

A Survey of Freshwater Turtles in Diaoluoshan Nature Reserve with Conservation Implications for the Endangered Big-Headed Turtle

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ABSTRACT. – We conducted population surveys of freshwater turtles in Diaoluoshan National Nature Reserve, Hainan Province, China, from 2012 to 2019, capturing 20 individuals belonging to 4 species, including 2 native species (big-headed turtle [*Platysternon megacephalum*] and Hainan four-eyed turtle [*Sacalia insulensis*]) and 2 alien species (red-eared slider [*Trachemys scripta elegans*] and common snapping turtle [*Chelydra serpentina*]). We captured 12 big-headed turtles and estimated their population density (1.6514 individuals/km) and abundance (14 individuals), both of which indicate that Diaoluoshan National Nature Reserve is an important area for turtle conservation, especially for big-headed turtles.

More than 60% of the world's turtle species are listed as critically endangered, endangered, or vulnerable by the International Union for Conservation of Nature (Rhodin et al. 2018). Unfortunately, there is a lack of basic ecological data for many endangered turtles, hindering conservation and management efforts (Rhodin et al. 2018). Population surveys have been conducted for only ~ 20% of Chinese turtle species (Shi 1998; Gong et al. 2005, 2006a, 2007, 2012; Wang et al. 2010; Sung et al. 2013); however, most of these surveys were spatially limited, and much of the distribution of these species remains understudied. Without population estimates throughout the distributions of species, it is difficult to assess their conservation status, formulate effective conservation strategies, and evaluate current conservation efforts for these species.

Hainan Island in Hainan Province is a tropical region with the richest biodiversity in China and is a global hot spot for biodiversity research and conservation (Gong et

al. 2003), including turtle biodiversity; a total of 8 turtle species are found on the island (Wang 2014). Diaoluoshan Nature Reserve is located in the southeast portion of Hainan Island and is one of the most biodiverse areas in Hainan Island because of its abundant rainfall and complex ecological environment (Jiang et al. 2006). Eight turtle species have been recorded in Diaoluoshan Nature Reserve (Wang 2014); however, most turtle species, except the Indochinese box turtle (*Cuora galbinifrons*) and keeled box turtle (*Cuora mouhotii*) (Lian 2009; Wang et al. 2011), are understudied in this area. Here, we conducted a survey of the freshwater turtles (except *Cuora* species and *Geoemyda spengleri* because they inhabit inland habitats) in Diaoluoshan National Nature Reserve to estimate their relative densities. The findings of the present study could aid the conservation efforts and management of endangered turtle species in this region.

Methods. — Field surveys were conducted at 9 streams in Diaoluoshan Nature Reserve, Hainan Province, within an altitude range of 500–1050 m. Two of these streams were below 900 m. Turtles were hand captured using visual searches (Hall et al. 1999) for a total of 7 d in April 2012 and 2013. Baited hoop nets (cages) were used to capture freshwater turtles in streams from 2015 to 2019 (Burgin and Ryan 2010). Cages were placed in 4 streams for 7 d in April 2015 and May 2016, 10 d in July 2018, and 5 d in October 2018. Additionally, in April and May 2019, we set cages for 20 d in 9 streams, which included the same 4 streams from previous years. We arranged these nylon cages (length = 60 cm, diameter = 33 cm, mesh size = 2 cm; baited with dried fish) along the stream at an interval of 30 m. The length of each stream surveyed was recorded by GPS. The total sample line length (5.45 km) was the sum of the distances traveled by each stream surveyed. All cages were checked daily during morning hours. At each cage, we recorded altitude, longitude, latitude, transect length, the number of turtle species and individuals captured, and the number of recaptures. We measured straight carapace length and recorded sex and age-group (i.e., adult, subadult, and juvenile) for all captured individuals. All native species were released at their point of capture, whereas the alien species were removed from the wild. All cages were removed to avoid harming the local wildlife. Captured big-headed turtles were marked on their marginal scutes (except in 2013) and released at their original capture location.

The population density of big-headed turtles was estimated as the relative population density (RPD; catch per unit fishing effort) and absolute population density (APD; total count) for only the 2019 data using the following formulas:

$$\text{RPD} = n/\text{cage} - \text{day} \quad [1]$$

$$\text{APD} = n/l \quad [2]$$

where n is the number of turtles caught, cage-day is the

Table 1. Species and number of individuals captured during field surveys in Diaoluoshan National Nature Reserve, Hainan Island, China, from 2012 to 2019. Numbers in parentheses after the age-group and date indicate the number of turtles. International Union for Conservation of Nature (IUCN) Red List status: LC = least concern; EN = endangered.

Species	Common name	No.	Age	Date	IUCN status	Capture method	Source
<i>Sacalia insulensis</i>	Hainan four-eyed turtle	1	Adult	2019	EN	Baited hoop net	Native
<i>Platysternon megacephalum</i>	Big-headed turtle	12	Adult (7) Juvenile (5)	2013 (1), 2015 (1), 2016 (5), 2018 (3), 2019 (9)	EN	Visual search and baited hoop net	Native
<i>Trachemys scripta elegans</i>	Red-eared slider	6	Adult (4) Juvenile (2)	2013 (3), 2019 (3)	LC	Visual search and baited hoop net	Alien
<i>Chelydra serpentina</i>	Common snapping turtle	1	Adult	2012	LC	Visual search	Alien

number of cages multiplied by the number of days, and l is the length of the survey sample line (stream length in kilometers).

Additionally, we estimated the population size of big-headed turtles using a mark–recapture method (Langtimm et al. 1996; Converse et al. 2005):

$$N = (M \times n) / m \quad [3]$$

where N is the population number, M is the number of turtles marked and released at the time of capture (first capture) (2015–2018), n is the total number of recaptured turtles (second capture) (2019), and m is the number of turtles marked at first capture and recaptured (second capture). Because of the small number of captured individuals across all streams, we combined all big-headed turtle captures and recaptures to estimate the population size for Diaoluoshan Nature Reserve.

Results. — A total of 20 individuals from 4 turtle species were found across the studied streams in Diaoluoshan Nature Reserve (Table 1). Two species were

native (i.e., big-headed turtle [*Platysternon megacephalum*] and Hainan four-eyed turtle [*Sacalia insulensis*; Lin et al. 2018, 2020]), and 2 were alien species (i.e., common snapping turtle [*Chelydra serpentina*] and red-eared slider [*Trachemys scripta elegans*]). In 2012 and 2013, 1 big-headed turtle, 1 common snapping turtle, and 3 red-eared sliders were found using the visual search method (Table 1). From 2015 to 2018, 6 big-headed turtles were caught using the baited hoop-net method (Table 1). From April to May 2019, 13 freshwater turtles (95 cages; 637 cage-days), including 9 big-headed turtles, 3 red-eared sliders, and 1 Hainan four-eyed turtle, were captured in streams (Table 1). The Hainan four-eyed turtle (straight carapace length = 134 mm), a female, was captured at an altitude of 939 m.

In 2019, 9 big-headed turtles were caught in 9 streams along a sample line of 5.45 km (Table 2), resulting in a relative population density of 0.0141 individuals/cage-day and an absolute population density of 1.6514 individuals/km. A total of 6 big-headed turtles were captured from 2015 to 2018, 4 of which were recaptured in their original streams in 2019 at a recapture rate of 67%. Using the mark–recapture method, the population number within the survey region was estimated to be 14. These individuals were distributed in the streams at an altitude greater than 900 m (Table 2), and no individuals were found between altitudes of 500–900 m.

Discussion: Native Species. — We found 12 big-headed turtles and 1 Hainan four-eyed turtle in the Diaoluoshan National Nature Reserve. The big-headed turtle is listed as an endangered species (Asian Turtle Trade Working Group 2000) and is included in Appendix I of the Convention on International Trade in Endangered Species (CITES 2019). The relative population density of the big-headed turtles in Diaoluoshan Nature Reserve (0.0141 individuals/cage-day) was higher than that reported for Jianfengling Nature Reserve (0.0036 individuals/cage-day; Gong et al. 2006b), Yinggeling Nature Reserve (0.0019 individuals/cage-day; Wan et al. 2015), and Limushan Nature Reserve (0.0014 individuals/cage-day; Gong et al. 2005) on Hainan Island. It was also higher than that previously reported for Dinghushan Nature

Table 2. *Platysternon megacephalum* captured during field surveys in Diaoluoshan National Nature Reserve, Hainan Island, China, from 2012 to 2019.

Turtle no.	Straight carapace length (mm)	Sex	Date	Altitude (meters above sea level)
1	144.35	Female	Apr 2015	> 900
	146.23		May 2016	> 900
	149.56		May 2019	927
2	107.02	Female	May 2016	> 900
	125.64		Jul 2018	942
	126.95		May 2019	942
3	164.40	Male	May 2016	> 900
	166.45		Jul 2018	935
	167.39		May 2019	943
4	—	Male	May 2016	> 900
	141.33		May 2019	932
5	85.91	Juvenile	May 2016	> 900
6	131.89	Male	Oct 2018	938
7	141.42	Male	Apr 2013	943
8	69.4	Juvenile	May 2019	932
9	60.65	Juvenile	May 2019	933
10	63.42	Juvenile	May 2019	929
11	63.19	Juvenile	May 2019	956
12	113.07	Female	May 2019	905

Reserve (0.0046 individuals/cage-day; Gong et al. 2012), Guangdong Province (0.0029 individuals/cage-day; Wang et al. 2010), and the Guangxi autonomous region (0.0005 individuals/cage-day; R.B., unpubl. data, 2014). Similar to relative population density, the number of recaptures of the big-headed turtle in Diaoluoshan Nature Reserve (67%) was higher than that in Hong Kong, China (47%; Sung et al. 2013). Moreover, the sex rate (1:1; Table 2) and age structure (7 adults, 5 juveniles; Table 1) of the big-headed turtle were normal in Diaoluoshan Nature Reserve; thus, it will be beneficial to maintain the population stability of this species.

The big-headed turtle is distributed mainly in high-altitude streams (800–1000 m) on Hainan Island (Gong et al. 2006b); our findings strongly confirmed this conclusion. No big-headed turtles were found between altitudes of 500 and 900 m in this study, but this species occurs at these altitudes elsewhere within the species' distribution (e.g., Guangdong; Wang 2010). One potential reason for the higher altitude on Hainan Island is the warmer climate of this region (Wang et al. 2011). Big-headed turtles prefer low-temperature habitats (Shi et al. 2011) and therefore must shift to higher altitude habitats to occupy a cooler thermal regime. Another possible reason is that the density at lower elevations was quite low, as seen in other reserves on Hainan (Gong et al. 2005, 2006a), and our trapping strength was inadequate in those areas.

The Hainan four-eyed turtle had some sympatric distribution with the big-headed turtle, but it prefers the low-altitude streams (170–470 m) on Hainan (Gong et al. 2005). To the best of our knowledge, this study is among the first to record Hainan four-eyed turtles at an altitude above 900 m in China. Previous investigations in Hainan found this species to be distributed within an altitude range of 170–800 m (Gong et al. 2005, 2006a, 2006b, 2007). The results of the present survey demonstrated an expansion of the known elevation range of this species in Hainan. It is possible that the high-altitude streams of the other reserves have not been fully investigated, and thus the scope of surveys should be expanded in the future.

There have been 2 other freshwater turtle species, the Asian yellow pond turtle (*Mauremys mutica*) and the Chinese striped-neck turtle (*M. sinensis*), recorded in the reserve (Wang 2014). However, we did not find them in the reserve at altitudes of 500–1050 m. This is because these species inhabit mainly ponds and rivers in low-elevation hills (Shi et al. 2011). Further surveys should strengthen the investigation of the low-elevation section of the reserve.

Discussion: Alien Species. — The red-eared slider is one of the most invasive species in the world (Invasive Species Specialist Group/Species Survival Commission [ISSG/SSC] 2001). After adapting to local climate conditions and successfully breeding in the wild, the species outcompetes native species for resources (e.g., food, shelter, and basking sites), causing population

declines (Cadi and Joly 2003; Polo-Cavia et al. 2008, 2009; Vilà et al. 2008; Zhao et al. 2013). In our study, 6 red-eared sliders (4 adults and 2 juveniles) were captured in Diaoluoshan Nature Reserve, which is not surprising because the species is known from other areas in Hainan (Ma and Shi 2017). Red-eared sliders are found mainly in hilltop streams; however, their populations have spread throughout the reserve. The capture of both adult (males and females) and juvenile red-eared sliders in the nature reserve suggests that there is an established breeding population or that there have been consistent releases of this invasive turtle into the wild by the visitors or inhabitants surrounding the nature reserve. Further studies are warranted to determine the impacts of red-eared sliders on the turtle community of Diaoluoshan Nature Reserve.

Another alien turtle species captured in the nature reserve was the common snapping turtle. The common snapping turtle is a known alien species in China and a common species within the pet trade in the region. We captured this species only in 2012 and have not recorded another capture in the nature reserve since that time. This result might imply that common snapping turtles are not released in high frequencies and/or that this species is not abundant in the evaluated high-elevation streams.

The captured specimens of this alien species were likely mercy releases because some specimens had Chinese letters on their carapaces. Mercy releases of alien turtles, especially in connection with religious activities, are common in Hainan Province and all over China. Moreover, alien turtles are often transported to nature reserves for release because some law enforcement officials cannot identify them and do not have expert identification and release procedures reviewed. These releases are detrimental not only to native turtle species but also to other forms of biodiversity (Ma and Shi 2017). Therefore, public awareness and conservation education campaigns are urgently needed within the communities neighboring the nature reserve to limit these types of releases.

Conclusions. — This study acknowledges the role of Diaoluoshan Nature Reserve as an important high-elevation habitat for the big-headed turtle. For example, this nature reserve supports the highest reported density for this species across its distribution. This endangered species is difficult to breed in captivity (Sung et al. 2014), further limiting the possibility of a human-assisted recovery (i.e., reintroductions of hatchery-reared individuals). Therefore, in situ conservation in this reserve is critical for this endangered species, and we have reason to believe that the reserve will protect these native freshwater turtles from illegal hunting from the point of view of conservation of the big-headed turtle. The presence of 2 alien turtle species, in particular red-eared sliders, at the evaluated high-elevation sites suggests the possibility for negative interactions with the native turtle species of the nature reserve. We recommend strength-

ening monitoring for any kind of release and potentially the removal of alien species from the nature reserve, but also the nature reserve might consider implementing outreach and education to inform visitors and the local communities about the negative impacts that any kind of release could cause to native wild.

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