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Studies on the Physiological Ecology of Incubation in *Chinemys reevesii* Eggs

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Abstract: -The length of incubation period in *Chinemys reevesii* eggs is 66.91±3.70, 62.29±9.00 and 56.57±2.85 days at 28°C, 30°C and 33°C, respectively. The values of effective accumulative temperature approximate to a range of constant (1871 to 1903 C-day) during different incubation temperatures. The mass of eggs buried in wet sand through incubation increased slightly about an average 0.48% to 3.66%. The mass of turtle hatchlings just after hatching at 28°C, 30°C, and 33°C averaged 59.76±6.85%, 59.12±5.33% and 56.31±5.36% of pre-incubation egg mass, respectively. The total lost rate of energy substances increased with the temperature of incubating and lost 25.92±9.67% at 28°C, 32.56±6.77% at 30°C, and 34.35±5.67% at 33°C. The metabolic rate of *C. reevesii* eggs was measured through incubation at 28, 30 and 33°C. The pattern of metabolic rate of embryonic development in *C. reevesii* is peaked, similar to the conditions of some other fresh water turtles. Maximum VO_2 occurred when its incubation is 65% to 80% of total incubation times. Total VO_2 of *C. reevesii* eggs was 94.61 mL/g at 28°C, 112.88 mL/g at 30°C and 152.22 mL/g at 33°C.

Key Words: Reptilia, Testudines, *Chinemys reevesii*, incubation period, hatchlings, effective accumulative temperature, oxygen consumption.

Introduction

The metabolic rate of developing embryos in reptiles reflects the energetic demands of both growth and maintenance (Wang et al., 1988). The ontogeny of embryonic metabolic rate in reptiles has been shown to have three patterns (Thompson, 1989). The metabolic rate during most of the incubation period has been reported in seven species of snakes (Clark, 1953; Dim'el, 1970; Black et al., 1984), ten chelonians (Lynn and von Brand, 1945; Ackerman, 1981a; Thompson, 1989; Gettinger et al., 1984), three crocodilians (Thompson, 1989; Whitehead 1987), and one lizard (Wang et al., 1988). So far, that of the common turtle (*Chinemys reevesii)* has not been reported.

Ecological and heat energy metabolic studies on adult turtles (*Chinemys reevesii)* have been reported before (Wang and Lu, 1985; Wang et al, 1988). In this paper, we will attempt to deal with the length of the incubation period and the metabolic rate of embryonic development inside the eggs of *Chinemys reevesii* in relation to ambient temperature from July to September, 1988 and 1989.

Methods and Materials

Fresh eggs of the turtle (*Chinemys reevesii)* were collected in the morning, after being laid in the sandbox of the turtle farm near our University. When each fresh egg was removed from the sandbox on the day of laying, it was marked and weighed with a torsion balance (±0.01 g) to determine the fresh egg mass. A total of 350 eggs were examined and divided into three groups in which the eggs of each group were buried in a dish of moist sand and incubated at temperatures of 28, 30 and 33°C, respectively. The relative humidity of sand in the dish was maintained at a range of 98 to 100%.

The determination of oxygen consumption of eggs in *C. reevesii* during the period of incubation was carried out with a small, simple and closed system respirometer that was described by Wang (1986). During determination, the ambient temperature also identified with the incubation temperature of each group. The experimental period was limited at 8:30 to 10:30 in the morning and each experiment lasted an hour with recording every five minutes. The carbon dioxide (CO_2) was absorbed by 10% NaOH solution. The

TABLE 1. T-test on differences of mean times from incubation to hatching in *C. reevesii* eggs at 28°C, 30°C, and 33°C

$T_{incub.}$ = C	Different values (days)	t	P
28 v. 30	4.63	18.37	<0.001
28 v. 33	10.34	11.37	<0.001
30 v. 33	5.71	8.66	<0.001

TABLE 2. Total temperature-day of requirement for embryonic development inside the egg of *C. reevesii*.

$T_{incub.}$ = C	28°C	30°C	33°C
N	98	103	54
Total temperature-day (C-day)	1882.61 (SD= 168.99)	1903.80 (SD= 116.62)	1871.27 (SD= 94.88)

TABLE 3. T-test on the different values of total temperature-day for embryonic development of *C. reevesii* at 28, 30, and 33°C.

$T_{incub.}$ = C	Different values (days)	t	P
28 v. 30	21.19	0.73	<0.005
28 v. 33	11.34	0.40	<0.005
30 v. 33	32.53	1.27	<0.005

TABLE 4. The changes of egg masses in *C. reevesii* throughout incubation period at 28, 30, and 33°C.

Days of incubation	28°C			30°C			33°C		
	N	M±SD	%	N	M±SD	%	N	M±SD	%
0	7	7.73±1.96	100	7	6.89±0.76	100	7	7.56±1.17	100
5	7	7.58±1.80	98.1	7	7.02±0.72	101.9	7	7.71±1.12	102
10	7	7.56±1.63	97.8	7	7.27±0.61	105.5	7	7.73±1.13	102.2
15	7	7.60±1.63	98.3	7	7.23±0.56	104.9	7	7.61±1.24	100.7
20	7	7.60±1.63	98.3	7	7.33±0.50	106.4	7	7.99±1.31	105.7
25	7	7.68±1.61	99.4	7	7.16±0.53	103.9	7	8.05±1.42	106.5
30	7	7.71±1.61	99.7	7	6.98±0.70	101.3	7	7.67±1.14	101.5
35	7	7.92±1.61	102.5	7	7.21±0.39	104.6	7	7.65±1.23	101.2
40	7	7.99±1.53	103.3	7	7.24±0.33	105.1	7	7.78±1.43	102.9
45	7	8.09±1.63	104.7	7	6.98±0.45	101.3	7	8.05±1.94	106.5
50	7	7.95±1.86	102.8	7	6.92±0.24	100.4	7	7.82±1.73	103.4
55	7	7.86±2.14	101.7						
Total (M±SD)		7.27±0.18	100.6 ±2.4		7.11±0.16	103.2 ±2.3		7.79±0.17	103 ±2.3

TABLE 5. A comparison between the mass of pre-incubation eggs and one of *C. reevesii* hatchlings during incubation at 28, 30, and 33°C.

Incubation Temp.	Pre-incubation			Just after hatching						
	Eggs, g	Standard rate %		Egg Shell			Hatchlings			Total lost rate, %
		Egg Shell	Egg Content	g	% of egg	>SRes %	g	% of egg	>SRc %	
28°C	8.23 SD=1.10 N=10	14.6 N=20	85.4 N=20	1.17 SD=1.17 N=10	14.31 SD=2.56 N=10	0.29	4.83 SD=0.74 N=10	59.76 SD=6.85 N=10	25.64	25.92 SD=9.67 N=10
30°C	7.86 SD=0.94 N=10	14.6 N=20	85.4 N=20	0.75 SD=0.30 N=10	9.44 SD=2.97 N=10	5.16	4.66 SD=0.78 N=10	59.12 SD=5.33 N=10	26.28	32.56 SD=9.68 N=10
33°C	7.28 SD=1.01 N=10	14.6 N=20	85.4 N=20	0.7 SD=0.17 N=10	9.51 SD=1.60 N=10	5.09	4.12 SD=0.83 N=10	56.37 SD=5.36 N=10	29.03	34.35 SD=9.67 N=10

Note: Standard rate (%) shows both eggshell % of and egg contents % of whole egg mass according to the average value of twenty fresh eggs.
SRes shows standard rate of egg shell.
SRc shows standard rate of egg content.
Total lost rate equals the mass of pre-incubation egg subtracting both masses of eggshell and of hatchlings just after hatching.

oxygen consumption was expressed as mL $O_2/h^{-1}g^{-1}$ or mL $O_2/day^{-1}g^{-1}$.

Results

Length of Incubation Period

The mean time of incubation to hatching for *C. reevesii* eggs was 66.91 days (SD=3.70, N=98), 62.29 days (SD=9.00, N=103) and 56.57 days (SD=2.85. N=54) during 29°C, 30°C and 33°C of incubation temperature, respectively. These differences of the mean values were compared by a t-test and the results indicate the significant differences (Table 1).

The length of the incubation period in turtle eggs decreased as the incubation temperature increased, that is a negative correlation of linear regression with the following equation: For Days = 124.2550 - 2.0550 (°C), r = -0.9985 (P>0.05).

Total Temperature-day of Requirement for Hatching

The total values of temperature-day for embryonic development in *C. reevesii* eggs during 28°C, 30°C and 33°C, is shown in Table 2. The different values of total temperature-day for embryonic development inside egg from Table 2 were compared by a t-test and the results of those show no significant differences in Table 3.

Changes of Egg Mass through Incubation

During the incubation of turtle eggs buried in wet sand, the average mass of those eggs increased slightly from 0.48% to 3.66% (Table 4).

Mass of Hatchlings

The mass of turtle hatchlings (*C. reevesii*) just after hatching at 28°C, 30°C and 33°C averaged 4.83 g (SD=0.74, N=10), 4.66 g (SD=0.78, N=10) and 4.12 g (SD=0.83, N=10) or 59.76% (SD=5.36,

TABLE 6. Equations relating VO_2 ($ml/g^{-1}day^{-1}$) to incubation days, total VO_2, and VO_2 of peak during incubation to hatching in *C. reevesii* eggs.

T-incub.	Before Peak in VO_2 = a +b days	r	After Peak in VO_2 = a +b days	r	Peak	Total O_2 ml/g
28°C	VO_2 = -2.0888+0.0356	0.944	VO_2 = 1.2524-0.0074	-0.931	2.06	94.61
30°C	VO_2 = 2.7922+0.0546	0.979	VO_2 = 1.3576-0.060	-0.695	2.52	112.88
33°C	VO_2 = 1.75+0.043	0.974	VO_2 = 1.7529-0.115	-0.811	2.72	152.22

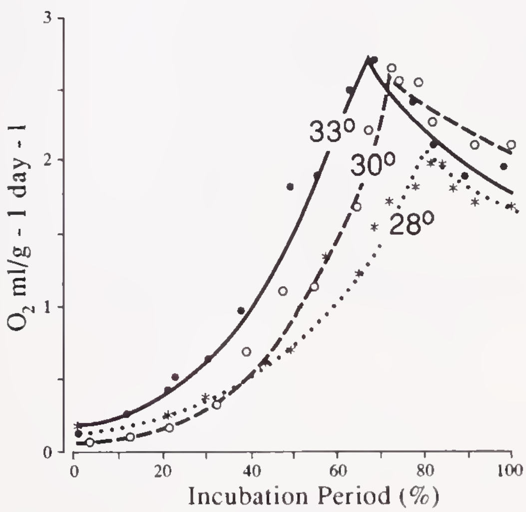

FIG. 1. Relationship between oxygen consumption of eggs and percentage of incubation period in *Chinemys reevesii*.

N=10), 59.12 % (SD=5.33, N=10) and 56.31% (SD=5.36, N=10) of pre-incubation egg mass, respectively. These values are 25.64%, 26.28% and 29.03% less than the standard rates of substance contents inside eggs during pre-incubation eggs of *C. reevesii* (Table 5).

Oxygen Consumption

The pattern of change in oxygen consumption (VO_2) of *C. reevesii* eggs was similar at 28°C, 30°C and 33°C. The VO_2 increased approximately exponentially and peaked to 65% of incubation period at 33°C, to 70% of that at 30°C, and to 80% of that at 28°C, and then declined to the time of hatching (Fig. 1). Total amount of O_2 consumed was calculated by integration of the equations (Table 6) over the interval from the first VO_2 measurement to the mean total incubation period at relevant temperature. The total VO_2 was 94.61 mL/g at 28°C, 112.88 mL/g at 30°C, and 152.22 mL/g at 33°C.

Discussion

Length of Incubation Period and Thermal Constant

Table 1 shows that the results of t-test for the average lengths of incubation period in *C. reevesii* eggs at 28°C, 30°C and 33°C exhibit the significant differences ($P<0.001$). The average lengths of those decreased with incubation temperature increased. This may be a characteristic of embryonic development in oviparous ectothermic animals. The thermal demands of embryonic development inside eggs is provided by the surroundings, so that, the developing velocity (days) of embryo in *C. reevesii* is affected by its surrounding temperature.

The relationship between length of incubation period and effective temperature of ectothermic embryonic development may be presented in an equation of effective accumulative temperature as follows: $K = D (t_1 - t_0)$, where K = effective accumulative temperature , it is a constant or total temperature-day (C-day), t_0 = developmental temperature, D= total time (days) of embryonic development. On developmental zero (t_0) of embryos in *C. reevesii*, it is supposed by 0°C, so that , the effective accumulative temperature amounted to 1882.61±168.99 C-day during incubation to hatching in the *C. reevesii* eggs at 28°C, 1903.80±116.62 C-day at 30°C, and 1871.27±94.88 C-day at 33°C (Table 2).

The average values of those were taken by t-test and the results of those exhibited no significant differences (Table 3), in other words, the values of effective accumulative temperature for the embryonic developing inside the egg of *C. reevesii* are approximately a range of constant during different incubation temperature.

Changes of Incubating Egg Mass

During incubation, *C. reevesii* eggs buried in wet sand (RH, 98-100%) increased slightly in mass (Table 4). This may be due to the intake of water through the egg shell from the substrate (wet sand) and egg shell type.

On changes of egg mass (weight) in *C. reevesii* during incubation which are considered due to a net water intake from surroundings or of export. For an egg to absorb water, the potential of water in substrate must exceed the algebraic sum of the pressure potential and the osmotic potential of the egg contents (Packard et al., 1977). So that the viable egg contacting wet substrates experienced net increases in mass during incubation, that reflects on net fluxes of water across its egg shell (Packard et al., 1977, 1982, 1985; Gutzke and Packard, 1987). The egg shell type of *C. reevesii* is a hard shell, so the water contents of intake from its wet substrates must be controlled or limited to its egg shell type.

The Ratio between Mass of Pre-incubation Egg and of Hatchlings

The mass of *C. reevesii* hatchlings just after hatching at 28°C, 30°C and 33°C averaged for 59.76%, 59.12% and 56.37% of pre-incubation egg mass (Table 5), respectively. The values of those were less 25.64%, 26.28% and 29.03% less than standard rate of pre-incubation egg mass (Table 5). However, the algebraic sum for mass of egg shell and of hatchling just after hatching in *C. reevesii* is also less than one of pre-incubation egg (Table 5). This suggests that a part of energy substances in egg is lost or consumed through incubation. The total lost rate of energy substances increased with the temperature of incubation. The losses were 25.92±9.67% at 28°C, 32.56±6.77% at 30°c and 34.75±5.67% at 33°C (Table 5).

Pattern and Rate of Metabolism

The ontogeny of embryonic metabolic rate has been reported with three patterns: peaked, sigmoid and exponential. The eggs of *C. reevesii* during incubation at three different temperatures had an extreme peaked pattern of oxygen consumption, similar to the conditions of some fresh water turtles (Gettinger et al., 1984; Lynn and von Brand, 1945; Thompson, 1989; Webb et al., 1986), *Crocodylus* (Whitehead, 1987), *Alligator* (Thompson, 1989), and some birds (Vleck et al, 1980) but different from many other reptiles (Ackerman, 1981; Black et al., 1984; Clark, 1953; Dmei'el, 1970; Wang et al., 1988).

The patterns of embryonic metabolic ontogeny appear to be associated with different patterns of growth, egg shell types and environmental conditions of incubation (Thompson, 1989; Whitehead and Seymour, 1990). However, peaked or sigmoid pattern of embryonic metabolic ontogeny may be due to the fact that the energy expenditure for embryonic growth is decreased as the growth rate of embryo in late incubation period declines. This can possibly facilitate synchronous hatching in clutches.

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