

Reproductive characteristics of the Burmese Narrow-headed Softshell Turtle, *Chitra vandijki*, in captivity

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Abstract.—The purpose of this study was to provide basic data for the breeding biology of *Chitra vandijki* and to contribute to the conservation of this species. The Burmese Narrow-headed Softshell Turtle, *Chitra vandijki*, is a CITES Appendix I-listed species, and biological information on wild and captive *C. vandijki* is relatively scarce. In 2019, we studied the reproductive biology of two *C. vandijki* specimens (a female and a male) that had been in captivity for approximately 25 years. The oviposition period of the domesticated female *C. vandijki* was from June to August. The female laid eggs at night, and no egg protection behavior was observed. The female *C. vandijki* laid five clutches of eggs in a year representing 564 eggs in total, with 100–131 eggs/clutch, and the interval between successive clutches was 9–28 d. The fertilization rate of *C. vandijki* was 90.4%, and the hatching rate was 38.6%. The eggs were spherical and rigid, with an average mass of 15.04 ± 0.65 g and an average diameter of 2.96 ± 0.22 cm. The average hatching period of *C. vandijki* was 65.3 d at 28.0–29.0 °C, and the average accumulated incubation temperature was 44,688.6 °C-h. The average mass of newly hatched neonates was 10.51 ± 0.57 g, and the average mass of juvenile *C. vandijki* reached 150.37 ± 53.86 g after one year of feeding live fry in a greenhouse.

Keywords. Burmese Narrow-headed Softshell Turtle; captive-breeding; *Chitra vandijki*; conservation; egg laying; juvenile; threatened species

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Introduction

The Burmese Narrow-headed Softshell Turtle, *Chitra vandijki*, is a large turtle with a straight-line carapace length of up to 1 m. It is listed on CITES Appendix I and classified as Critically Endangered (CR) on the *IUCN Red List of Threatened Species* (Rhodin et al. 2018; Platt et al. 2021). It is mainly distributed in rivers in Myanmar and Thailand (Platt et al. 2014). The abundance and distribution of *C. vandijki* have been sharply reduced because of human hunting and habitat destruction (Kuchling et al. 2004; Platt et al. 2005, 2014). Because little is known about the turtle's ecological habits, successful cases of artificial breeding are very few and knowledge of its breeding biology is extremely lacking (Platt et al. 2018, 2020).

Because the external appearance of *C. vandijki* is similar to the Asian Giant Softshell Turtle, *Pelochelys cantorii*, and given the demands of the Chinese wild

animal market, C. vandijki has been illegally traded to China as food or for rearing in the last century. Although P. cantorii in China is Critically Endangered (Gong et al. 2017; Hong et al. 2019; Wu et al. 2020), we have successfully carried out artificial breeding of six P. cantorii (three females and three males) turtles since 2014 (Zhu et al. 2015; Hong 2020). At present, we have bred more than 800 P. cantorii between 1 and 6 years old (Ministry of Agriculture and Rural Affairs of People's Republic China 2020). Based on our successful experience in the artificial breeding of captive P. cantorii, we carried out research on the reproductive biology of two captive C. vandijki, and the mitochondrial genomes of the individual hatched offspring confirmed their identity as Burmese Smallheaded Turtles (Chen et al. 2021). The findings of this study enrich the basic biological data of C. vandijki and provide a theoretical basis for its conservation biology.

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Materials and Methods

Captive Care, Conditions, and Management

Two *C. vandijki*, one male and one female, were accidentally captured and rescued from the waters of the Mekong River at the border between Myanmar, Laos, and Guanli Town, Mengla County, Xishuangbanna Prefecture, Yunnan Province, China in 1995. Their body weights at the time of capture were approximately 3 kg and 7 kg, respectively.

The two C. vandijki were raised in an outdoor fish pond at an elevation of 570 m asl (21°35'40.84"N 101°14'5.02"E) in Xishuangbanna Prefecture, Yunnan Province, China. This region has a north tropical and south tropical humid monsoon climate, which includes a long summer without winter. The annual average temperature is between 18.6 and 21.9 °C, and the annual average precipitation is between 1,200 and 1,700 mm. The dimensions of the pond were 30 m \times 25 m, the water depth was 1.2 m, and the bottom mud was 30-40 cm thick (Fig. 1A). In 2012, a 25 m \times 2 m nesting sand pond with a depth of 60 cm was built on the side of the main pond, and there was a shed above the sand pool for shade. Tiles were used to build an incline of about 30° so the turtles could climb up from the water to the sand pond (Fig. 1A–B). In the pond, Tilapia Oreochromis niloticus, Carp Cyprinus carpio, and Crucian Carp Carassius auratus were cultured together, and the C. vandijki lived by feeding on these fish.

In December 2018, the fish pound was cleared, and the large fish were removed. From February to May 2019, 200 kg of live fish fry, including Carp, Crucian Carp, and Mud Carp (*Cirrhinus molitorella*) with body lengths of 3–5 cm, were regularly added to the pond to serve as the food for improving the cultivation of *C. vandijki*.

Collection and Hatching of Eggs

In April 2019, the stones and plants in the spawning sand pool were cleared, and the sand was raked loose and sieved. From May to July, water was sprayed irregularly into the spawning sand pond to ensure that the sand remained damp. A surveillance camera was installed above the spawning pool to observe the oviposition activity of *C. vandijki*. For the first clutch, the eggs were incubated *in situ* for 25 days, the clutch was dug manually, and artificial incubation was continued. For the other clutches, within 16–24 h after the turtle had laid the eggs, they were collected by excavating the nest. The numbers of eggs and fertilized eggs were counted and recorded. The diameter of each egg was measured with a Vernier caliper (\pm 0.01 cm), and the egg weight was measured using an electronic balance (\pm 0.01 g).

The incubators for fertilized eggs were plastic boxes with dimensions of $57 \times 41 \times 36$ cm. The medium was sieved fine river sand, and the moisture content of the sand was 8–10% (weight ratio, Fig. 1C). The thickness of the sand pile was approximately 15 cm. The fertilized eggs in a given clutch were arranged on the sand pile in

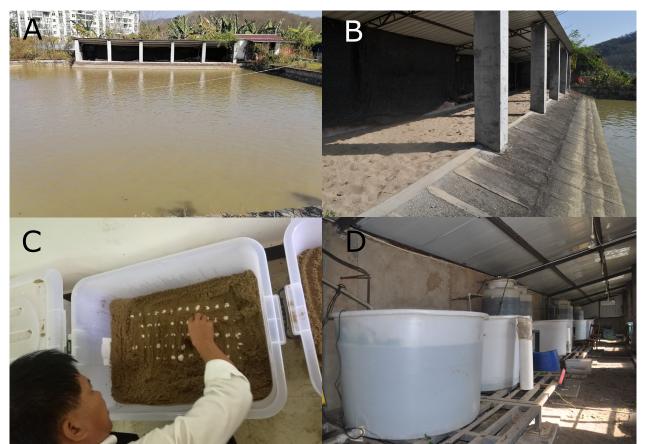


Fig. 1. Artificial rearing facility of Burmese Narrow-headed Softshell Turtles: (A) breeding pond, (B) nesting area, (C) incubation box, (D) rearing facilities.

the incubator and covered with 2 cm of fine sand with the same dampness. The incubator was then covered. The temperature of the incubator was controlled by an indoor air conditioner, and maintained at 28.0–29.0 °C. Water was sprayed regularly onto the sand to control the humidity.

Cultivation of Hatchlings

After emergence, the hatchlings were observed and photographed. The body mass of each hatchling was obtained using an electronic balance $(\pm 0.01 \text{ g})$. The length and width of the carapace and the length and width of the snout of each juvenile were measured using a caliper (± 0.01 cm). Hatchlings were reared according to the rearing method of Asian Giant Softshell Turtle (P. cantorii) hatchlings (Hong et al. 2018), and cultured in six custom-designed round buckets with a diameter of 1.2 m and a water depth of 0.5 m. The cultivation density was 25-30 individuals/m², the bottom of the bucket contained 15 cm of fine sand, and the water was filtered through circulation (Fig. 1D). The pH of the water was measured regularly and adjusted to 7.0-7.5 with quicklime. The neonates were fed live Mosquito Fish, Gambusia affinis. The temperature was controlled by air conditioning and the water temperature was maintained at 26.0-31.0 °C. In July 2020, five juvenile

C. vandijki were randomly selected from each barrel. The body mass and length and width of the carapace of 30 hatchlings were measured.

Statistics

The data shown below and labeled as "this study" are expressed as the mean \pm SD, and were compared and analyzed using ANOVA. Statistical analysis was conducted using IBM SPSS 23.0 software. All statistical tests were two-tailed, and the significance level was set as *P*<0.05.

Results and Discussion

Morphology of the Parents

In 2012, the body mass of the female *C. vandijki* was 38.0 kg. On 6 December 2018, the body masses of the female and male parental *C. vandijki* were 59.2 kg and 40.0 kg, respectively.

There were irregular, slightly fuzzy yellow stripes on the adult carapace (Fig. 2A). The longitudinal stripes on the neck and back merged behind the head, and the neck stripes were more obvious than the stripes on the back (Fig. 2B). The neck was not obviously separated from the anterior edge of the carapace. The neck of the

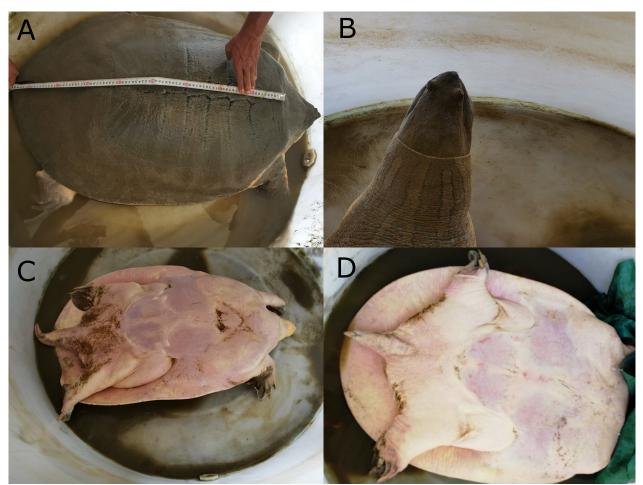


Fig. 2. Characteristics of Burmese Narrow-headed Softshell Turtles: (A) back; (B) head and neck, close-up; (C) male, ventral view; (D) female, ventral view.

Date of egg laying	Clutch size	Number of fertilized eggs/ clutch	Fertilization rate (%)	Number of hatchings/ clutch	Hatching rate (%)
3 June 2019	101	69	69.3	43	62.3
1 July 2019	131	117	85.4	80	80.0
18 July 2019	110	105	95.5	54	68.4
27 July 2019	122	120	98.4	20	9.1
9 August 2019	100	99	99.0	20	
Total	564	510	90.4	197	38.6

adult *C. vandijki* was slightly short and could not turn to the middle or rear of the carapace. The front edge of the carapace was flat, without folds or warts. The head of the adult *C. vandijki* was small, and the snout was short (Fig. 2A–B).

The main morphological difference between the males and females was the tail. The tail of male *C. vandijki* was thick and long, extending out from the edge of carapace, while the tail of female *C. vandijki* was thin and short, extending no longer than the edge of the carapace (Fig. 2C–D).

Egg Laying

In 2004 and 2006, 10 and 20 eggs were laid, respectively, in the culture pond water of the parent *C. vandijki*. After the construction of the spawning sand pond, 98 *C. vandijki* hatchlings were collected from the fish pond in September 2018. However, they all died within 30 d of captive feeding following the cultivation method of the Chinese Softshell Turtle *Pelodiscus sinensis* (Zhao et al. 1997).

Beginning in June 2019, the female turtle was observed climbing up the sand dunes at night, looking for nesting sites. On the nights of 3 June to 9 August, the female turtle laid five clutches of eggs, for a total of 564 eggs. The four intervals between the five clutches were 28 d, 17 d, 9 d, and 13 d, respectively (Table 1), and the average interval was 16.75 ± 8.18 d.

The *C. vandijki* eggs were nearly round and rigid, and the calcareous layer of the eggshell was thin. The number of eggs in each clutch varied from 100 to 131 (Table 1), with an average of 112.8 ± 13.5 eggs/clutch. We randomly selected 40 eggs from the first clutch and another 40 eggs from the second clutch for measurements. The egg masses were 13.37–16.47 g (15.04 ± 0.65 g) and egg diameters were 2.76–3.15 cm (2.96 ± 0.22 cm). According to the average egg weight, the total weight of the five clutches of eggs could be estimated as 8,482.56 g, accounting for 14.335% of the maternal body weight.

Hatching and Characteristics of Hatchlings

The five clutches included 510 fertilized eggs, and the fertilization rate was 90.4%. In total, 197 hatchlings emerged, for a hatching rate of 38.6% (Table 1).

The average incubation period of the fertilized eggs was 65.3 ± 5.4 d, and the average accumulated incubation temperature was 44,688.6 °C-h at a room temperature of 28.0–29.0 °C (28.51 °C on average) based on the hatching data of the second clutch. Under artificial conditions, the hatching rate for the last four clutches of eggs was 34.9%.

The carapace of the newly hatched neonate *C. vandijki* was approximately round, with obvious yellow stripes on the neonate's back, neck, and limbs. The carapace was covered with small protuberances and the posterior edge was yellow without stripes (Fig. 3). The newly hatched neonate *C. vandijki* weighed 9.44–11.75 g (10.51 ± 0.57 g, n = 60, 30 neonates in the first clutch and another 30 neonates in the second clutch). The length of the neonate *C. vandijki* carapace was 4.18-4.70 cm (4.41 ± 0.13 cm), and the width of the carapace was 3.75-4.24 cm (4.01 ± 0.10 cm). The length of the snout of neonate *C. vandijki* was 0.14-0.22 cm (0.16 ± 0.02 cm) and the width of the snout was 0.15-0.24 cm (0.19 ± 0.02 cm).

The reproductive biology data for four species of softshell turtles bred in captivity are shown in Table 2. *C. vandijki*, *P. cantorii*, and the Siamese Narrow-headed Softshell Turtle (*Chitra chitra*) are all large Trionychidae

Table 2. Comparison of the reproductive biology of four species of softshell turtles in captivity.

	Burmese Narrow- headed Softshell Turtle (this study)	Siamese Narrow- headed Softshell Turtle (Kitimasak et al. 2003)	Asian Giant Softshell Turtle (Hong et al. 2018; Hong 2020)	Chinese Softshell Turtle (Yang et al. 1999; Zhou 2004)
Parent sample size (\bigcirc, \Diamond)	1, 1	2, 4	2, 2	>800, >100
Number of clutches/year	5	3–4	4–6	5–7
Clutch size	100-133	40-88	32–55	8–25
Egg diameter (cm)	2.96 ± 0.22	3.32 ± 0.15	3.10 ± 0.18	2.00-2.40
Egg mass (g)	15.04 ± 0.65	19.00 ± 1.67	16.82 ± 1.99	3.55-6.77
Mass of neonate (g)	10.51 ± 0.57	13.10 ± 1.03	13.60 ± 0.85	2.33-4.83
Accumulated incubation temperature (°C-h)	44,688.60	Not reported	44,886.50	36,000



Fig. 3. Hatchling of Burmese Narrow-headed Softshell Turtle.

animals with similar breeding biology, but they are very different from the Chinese Softshell Turtle, Pelodiscus sinensis. There are very limited breeding data for wild C. vandijki. Platt et al. (2020) reported that the numbers of eggs in four collected clutches were 58, 76, 89, and 102; and the diameter of the eggs was 2.01-3.66 cm (2.60-2.95)cm on average). The length of the carapace of the hatchlings was $2.73-4.10 \text{ cm} (3.52 \pm 0.35 \text{ cm})$, and the width of the carapace was 2.75-3.86 cm $(3.39 \pm 0.26$ cm). However, data for the parent C. vandijki were not reported in that study. In general, in our study, the clutch size, diameter of eggs, and body size of neonate C. vandijki were all higher than those reported by Platt et al. (2020), suggesting that the maternal size in our study might be larger and/or that the nutritional status of C. vandijki in captivity is better than that in the field (Gibbons et al. 1990; Litzgus et al. 2008; Hong et al. 2018).

Based on the egg laying and hatching data, we found that the number of eggs in each clutch was relatively constant and the fertilization rate remained at a high level. However, the hatching rates of the last two clutches were relatively low (Table 1). The total mass of the five clutches of eggs accounted for approximately 14% of the body mass of the female C. vandijki. We considered that the low hatching rate of the last two clutches may be due to the influence of oviposition frequency and the availability of reproductive resources for the female C. vandijki (Jackson and Prange 1979; Ferguson et al. 1982). We speculate that too much of the energy of the female C. vandijki had been consumed by the late stage of oviposition, and the spawning intervals between the last two clutches were short (9 d and 13 d), which may have resulted in an insufficient energy supply for the development of the eggs, leading to improper development of the embryos. This phenomenon has been reported for P. cantorii (Hong et al. 2018).

Growth of Juveniles

By July 2020, 180 juvenile *C. vandijki* survived, for a survival rate of 91.4%. The body weight of juvenile *C.*

vandijki was 49.80–311.10 g (150.37 ± 53.86 g, n = 30), the length of the juvenile carapace was 7.54–13.19 cm (11.05 ± 1.66 cm), the width of the carapace was 7.56–12.87 cm (10.84 ± 1.53 cm), the length of the juvenile snout was 0.22–0.40 cm (0.29 ± 0.05 cm), and the width of the snout was 0.23–0.43 cm (0.31 ± 0.06 cm). The living habits of *P. cantorii* and *C. vandijki* are similar; thus, the juvenile breeding method of *P. cantorii* is also suitable for *C. vandijki* and is ideal in terms of survival rate and growth rate.

P. cantorii is endangered due to overhunting and habitat destruction, and China has increased its artificial conservation efforts to gradually restore wild resources, which has achieved initial results (Ministry of Agriculture and Rural Affairs of People's Republic China 2020). Currently, the *C. vandijki* population has been greatly reduced, and so it is also in a critical condition state. For this reason, referring to the Chinese protection strategy for *P. cantorii*, we can use the limited captive population of *C. vandijki* to carry out conservation biological research in order to achieve the artificial conservation of this species, and subsequently release the captive turtles into the wild to restore the wild population.

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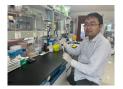
Reproductive characteristics of the Burmese Narrow-headed Softshell Turtle



Hong Xiaoyou completed his B.A. degree at Huazhong Agriculture University, People's Republic of China, and his M.S.A. and Ph.D. degrees in Conservation Biology from Shanghai Ocean University, People's Republic of China. In 2011, he joined the Pearl River Fisheries Research Institute, Chinese Academy of Fishery Sciences in Guangzhou, where his research is focused on the exploitation, conservation of genetic resources, artificial breeding, and genetic improvement of turtles. In particular, Dr. Hong has successfully bred *Pelochelys cantorii* in captivity for the first time in China.



Zhu Xinping received his Ph.D. degree in Genetics from the Institute of Hydrobiology, Chinese Academy of Sciences, People's Republic of China, in 2004. He has worked at the Pearl River Fisheries Research Institute, Chinese Academy of Fishery Sciences in Guangzhou since 1988. Prof. Zhu is now the Deputy Director of the Research Institute. The research scope of the team he leads focuses on conservation and breeding of *Pelochelys cantorii*, the developmental mechanisms of turtle germ cells and transplantation technology, breeding of high-fecundity *Mauremys mutica*, and early propagation and breeding of fast-growing *Pelodiscus sinensis*.



Chen Chen completed his B.A. degree at Ludong University, People's Republic of China, and his M.S. and Ph.D. degrees at the Ocean University of China, People's Republic of China. After graduation, Dr. Chen joined the Laboratory of Aquatic Germplasm Resources and Genetic Breeding at the Chinese Academy of Fishery Sciences, Pearl River Fisheries Research Institute in Guangzhou. Recently, his research direction is in turtle population genetics, focusing on the allele distributions of turtle populations and metapopulation dynamics.



Cai Xiaodan received her M.S. degree in Fishery Resources at Nanjing Agricultural University, People's Republic of China. She joined the Pearl River Fisheries Research Institute, Chinese Academy of Fishery Sciences in Guangzhou in 2007, where her work is focused on river fisheries management and aquatic life protection, with a special interest in Endangered aquatic wildlife protection.



Li Yongming graduated in 1988 from the Yunnan Agricultural School, People's Republic of China, majoring in Freshwater Aquaculture, and has been engaged in the domestication and breeding of indigenous fishes in the Lancang River. As a government employee, Mr. Li conducts fishery supervision and management as well as aquatic wildlife protection work.



Li Xinping is a farmer who cultivates aquatic seedlings, with a special interest in the protection and breeding of turtles. Mr. Li has been rearing the two captive *Chitra vandijki* specimens used in this study for more than 25 years.