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Nesting Activity of the Chinese Softshell Turtle, *Pelodiscus sinensis*, on the Yellow River, Northwestern China

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ABSTRACT. – The nesting activity of the Chinese softshell turtle (*Pelodiscus sinensis*) in its natural habitat is largely undocumented despite its broad geographical distribution across East Asia. Here, we report on female nesting behaviors, egg incubation, and hatchling emergence of *P. sinensis* on the Yellow River in northwestern China. In this region, the turtle is currently threatened by overharvesting, and successful conservation strategies should include strict monitoring of hunting and trade activities.

The nesting season, which includes terrestrial oviposition, egg incubation, and hatchling emergence, represents an essential yet vulnerable period for aquatic turtles (Tornabene et al. 2018). Nesting in terrestrial environments entails great risks for adult turtles due to increased exposure to predators and temperature extremes (Plummer and Doody 2010). Additionally, the eggs are vulnerable to predators and flooding events (Tornabene et al. 2018), and hatchlings are susceptible to predation and desiccation (Plummer 2007; Ghaffari et al. 2013). Studying the behavioral aspects of the nesting of adult turtles, as well as hatchling emergence, may provide important insights into the choices that optimize their fitness and improve the success of the nest (Hughes and Brooks 2006; Plummer 2007; Doody et al. 2009).

The Chinese softshell turtle (*Pelodiscus sinensis*) is widely exploited for food and traditional medicine in East Asia (Cheung and Dudgeon 2006) and has a geographical distribution ranging from Japan, through eastern, central, and southern China to Vietnam (Turtle Taxonomy Working Group [TTWG] 2017). Wild populations of *P. sinensis* have suffered a continuous decline in recent decades because of overharvesting and habitat destruction (Kong 2020), and this species is classified as vulnerable on the IUCN Red List (TTWG 2017). The nesting activity of *P. sinensis* in its natural habitat is largely unknown, with only a few observations of reproductive behaviors and incubation available in captivity (Li and Tang 1999; Si et al. 1999). This study provides the first report on nesting behaviors, egg incubation, and hatchling emergence of *P. sinensis* in a natural environment.

Methods: Study Site. — Field studies were conducted from April 2017 to October 2018 along the Yellow River in Dali County, Shaanxi Province, China (lat 34.9°N, long 110.2°E). The Yellow River flows southward in this area and forms multiple branches that pass through 120 ha of sandy wetlands. Approximately 1.5 km from the Yellow River, multiple fishponds are located close to the river branches. The sparsely vegetated sandbanks of these wetlands and ponds provide ideal nesting sites for *P. sinensis* (Kong 2020). Dali County has a dry climate with hot summers, and during the study period, the mean ambient temperature in summer afternoons often exceeded 36°C (data obtained from the local meteorological bureau, 2017–2018). In both years, the Yellow River entered its peak flood stage in July, and the wetlands were inundated by rising river water for approximately 20 d/yr.

Data Collection. — Turtles were captured in the Yellow River by net traps. Before radio tracking, interview surveys were carried out with experienced *P. sinensis* hunters who suggested that gravid *P. sinensis* might be recognizable because of their relatively domed carapaces. Eighteen female individuals that had this characteristic and were suspected to be gravid were outfitted with radio transmitters and released at their capture sites (for detailed information concerning capture methods, models of the transmitters, and attachment methods, see Kong 2020). Radiotelemetry was conducted every 2 hrs for 24 hrs, 7 d per week, until the females nested. When an individual was encountered on land, nesting behaviors were recorded from a distance of 7–8 m. After the turtle laid its eggs and returned to the river, the nest was excavated to record the clutch size, egg measurements, and dimensions of the nest chamber (provided no damage had occurred during excavation). Developing eggs were easily identified by the presence of a white spot on the eggshell. After the measurements, the eggs were reburied in the nest. To observe the emergence behavior of hatchlings, the nests were checked twice a day from 10 d before the estimated date of emergence.

Results: Timing and Behaviors of Nesting. — Out of 18 female turtles, 11 individuals with a mean weight (± SD) of 1376.32 ± 561.47 g (range, 574–2461 g) and carapace length of 22.41 ± 4.22 cm (range, 17–32.2 cm) were successfully tracked. Tracking of the remaining 7 individuals failed because the signals were lost. Eight of the tracked turtles nested adjacent to the wetlands, and 3 nested adjacent to the ponds. The nesting activities lasted from 26 May to 20 August in 2017 and from 10 May to 22 July in 2018. Consecutive tracking and observation

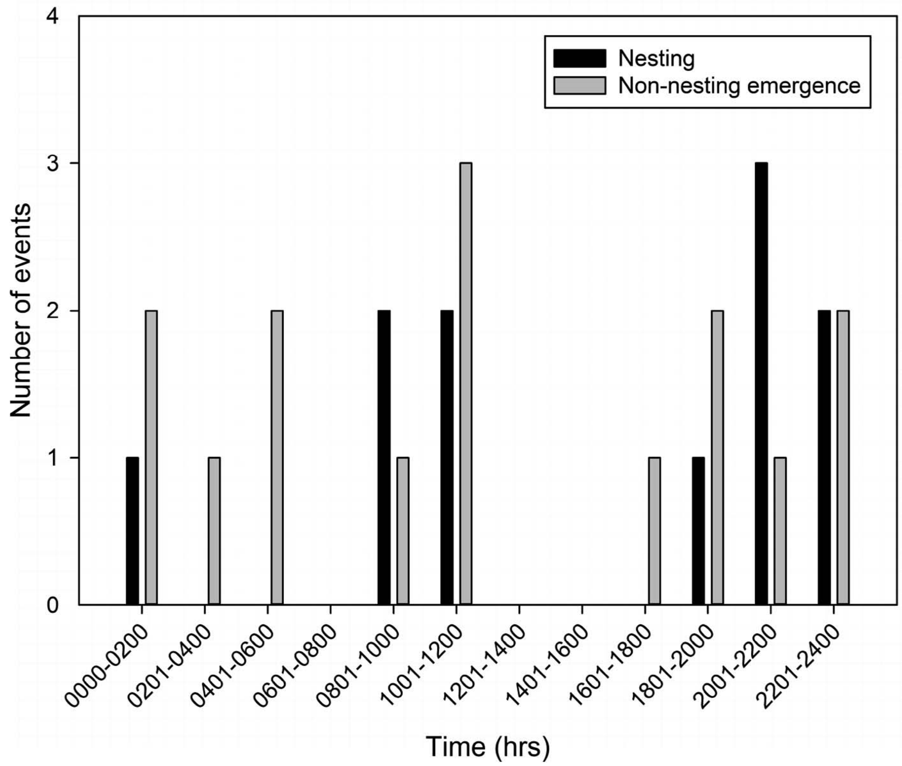

Figure 1. Temporal distributions of nesting events (n = 11) and nonnesting emergence (n = 15) of *Pelodiscus sinensis*.

revealed that most turtles emerged onto sandbanks either in the morning or at night, with the exception of 1 individual that emerged in the afternoon (Fig. 1). Turtles did not nest during the first emergence onto land. Nesting occurred in the morning, or in the night of the second or third appearance within the following 3 d. Four components of nesting behavior—nest-site selection, chamber excavation, egg-laying, and filling—were recorded for 4 individuals. The remaining 7 turtles were already excavating the chambers or laying eggs by the time they were encountered on land.

During nest-site selection, females displayed intermittent movements in the potential nesting areas and would often elevate the head a few centimeters above the carapace, which possibly corresponded to vigilance behavior.

Chamber excavation involved 2 steps: scraping and excavation. Turtles initially removed surface sand with their forelimbs and scraped a shallow pit slightly larger than their bodies, then initiated the excavation by digging with alternating hind limbs. During excavation, the turtle's body swung left and right, corresponding to the alternation of hind limbs, making the nest entrance roughly symmetrical and creating an inclined nest chamber. The mean vertical distance from the bottom of the nest chamber to the surface was 10.45 ± 1.72 cm (range, 9.3–14.2 cm, n = 6), and the diagonal distance was 13.40 ± 2.53 cm (range, 11–18.8 cm, n = 6) (Fig. 2).

Egg laying occurred immediately after excavation. The posterior parts of the females' carapace moved up and down accompanying the deposition of eggs.

After laying the eggs, the turtles swept the sand gathered behind the chamber into the nests with alternating hind limbs. Once the chamber was filled, the turtles flattened the shallow pit using their hind limbs; there was no direct contact between the plastron and the surface. Almost all traces of nesting were removed, which made it difficult to find the nests without the help of radio tracking (Fig. 2). Once this process was finished, the females quickly returned to the water.

Incubation and Hatchling Emergence. — The 11 nests contained a total of 196 eggs (10–32 eggs per nest), 7 of which were broken during excavation. Of the remaining 189 eggs, which had a mean weight of 4.47 ± 0.91 g (range, 2.6–6.9 g) and a mean diameter of 19.60 ± 1.36 mm (range, 16.59–23.07 mm), 180 were developed (95.2%, 78.9–100% per nest). Eggs in 3 nests located in the wetlands failed to hatch due to the inundation of these nests by rising water. A total of 106 hatchlings emerged from the remaining 8 nests, which contained 119 eggs, after a mean incubation period of 54.8 ± 7.8 d (range, 46–68 d). Hatchlings had dark black carapaces with widely distributed tubercles, oval yellow plastrons with large black patches, and white speckles that were shaped like dots and stripes on their necks (Fig. 3).

Hatchlings emerged from the nests singly after sunset or in the morning, presenting a typical flattened softshell profile. After emergence, they displayed brisk movements

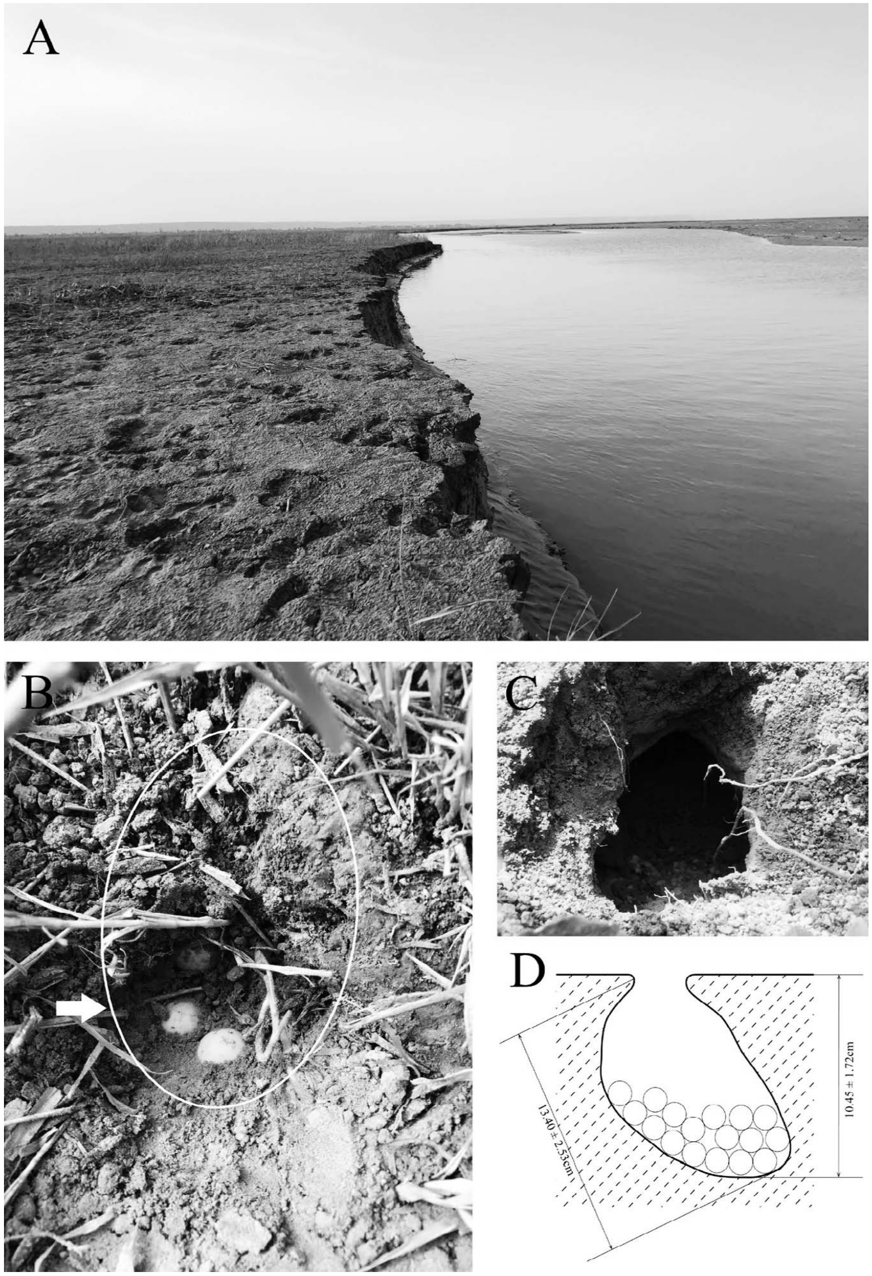

Figure 2. (A) Nesting habitat of *P. sinensis* on the Yellow River. (B) Subtle difference between the nest-site surface and its surrounding environment. (C) Nest digging with alternating hind limbs created a roughly symmetrical nest entrance. (D) Graphical representation of the inclined nest chamber. Photos by Qingjun Zhu.

on the surface and dispersed in the direction of the nearby water. No predators were found in this study, probably because of the researchers' presence. However, the hatchlings displayed a terrestrial burrowing behavior when the researchers approached them (which may be interpreted as a potential predator avoidance behavior) (Fig. 3). The hatchlings then stayed stationary beneath the surface. Presumably, the researchers' departure cued their reappearance.

Discussion. — The diel timing of nesting might influence the fitness of the adults because of daily variation in ambient temperature and predation risk (Doody et al. 2009). Softshell turtles, such as *Apalone spinifera* and *Apalone mutica*, generally nest during the day (Plummer 1976; Doody et al. 2009), mostly in the afternoon (Tornabene et al. 2018). In contrast, on the Yellow River, *P. sinensis* emerged from the water mainly either in the morning or at night, and no turtles nested in the afternoon (Fig. 1). Since the ambient temperature in Dali County often exceeds 36°C during the breeding season of *P. sinensis*, nesting on riverbanks in these conditions has an associated risk of heat stress exposure. Therefore, this apparent preference for nesting during cooler times of the day probably represents an adaptation to minimize this risk.

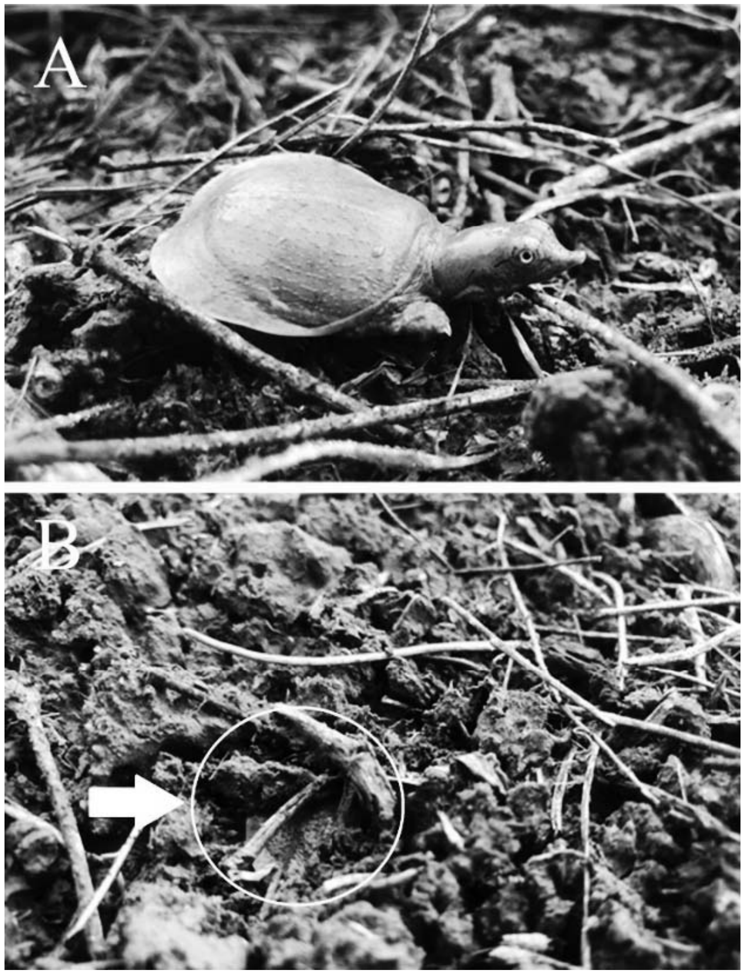

Figure 3. (A) A hatchling emerging from the nest and crawling to the water. (B) The hatchling exhibiting terrestrial burrowing when the researchers approached it. Photos by Qingjun Zhu.

Additionally, the time of nesting may be related to historical or current predation events or perceived predation risk (Spencer 2002; Doody et al. 2009). Consistent with this idea, captive individuals of *P. sinensis* nest only at night, when there are no visitors present (Li and Tang 1999; Si et al. 1999). In Dali County, *P. sinensis* is overharvested by indigenous people for food and trade. During our investigations, a professional hunter reported an exceptionally large harvest of 30 kg/d, with a trade price of 160 RMB/kg (Fig. 4). This lucrative return had prompted people to professionally hunt *P. sinensis* almost every day during the nesting season. Such long-term overharvesting may also disturb nesting turtles, leading to a higher incidence of nesting at night (Fig. 1), when there is less anthropogenic disturbance (Vogt 2008; Doody et al. 2009). Comparative analyses of populations in different areas would be necessary to determine how natural and anthropogenic factors affect this widely distributed species.

In general, most nesting behaviors appear to be similar among trionychid turtles (Plummer 1976; Hossain et al. 2010; Tornabene et al. 2018); however, there are important differences between *P. sinensis* and other softshell turtles. For instance, nesting *P. sinensis* females do not exhibit terrestrial burrowing (commonly observed among nesting *Apalone* spp.; Plummer 1976; Plummer and Doody 2010), as adult *P. sinensis* return to safer waters immediately after nesting. Moreover, *P. sinensis* individuals remove the traces left by nesting with their hind limbs, making the actual nest-site locations indistinguishable from the surrounding environment (Fig. 2). However, *A. spinifera* (Tornabene et al. 2018) and *Lissemys punctata* (Hossain et al. 2010) exhibit trace removal behaviors using their plastrons, sometimes leaving obvious troughs after nesting (Plummer and Doody 2010). Collectively, these maneuverability patterns of *P. sinensis* are important not only for increasing the survival of nesting females but also for protecting nests from predation.

In the present study, crawling *P. sinensis* hatchlings displayed a terrestrial burrowing behavior when researchers approached them (Fig. 3). Nesting females of *Apalone* spp. (Plummer and Doody 2010) and *Kinosternon* spp. (Iverson 1990; Burke et al. 1994) also perform terrestrial burrowing, which may provide temporary shelter when they are unable to return to the water immediately

Figure 4. A common harvesting tool in Dali County called the "softshell turtle gun." The hunter threw out the bait with hooks, caught the turtle that came out of the water to breathe, and pulled it back using the wheel. The professional hunter reported an exceptionally large harvest of 30 kg/d with this tool. Photos by Qingjun Zhu.

(Plummer and Doody 2010); however, this phenomenon has not been detected previously in trionychid hatchlings. Our study showed that terrestrial burrowing behavior is already displayed by hatchlings at the time of nest emergence and may be an important mechanism for hatchlings to cope with a dangerous terrestrial environment, which involves predators, temperature extremes, and desiccation (Janzen et al. 2000; Plummer 2007; Ghaffari et al. 2013).

Freshwater ecosystems (e.g., rivers, tributaries, ponds, and wetlands) in the middle and lower reaches of the Yellow River are important habitats for *P. sinensis*, so much so that, in 2012, China established the Chinese Softshell Turtle National Aquatic Germplasm Reserve of Yellow River Beach in Dali County. However, during this investigation, overharvesting and trade activities were frequently observed in this reserve (Fig. 4). In an attempt to successfully sustain viable populations, an enormous number of juvenile turtles purchased from farms were released into the Yellow River by the reserve's management department (Aquaculture 2014). However, evidence shows that long-term farm breeding of Chinese softshell turtles (*Pelodiscus* spp.) results in massive erosion of biodiversity (Gong et al. 2018). Consequently, the release of captive individuals into wild populations is antithetical to the purposes of conservation. In addition, this action further promotes the exploitation of wild populations since these are considered to have better nutritional value and taste and therefore offer much higher prices (Shi et al. 2007).

In conclusion, our results show that *P. sinensis* exhibits adapted behaviors at both life stages (hatchling and adult) to avoid predation and temperature extremes and has high egg development and hatchling rates. Together with the possible production of more than 1 clutch of eggs each year (Si et al. 1999), these factors indicate that *P. sinensis* may have a relatively stable intrinsic rate of population increase in the field. As such, we suggest that conservation strategies for *P. sinensis* include strict monitoring of hunting and trade activities to be likely to succeed reasonably.

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