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Effects of Incubation Temperatures on Hatching Period and Growth in Korea Reeves' Turtle(*Mauremys Reevesii*)[1]

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한국산 남생이의 부화와 성장에 부화 온도의 영향[1]

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ABSTRACT

This study was conducted to determine whether the difference in egg hatching temperature of Korea Reeves' turtle (*Mauremys reevesii*) at the artificial nursery of Seoul National Grand Park affects the incubation period and growth. A total of 201 eggs were incubated at 26 ℃ (n=89), 28 ℃ (n=75) and 32 ℃ (n=37). The incubation period of eggs showed significant differences according to the hatching temperature. In this study, the higher the hatching temperature, the higher the hatching rate. The incubation period of the eggs hatched at 26 ℃, 28 ℃ and 32 ℃ was 66.1 (±4.0, n = 52) days, 65.3 (±3.3, n = 44) days and 58.8 (±7.7, n = 31) days, respectively. Eggs incubated at 32 ℃ (83.8%) had a higher hatching success than those at 26 ℃ (58.4%) and 28 ℃ (58.7%). The body mass of 14-day-old hatchlings incubated at 32 °C was greater than those incubated at 26 ℃ and 28 °C. However, there was no significant difference in the mean body mass of 180 and 270-day-old turtles hatched at these different temperatures. This study showed that the hatching temperature significantly affected the incubation period and body mass in the early life of the Korea Reeves' turtle (*M. reevesii*).

KEY WORDS: Turtle Conservation, Incubation Temperature, Post-hating Growth, Thermoregulation

요 약

본 연구는 서울대공원 인공사육장에서 남생이(*Mauremys reevesii)* 알의 부화온도 차이에 따른 부화기간과 성장에 영향을 주는지 알아보고자 연구했다. 전체 201개의 남생이 알을 26(n=89)℃, 28(n=75)℃ 그리고 32(n=37)℃에서 각각 부화시켰다. 남생이 알의 부화온도와 부화기간은 온도에 따라 유의미한 차이를 나타내었다. 본 연구에서 부화 온도가 높을수록 부화율이 증가하였다. 26℃, 28℃, 그리고 32℃에서 부화한 알의 부화 기간은 각각 66.1(±4.0, n=52)일, 65.3(±3.3, n=44)일 그리고 58.8(±7.7, n=31)일이었다. 부화율은 32℃(83.8%)가 26℃(58.4%)와 28℃(58.7%)보다 상대적으로 높았다. 32℃에서 부화한 14일 된 새끼의 몸무게가 26℃와 28℃에서 부화한 14일 된 새끼의 몸무게보다 높았다. 그렇지만 부화 후 180일과 270일에 새끼의 몸무게는 부화 온도에 따라 큰 차이가 없었다. 본 연구에서 한국산 남생이(*M. reevesii*) 알의 부화 온도가 부화 기간과 성장 초기 남생이의 체중에 유의한 영향을 미치는 것으로 나타났다.

주요어: 남생이 보전, 부화 온도, 초기 성장, 온도조절

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Introduction

Environmental conditions affect biological processes in animal species. Some studies have reported that incubation temperature has a significant influence on embryonic development (Packard and Packard, 1988; Booth 1998; Grant *et al*., 2003; Zhu *et al*., 2006; Du *et al*., 2007). In reptiles, temperature, which is an important ecological factor, affects the incubation period (Booth *et al*., 2004; Zhu *et al*., 2006), sex determination (Bull, 1980; Booth *et al*., 2004), growth rates (Fordham *et al*., 2007; Du *et al*., 2007), hatching success (Spotila *et al*., 1994; Bell *et al*., 2003) and embryonic development (Booth, 1998; Grant *et al*., 2003; Zhu *et al*., 2006). In turtles, incubation temperature has been reported to have profound effects on embryonic development (Plummer *et al*., 1994; Du and Ji 2003; Zhu *et al*., 2006). Effects of incubation temperature on incubation period have been reported in marine turtles (Godley *et al*., 2001), Crocodile (Piña *et al*., 2003), lizards (Castilla and Swallow, 1996), and snakes (Burger, 1991; Lin *et al*., 2005). In a previous study, the incubation period varied with the incubation temperature of eggs of soft-shell turtles (*Tryonix sinensis*, Du and Ji, 2003) and loggerhead sea turtles (*Caretta caretta*, Matsuzawa *et al*., 2004), but did not vary in Hermann's tortoises (*Testudo hermanni*, Eendebak 1995) and with hydric environmental conditions of Chinese three-keeled pond turtles(*Chinemys reevesii,* Du and Zhen, 2004).

The Reeve's turtle is a widely distributed aquatic emydid found in China, Taiwan, Korea, and Japan (Dancik, 1974). However, few detailed reports have been published on the effect of incubation temperature on the incubation period and body mass of Chinese three-keeled pond turtle (*C. reevesii*) hatchlings (Zheng *et al*., 2006; Du *et al*., 2007). In the Korea Reeves' turtle which is endangered species in Korea, Some studies reported on morphology and genetic diversity (Eo, 2006; Oh *et al*., 2017), habitat status(Jo et al., 2017; Koo *et al*., 2019), behavior(Kim *et al*., 2023), and physiology(Jung *et al*., 2016). In this study, we investigated the hypothesis that hatching period is affected by different constant incubation temperatures in the Korea Reeves' turtle. We predicted that at high temperatures, the hatching period would be shorter and body mass would be heavier than those at low temperatures in Korea Reeves' turtle. We investigated the effects of incubation temperature on hatching success, incubation period, and body mass of hatchlings during the growth period in the Korea Reeves' turtle.

Materials and Methods

Twenty-three adult Korea Reeves' turtle donated by Mr. SH Son who raised the turtles as a hobby have been breeding in an outdoor terrarium for the turtles in Seoul Grand Park Zoo since 2004. A keeper in Seoul Grand Park Zoo checked and recorded all signs of illnesses and injuries each day in the early morning. If a turtle was ill or injured, it was treated immediately with the aid of a veterinary surgeon after removal from the outdoor terrarium. However, no illness or injury occurred during this study. The keeper also collected eggs from the sand every day in the early morning and introduced them into an artificial incubator. Turtles were fed 2% body weight every 24 h with a diet consisting of a fresh mudfish in small size, beef slices, peas, corn, and berries at approximately 10:00 AM every day. The remaining items were removed near dawn every day. There was no competition to eat the items among the turtles. Thus, all turtles appeared to have been adequately fed.

The incubation box containing the eggs was placed in a constant-temperature culture box in an door. One mercury thermometer was placed in water at the bottom of the incubation box and served as the basis for adjusting the incubation temperature. The temperature of the automatically regulated incubator was maintained at 28°C (±0.5°C) and 32°C (±0.5°C) in 2006 and at 26°C (±0.5°C) in 2007 until the eggs hatched. The eggs were randomly selected from different clutches when the eggs were incubated at different constant temperatures in 2006 until they hatched. The average humidity of the incubator was 70% (±5) during incubation in 2006 and 2007. Two-thirds or more of the eggs were placed under sand within the incubator and covered with moss to maintain constant humidity during incubation. The interior of the artificial incubator was kept dark to prevent the effects of electric light.

The keeper checked the temperature and humidity of the artificial incubator every day in the early morning. The keeper also periodically checked for signs of hatchling emergence in the early morning and again at dawn; after 40 days of incubation, the keeper checked for signs of hatchling emergence every day. When a turtle hatched, the keeper recorded the number of the egg from which the turtle hatched to identify the hatching period. The turtles were weighed upon hatching and raised individually for 5 days in a small plastic box containing approximately 2–3 cm of tap water at 24°C under natural photoperiod. The hatchlings were then transferred into 15 plastic containers containing water at a constantly maintained temperature for 4–7 days. The hatchlings were not individually marked, but were divided into separate containers when they hatched; those that were born within ≤3 days of one another were maintained in the same container. Thus, each container had hatchlings of different ages, but all were within 2–4 days of age. The hatchlings that hatched at different constant temperatures were maintained in different containers. Body mass was checked at hatching and at 14, 180, and 270 days of age for hatchlings that hatched at 28℃ and 32°C in 2006; body mass was not checked at hatching for hatchlings that hatched at 26°C in 2007.

Results

A total of 201 eggs were incubated in this study. Of the 201 eggs, 89, 75, and 37 were incubated at temperatures of 26℃, 28℃, and 32°C, respectively. There was a significant difference in hatching success among these constant incubation temperatures (Chi-square test, $\chi^2 = 7.07$, $P = 0.008$), resulting in an increase in hatching success as incubation temperature increased. Of 127 eggs, 52 (52/89, 58.4%) were incubated at 26°C, 44 (44/75, 58.7%) were incubated at 28°C, and 31 (n = 31/37, 83.8%) were incubated at 32°C(Fig. 1). The mean initial egg mass at incubation temperatures of 26°C, 28°C, and 32°C was 9.40 (±0.96, n = 89), 9.17 (±0.83, n = 75), and 9.24g (±1.04, n = 37), respectively. The initial egg mass did not differ significantly among incubation temperatures (ANOVA, F = 2.54, $P = 0.08$). In addition, the initial egg mass did not affect hatching success (ANOVA, F = 1.33, $df = 1$, $P = 0.25$), implying that initial egg mass is not important for hatching success.

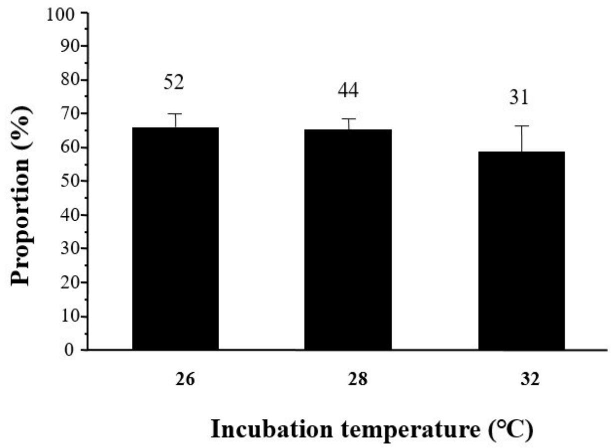

Figure 1. Incubation period at different constant temperatures in the Korea reeves' turtle (*M. reevesii*). The numbers over bar are sample sizes.

There was a significant difference in the incubation period of eggs among different constant incubation temperatures (ANOVA, F = 25.25, $df = 2$, $P < 0.0001$, Fig. 1). The incubation period of eggs at 26℃, 28℃, and 32°C was 66.1 (±4.0, n = 52), 65.3 (±3.3, n = 44), and 58.8 days (±7.7, n = 31), respectively. The incubation period decreased as the incubation temperature increased for Korea Reeves' turtle.

Figure 2 compares the growth of hatchlings that hatched at different constant incubation temperatures. During the growth experiment, 4 hatchlings incubated at 32°C and 3 hatchlings incubated at 28°C died and were thus excluded from subsequent analysis. There was no significant difference in body mass between hatchlings that hatched at different constant temperatures (ANOVA F = 3.15, $P < 0.08$). The body mass of 14-day-old turtles incubated at 32°C was greater than that of 14-day-old turtles incubated at 28°C (ANOVA F = 27.15, $P = 0.0001$). However, there was no significant difference in mean body mass of 180- (ANOVA F = 2.23, P = NS) and 270-day-old (ANOVA F = 0.23, P = NS) turtles hatched at these different temperatures, although the mean body mass of turtles incubated at 32°C was slightly higher than that of turtles incubated at 28°C.

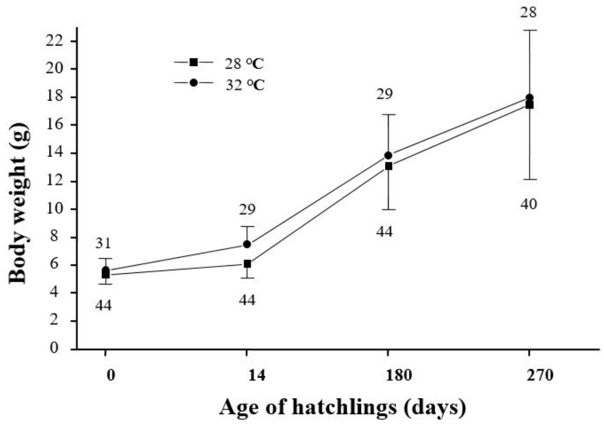

Figure 2. Growth of the Korea reeves' turtle (*M. reevesii*) hatchlings incubated at two different constant temperatures at hatching, 14, 180, and 270 days old(±2 days). The numbers over and below bar are sample sizes. Data are means ± standard errors.

Discussion

These results are consistent with those of Booth (1998) and Booth *et al*., (2004) in that the incubation period of eggs of the Brisbane river turtle, *Emydura signata*, decreased as the incubation temperature increased. In the Chinese three-keeled pond turtle, *Chinemys reevesii*, Du *et al*., (2007) reported that the incubation period decreased nonlinearly with increasing temperatures. In marine turtles, the incubation period also decreased significantly as the temperature increased (Godley *et al*., 2001). Similarly, the incubation period decreased as the incubation temperature increased in a prolonged egg-retaining snake (*Deinagkistrodon acutus*, Lin *et al*., 2005).

Nest site selection by females may also entail taking advantage of natural heat sources in turtle. Some researchers reported that incubation temperature affected hatchling body mass and growth rate. For example, in Brisbane river turtles (*Emydura signata*), incubation temperature has a potential influence on hatchling fitness (Booth *et al*., 2004). Furthermore, hatchlings incubated at 30°C grew faster than hatchlings incubated at 24°C and 27°C. In the present study, incubation temperature did not have a significant effect on hatchling body mass but had a clear effect on body mass in the early stages of turtles' lives. The body mass of hatchlings that hatched at 32°C was significantly greater than that of 14-day-old turtles incubated at 28°C. However, just after incubation, Chinese three-keeled pond turtle, *C. reevesii*, hatchlings incubated at 24°C, 26°C, and 28°C were larger and heavier than those incubated at 30°C, 32°C, and 34°C (Du *et al*., 2007).

In reptiles, parents select nest sites to increase their fitness because environmental factors such as temperature influence embryo survivorship, hatching quality, and sex ratio (Wood and Bjorndal 2000; Piña and Larriera, 2002; Georges *et al*., 2005). In turtles, nest temperature also affects the rate of embryonic development (Ackerman 1997). For example, in Chinese three-keeled pond turtles (*C. reevesii*), Zheng *et al*., (2006) reported a moderate incubation temperature of 27–30°C may improve embryonic utilization of energy from the yolk and eggshell and facilitate the production of well-developed hatchlings from eggs. Considering that the incubation period decreased as the temperature increased in this study, we suppose that the ideal moderate temperature for the production of well-developed hatchlings is at 32°C in the Korea Reeves' turtle (*M. reevesii*). In contrast, a study on freshwater turtles, *Chelodine rugosa*, showed that embryonic survival was greatest at 26°C and steadily declined as the temperature increased to 32°C (Fordham *et al*., 2007). In the Asian yellow pond turtle, Zhu *et al*., (2006) reported that an incubation temperature of 29.0°C (±0.5) was optimal for embryo survival and development and that an incubation temperature of 33.0°C (±0.5) was harmful for embryonic development. The previous studies reported that the optimal temperature for increased fitness in turtles differs among species.

Those results showed that in the early life stages, growth rates of hatchlings incubated at high temperatures were higher than those of hatchlings incubated at lower temperatures, although a statistically significant difference was not seen at 180 or 270 days of age. It is similar to my previous study that showed that after the age of 3 months, hatchlings from higher incubation temperatures grew faster than did those from lower incubation temperatures (Du *et al*., 2007). This suggests that hatchlings with high growth rates will more quickly achieve sexual maturity than will hatchlings with lower growth rates; thus, increased lifetime reproductive output is based on the results that sexual maturation in freshwater turtles is size-dependent, and on the "bigger is better" hypothesis (Congdon *et al*., 1999). In a similar study, Booth *et al*., (2004) reported that males incubated at 26°C were consistently poorer swimmers than were males and females incubated at warmer temperatures. It has also been suggested that faster-growing and swimming turtles have a lower risk of predation based on previous research showing that the rate of predation on small turtles is greater than that on larger turtles (Janzen *et al*., 2000).

This study revealed that incubation temperature affected the incubation period and slightly influenced body mass at hatching and the growth of hatchlings in the early stages of their lives. An understanding of the results of this study is helpful for determining the most suitable conditions for incubation and growth after incubation to increase fitness; this study also has important implications in terms of husbandry and conservation. Thus, the present results suggest that incubation temperature can influence the fitness of Korea Reeves' turtle hatchlings.

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