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Reproduction and Nesting of the Endangered Keeled Box Turtle (*Cuora mouhotii*) on Hainan Island, China

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ABSTRACT. – We studied reproduction and nesting of the keeled box turtle (*Cuora mouhotii*) during 2003 and 2009 on Hainan Island, China. We found the first gravid turtle on 28 April and nesting was from May until July, with a peak in mid-June. We observed the first fresh nest on 1 June and last fresh nest on 27 July. Some females laid eggs from 1 clutch in 2 batches. Clutch size ranged from 1 to 5. Mean egg measurements were as follows: length, 4.5 cm; width, 2.7 cm; and mass, 19.8 g. There was no significant relationship between turtle body size (mass, length, height, and width) and clutch size or egg size (mass, length, and width). Clutch size was significantly correlated with the egg mass and egg width. Possible low fertility, low hatching rate, and the long period to reach maturity imply that *C. mouhotii* has a low intrinsic rate of population increase. This, combined with overcollecting and habitat destruction, requires development of more effective conservation strategies for this endangered species.

KEY WORDS. – Reptilia; Testudines; Geoemydidae; turtle; *Cuora mouhotii*; reproduction; nest; eggs; Hainan Island; China

Evidence suggests that Asia's turtles are being systematically extirpated to feed the insatiable demand for food and traditional uses (van Dijk et al. 2000). Of an estimated 90 species that are native to the region, more than 50% are listed as critically endangered or endangered on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Asian Turtle Conservation Network 2010). The keeled box turtle (*Cuora mouhotii*) is one such species found in China (Hainan, Guangdong, Guangxi, and possibly southern Yunnan), northern and central Vietnam, Laos, northern Cambodia, Thailand, Myanmar, and the state of Assam in India (Ernst and Barbour 1989; Zhao and Adler 1993; Turtle Taxonomy Working Group 2010). Overcollection, illegal trade, and habitat destruction have resulted in drastic reductions in the wild populations (De Bruin and Artner 1999; Lau and Shi 2000; Gong et al. 2006), and *C. mouhotii* is now classified as an endangered species by the IUCN Red List (IUCN 2010).

Most previous research focused on the taxonomy and systematics of this species (Gray 1862, 1864; Honda et al. 2002; Shi et al. 2005; Spinks and Shaffer 2007; Zhang et al. 2008), but little is known about its biology in the wild. Even basic reproductive data are lacking, such as the time of egg laying or clutch size. This situation hinders efforts to protect this endangered species.

Our objectives were to acquire basic ecological information on female body size, clutch size, egg size, nesting season, incubation period, incubation temperature, and nest predation of *C. mouhotii*. Our study provides some of the first ecological data on the reproduction and nesting of *C. mouhotii.*

METHODS

From 20 February to 15 August 2003, we visited markets and turtle collectors once per week (total 23 weeks) to gather information on the turtle trade in Nanmao and surrounding villages (Fig. 1) in east-central Hainan Island, off the coast of southern China. We obtained some reproduction information on *C. mouhotii* during that survey (see Gong et al. 2006).

From February to December 2009, we studied *C. mouhotii* at Diaoluoshan, located in southeastern Hainan (lat 18°43′538″N, long 109°52′109″E, elevation 914 m; Fig. 1). The field site was approximately 70,700 ha of rainforest. January is the coldest month, with an average temperature of 15.4°C, and the hottest month is July, with an average temperature of 28°C. Rainfall is plentiful with 1870–2760 mm of annual precipitation, mostly in a distinct rainy season (May to October).

Turtles were captured in traps baited with salty fish or rancid pork skin. We marked turtles by engraving their marginal scutes. Straight-line carapace length, carapace width, and body height were measured to the nearest millimeter with tree calipers. Body mass was measured to the nearest 0.1 g using a portable electronic balance. All females captured in the field were palpated to determine whether they were gravid or not. Any females suspected

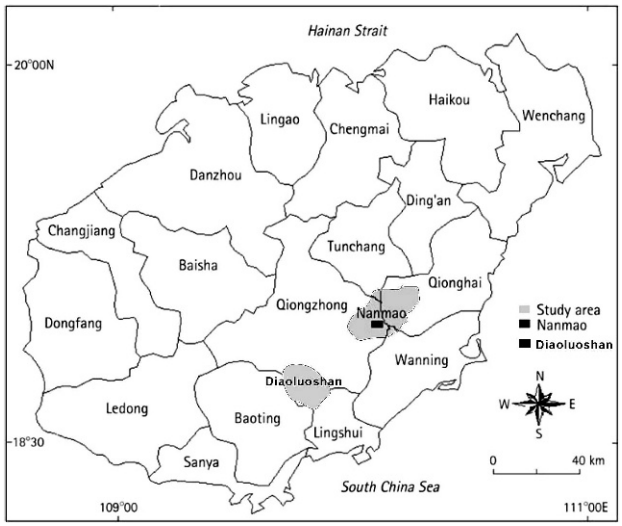

Figure 1. The locations of the study site, Diaoluoshan and Nanmao areas (shaded), on Hainan Island, China.

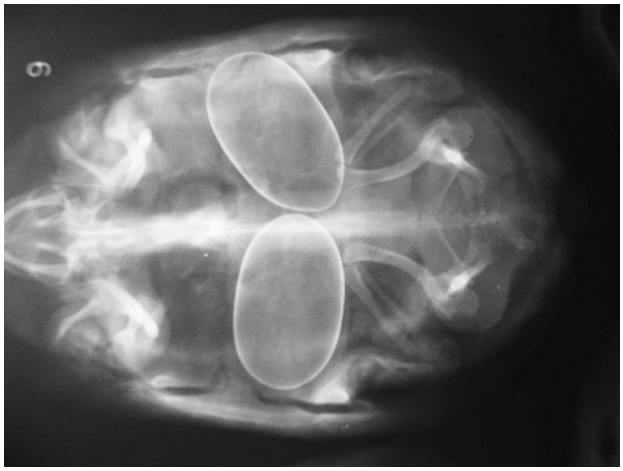

Figure 2. Radiographs of a gravid *Cuora mouhotii* female with 2 eggs.

to be gravid were taken to a field research site where we took radiographs. We outfitted turtles with radiotransmitters (Holohil Systems Ltd., Carp, ON, Canada RI-2B, 6 g) and released them at the site of capture. Female turtles with radios were radiographed every 20–25 days from March to October. Gravid females were easily identified by palpation and from the eggshell images in the radiographs (Kuchling 1999). Once female turtles were gravid, we radiotracked them every hour until they deposited eggs. We collected the eggs 2–3 days after oviposition. Nest cavity depth and diameter were measured when no damage to these dimensions had occurred while excavating the nest cavity. All eggs were weighed and measured at the nesting site, and then reburied in the nest cavity. For each egg, we recorded egg length and egg width, both measured to the nearest 0.1 mm, and egg mass, measured to the nearest 0.1 g. Relative clutch mass (RCM) was calculated as RCM = clutch wet mass/female body mass before oviposition. Nests were protected by wire mesh folded into an open box and buried open-side down over the clutch up to ca. 3 cm below the soil surface. Temperatures were monitored for one nest in 2009 with a Watchdog-100 data logger (Spectrum Technologies). The data logger was programmed to record nest temperatures at 1-hour intervals throughout the incubation period and was sealed in a plastic container and buried beside the nest cavity.

RESULTS

Nanmao Area. — In 2003, we found 136 *C. mouhotii* in villages or markets, representing 43 females, 20 males, and 73 juveniles/subadults that could not be sexed externally by relative tail lengths. The smallest one caught by local people was only 17 g. Some of the turtle owners would not cooperate fully with our research project. As a result we could not collect measurements for all variables and all turtles caught by local people, resulting in different sample sizes for our analyses. Measurements for female turtles were as follows (mean ± SD, range, n): female body mass, 505.6 ± 213.0 g SD (200–800 g, $n = 16$); carapace length, 15.8 ± 2.2 cm SD (10.3–18.4 cm, $n = 28$); carapace width, 10.8 ± 1.3 cm SD (8.0–12.6 cm, $n = 27$); and body height, 6.4 ± 1.0 cm SD (4.3–7.8 cm, $n = 27$). The following measurements were made for male turtles: male body mass, 491.3 ± 19.6 g SD (225–850 g, $n = 12$); carapace length, 15.0 ± 2.5 cm SD (11.1–18.2 cm, $n = 13$); carapace width, 10.6 ± 1.5 cm SD (8.4–12.5 cm, $n = 13$); and body height, 6.0 ± 1.0 cm SD (4.9–7.4 cm, $n = 13$). We compared body characters between males and females (Student *t*-test); there were no significant differences in their mean body mass, carapace width, length, or height ($p > 0.05$).

Among 43 female turtles from villages or markets, 12 were gravid by palpation. The first gravid turtle was found on 28 April. The other gravid turtles were found during May and June. We checked 12 gravid females by radiography (Fig. 2) and recorded 29 shelled eggs. Mean clutch size was 2.4 eggs, and clutch sizes were varied: 1 egg (8.3%), 2 eggs (50%), 3 eggs (33.4%), and 4 eggs (8.3%).

We took 12 gravid turtles back to the field site where they were fed. Most ($n = 9$) deposited 23 intact eggs. The mean, SD, and range measurements of these eggs were as follows: egg length, 4.6 ± 0.3 cm SD (4.1–5.2 cm, $n = 23$); egg width, 2.7 ± 0.1 cm SD (2.5–3.0 cm, $n = 23$); and egg mass 22.5 ± 3.2 g SD (17.2–28.7 g, $n = 23$). There was significant correlation between egg mass and length, and egg width and mass (all $p < 0.01$). Keeled box turtles from the Nanmao area had a mean RCM of 8.5% (5.3%–11.8%; Table 1).

Eggs were buried underground and the soil temperature ranged from 22.0°C to 28.0°C. About 120 days later, only one hatchling emerged from the nests.

Table 1. Measurement data of gravid *C. mouhotii* and eggs. NM = Nanmao area, DLS = Diaoluoshan area, RCM = relative clutch mass.

Turtle ID	Female body mass (g)	Female carapace length (cm)	No. of oviductal eggs	No. of eggs laid	Mean egg mass (g)	Mean egg length (cm)	Mean egg width (cm)	RCM
NM1	800.0	16.2	2	2	21.4	4.6	2.6	5.4%
NM2	600.0	16.2	3	3	23.6	4.7	2.8	11.8%
NM3	650.0	17.2	3	3	20.4	4.5	2.6	9.4%
NM4	550.0	18.4	2	0	-	-	-	-
NM5	712.0	18.0	3	0	-	-	-	-
NM6	688.0	17.6	2	2	28.6	5.0	2.9	8.3%
NM7	647.0	16.4	2	2	24.8	4.9	2.8	7.7%
NM8	653.0	16.7	1	0	-	-	-	-
NM9	662.0	15.9	2	2	27.7	5.1	2.9	8.4%
NM10	638.0	16.0	3	3	21.7	4.6	2.7	10.2%
NM11	774.0	17.4	2	2	20.7	4.4	2.7	5.3%
NM12	726.0	16.4	4	4	18.1	4.1	2.7	10.1%
DLS1	594.0	16.8	2	2	20.5	5.2	2.6	6.9%
DLS2	625.0	18.5	4	4	22.8	4.9	2.8	14.6%
DLS3	580.0	18.2	4	4	17.4	4.3	2.5	12.0%
DLS4	700.0	17.5	4	4	16.0	4.6	2.5	9.1%
DLS5	698.0	16.7	4	4	19.0	4.6	2.6	10.9%
DLS6	512.0	17.0	4	1	17.4	4.1	2.7	-
DLS7	675.0	16.7	4	3	18.1	4.2	2.6	-
DLS8	495.0	15.6	5	4	14.2	3.9	2.6	-
DLS9	632.0	17.4	4	1	16.5	3.9	2.7	-
DLS10	595.0	15.9	4	4	17.2	3.7	2.7	11.6%

Figure 3. *Cuora mouhotii* depositing eggs in the field. Photo by Jichao Wang.

Diaoluoshan Area. — In 2009, we trapped 17 female *C. mouhotii* in the field and 10 were gravid. Measurement data of these 10 gravid turtles were (mean ± SD, range): body mass, 610.6 ± 70.7 g SD (495–700 g); carapace length, 17.0 ± 0.91 cm SD (15.6–18.5 cm); carapace width, 11.7 ± 0.6 cm SD (11.8–12.8 cm); and body height, 6.7 ± 0.7 cm SD (6.1–8.4 cm).

The clutch size was 3.9 ± 0.7 SD (2–5, $n = 10$), with clutch sizes of 2 (10%), 4 (80%), and 5 (10%). We found the first gravid female on 6 May. Oviposition occurred from early June to late July. The first fresh nest was found on 1 June and the last fresh nest on 27 July. Four of the females deposited a partial clutch and retained the remaining eggs for one or several days. Eggs were smooth, and characterized by a white shell and oval shape. The egg mass was 18.1 ± 2.7 g SD (11.3–23.0 g, $n = 31$), egg length was 4.4 ± 0.4 cm SD (3.6–5.3 cm, $n = 31$), and egg width was 2.6 ± 0.1 cm SD (2.4–2.8 cm, $n = 31$). Turtles from the Diaoluoshan area had a mean RCM of 10.9% (6.9%–14.6%; Table 1).

We recorded nesting for 2 radiotracked gravid turtles. At 1230 hours, 20 June, turtle no. 6478 was moving on the forest floor. At 1330 hours, the turtle remained in one place for 2 hours. Around 1530 hours, it began to dig a hole with its left rear leg. The turtle stopped for 2–3 minutes every 4–5 minutes; both legs were used alternately to dig the cavity. At 1557 hours, the nest hole was completed. At 1600 hours, the female deposited the first egg; 3 minutes later, the second one appeared; at 1607 hours, the third egg was deposited (Fig. 3), and 4 minutes later, the last one was deposited. The duration of oviposition was about 12 minutes, after which the female rested for about 4–5 minutes and then began to bury the eggs. At 1720 hours, nesting was completed and the female left the nesting site.

Among 5 gravid turtles, 3 dug nest cavities and covered the eggs completely with soil. One gravid turtle placed the nest in sand; the remaining one did not dig a hole and deposited eggs under fallen leaves. Two nest sites were located beside the roots of herbaceous plants and other nests were located in open areas. Some turtles buried themselves under fallen leaves while nesting. Measurements for the nest cavities were as follows: length 10.6 cm (9.0–13.0 cm, $n = 3$), width 8.0 cm (7.9–8.1 cm, $n = 3$), and depth 6.9 cm (5.6–9.0 cm, $n = 3$).

Of 16 eggs checked, only 3 were fertilized eggs as determined by the presence of cicatricula in the yolk of the egg. All eggs were incubated at the nesting site, but no hatchings emerged after 4 months, at which time the eggs were moldy and rancid. The soil temperature of the nesting site varied from May, mean 19.3°C (range 15.0°–24.0°C); June, 20.7°C (19.0°–23.3°C); July, 20.6°C (18.7°–23.1°C); August, 20.8°C (18.5°–23.0°C); September, 20.7°C (18.5°–24.0°C); October, 19.3°C (17.5°–21.0°C); November, 17.9°C (15.0°–20.0°C).

Comparison. — Body size of gravid *C. mouhotii* did not differ significantly between Nanmao and Diaoluoshan (all measurements $p > 0.05$). There was no significant difference in egg length between Nanmao and Diaoluoshan ($p > 0.05$), but there was a significant difference of clutch size, egg width, and egg mass (all $p < 0.01$).

DISCUSSION

Reproductive Season. — Rainfall and air temperature variation are weather components that demarcate yearly seasons and thus freshwater turtles often exhibit distinct activity peaks during the year, mainly during spring and summer seasons (Souza and Abe 2001). According to our data, the breeding season of *C. mouhotii* occurs from May to July, coincident with the rainy season in Nanmao and Diaoluoshan, when mushrooms, earthworms, and other foods favored by the species are in abundance.

Clutch Size and Frequency. — Turtle body size has been shown to influence reproductive potential in female turtles (Valenzuela 2001), as the area of the pelvic girdle is correlated with female size and may constrain the size and number of eggs an individual can oviposit (Bowden et al. 2004). Within each species of turtle, larger females generally have larger clutches than smaller females (Gibbons et al. 1982). For the gravid turtles in the Nanmao and Diaoluoshan areas, we did not find a relationship between clutch size and gravid turtle body size, but there were significant differences in egg width and egg mass in relation to clutch size. The turtles in Diaoluoshan in 2009 had more and smaller eggs than in Nanmao area in 2003. However, further studies are needed to determine the regional and temporal difference due to the small sample size in this initial study. This species has relatively few eggs per clutch (1–5 eggs) but so do other turtles of that approximate size. *Cuora mouhotii* RCM from the Nanmao area ranged from 5.3% to 11.8%, whereas those from the Diaoluoshan area ranged from 6.9% to 14.6%. Their RCM is fairly equivalent to other freshwater turtles because *C. mouhotii*

eggs, although few in number, are relatively large (in mass and length). For comparison, RCM for the yellow-margined box turtle (*Cuora flavomarginata*) ranges from 2.3% to 9.2% (Chen and Lue 1999). In the painted turtle (*Chrysemys picta*), RCM ranges from 4% to 32%; the pond slider (*Trachemys scripta*) RCM ranges from 3% to 17%, and the wood turtle (*Glyptemys insculpta*) RCM ranges from 9.0% to 16.3% (Ernst and Lovich 2009). RCM varied significantly across clutch size and clutch frequency in our specimens, but accurate determination of clutch frequency within and among years remains a problem in turtle studies (Iverson and Smith 1993).

Relatively little reproductive information is available for other species of *Cuora*. Chen and Lue (1999) reported that *C. flavomarginata* laid 1–2 clutches and clutch size varied from 1 to 3. We observed that *C. mouhotii* females sometimes deposited a portion of their clutch one time and retained other eggs for one or several days before depositing the remainder at a different time and place. However, we did not document multiple clutches in one breeding season. Perhaps the extensive handling of females during our study had an effect on their reproduction.

Incubation Temperature and Period. — In the Nanmao area, the soil temperature during incubation ranged from 22°C to 28°C. About 120 days later, 1 *C. mouhotii* hatchling emerged. In the Diaoluoshan area, the highest soil incubation temperature was only 24°C, the lowest was 15°C and the average temperature for the month ranged from 17.9°C to 20.8°C, indicating that incubation temperatures at Diaoluoshan were much lower than at Nanmao. Lower incubation temperatures result in longer incubation periods for the turtle eggs (Ewert 1985). If the incubation period at Diaoluoshan area was longer than 120 days, hatchlings would emerge in October or November. The next 3 months are the dry season and temperatures are the lowest of the entire year; we do not know whether hatchings stay in the nest or emerge to find other cover sites.

The sex of most turtle species is dependent on incubation temperature (George et al. 2001), but there is no information on the sex-determination mechanism of *C. mouhotii*. According to the soil temperature data for nesting sites in Diaoluoshan, the highest temperature was 24°C, which is much lower than the critical sex differentiation temperatures of other *Cuora* species (Li and Tang 2002; Huang et al. 2009).

Egg Fertilization Rate. — The egg fertilization and hatching rates of *C. mouhotii* were very low in this study. At Diaoluoshan only 3 out of 16 checked eggs could be diagnosed as fertilized. At Nanmao only 1 hatchling emerged out of 23 eggs and at Diaoluoshan none emerged from 31 eggs. The factors that lead to low egg fertilization and hatch rate remain unresolved. Although we handled eggs carefully, we did not exclude the possibility that our methods could give rise to negative effects on hatching rate.

Conservation Implications. — Overcollecting and habitat destruction have been threatening wild populations of *C. mouhotii* (Gong et al. 2006). Our studies suggest that deposition of fertilized eggs and the hatching rate of *C. mouhotii* in the field are both low, assuming that our egg handling effects were minimal. These factors, plus the many years required to reach maturity, imply that *C. mouhotii* has a low intrinsic rate of population increase. It is urgent that more effective conservation strategies be adopted to protect populations of this endangered species.

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