores among the three pieces of shell (ANOVA analysis), and data from the three samples were pooled.

Statistical analysis

Data were first analyzed by computer with ANOVA and Duncan's multiple range tests. There were no significant differences among infertile eggs (Groups 7–10) in any of the parameters, allowing their combination as one group (Group 7). Linear regression analyses were then performed. An analysis of covariance was used to determine differences between slopes and intercepts of the regression equations (Snedecor and Cochran, '80).

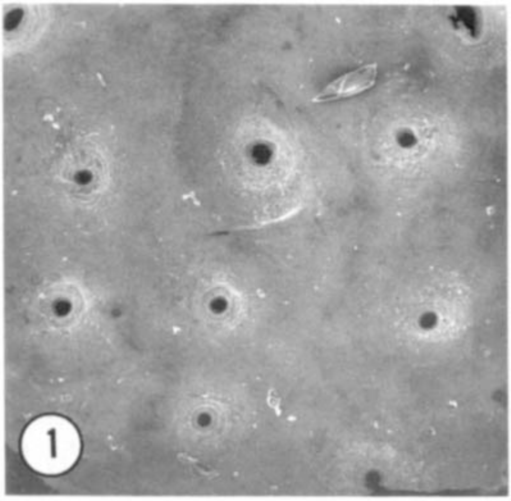
RESULTS

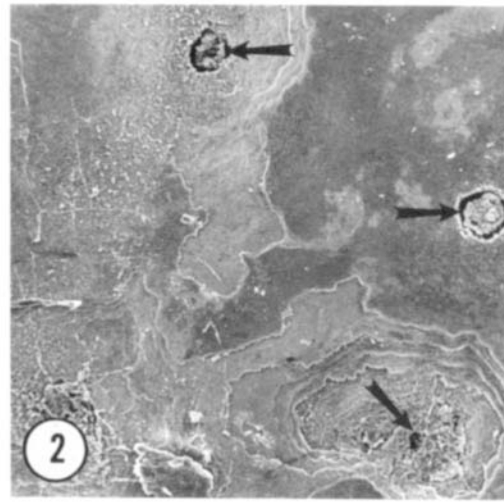
Table 1 summarizes the results of the ANOVA and Duncan's tests. Group 6 (captive, fertile,

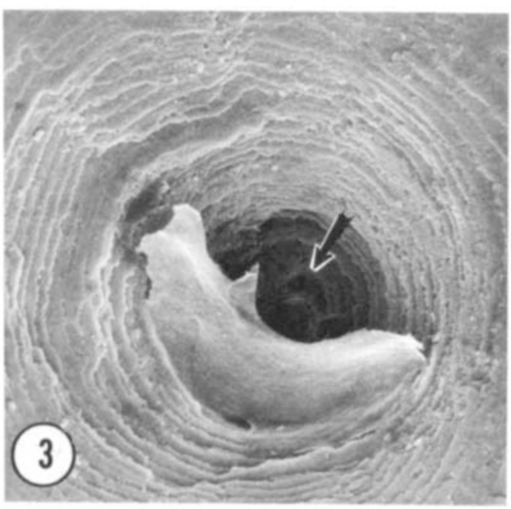
TABLE 1. Volume of eggs, number of open pores on outer shell surface, shell thickness, and presence of deposits[a]

Group	N	Volume (ml^3)	No. pores	Thickness (μm)	Deposit score[b]
1. Wild, fertile, unincubated	7	631.08 ± 28.49	$10.3 \pm 3.2^{2-4,7}$	413.5 ± 23.7	2.0 ± 0.0
2. Captive, fertile, unincubated	7	695.72 ± 84.94	$4.6 \pm 2.9^{1,4-7}$	431.4 ± 23.6	1.9 ± 0.4
3. Wild, fertile, incubated, early embryonic death	7	643.38 ± 48.54	$3.6 \pm 1.7^{1,4-7}$	406.0 ± 21.2	2.0 ± 0
4. Captive, fertile, incubated, early embryonic death	7	656.81 ± 80.88	$0.7 \pm 1.1^{1-3,5,6}$	410.1 ± 25.8	$1.3 \pm 0.5^{1-3,5}$
5. Wild, fertile, incubated, full-term embryo	7	600.36 ± 34.02	$7.9 \pm 2.5^{2-4,7}$	$364.7 \pm 21.9^{1-7}$	2.0 ± 0
6. Captive, fertile, incubated, full-term embryo	8	$703.82 \pm 39.62^{1,5}$	$7.1 \pm 2.9^{2-4,7}$	397.6 ± 31.6^{2}	1.6 ± 0
7. Infertile[c]	24	681.52 ± 63.02	$1.0 \pm 1.3^{1-3,5,6}$	411.0 ± 27.0	1.5 ± 1

[a]Values are listed as mean ± SD. Superscripts indicate significant differences between a particular numbered group and other numbered groups (1–7) with respect to the listed parameter; $P < 0.05$.
[b]A deposit score of 1 denotes rough eggs; a score of 2 indicates smooth-shelled eggs.
[c]Group 7 in this table applies to all infertile eggs (Groups 7–10) examined.







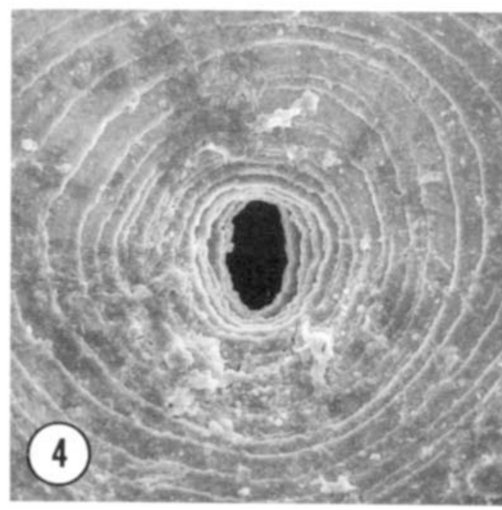


Fig. 1. Scanning electron micrograph of the outer surface of an eggshell from a wild alligator. Note multiple open pores. ×35.

Fig. 2. Scanning electron micrograph of the outer surface of the shell from an egg with early embryonic death from a captive alligator. Note sparsity of pores occluded with deposits and/or pore plugs (arrows). ×50.

Fig. 3. Scanning electron micrograph of a pore from the shell of an egg with early embryonic death from a captive alligator. Note material occluding pore opening (arrow). ×200.

Fig. 4. Scanning electron micrograph of a typical pore from the eggshell of a wild alligator. ×350.

incubated, full-term embryo) had the largest eggs, the eggs of Group 4 (captive, fertile, incubated, early embryonic death) had the roughest shells, and Groups 5 and 6 (wild and captive, fertile, incubated, full-term embryos) had the thinnest shells. The number of pores on the outer surfaces of shells was lowest in Groups 4 and 7 (captive, fertile, incubated, early embryonic death, and infertile), intermediate in Groups 2 and 3 (captive, fertile, unincubated, and wild, fertile, incubated with early embryonic death), and highest in Groups 1, 5, and 6 (wild, fertile, unincubated, and wild and captive, fertile, incubated, full-term embryos). Most pores on shells of eggs from wild alligators appeared to be open and unobstructed (Figs. 1, 4), as many of those from pen-reared alligators were either partially obstructed or completely filled with material (Figs. 2, 3).

Linear regression analysis indicated a significant negative correlation between thickness of shells and number of pores on the outer surfaces

($r = -0.24$, $P < 0.05$) in all the groups. There were no other significant correlations between parameters among the groups.

DISCUSSION

The morphology of the eggshell of the alligator has been described in detail by Ferguson ('82). It consists of an outer densely calcified layer (100–200 μm thick) made up of layers of vertically stacked calcite crystals, a honeycomb layer (300–400 μm thick) of horizontally stacked calcite crystals, an organic layer (10 μm thick) containing a relatively greater percentage of organic matrix to calcite crystals than other layers, and a mammillary layer (20–30 μm thick), consisting of cones of calcite crystals which have their bases on the organic layer and their tips on the innermost layer, the eggshell membrane (150–250 μm thick). Pores extend from the outer surface of the eggshell through the calcified layers to end between mammillae on the eggshell membrane. The outer surfaces of the pores are capped by plugs of material of unknown composition which become dislodged or degraded during incubation. It is generally agreed that water and gas exchange between the developing embryo and the nest environment occurs by diffusion through the pores of the shell in reptilian eggs (Ackerman and Prange, '72; Lomholt, '76; Lutz et al., '79; Ferguson, '82; Whitehead, '87; Manolis et al., '87) as well as in avian eggs (Wagensteen et al., '71; Ar et al., '74; Tullett, '75). Tullett and Burton ('85) noted that variations in shell porosity may be responsible for the wide variation in physical properties of blood among embryos at the same stage of incubation.

In the present study decreased porosity of the eggshell appeared to be a feature of eggs with early embryonic death and infertile eggs (Table 1). It may be that decreased porosity contributed to early embryonic death, possibly due to decreased respiration of the embryo. A second possibility is that decreased porosity is a feature of abnormal and infertile eggs and that the mechanisms involved in decreased porosity as well as those responsible for early embryonic death have not yet been determined. Acquisition of normal porosity might require the presence of a living embryo at some rather developed stage. If so, then infertility and early mortality would be necessarily associated with low porosity.

The poultry industry has long associated smooth eggshells with good shell quality (Hess, '86), and some studies have demonstrated a negative correlation between rough shells and hatchability of fertile turkey eggs (Nestor and Bacon, '81). In the present study rough shells appeared to be a feature of eggs of penned animals and of infertile eggs (Table 1). Because the hatch rate of eggs from captive alligators is lower than that of wild alligators (Joanen and McNease, '87) and eggshells of captive alligators are rougher than those of wild alligators, it may be that the quality of the eggshell is an indicator of hatchability. However, eggshells from Group 3 (wild, fertile, incubated, early embryonic death) had smooth shells, indicating that eggshell quality could not be the only indicator of the hatchibility of alligator eggs. Occlusion of pores on shells of eggs from captives (Figs. 2, 3) may have been caused by the rough, knobby deposits on the shells.

There are two possible, but not mutually exclusive, explanations for the differences in porosity and quality of eggshells of fertile eggs between the wild and captive alligators of this study. Alligators in the pens were more crowded than those in the wild. It has been shown that laying hens kept in cages at high stocking densities were more stressed than those housed in low stocking densities (Mashaly et al., '82; Koelkebeck and Cain, '83; Craig and Craig, '85). The stressed hens laid abnormal eggs with extraneous calcareous deposits on the outer surfaces of the shells, which was thought to be caused by the retention of the eggs in the body beyond the normal time of oviposition (Sykes, '55; Hughes and Gilbert, '84). In general, captive alligators lay their eggs 1–2 weeks later than in the wild. It is possible that crowding stress of the captive animals inhibits or delays oviposition, causing extraneous deposits on the eggshells (Joanen, personal communication).

Another possible explanation for the abnormal shells is diet. Alligators in pens were fed a diet of ground nutria, but wild alligators eat a more varied diet of fish, nutria, birds, etc. Perhaps both diet and crowding contributed to the differences in porosity and quality of the shells.

It is not difficult to understand why the eggshells of Groups 5 and 6 (wild and captive fertile, incubated with full-term embryos) were thinner than those of the other groups. As incubation progresses, there is extrinsic acidic degradation of the eggshell by the nesting material (Ferguson, '82), and intrinsic degradation via mobilization of calcium from the eggshell by the growing embryo (Jenkins, '75; Ferguson, '82). All incubated eggshells of this study were thinned somewhat by extrinsic degradation, but only shells of eggs with full-term embryos were thinned significantly by both intrinsic and extrinsic degradation. Linear regression analysis showed that as the shells were degraded and thinned, the pores increased in number and were enlarged. That there were no other significant correlations between parameters indicates that size (volume) of eggs did not determine porosity, thickness, or quality of shell.

In conclusion, our study suggests that decreased porosity of the alligator eggshell may be associated with early embryonic death, is more prevalent in eggs of captive than of wild alligators, and may, therefore, be related to poor hatchability among captive animals.

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LITERATURE CITED

Ackerman, R.A., and H.D. Prange (1972) Oxygen diffusion across a sea turtle (*Chelonia mydas*) egg shell. Comp. Biochem. Physiol. *43A*:905–909.

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.

Beyer, W.H. (1981) CRC Standard Mathamatical Tables. Boca Raton, Florida: CRC Press, Inc.

Craig, J.V., and J.A. Craig (1985) Corticosteroid levels in white leghorn hens as affected by handling, laying-house environment, and genetic stock. Poult. Sci. *64*:809–816.

Ferguson, M.W.J. (1982) The structure and composition of the eggshell and embryonic membranes of *Alligator mississippiensis*. Trans. Zool. Soc. Lond. *36*:99–152.

Hess, J.B. (1986) Influence of Nutrition and Forced Molting on Eggshell Pimpling in White Leghorn Hens. Thesis for Master of Science Degree, University of Georgia, Athens, Georgia.

Hughes, B.O., and A.B. Gilbert (1984) Induction of eggshell abnormalities in domestic fowls by administration of adrenalin. IRCS Med. Sci. *12*:969–970.

Jenkins, N.K. (1975) Chemical composition of the eggs of the crocodile (*Crocodylus novaegiunea*). Comp. Biochem. Physiol. *51A*:891–895.

Joanen, T., and L. McNease (1976) Culture of immature American alligators in controlled environmental chambers. Proc. World Maricult. Soc. *7*:201–211.

Joanen, T., and L. McNease (1977) Artificial incubation of alligator eggs and post hatching culture in controlled environmental chambers. Proc. World Maricult. Soc. *8*:483–490.

Joanen, T., and L. McNease (1981) Incubation of alligator eggs. Proc. Alligator Production Conf. *1*:117–128.

Joanen, T., and L. McNease (1987) Alligator farming research in Louisiana, USA. In G.J.W. Webb, S.C. Manolis, and P.J. Whitehead (eds): Wildlife Management: Crocodiles and Alligators. Sydney, NSW: Surrey Beatty and Sons Ltd., in association with the Conservation Commission of the Northern Territory, pp. 329–340.

Koelkebeck, K.W., and J.R. Cain (1983) The influence of management alternatives on aspects of laying hen welfare. Poult. Sci. *62*:1449–1550.

Lomholt, J.P. (1976) The development of oxygen permeability of the avian eggshell and its membranes during incubation. J. Exp. Zool. *198*:177–184.

Lutz, P.L., T.B. Bentley, K.E. Harrison, and D.S. Marszalek (1979) Oxygen and water vapour conductance in the shell and shell membrane of the American crocodile egg. Comp. Biochem. Physiol. *66A*:335–338.

Manolis, S.C., J.W. Webb, and K.E. Dempsey (1987) Crocodile egg chemistry. In G.J.W. Webb, S.C. Manolis, and P.J. Whitehead (eds): Wildlife Management: Crocodiles and Alligators. Sydney, NSW: Surrey Beatty and Sons, Ltd., in association with the Conservation Commission of the Northern Territory, pp. 445–472.

Mashaly, M., M. Webb, and W. Roush (1982) Adrenal gland response of laying hens to different cage densities. Poult. Sci. *61*:1506.

Nestor, K.E., and W.L. Bacon (1981) Pimpling of eggshells and hatchability in several strains of turkeys. Poult. Sci. *60*:1939–1943.

Shirley, M. (1982) Shell Thickness of Alligator Eggs as Affected by Diets. Annual Progress Report. La. Dept. of Wildl. and Fish, Grand Chenier, La. 3 pp.

Snedecor, G.W., and W.G. Cochran (1980) Statistical Methods, 7th Ed. Ames, Iowa: Iowa State University Press.

Sykes, A.H. (1955) The affect of adrenalin on oviduct motility and egg production in the fowl. Poult. Sci. *34*:622–628.

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

Tullett, S.G., and F.G. Burton (1985) The effects of eggshell porosity on blood-gas and acid-base status of domestic fowl embryos within eggs of the same weight. Comp. Biochem. Physiol. *81A*:137–142.

Wagensteen, O.D., D. Wilson, and H. Rahn (1971) Diffusion of gases across the shell of the hen's egg. Respir. Physiol. *11*:16–30.

Whitehead, P.J. (1987) Respiration of *Crocodylus johnstoni* embryos. In G.J.W. Webb, S.C. Manolis, and P.J. Whitehead (eds): Wildlife Management: Crocodiles and Alligators. Sydney, NSW: Surrey Beatty and Sons, Ltd., in association with the Conservation Commission of the Northern Territory, pp. 473–497.