

Research on the Sex Sensitive Period During the Incubation of Chinese Alligator Eggs

ZHENG-DONG ZHANG

Anhui Research Center of Chinese Alligator Reproduction, Xuanzhou, Anhui, China

Abstract. -The sex of Chinese Alligators (*Alligator sinensis*) is determined by the temperature that incubating eggs are exposed to. There is a sex sensitive period between the 14th and 27th day of incubation. Eggs treated at temperatures above 34° C produce males.

Key words: Reptilia, Crocodylia, Alligatoridae, *Alligator sinensis*, China, incubation, sex sensitive period.

Introduction

It is generally understood that the sex distinction of the majority of reptiles (including crocodiles and alligators) is determined by the environmental temperature during incubation. We have conducted several experiments on the incubation of Chinese Alligator (*Alligator sinensis*) eggs at different temperatures.

At present, there has not been a study which reports the existence of the "sex sensitive period" in the incubation of alligator eggs. Ferguson (1982) reported on the sex exchange during the entire period of incubation for *Alligator mississippiensis* eggs at different temperatures. In regard to the "sex sensitive period" of *Alligator mississippiensis* (Ferguson called it "temperature sensitive period"), it is believed that the sex determination occurs in the period between the second and third week of incubation. In 1988, we visited the United States and learned that an approach had been in progress to jointly study the "sex sensitive period" of *Alligator mississippiensis* by American and British scientists in London using more accurate means. Obviously such an approach in China still remains blank at the time of writing.

For the purpose of initiating an approach to determine whether there really exists a "sex sensitive period" during Chinese Alligator egg incubation, the Anhui Research Center of Chinese Alligator Reproduction has been conducting experimental studies since 1988 in search of factual understanding. The results of the

two-year study (1988-1989) are reported here.

Materials and Methods

The eggs for the experiment were selected from captive reproduction at the Anhui Research Center. It is imperative to know the exact time when the eggs are laid. The time difference for the laying of the experimental eggs should not be more than 6 hours, nor remain in the natural environment over 12 hours. It is essential that the entire brood be collected immediately and then the eggs divided into separate groups to be hatched under artificial temperature control.

Each experimental group is made up with a corresponding number of eggs from each pre-determined brood to erase the influence which may originate from the different broods.

In 1988, three experimental groups were organized: 88-1, 88-2 and 88-3 together with a control group. The time to undergo the high temperature treatment was pre-arranged with Group 88-1 from the 2nd to the 15th day after the eggs were laid, Group 88-2 from the 16th to the 29th day, and Group 88-3 from the 30th to the 43rd day. All the high temperature treatments were conducted in a constant temperature box. For the rest of the time, each group was taken to the incubation room under the normal temperature. The average temperature received by the groups at the various stages are listed in Table 1 (The temperature appearing on the list and all lists hereafter is in Centigrade).

TABLE 1. The incubation temperature of experimental groups and control group at various time periods in 1988.

Group No.	No. of eggs	In incubation, the average temperature of various time periods			
		2nd-15th day July 2-15	16th-30th day July 16-30	31st-46th day July 31-Aug. 15	47th-54th day Aug. 16-23
88-1	20	34.5±0.8°C	39.5±0.2°C	31.7±0.2°C	32.2±0.2°C
88-2	20	31.6±0.2°C	35.0±0.8°C	*	
88-3	20	31.6±0.2°C	31.5±0.2°C	31.7±0.2°C	32.2±0.2°C
Control group	20	31.6±0.2°C	31.5±0.2°C	31.7±0.2°C	32.2±0.2°C

*At 2nd day of the high treatment, the constant temperature box was bad and the embryos were all dead.

TABLE 2. The incubation temperature of experimental groups in 1989.

Group No.	Average temperature and time periods for high temperature treatment		Average temperature of stages in incubation room	
	Time period of treatment	Average Temperature	Incubation stages	Average Temperature
89-1	14th-18th day	34.5±0.3°C	1st-9th day (July 7-15)	30.5±0.1°C
89-2	15th-19th day	34.5±0.3°C		
89-3	17th-21st day	34.3±0.6°C		
89-4	19th-23rd day	34.5±0.6°C	10th-25th day (July 16-31)	31.0±0.4°C
89-5	21st-25th day	34.6±0.4°C		
89-6	23rd-27th day	34.6±0.1°C		
89-7	16th-20th day	34.3±0.6°C	26th-40th day (Aug. 1-15)	31.5±0.1°C
89-8	18th-22nd day	34.5±0.6°C		
89-9	20th-24th day	34.6±0.4°C		
89-10	24th-31st day	34.7±0.2°C	41st-54th day (Aug. 16-29)	31.3±0.1°C

Attention must be paid to comparing the growth of young alligators in the first 8 months after hatching.

Based on the experiments achieved from Group 88-2, 9 groups were established in 1989. The time for the high temperature treatment of various groups was shortened to 96 hours. The time threshold for the treatment of each group was to be alternated from one to two days. Because of some unanticipated causes, the experiment on Group 88-3 was not satisfactorily accomplished. Another group, No. 10, was then set up, to pass through the prolonged alternate high temperature treatment for 7

days. The average value of the treatment group to various groups and the temperature at various stages at other times are listed in Table 2.

Care is also required to note the comparison among the hatching results of various groups in 1989, and also the cause of mortality of young alligators in the first ten months of growth.

Tissue-section tests were used for sex identification in order to determine the correct sex without error.

TABLE 3. The incubation results, growth comparisons and sex ratio of the 1988 groups (weight in grams and length in centimeters).

Incubation results				Growth comparison of the first 8 months					
Group No.	No. eggs	No. dead	No. hatched	(7th day)		(hibernation begins)		(hibernation begins)	
				Aug. 30, 1988		Nov. 25, 1988		Dec. 13, 1988	
				weight (\bar{x})	length (\bar{x})	weight (\bar{x})	length (\bar{x})	weight (\bar{x})	length (\bar{x})
88-1	20	14	6	21.1±0.6	21.2±0.6	45.8±6.2	25.1±1.3	48.0±5.0	25.5±1.4
88-2	20	1	19	24.1±1.9	22.2±0.6	40.1±5.1	23.7±0.9	43.7±5.6	23.5±1.2
Comp.	20	0	20	23.3±1.1	22.2±0.5	43.3±4.7	25.1±0.8	45.6±5.9	24.9±0.9
Sex ratio				(hibernation finishes)				No. dead or weak	
No.	No.			Mar. 22, 1989		April 30, 1989		No.	No.
?	!			weight (\bar{x})	length (\bar{x})	weight (\bar{x})	length (\bar{x})	Dead	Weak
88-1	1	5		44.9±6.1	25.2±1.9	65.2±8.0	27.9±1.5	0	0
88-2	19	0		40.7±6.2	23.4±1.2	53.0±11.5*	35.4±1.7	2	0
Comp.	5	15		43.7±6.0	25.4±0.9	54.7±9.2**	27.0±1.5	1	2

Note: * n=17, ** n=19

TABLE 4. The incubation results, growth comparisons and sex ratio of the 1989 groups.

Group No.	Incubation result				Cause of mortality		Sex proportion		
	No. of eggs	No. of embryos	No. hatched	Artificial Midwifery	No. dead	No. growing badly	No. probed	No. ?	No. !
89-1	10	0	10	7	0	4	10	10	0
89-2	10	0	10	5	0	4	3	3	0
89-3	10	0	10	7	0	4	10	10	0
89-4	10	0	10	8	0	3	2	2	0
89-5	10	1	9	6	1	1	8	8	0
89-6	10	1	9	5	0	4	9	9	0
89-7	10	1	9	7	0	3	9	9	0
89-8	10	1	9	7	1	3	9	9	0
89-9	10	1	9	7	1	2	9	9	0
89-10	16	1	15	6	0	1	15	13	2

Results

The main results from the 1988 experiment are shown in Table 3, and the main results from the 1989 experiments are shown in Table 4.

Discussion

According to the incubation results from Group 88-1, it is understood that in the first

14 days of incubation was the "high temperature sensitive period" of the alligator's embryo. The embryo is easily damaged and even death occurs in the high temperature for experimental purposes.

It is worthy to note that in another group which underwent the experimental high temperature simultaneously, all the tested embryos were found dead in the first 14 days, because of the application of

36±0.5°C to the temperature environment (The data relative to this test is not presented here). Therefore, the range of temperature control at this stage for Group 88-1, in fact, reached the high temperature limit. In comparison, the average temperature applied to Group 88-2, which was raised 0.5°C higher than that of Group 88-1, safety could be ensured at that time threshold.

From data gathered from various groups in 1988 on the growth of young alligators, it indicated that although among the three groups, the weight of young alligators in Group 88-2 was the largest, those in Group 88-1 had the smallest weight. However, after 90 days (Nov. 25), the young alligators of Group 88-1 overtook the other two groups, and the weight of Group 88-2 became smallest among the three groups. Such condition was maintained until the conclusion of the experiment. The average length of the young alligators in the three groups showed a similar phenomenon (See Table 3). This suggests that the young alligators, which were under the treatment of the special temperature at the "sex sensitive period", have become comparatively weak.

The sex proportion of Group 88-1, 88-2 and the control group revealed that Group 88-1 was almost the same as the sex proportions of wild Chinese Alligators which we have studied (M:F=5:1). The time threshold of high temperature treatment for Group 88-2 was just at the "sex sensitive period". All of them were males. Those in the control group showed sex differences somewhere between the other two groups. The cause of the latter condition is still unknown (Table 3).

In the experimental test in 1989, the sex proportion from the various groups was much beyond expectation. It provided the understanding that the time threshold for the "sex sensitive period" in the incubation of the Chinese Alligator is quite wide in range. Direct study on experiments and Group 88-2 pointed out that the time threshold must be from the 14th to the 27th day during the time of incubation.

Based on the incubation results of young alligators in various groups as well as the cause of mortality in the first 10 months, it was determined that the physical condition of young alligators in Groups 89-1 to 89-9 was considerably weak, corresponding with the result obtained in 1988. Comparatively speaking, those in Group 89-10 proved to be better. The materials relative to their sex distinction revealed that the time threshold for high temperature in this group has somewhat deviated from the "sex sensitive period". Therefore, during the time of the "sex sensitive period", the unisexual offspring that we obtained from treatment of artificial temperature control were considerably weak. At the initial stage of growth, they require particular care.

Direct study has discovered that there may exist some other factors which influence the "sex sensitive period" of the Chinese Alligator. At present, we are deep in our research on this field.

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