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## Reproduction of endangered Big-headed Turtle, *Platysternon megacephalum* (Reptilia: Testudines: Platysternidae)

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**Abstract.** The big-headed turtle (*Platysternon megacephalum*) is heavily harvested to support tremendous demands from food and pet markets, and thus its ecology remains poorly understood. The presence of self-sustaining populations in Hong Kong (22°09'-22°37'N, 113°50'-114°30'E) provides important opportunities to advance our understanding of this species. We employed mark-recapture surveying, radio-tracking of two gravid females, and directed streamside searches to document the reproductive ecology of the species between September 2009 and June 2011 in Hong Kong. We found seven gravid females between 20-27 June 2010 and 2011, and which subsequently oviposited on average three eggs (range 2-8), with mean length and width of 36 mm and 21 mm, in early July. There was positive correlation between the size of females and clutch sizes. We found one clutch in leaf litter 1.6 m away from the stream, which hatched between 14 to 18 October. The incubation period was estimated to be between 103 and 110 days. The results of this study provide important information to formulate conservation plan for this endangered species.

**Keywords.** Asian turtle crisis, incubation, oviposition, turtle conservation.

Big-headed turtles (*Platysternon megacephalum*) is currently listed as Endangered in the IUCN Red List (IUCN, 2013) and was up-listed to Appendix I of CITES (CITES, 2011). However, turtles are still captured and commonly sold in food and pet markets (Cheung and Dudgeon, 2006; Gong et al., 2009). It is evident that populations of *P. megacephalum* have been heavily depleted and are in drastic decline in China (Hendrie, 2000; Tana et al., 2000; Gong et al., 2006). The IUCN Red List Status of this species was recently proposed to be changed from Endangered to Critically Endangered to highlight the immediate need to conserve this species (Horne et al., 2012).

Given the acute nature of declines of *P. megacephalum* throughout its range (Horne et al., 2012), the presence of self-sustaining populations in Hong Kong provides important opportunities to advance our understanding of this species (Sung et al., 2013). To our knowl-

edge, the reproductive biology of wild *P. megacephalum* such as clutch size and timing of reproduction has not been documented. In this study, we aimed to investigate the reproductive biology of *P. megacephalum* to answer the following questions: 1) When is the reproductive season, including when do they lay eggs and their eggs hatch? 2) What are the clutch sizes and egg sizes? 3) Where do they lay eggs? 4) Is the number of eggs by clutch related to the size of females? Our overarching goal in delineating the reproductive ecology of the species was to generate information that is useful to design conservation plans for this species.

We conducted this study within the Hong Kong Special Administrative Region, China (22°09'-22°37'N, 113°50'-114°30'E) from 1 September 2009 to 27 June 2011. Study sites included four streams located within national parks and a stream in Kadoorie Farm and

Botanic Garden (KFBG), a private reserve in which access by the public is controlled. The elevation of the study sites are between 250 to 800 m above sea level. In order to investigate the reproductive ecology of *P. megacephalum* we employed three methods: radio-tracking of gravid females, mark-recapture surveys, and daytime searches for oviposition sites.

We conducted a mark-recapture study, regularly surveying for turtles using baited hoop traps and visual encounter surveys in all five study sites following methods in Sung et al. (2013). We made 261 captures of 138 individuals, of these, 26, 30 and 82 were adult males, females and juveniles, respectively. Female turtles achieve maturity when they reach 100 mm in carapace length (Sung et al., in press), thus we regarded all female turtles over 100 mm in carapace length as adults. For every adult female *P. megacephalum* captured, we assessed the presence of eggs by palpation (Kuchling, 1999), and potentially gravid females were transported to the Wild Animal Rescue Centre of Kadoorie Farm and Botanic Garden for X-ray radiography to confirm the presence of oviductal eggs and document clutch sizes. We conducted linear correlation analysis between the clutch size and the carapace length of females using software R (R Core Team, 2014). The sizes of eggs were measured from radiograph images using ImageJ software (National Institutes of Health; <http://rsb.info.nih.gov/ij/>). A total of 25 eggs were measured. We used the straight-line carapace length as reference for measurement. We compared the carapace length and plastron length between gravid females and nongravid females by t-test using software R. Data were log-transformed to meet the assumption of normality.

In June 2010, we attached radio-transmitters (LF-1-2477-RS-T, L.L. Electronics, USA) on the rear side of the carapace of two gravid females using epoxy putty glue and released them back to their original locations within 24 hours of capture. We used a communication receiver (IC-R10, ICOM Inc., USA) and a flexible 3-element Yagi antenna (Biotrack Ltd., UK) to relocate the radio-tracked females every evening after release in an attempt to locate egg laying sites. We palpated the turtles to determine whether turtles had laid eggs.

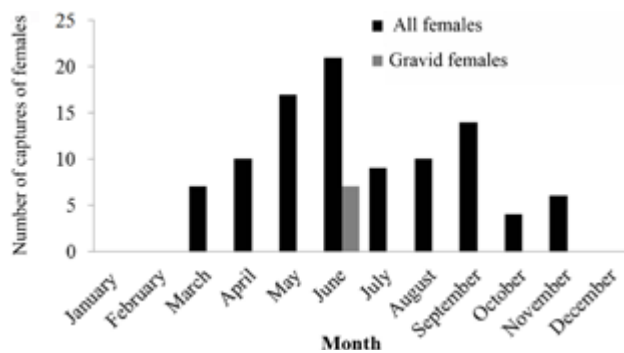
We conducted opportunistic and systematic daytime searches for eggs in potential nesting sites, including in leaf litter and soil within 0.5 to 20 m from streams during the wet season (i.e. between 27 April 2011 to 2 September 2011). Searching effort for nests totaled 15 person-hours over the study period. The sizes of eggs discovered in the field were carefully measured to the nearest 1 mm by caliper (Mitutoya Corporation, Japan) and weighed to the nearest 1 g with a spring balance (Pesola, Baar, Switzer-

land). We visited identified nests every three to four days to determine the time of hatching.

During our mark-recapture study, which captured a considerable number of females from March–October, we only detected gravid females by palpation in June (Fig. 1). Among 105 captures of females all year round, we found a total of seven different gravid females between 20 June to 27 June in both 2010 and 2011. The average straight-line carapace length and plastron length of gravid females were 125.1 mm (SD = 14.0, range 110.5–152.9 mm,  $n = 7$ ) and 103.7 mm (SD = 11.6, range 90.3–127.2,  $n = 7$ ), respectively, and the average weight was 393.3 g (SD = 121.9, range 276–624,  $n = 7$ ). The average straight-line carapace length and plastron length of nongravid females were 119.7 mm (SD = 12.0, range 105.6–153.2 mm,  $n = 23$ ) and 100.8 mm (SD = 10.1, range 88.8–127.1,  $n = 23$ ), respectively, and the average weight was 326.3 g (SD = 105.3, range 180–595,  $n = 23$ ). There were no significant differences between females that we found gravid and nongravid females in carapace length ( $t = -1.50$ ,  $P = 0.18$ ) and plastron length ( $t = -0.89$ ,  $P = 0.42$ ). Average clutch size was 3.6 (SD = 2.0, range 2–8,  $n = 7$ ). The clutch size was positively correlated with the carapace length of females ( $r^2 = 0.70$ ,  $P = 0.02$ ). Analysis of X-ray radiographs revealed that the mean length of oviductal eggs was 35.8 mm (SD = 0.8, range 27.4–43.7,  $n = 25$ ) and the average width of eggs found in nests was 20.5 mm (SD = 1.1, range 18.0–21.9,  $n = 25$ ).

We radio-tracked two gravid females in June and July 2010, however, they were relocated to the same place where we captured them. They were gravid until we found that they laid their eggs on 1 or 2 July and on 3 or 4 July. Despite extensive searching during radio-tracking, we were unable to find these nests.

During daytime searches for nest sites, we were only able to find one clutch containing two eggs in leaf litter at 5 cm soil depth, 1.6 m from the stream edge, and at



**Figure 1.** Number of captures of all females and gravid females *Platysternon megacephalum* in Hong Kong between 2009 and 2011.

689 m elevation on 26 July 2011. Unfortunately, one of the eggs was damaged during searching and thus measurements were only taken for one egg. The egg measured  $38.5 \times 21.6$  mm in size and weighed 11.0 g. The egg hatched between 14 October and 18 October 2011. Based on our observations in the field, at least some female *P. megacephalum* deposit eggs in early July (1-4 July in this study) and the embryonic developmental period may be between 103 and 110 days.

This is the first study to document a nest of wild *P. megacephalum*. Some freshwater turtles travel large distances on land to seek out suitable nesting sites (Congdon et al., 1987). However, it appears that some *P. megacephalum* nest within 2 m of streams. A radio-tracking study found that females remained near (i.e. less than 5 m) streams during reproductive seasons (Y.H. Sung, unpublished data), and another radio-tracking study of relocated adults showed that the maximum distance from the streams was 5.8 m (Shen et al., 2010). These provided further evidence that females likely lay eggs near streams. Females apparently dig a shallow nest and lay eggs in leaf litter. Our observations indicated that much of the riparian area along the study streams appears to be suitable for nesting. There may be other suitable nesting sites that are more obscure and difficult to find, such as rock crevices and deeper within the soil.

*Platysternon megacephalum* in Hong Kong appears to have consistent and synchronized breeding seasons during the wet season, as we only detected gravid females in late June. In addition, we observed males and females inhabiting the same small pool on multiple occasions (over five times) in May from a mark-recapture study (Y.H. Sung, unpublished data). Such observations were rare in other times of the year, and suggest that pairing and copulation may occur in May. As with many other turtle species, the onset of reproduction, and the period between mating and egg laying, of *P. megacephalum* is concordant with the wet season, when food is abundant for gravid females (Wang et al., 2011). However, it is not known if females in this species store sperm and if fertilization of eggs can be accomplished from previous years' mating. Females of many species of turtles are capable of sperm storage (Pearse and Avise, 2001), and this is a possibility for *P. megacephalum*.

The breeding season among individual *P. megacephalum* is relatively consistent because we were only able to detect gravid females in late June and early July, despite considerable number of females captured throughout the wet season (Fig. 1). Females of other Asian turtle species are gravid for a longer period of time, for example, between late April to June for *Cuora mouhotti* (Wang et al., 2011) in Hainan and mid-April to mid-July for *Cuora*

*flavomarginata* in Taiwan (Chen and Lue, 1999). We did not X-ray females regularly to detect oviductal eggs when they could not be detected by palpation. Regular X-raying of females in the wet season may help us to better understand the reproductive season of *P. megacephalum*.

The single clutch of eggs located in the field in this study hatched in mid-October, which was concordant with observations of newly-hatched neonates in October and November in Hong Kong (Kendrick et al., 2011). However, there was an exceptional capture of newly-hatched neonates in April (Kendrick et al., 2011). There are two possible explanations for this observation. One explanation is that in some cases embryos may develop over the winter period or eggs may hatch and hatchlings may remain in nests until spring. An alternative explanation is that *P. megacephalum* lays eggs during the winter. The latter is unlikely as we have documented through radio-telemetry that most *P. megacephalum* are inactive during winter (Y.H. Sung et al., unpublished data).

In captive situations, female *P. megacephalum* lay one to three eggs as a single clutch per year (Bonin et al., 2006; Zhou and Wang, 2009), and we confirmed that the clutch sizes of most females were three. Notably, one female at a protected site contained eight eggs, and it was the largest female captured in this study (plastron length = 127.5 mm). The largest female caught among the four other sites (plastron length = 105.5 mm), where illegal harvesting has occurred, were at least 15% smaller (Sung et al., 2013). The absence of large females at unprotected sites was likely caused by past illegal harvesting of turtles (Sung et al., 2013). We found that clutch size was positively correlated to the size of females, removal of large females severely reduces the resilience of populations to over-exploitation (Paitz et al., 2007). This may explain why populations of *P. megacephalum* decline quickly in places where harvesting occurs.

The size of eggs may be underestimated as the eggs may be tilted inside the turtles during X-ray, however, this was still a valuable method because this was the only non-invasive way to provide information of egg size of this species.

For many imperiled turtle species under extremely high pressure from harvesting, captive breeding is believed to be valuable to help their conservation (Horne et al., 2012). Populations of *P. megacephalum* in many places have been heavily depleted because of unflagging demand in the food and pet markets, as enforcement has been inadequate to stop illegal harvesting. Captive breeding has been suggested as a means to conserve *P. megacephalum*, yet it has rarely been successful (Shi et al., 2007). One major reason is the lack of knowledge about their natural history, including reproductive biology, diet,

and behavior. We hope the information yielded from this study in Hong Kong, where relatively healthy populations remain, may improve methods for captive breeding of *P. megacephalum*. Given the currently unresolved taxonomy of *Platysternon* across its broad distribution and severe harvesting pressure throughout much of its range, we recommend that increased effort be made for in-situ conservation and research. A major constraint to our ability to conserve Asian freshwater turtle species has been that populations are so severely diminished by over-collection that it becomes impossible to conduct ecological research that would provide a basis for conservation actions, as has been the case with many *Cuora* species and has nearly been the case with *P. megacephalum*. While fairly robust populations of some of these threatened turtles remain, it is imperative that we begin collecting baseline data to inform conservation programs as we work to reduce the threat that harvesting presents to freshwater turtle populations.

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