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Preliminary Research on the Function of the Eggshell in the Chinese Alligator (*Alligator sinensis*)†

BIHUI CHEN[1] AND BAODONG LIANG[2]

[1]*Department of Biology, Anhui Normal University, Wuhu, Anhui, China*
[2]*Anhui Research Center of Chinese Alligator Reproduction, Xuancheng, Anhui, China*

Abstract. -There is a layer of mucous material on the eggshell of freshly laid Chinese Alligator eggs. Alligator eggshells have different functions in different periods of embryonic development. Over 95% of the eggshell consists of calcite and calcium carbonate. The freshly laid egg is strong and rigid to withstand the weight of the adult female alligator crawling over the closed and compacted nest. As incubation proceeds, erosion craters and cracks appear on the surface of an eggshell. This change of the eggshell is adapted to more efficient oxygen requirements and prevents the egg from dehydration or from too much water flowing into the egg, as the embryo rapidly develops. The eggshell does not provide calcium to supply embryonic developmental demands. The eggshell membrane plays an important role in the antimicrobial defence of the egg.

Key words: Reptilia, Crocodilia, Alligatoridae, *Alligator sinensis*, China, eggshell function

Introduction

Ecological examination in the field and long term artificial culture has revealed that a series of changes appear in the Chinese Alligator's (*Alligator sinensis*) eggshell as the egg develops. These changes seem to relate closely with the alligators' embryonic development and climatic conditions. In order to study this relationship, in 1976 we made some observations and experiments on the change of the Chinese Alligator's eggshell in the course of incubation. In 1987 and 1988, we made some additional experiments.

Methods

Experiments concerning the mucous material covering freshly laid eggs investigate its influence on egg incubation. Four clutches laid by a field alligator were marked as A, B, C, and D respectively. There were 21 eggs in clutch A (with 1 broken and 1 infertile), 17 eggs in clutch B, 20 eggs in clutch C and 18 eggs in clutch D (with 1 infertile). The eggs of clutches A and B were laid in the same day. Eleven eggs from clutch A were selected randomly for the experiment, with 10 eggs for the control. Eight eggs of clutch B were selected randomly for the experiment, with 9 eggs for the control. The experimental eggs of clutches A and B were put together into group I. Control eggs were put together into group II. The eggs were laid one day earlier in clutch C than in clutch D. Each half of the eggs of clutch C and D used were put together in group III, the other half of the eggs were used for a control and were put together into group IV. Each egg of group I and III was washed slightly with gauze in 26°C distilled water, had its mucous material cleaned and was wiped dry. The eggs of group III and IV were submerged in oxygenated distilled water (27-28°C) for 8 hours. Then the four groups were set in an environmental chamber in the same condition and incubated at 30-32°C.

The next experiment investigated the influence of humidity on egg incubation. Eighty-seven eggs, which were laid by an artificially cultured alligator in the Anhui Research Center of Chinese Alligator Reproduction (ARCCAR), were selected randomly and divided into three groups. In group 1, humidity was maintained at 95% from 0 to 21 days after laying, at 80-85%

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TABLE 1. The influence of the mucous material surrounding Chinese Alligator (*A. sinensis*) eggshells during incubation.

Groups	Eggs	Preparative treatment	Hatchlings	Average hatching (days)	Average time from puncture to emergence (hours)
I	19	Mucous material cleaned	16	62	10.5
II	19	None	18	58	4.6
III	19	Mucous material cleaned, submerged in distilled water 8 hours	3	64	31
IV	19	Not cleaned, directly submerged in distilled water for 8 hours	8	63	32

humidity from 22-40 days, and at about 90% humidity from 41 days to hatching. In group 2, the humidity of incubation was maintained at 95-100% humidity from the beginning to the end. In group 3, humidity was maintained at 85-95% and increased to nearly 100% all day respectively on the 10th, 30th, and 40th days of incubation. Generally, the embryonic developmental state was examined with lamp light at 20 day intervals. After group 3 was treated with high humidity, examinations were increased. Except for humidity, the rest of the incubation conditions of the three groups were similar, and temperature was maintained at 31-32°C. The method of determining the calcium and magnesium contents in the alligator's eggshell used by Gu et al. (1987) was adopted. The observations on morphological change of the alligator's eggshell in the course of incubation was primarily in ARCCAR.

Results

The influence of mucous material around the alligator's eggshell on incubation is shown in table 1.

The Joanen and McNease (1977) experiment regarding the influence of washed and unwashed eggs in *Alligator mississippiensis* on egg incubation suggested that washed eggs had no influence on hatching rate, but their emergent duration was extended. Our results on Chinese Alligator eggs show that washed eggs have an 84.2% hatching rate, while unwashed eggs have a 94.7% hatching rate (with the exception of infertile eggs). Incubation periods of washed eggs is delayed an average of 4 days and emergent duration is delayed an average of 5.9 hours. The experiments of groups III and IV indicated that after the eggs were submerged in distilled water for 8 hours, their hatching rates, hatching time and hatchling's emergent duration are very much influenced. Simultaneously, such hatchlings after emergence are weaker and grow slower.

Eggs in group III, in which mucous material was cleaned, have far lower hatching rates (15.8%) than unwashed eggs (42%) in group IV. We consider that this phenomenon probably is related to the mucous material around the eggshell. The mucous material possesses functions that protect the egg from both dehydration and too much environmental water flowing in. This may protect the early embryo from being effected by bad weather.

Clearly, this is of ecologically important significance, because female alligator's lay their eggs in an egg cavity piled with grasses. The initial constructed nest is loose, with free air circulation. When it is a fine day and temperatures are higher, water rapidly evaporates easily leading to dehydration. Conversely, during continuous cloudy and rainy days, the rain easily permeates loose nests into the egg cavity and influences normal egg development. A small opaque white patch was observed on the top surface of the eggshell of a freshly laid Chinese Alligator egg. As incubation progressed, the patch expanded in width around the shell center and in length towards the ends of the shell. About one day after egg laying, it expands approximately 2/5 around the shell, 3/4 around in two days, and completely around the shell after three days. This band slowly extends in length, completely reaching the ends of the shell after about one month.

Ferguson (1982) has reported a similar change for *A. mississippiensis* eggshells and suggested a variety of explanations. One of these explanations is the development of erosion craters that rendered the calcite opaque, altering the optical properties of the shell. Another is a drying out of the eggshell due to polarization of the watery albumin towards the ends of the egg and an increase in porosity of the shell. We quite agree with his explanations. Erosion craters on the Chinese Alligator eggshell increased with the advance of incubation and then progressively became cracks. Some longitudinal cracks were observed on the eggshell around the third week of incubation. They became progressively more extensive in number and diagonal cracks appeared around the fifth week. Eventually these cracks became more and more extensive in size, number, and distribution up until hatching.

In chemical analysis of the Chinese Alligator's eggshell, calcium carbonate in calcite form reached above 95% (Gu et al., 1987). This is similar to *A. mississippiensis* eggshells (Ferguson, 1982). During examination of Chinese Alligator nests in the field, broken eggs were observed in nest cavities less than 1% of the time. Apparently, the freshly laid egg is strong and rigid to withstand the weight of the adult female crawling over the closed and compacted nest. In the initial stage of incubation, due to nest material decay, the resulting acidic effect produces erosion craters on the eggshell surface. The eggshell's strength decreases, but at that time, the frequency of the female alligator crawling over nest tops decreases, so the eggshell isn't damaged. Moreover, there is better air circulation to allow more efficient oxygen requirements during rapid embryonic development. In addition, there is an increase of exchange between interior and exterior water of the egg. At that time, the average atmospheric temperature remains at approximately 29-30°C. It is uncertain whether water loss from the egg should be present or not. In order to explore the relations between incubative humidity and eggshell change, we made some experiments on the hatching rate of alligator eggs versus humidity.

As shown in table 2, group 1 had the best efficiency, and the hatching rate was 100%. Group 2 was hatched under high humidity throughout the incubative period. After the inter-period of incubation, there were 9 eggs which took in water, the eggshell swelling, and cracking. Four eggs were particularly swollen, and the eggshells membrane was cracked. Nine swollen eggs were approximately 6.13±0.29 cm in diameter, and 4.46±0.21 cm in width. Fifteen normal eggs, from the same incubative period, were randomly selected and measured 6.14±0.27 cm in diameter, and 3.48±0.12 cm in width. Both were similar in diameter, but the width of the former is about one centimeter larger than the latter.

Packard et al. (1979) reported that the water conductance of alligator eggs is five times higher than that of birds eggs, which is in keeping with the porous nature of the late alligator eggshell. Our experimental results agree with their report. The humidity of group 3 was 85-95% throughout the incubation period, and only on 10th day increased to nearly 100%. There were no unusual phenomenon to be observed. On the 30th and 40th day there was one and three dead embryos respectively. The above experiments indicate that high humidity in the inter-

TABLE 2. The effect of humidity on *A. sinensis* egg development.

Groups	Eggs	Humidity (%) Ante-period	Inter-period	Post-period	Hatchlings	Hatching rate (%)	Comments
1	29	95	80-85	90	29	100	Normal hatching
2	27	over 95	Over 95	Over 95	23		9 swollen eggs eggshells cracking
3	31	85-95	85-95	85-95	27	87.1	One dead embryo after humidity increase on 30th day, three more dead after increase on 40th day

Note: Ante-period- from the beginning of incubation to 21st day; Inter-period- 22nd day of incubation to 40th day; Post-period- 41st day of incubation to hatching.

TABLE 3. The calcium and magnesium contents of Chinese Alligator (*A. sinensis*) eggshells.

		Mg content		Ca content	
Number	Sample	Number of tests	Means (%)	Number of tests	Means (%)
1	After hatch	4	0.04±0.02	4	38.6±0.1
2	After hatch	4	0.06±0.05	4	38.0±0.0
3	Infertile	4	0.18±0.05	4	38.7±0.1
4	Infertile	4	0.21±0.06	4	38.7±0.01
5	Infertile	4	0.22±0.04	4	38.4±0.0

period is adverse to embryonic development. In ARCCAR, incubative humidity was maintained at over 95% (hatching rate about 90%) throughout the incubation period before 1986. In 1987 and 1988, humidity remained at about 95% in the ante-period and was decreased to 80-90% during the inter- and post-period. The hatching rate was over 95%, and hatchlings were strong. Both survival rate and growth state were better. In addition, according to observations in the field which happened to be during the rainy season in the ante-incubation period, nest humidity remained at more than 95%. High humidity within the nest can prevent water loss in the embryo.

Weather in the wild changed, with clear days and decreasing rainfall after the third week of incubation (inter- and post-period). Humidity within nest cavities measured in the field averaged about 80-85%, and the atmospheric temperature averaged 30-31°C. At that time, some longitudinal cracks appeared on the eggshells. As incubation proceeded, the cracks became progressively more extensive in size and number. Then the exchange between interior and exterior water of eggs increased as humidity within the nest cavity decreased. But at that time, the corneous layer within the skin of the alligator embryo was well developed allowing it to depend less on the surrounding humidity. Clearly, such incubative humidity is adapted to the change of the eggshell. In addition, the cracks progressively increased in size and number to let more and more air through during the growth and development of the alligator embryo. Thus, these changes of the eggshell are not only to satisfy developmental needs, but also to adapt to environmental climatic conditions. The eggshell has different functions at different periods of embryonic development.

Jenkins (1975) estimated that embryos of *Crocodylus novaeguinae* obtains between 1.7 and 2.4 times as much calcium from the shell as from the egg contents. Ferguson (1982) has reported the fact that embryos of *A. mississippiensis* obtains much less calcium from the eggshell than either birds or turtles, indicating that it should be possible to grow normal alligators using shell-less culture techniques.

Gu et al. (1987) have analyzed the calcium and magnesium contents of Chinese Alligator eggshells (Table 3). As shown in table 3, calcium contents in eggshells after hatching are similar to infertile eggs not incubated. This indicated that the calcium content in eggshells which underwent incubation for about two months did not decrease. This is greatly different from bird embryos which obtain a large amount of calcium from eggshells. The experiment proved that alligator eggshells provide very little or no calcium for embryonic developmental. Thus, the eggshell does not store calcium. A lot of experiments of artificial incubation made in ARCCAR indicate that if Chinese Alligator eggs are damaged due to various causes, so long as the eggshell membrane is not breached, and temperature and humidity is well controlled, the eggs will develop normally; their hatching rate still reached over 80%. But if the membrane is punctured, microbes easily invade and the eggs rot and stink. This suggests that the eggshell plays an important role in antimicrobial defense of the egg.

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