

Effects of incubation temperatures on embryonic development in the Asian yellow pond turtle

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Abstract

The Asian yellow pond turtle, *Mauremys mutica* (Cantor), is a potential aquaculture target in China owing to the higher values for food and remedy than other species of turtle. In this study, color and morphological changes of fertilized eggs were observed during embryogenesis, and the effects of incubation temperature on embryonic development were analyzed. Both calcium layer and membrane layer are thicker in the middle portion of egg-shell than that in the terminal portion, and become thinner after embryo hatching than before embryonic development. Significant change in the white spot and subsequent white ring on the egg-shell occurs during embryonic development. Of five different incubation temperatures used to investigate the effects of incubation temperatures on embryonic development, 29.0 ± 0.5 °C was optimal for embryo survival and development. Moreover, the incubation temperature of 33.0 ± 0.5 °C was harmful effect to embryonic development. The data provide important and useful information for husbandry and management of the Asian yellow pond turtle.

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1. Introduction

The Asian yellow pond turtle, *Mauremys mutica* (Cantor), is a member of the family Bataguridae (Joyce et al., 2004). Owing to the higher values for food and remedy than other species of turtle, it has become one of the most commonly and highly traded turtles in Asia (Zhao, 1997, 1998; Hendrie, 2000; Lau and Shi, 2000; Shi and Pharham, 2001; Pharham and Shi, 2001) and, recently, its husbandry and management has been a focus of

aquaculture practice (Zhu et al., 2001a,b,c). Only 2.8 eggs on average are laid per clutch, and one mature female has only 2.1 nests on average each year (Zhu et al., 2001a, b). Therefore, it is very important to determine how to undertake incubation and to achieve optimal incubation conditions for aquaculture.

In reptiles, temperature is an important ecological factor, and incubation temperature has been demonstrated to have profound effects on embryonic development and husbandry outcomes (Piña and Larriera, 2002; Georges et al., 2005). Significant data on the influence of incubation temperature on embryonic development are available in some turtles (Packard and Packard, 1988; Congdon and Gibbons, 1990; Booth, 1998; Grant et al., 2003), but no

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studies of the Asian yellow pond turtle have been undertaken. To reveal basic characters of fertilized eggs and to provide further information for husbandry and management, we investigated the effects of incubation temperature on hatching success, hatchling survival, and color and morphological changes of fertilized eggs during embryogenesis in the Asian yellow pond turtle.

2. Materials and methods

2.1. Experimental materials

Mature Asian yellow pond turtles were collected at markets from Qinzhou city of Guangxi Province. They were maintained in captivity during the reproduction season at the Pearl River Fisheries Research Institute in Guangzhou (Zhu et al., 2001a,b). A total of 876 turtles, including 430 males and 446 females, were used in this study.

2.2. Observation of morphological traits of fertilized eggs in incubation

To accommodate variation among clutches, one fertilized egg was selected at random from each of 30 clutches for morphological analysis. The fertilized eggs were incubated at 29.0 ± 0.5 °C. Mass (g), length and width (mm) were measured before and after egg incubation. During incubation, the development of the white spot (Ewert, 1985) was recorded by measuring its relative curved length and width.

2.3. Measurement of egg-shell thickness

Before incubation, one fresh egg was randomly taken from each of 25 clutches, opened and the middle and terminal portions of egg-shells were cut into small pieces under a light microscope. Thicknesses of the maternal calcium and maternal membrane layers were each measured with a micrometre scale fitted to the eyepiece of the microscope and calibrated against a standard graduated slide. An additional fertilized egg was randomly taken from each of the 25 clutches, and incubated at a constant 29.0 ± 0.5 °C. After hatching, the egg-shells were also cut into small pieces and measured under a light microscope as described above.

2.4. Incubation of fertilized eggs under different temperatures

A total of 200 fertilized eggs were randomly collected from the eggs laid in one day, and divided into 5 groups (40 fertilized eggs/each group). They were allocated at

random to each of five different temperatures (25.0 ± 0.5 °C, 27.0 ± 0.5 °C, 29.0 ± 0.5 °C, 31.0 ± 0.5 °C, 33.0 ± 0.5 °C) and incubated. The experiment was duplicated (20 eggs per replicate). The clutch of origin was not identified in our analyses, and so the component of variation attributed to differences among clutches was incorporated into the error in subsequent statistical analyses.

Incubation management was performed as described by Zhu et al. (2001a). Briefly, the boxes containing the eggs were put in a constant temperature culture box (TSE-Incubator MIR-151, Sanyo, Japan). One mercury thermometer (calibrated by a certified thermometer from the National Center of Metrology in Guangzhou) was inserted in the sand layer with eggs in each culture box and used as the basis for adjusting the incubation temperature. The hatch time, hatching rate and embryonic development rate were recorded, and the cumulative temperature unit (CTU) was calculated as incubation time (h) \times temperature (degree).

3. Results

3.1. Thickness change of egg-shell before incubation and after hatching

Egg-shell of the Asian yellow pond turtle consists of two layers. The outer is rigid maternal calcium layer and inner is maternal egg membrane layer. Both the calcium layer and the membrane layer were thicker in middle portion of egg-shell than that in terminal portion ($t=7.0$, $df=48$, $P<0.001$, and $t=3.0$, $df=48$, $P<0.005$ respectively; and both were thinner after hatching than before egg incubation ($t=2.7$, $df=48$, $P<0.001$, and $t=7.0$, $df=48$, $P<0.001$ respectively) (Table 1).

3.2. Color and morphological changes of fertilized eggs during embryo development

Color change of egg-shell surface is one important character of fertilized eggs in the Asian yellow pond

Table 1
Measurement of egg-shell thickness of the Asian yellow pond turtle

Status of egg-shell	Middle portionpart (mm)		Terminal portionpart (mm)	
	Calcium layer	Membrane layer	Calcium layer	Membrane layer
Egg-shell before incubation (29.0 ± 0.5 °C)	0.26 ± 0.02	0.13 ± 0.02	0.20 ± 0.02	0.10 ± 0.01
Egg-shell after hatching	0.21 ± 0.02	0.14 ± 0.02	0.19 ± 0.02	0.11 ± 0.02

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