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BREEDING BIOLOGY OF NORTHERN RIVER TERRAPIN *BATAGUR BASKA* IN CAPTIVITY IN BANGLADESH

EBTISAMUL ZANNAT MIM, MOHAMMAD FIROJ JAMAN, AGJ MORSHED[1], MD MAHABUB ALAM* AND NASRIN AKTER BRISTY

Department of Zoology, University of Dhaka, Dhaka-1000, Bangladesh

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Abstract

Northern River Terrapin, *Batagur baska,* is an ecologically extinct species in Bangladesh. The 'Project Batagur' has undertaken some important initiatives to resuscitate the nearly deceased population through captive breeding at Bhawal National Park, Gazipur, Bangladesh. We conducted a study on breeding biology of *B. baska* from May 2016 to June 2017 in the project site. Four adult females and three adult males comprised breeding pair in the study site. Male *Batagur baska* developed black colouration on head and neck, with rich crimson on dorsal portion of the neck during breeding season. During our study, we observed paired basking behaviour rather courtship and parental care. We found three nests from mid-March to first week of April. Females laid eggs at night and a total of 67 eggs were extracted from three nests; eggs were elliptical and whitish in colour. Clutch size varied from 18 to 28 eggs. There was significant positive correlation between clutch size and nest diameter ($p = 0.03$). The relationship was also positive and significant between egg weight and diameter ($p = 0.01$).The size of the egg was not related with the size of breeding females ($r = 0.143$).Average incubation period was (68.26 ± 5.23) days and there was significant negative relationship with temperature ($p= 0.000$), clutch size ($p = 0.001$), and hatching success ($p = 0.004$). Hatching success was also negatively co-related with temperature ($p= 0.001$). Hatching started in May and ended in the first week of June 2017 with mean hatching success of 91%. Simple linear regression showed positive relationship between egg length and hatchlings' carapace length, plastron length, and height. We also found positive relationship between egg diameter and hatchlings weight, and carapace width. This study can be used as a protocol for captive breeding and rearing activities of threatened chelonians around the world.

Introduction

The genus *Batagur* has three species in Bangladesh, *Batagur baska*, *Batagur kachuga,* and *Batagur dhongoka*(1). *Batagur baska* has a distribution that includes India, Bangladesh, and Myanmar(2-6). During the 19th and 20th centuries, this species was abundant from the

*Author for correspondence: <mahabub.zoo@du.ac.bd>.[1]Project-Batagur, Vienna Zoo, Maxingstraße13b, 1130 Vienna, Austria.

Hugli river of India to the Ayeyarwady and the Bago Rivers of Myanmar[2-3,5]. In Bangladesh, Northern River Terrapin is known to occur in large rivers and estuaries in the coastal districts of Noakhali, Barisal, and Khulna[7]. The global wild population of *Batagur baska* has decreased over the last two centuries with less than 100 mature remaining individuals[4]. Population of this species have also been decimated from the wild in Bangladesh[5,6,8]. The causes of decline include removal of mangrove forests and sand mining[5,9-11], exploitation for eggs and adults, habitat loss, degradation and fragmentation[4,6,10-12]. Illegal turtle trade[4,13], incidental capture in fishing nets and traps, accidental death by collision with motor-boats, watercrafts, loss of nesting beaches, destructive fishing practices and unseasonal floods[10] are also responsible for the decline. These activities pose serious threats to this species globally including Bangladesh[4,5,10]. *B. baska* is now globally Critically Endangered (CR)[4,5], and regarded as one of the tropical Asia's most threatened chelonians[14,15]. It is also listed in the CITES Appendix I, where commercial trade of the species is completely prohibited[4].

Wild populations of Northern River Terrapin have been decimated to such an extent that the species is considered as ecologically extinct[5-6]. Spitzweg *et al*.[16] also reported that wild population experienced a grave decline long time ago confirmed by genetic studies of this species. Thus, the species is unable to survive without major *ex situ* conservation efforts[6]. First successful captive breeding initiative of *B. baska* was taken at Bronx Zoo in 1990[17]. In 2010, the first two captive-bred batches of juveniles hatched under a project at Vienna Zoo, Austria. This breakthrough presented an opening to call for attention to this Critically Endangered species[6]. In Bangladesh, 'Project Batagur' initiated with combined efforts with Vienna Zoo, Austria to promote *ex situ* conservation project in Bhawal National Park. In between 2012 and 2019, there were 265 juveniles hatched and fostered in captivity in Bangladesh.

To date, reproductive activities of *B. baska* are very little known in literature. The study aims to investigate breeding behaviour and breeding biology of the species in captivity. The study also provides the baseline information and enhances scientific knowledge that will provide insights for future conservation efforts in Bangladesh.

Materials and Methods

Study area: We conducted this study at 'Project-Batagur' site in Bhawal National Park (24°04'31.2"N 90°23'59.4"E), Gazipur from May 2016 to June 2017. The project facility includes a large non-breeding pond, a small breeding pond, and seven rearing tanks for hatchlings of *Batagur baska*. The rectangular non-breeding pond (48.786 m × 24.384 m) is accommodated with a sandy beach. The sandy beach has two iron cages with similar measurement (1.83 m × 1.83 m). Slope of the large pond is straight with 10.3 m in length. The peak height of the slope was 1.13 m from the water level. On the other hand, size of small breeding pond is 24.38 m × 18.3 m in measurement. This pond has 11.9 m long

sandy beach. The steepness of beach is gradual and slightly convex. The half-circle beach is encompassed by 0.69 m tall brick wall. The vertical length of the beach is 2.13 m from the water level. The water level of both ponds varies depending on the season. Water level goes down to 1.4 m during winter season. Seven well-constructed rearing tanks are placed linearly beside the non-breading pond for rearing hatchlings and juveniles. The larger tanks with similar sizes (4.3 m × 3.05 m × 0.9 m) while the smaller tanks also have the similar size of each (1.8 m × 1.8 m × 0.9 m). The water level of both larger and smaller tanks was 0.8 m.

Data collection: Data on breeding activities were collected twice a week during breeding season (September 2016 - June 2017), and once a week during non-breeding season (July-August 2016) that allowed us to compare turtle's breeding behaviour between these two seasons. There were four adult females and three adult males of *B. baska*, which comprised a breeding group in the project site. These individuals were kept in a very organized housing system and well-maintained breeding ponds. During the study, we confirmed identification of mature females by following Bhupathy[18]. Mature females in the study had mean carapace length (CL) of 500 mm, Carapace width (CW) of 400 mm, and body mass was 17 kg. We also followed Rashid & Swingland[19] to confirm adult males, that had CL of 421 mm, CW of 214 mm, plastron length (PL) of 378 mm, height (H) of 161 mm, and body weight 8.87 kg. We observed the ponds every morning carefully to trace the females' footprints to find the nesting places as well as any nest markings. The time and date of breeding activities were documented in order to determine breeding season in captivity. Breeding behaviour such as courtship, breeding colouration, paired basking, nest making and egg laying activities were observed and documented. We spent five to six days each month in the project site. In our absence, the caretaker of the project recorded breeding data according to our prescribed instructions for the rest of the days and we collected it afterward. When we found any nest, the layers of sand on the nest were removed carefully, and eggs were collected by bare hand. Clutch size was calculated by enumerating the eggs laid in nests. Weight, length, and diameter of eggs were measured immediately after the collection. The eggs from the natural nest were kept in anti-ant plastic boxes with similar measurements (57.5 cm × 37.5 cm × 22.5 cm) provided by the project authority following Weissenbacher *et al*.[6]. A sand layer was made inside the boxes and the eggs were kept in two to three rows above the layer and then covered again by another sand layer. The boxes were well facilitated to pass air and moisture. A digital Hobo thermometer was attached to the boxes to record temperature. The incubation period was monitored in the anti-ant boxes fitted well under the sand. The incubation period was calculated from the day of keeping the eggs in the boxes. Both nest and boxes were protected by an iron cage to keep it safe from predators such as *Varanus* spp. and *Herpestes* spp.[6]. Hatching success was calculated by enumerating the number of hatchlings in relation to the eggs laid per clutch.

Morphometric data of adult *B. baska*, eggs, and hatchlings were recorded in log book during field visits. The eggs, hatchlings, and adults were weighted by using digital weight machine. Slide calipers were used to measure egg length (EL), egg diameter (ED), as well as CL, CW, PL, H of hatchling. The size of adults, nest depth, diameter, and nest distance from water level were measured using measuring tape (about 15.5 m long). Egg shape was defined by the ratio of its length, and diameter. The ratio of spherical eggs varied from 1.00 to 1.10, elliptical eggs between 1.11 and 1.99, and oblong eggs had at least twice of its diameter[20]. The hatchlings were transferred carefully into the rearing tanks immediately after hatching out. Hatching success was calculated by considering the total number of eggs laid, the young hatched and rearing of hatchlings.

Data were processed in computer software in combination with Statistical Package for Social Science (SPSS version, 17.0), and MS Excel. We performed simple linear regression and correlation to assess the relationship between breeding parameters. Significance was tested at $p< 0.05$.

Results and Discussion

Observation of breeding behaviour: Breeding behaviour of *B. baska* was started in early September. Head colouration of breeding male was observed first time in September 2016 and fainted in February 2017. In breeding male, *B. baska* showed black colouration on head and neck, dorsal portion of the neck was also rich crimson in colour[5,16,21]. These seasonal colourations were shown by males presumably associated with breeding[22]. Females didn't show any breeding colouration due to difficulty to recognize their less colourful nature during breeding season[5,21]. *B. baska* also showed the highest breeding activities due to breeding season that match with the previous study done by Sadat[23]. We observed head swaying and throat pumping behaviour rather courtship to perform breeding activities[17]. Paired basking was observed in breeding pond about a month before the nest building and ended a week before laying eggs. Female turtles were found basking just behind the male.

Nesting sites, nest building and egg laying: Three circular nests were made on sandy bank with the help of head and sharp claws of forelimbs. We found first nest on 13 March, 2017; the second nest on 20 March, 2017 and the third nest on 02 April, 2017. The distance of the first, second and third nests were 640 cm, 716 cm and 692 cm (mean 682.67 ± 38.85 cm), respectively, from the water level. *Batagur baska* builds nest on island shores, beaches of the river mouth and along the coastlines[5,22,24]. Our result also corroborated previous studies suggesting that *B. baska* made nests at the end of January through late March[7,18,24-26]. Weissenbacher *et al*.[6] also stated that *B. baska* nested in spring during mid-February to mid-April. The depth of the nests was 28 ± 3 cm (range 25 - 31 cm) and diameter was 46±4 cm (range 42 - 50 cm) from the land surface (Table 1). Nest depth and diameter of *B. baska* varied due to the different sizes of eggs laid in the nests. The nests

were covered by sand after the female laying egg. Female turtles laid only one clutch of eggs in one breeding season between March and mid-April 2017. Eggs were laid at night and we found a total of 67 eggs from three nests. It could not be confirmed whether female laid eggs probably due to nest-building or egg-laying behaviour of *B. baska* in dark[5,24]. The clutch size was 22.33 ± 5.13 eggs (range 18 - 28 eggs) (Table 1).

Table 1. Measurements of nests (n = 3) and eggs (n = 67) of *Batagur baska* from May 2016 to June 2017 in captivity in Bangladesh.

Nest no.	Nest depth (cm)	Nest diam. (cm)	Clutch size	Egg weight (g)		Egg length (mm)		Egg diameter (mm)	
				Range	Mean± SD	Range	Mean ± SD	Range	Mean ± SD
First	28	46	21	75.15-84.2	78.99±2.05	67.9-78.59	71.29±2.44	43.29-44.86	44.17±0.37
Second	31	50	28	67.9-77.5	71.32±2.06	68.65-76.76	73.79±2.05	38.57-42.13	40.97±0.68
Third	25	42	18	64-78	69.44±3.27	56-76	67.88±4.45	39-44	41.05±1.16

The relationship between clutch size and nest diameter was significantly correlated (r = 0.974, t = - 6.30, df = 4, p = 0.03) and insignificantly correlated with nest depth (r = 0.974, t = -1.65, df = 4, p = 0.17). The greater the clutch sizes the deeper and wider the nest was. Simple linear regression also showed the positive relationship between clutch size and nest diameter (Y = 29.038 + 0.7595 × egg weight, R^2 = 0.949) and the nest depth (Y = 15.278 + 0.5696 × egg weight, R^2 = 0.949) (Fig. 1).

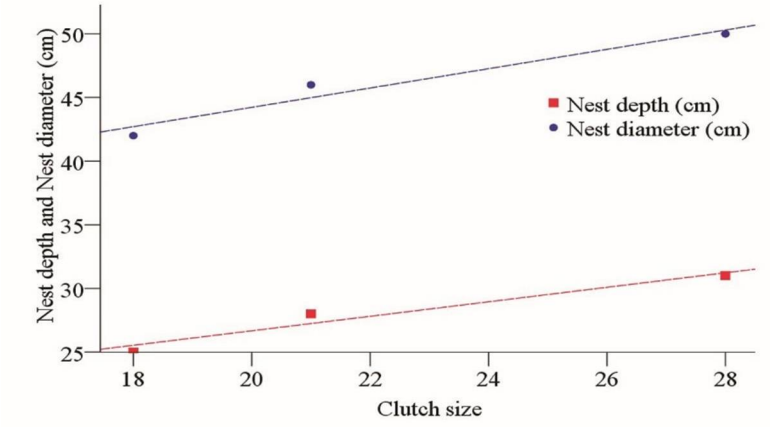

Fig. 1. Clutch size in relation to the depth and diameter of the nest of *Batagur baska.*

Our observed clutch size was almost similar with the clutch size 10 - 30 in captive population of *B. baska* of Bangladesh[6,7,24]. Weissenbacher *et al.*[6] also recorded clutch size of 21 - 24 eggs at the Vienna Zoo, Austria. The number of observed eggs in a clutch was

larger than the average 50 eggs in a clutch reported by Daniel[25] while Duli[22] stated 26 eggs, and Moll[27] described the clutch size 19 - 48 eggs. Variation in egg size and shape may be associated with clutch size and female anatomy[28,29].

Shape, colour and measurements of eggs: Eggs shape of *Batagur baska* was elliptical with an average EL and ED ratio 1.68. The shell was delicate and fine with whitish in colour. The adult females had mean weight of 20.29 kg, CL of 477.5 mm, CW of 396.2 mm and H of 218.7 mm. The weight, length and diameter of individual eggs was 73.21 ± 4.66 g (range 64 - 84.2 g, n = 67), 71.42 ± 3.80 mm (range 56-78.59 mm) and 41.99 ± 1.66 mm (range 38.57 - 44.86 mm), respectively. The size of the egg (EL) was not related with the size of breeding females (female CL) (r = 0.143). We also found variation of weight, length and diameter of eggs in each clutch of three nests (Table 1). The relationship between the eggs weight and ED was positively significantly correlated (r = 0.978, n = 67, t = 10.043, df = 4, p = 0.01). There was no relationship between the egg weight and EL (r = 0.272, t = 0.668, df = 4, p = 0.540). The relationship between egg weight and diameter maintained linearity. Simple linear regression showed the positive relationship between egg weight and ED (Y= 16.215 + 0.353 × egg weight, R^2 = 0.957). There was no relation between egg weight and EL (Y = 59.30 + 0.160 × egg weight, R^2 = 0.074) (Fig. 2). Variation of EL and ED of *B. baska* was observed due to the different sized egg in nests. For instance, *B. baska* had EL of 68-75 mm, ED of 39 - 45 mm with mean weight 69 - 80 g[24,27,30]. These previous studies support our findings.

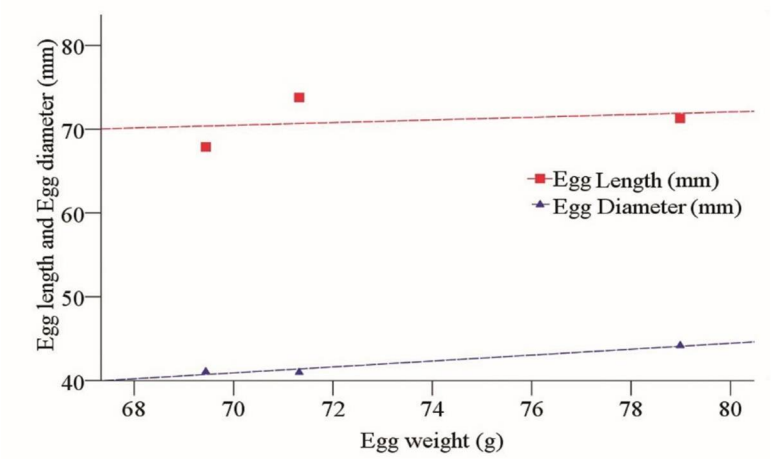

Fig. 2. Relationship between egg diameter and egg length with egg weight *Batagur baska*.

Incubation and temperature of nests: The mean incubation period was 68.26 ± 5.23 days (range 57 - 80 days). The variation of incubation period for first clutch was 75.63 ± 1.46 days (range 74 - 80 days), 65.42 ± 0.85 days (range 65 - 68 days), and 64.12 ± 2.15 days (range 57 - 65 days) for second and third clutch, respectively. Egg incubation period of *B.*

baska was previously documented 57 - 74 days in Bangladesh[7]. Maxwell[24] also recorded that the average egg incubation period was 70 days while Weissenbacher *et al.*[6] reported 59 - 77 days for this species in the same country. The number of days required for hatching revealed the similar result with our study. The mean temperature of three boxes was 29.73 ± 1.88°C (range 23.97 - 32.7°C). The temperature of first clutch was 29.04 ± 2.2°C (range 23.97 - 32°C), second clutch 29.66 ± 1.55 °C (range 24.5 - 32.04°C), and third clutch 30.55 ± 1.45°C (range 25.53 - 32.7°C). We found high temperature shortened incubation periods. Eggs of the first clutch took longer period with minimum average temperature of 29.04°C. We found significant negative correlation between the incubation period with the temperature (r = - 0.866), clutch size (r = - 0.123), and hatching success (r = - 0.011) (Table 2). The relation between temperature and hatching success was also negative, and significant (r = - 0.490, t = -49.41, df = 4, p = 0.000). We also performed simple linear regression between the incubation with the temperature, clutch size, and hatching success (Table 2, Fig. 3). Variation in temperature might influence the egg incubation of *B. baska*[17,31]. We found the mean incubation temperature 29.73°C, which corroborated the previous recorded temperature 23 - 33°C[7,31].

Table 2. Relationship between incubation periods with other reproductive parameters of *Batagur baska* from May 2016 to June 2017 in captivity in Bangladesh.

	Parameters	r value	t- statistics (calculated)	df	p value	Simple linear regression	
						R^2	Regression equation
Incubation period	Temperature	-0.866	10.541	4	0.000	0.75	36.883 - 0.104 × incubation period
	Clutch size	-0.123	9.814	4	0.001	0.015	29.201 - 0.100 × incubation period
	Hatching success	-0.011	-5.855	4	0.004	0	90.984 - 0.004 × incubation period

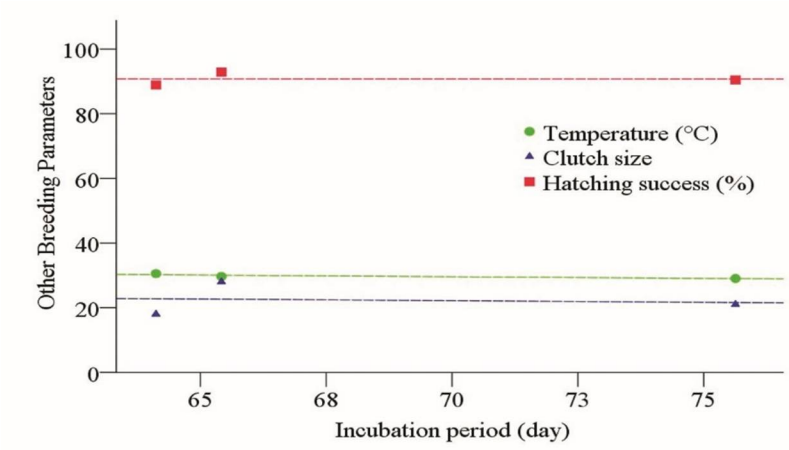

Fig. 3. Relationship between incubation periods with other breeding parameters *Batagur baska*.

Hatching time, hatching and breeding success: Hatching started in May and ended in June, 2017. The eggs of both first and second clutches took four days to hatch completely. When hatching started, all the eggs of the third clutch completed hatching within 9 days. A total of 61 healthy hatchlings emerged out of 67 eggs from three artificial boxes, and hatching success was 91%. Two eggs from each box totaling six among the 67 eggs were not developed for unknown reason. Hatching success was 19 (90.48%) for first clutch, 26 (92.86%) and 16 (88.89%) for second and third clutch, respectively (Table 3). Hatchlings hatched out independently to the sand surface after breaking egg shell from the boxes. We did not find death of any hatchlings during our study period.

Table 3. Clutch size, hatching time and hatching success rate of *Batagur baska* from May 2016 to June 2017 in captivity in Bangladesh.

Nest no.	Clutch size	Mean incubation period (day)	Mean temp. (°C)	Hatching time	Hatchlings number	Hatching success (%)
First	21	75.63	29.04	25-28 May 2017	19	90.48
Second	28	65.42	29.66	23-26 May 2017	26	92.86
Third	18	64.12	30.55	28 May-05 June 2017	16	88.89

The hatching success was 91% in the present study higher than 40% reported by Sanyal and Seth[26] and matched accurately with previous hatching success 91%[7]. Congenial environment with constant nursing in the project site might contributed to the higher hatching success[7]. Furthermore, the eggs were kept in the plastic boxes, which provided strong protections from the predators especially red ants, *Varanus* spp. and *Herpestes* spp.[6], consequently contributed to the higher hatching success.

Hatchlings growth measurement: Weight, CL, CW, PL and H of all hatchlings were measured after hatching out from the nests. Hatchlings weight was 49.16 ± 1.38 g (range 48 - 52 g, n = 61), CL was 62.61 ± 0.98 mm (range 61 - 64 mm), CW 59.79 ± 1.75 mm (range 57 - 62 mm), PL 58.05 ± 1.18 mm (range 57 - 61 mm), and H was 27.21 ± 1.23 mm (range 24 - 29 mm) for first box; whereas, the second box had hatchlings weight 46.54 ± 1.65 g (range 44 - 50 g), CL 62 ± 1.17 mm (range 60 - 64 mm), CW 56.77 ± 3.28 mm (range 49 - 65 mm), PL 57.42 ± 1.24 mm (range 56 - 60 mm); and H 27.38 ± 1.17 mm (range 25 - 30 mm); in the third box hatchlings weight was 43.88 ± 1.54 g (range 40 - 46 g), CL 56.19 ± 4.44 mm (range 51-63 mm), CW 54.31 ± 4.19 mm (range 47 - 62 mm), PL 54.31 ± 4.19 mm (range 39 - 57 mm), and H was 26.19 ± 0.75 mm (range 25 - 27 mm).

When the hatchlings weight, CL, CW, PL and H were examined via regression in relation to EL, there were positive relationship for CL (R^2 = 0.999), PL (R^2 = 0.686) and H (R^2 = 0.910). No relationship was observed between hatchlings weight and CW (Table 4, Fig. 4). Among the growth measurements, only CL showed significant relationship (p = 0.023) with EL (Table 4).

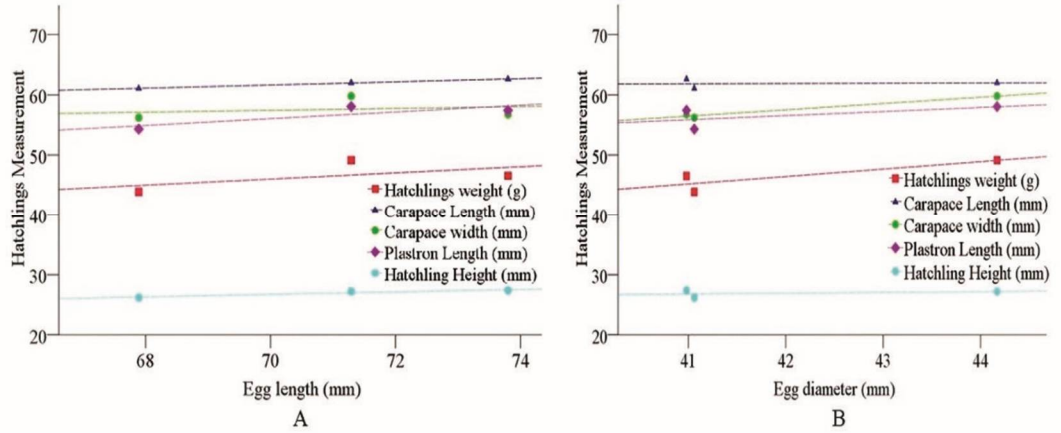

Fig. 4. Hatchlings growth in relation to egg size. (A) Egg length and (B) Egg diameter of *Batagur baska*.

Table 4. Regression analysis shows the relationship between EL and ED with hatchlings growth measurements *Batagur baska* from May 2016 to June 2017 in captivity in Bangladesh.

	Hatchlings growth	Simple linear regression		p value
		R^2	Regression equation	
Egg length	Weight	0.333	10.085 + 0.513 × egg length	0.609
	CL	0.999	43.213 + 0.263 × egg length	0.023
	CW	0.055	46.707 + 0.153 × egg length	0.849
	PL	0.686	16.900 + 0.559 × egg length	0.379
	H	0.910	12.230 + 0.207 × egg length	0.194
Egg diameter	Weight	0.727	-5.526 + 1.237 × egg diameter	0.350
	CL	0.010	60.082 + 0.043 × egg diameter	0.936
	CW	0.971	13.545 + 1.047 × egg diameter	0.110
	PL	0.376	28.216 + 0.675 × egg diameter	0.580
	H	0.130	21.556 + 0.128 × egg diameter	0.765

We also performed regression to assess the weight, CL, CW, PL, and H of hatchlings with ED (Fig. 4). We found positive relation only for hatchlings weight (R^2 = 0.727) and CW (R^2 = 0.971). The relationship was not significant for all growth measurements of hatchlings in relation to ED (Table 4). The weight of the eggs showed strong and positive relationship (r = 0.94, t = 8.11, df = 4, p = 0.001) with hatchlings weight. We found that heavier and larger hatchlings came out from heavier and larger eggs[7]. When egg size was small, the hatchlings size was also shortened and became lighter in weight. Sanyal and Seth[26] reported that smaller eggs produced lighter hatchling of 45 g which is almost similar to hatchlings weight of 49.16 g with the present study.

In conclusion, northern River Terrapin is considered as ecologically extinct species due to very low number of individual in Bangladesh[5,6]. It is unable to survive without stringent *in situ* and *ex situ* conservation efforts. The results of our study summarize breeding season and reproductive behaviour like nest building, egg laying, and incubation; which can be used as a protocol for wide ranging captive breeding and rearing activities of threatened chelonians elsewhere in the world. Furthermore, we found higher hatching success, and healthy hatchling in our study. This information might be helpful and provide a hope to reintroduce this species in nature. Considering their threatened status, we emphasize more researches on various ecological and conservation aspects of this species across sits distribution range.

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