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Conservation breeding of the Northern river terrapin *Batagur baska* at the Vienna Zoo, Austria, and in Bangladesh

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Wild populations of the Northern river terrapin *Batagur baska* have been decimated to such an extent that the species can be considered as ecologically extinct. Harvesting and habitat reduction are the main reasons for the drastic demise of *B. baska*, which formerly inhabited rivers and estuaries in East India, Bangladesh and Myanmar. A cooperative *in situ* and *ex situ* conservation project was established to secure the survival of this large river terrapin. In 2010, at Vienna Zoo, Austria, the first two captive-bred juveniles of the project hatched and presented an opportunity to call attention to this Critically Endangered species. With combined efforts a breeding population has been assembled in Bangladesh's Bhawal National Park and 84 juveniles have been reared in the past 2 years. Project-Batagur demonstrates how zoos can play a key role in sustainable long-term conservation of threatened species.

Key-words: breeding; conservation; *ex situ*; Geoemydidae; *in situ*; northern river terrapin; Project-Batagur.

INTRODUCTION

On 3 November 2013, as occurs annually, over 50 000 turtles were slaughtered in the streets of Dhaka's market and their meat was sold to worshippers of the Hindu Goddess Kali. During the Kali Puja festival it is a tradition to eat turtle meat. Although many of the turtles are classified as threatened, according to the International Union for Conservation of Nature (IUCN) Red List, and feature on Schedule 1 of the Indian Wild Life (Protection) Act 1972 (amended to 2013) and the Bangladesh Wildlife (Conservation and Security) Act 2012, the slaughter is often

overlooked by the authorities in the name of religion. Appraisals of the market trade, including estimates of the number of slaughters carried out per trader and the number of bags of turtles brought to the market, were monitored by R. Ghosh and A. G. J. Morshed during the festival in 2013, and account for at least 7000 individuals each of Indian roofed turtle *Pangshura tecta* [Least Concern (LC): IUCN, 2014] and Indian flapshell turtle *Lissemys punctata* (LC; IUCN, 2014), as well as 1000 Indian eyed turtle *Morenia petersi* [Vulnerable (VU): IUCN, 2014], and 9000 individuals each of Indian peacock softshell turtle *Nilssonina hurum* (VU: IUCN, 2014) and Indian softshell turtle *Nilssonina gangetica* (VU: IUCN, 2014). The Crowned river turtle *Hardella thurjii* (VU: IUCN, 2014) and the Northern river terrapin *Batagur baska* [Critically Endangered (CR): IUCN, 2014] are no exception to this annual cull during the Hindu festival and because of their rarity the market price per kilogram dramatically increases.

Wild populations of the Northern river terrapin have been decimated to such an extent that the species can be considered as ecologically extinct and unable to survive without major *in situ* and *ex situ* conservation efforts. The dramatic demise is the result of over exploitation of the flesh and eggs of the terrapin, as well as large-scale export to China, and habitat destruction and alterations (Moll,

D., & Moll, 2004; Kalyar & Thirakhupt, 2007; Platt *et al.*, 2008). The extant population of *B. baska* is assumed to be extremely small. Unless a previously unknown viable population is discovered, it is deemed fundamental that the remaining wild individuals are captured to create a breeding group. Dealing with such a small number of animals, an *ex situ* breeding programme, established with the last remaining specimens, will probably provide the only opportunity to ensure the survival of the species into the future (Magin *et al.*, 1994; Snyder *et al.*, 1996).

In a recovery programme for the Endangered Burmese roofed turtle *Batagur trivittata*, which was established at the Mandalay Zoo in Myanmar, different conservation methods were employed. Nests and egg-deposition sites of a small remnant population of *B. trivittata* were protected along the upper Chindwin River. The combination of nest monitoring of the wild population and local head-starting of hatchlings has led to a steadily increasing number of Burmese roofed turtle individuals (Kuchling *et al.*, 2006; Platt *et al.*, 2013).

In the last few decades the role of zoos has dramatically changed from mere animal exhibitors to educational facilities with an emphasis on captive-breeding programmes (Lee *et al.*, 2006; McFadden *et al.*, 2008; Browne *et al.*, 2011). In recent years, the focus broadened further by strongly encouraging on-site scientific research via staff and collaborations with universities, and by leading the way to *in situ* field conservation. Collaboration efforts and a fast response in conservation initiatives have previously yielded positive results in species preservation (Gagliardo *et al.*, 2008). Specialized efforts for *ex situ* conservation and reintroduction are under way for several taxa. In particular, the worldwide collapse of amphibian populations triggered several zoo and non-zoo based breeding programmes (Conway, 2011). However, the long-term sustainability of the populations of wild animals managed in zoos, especially in regard to genetics, demography and the carrying

capacity for threatened species, was called into question in a recent study focusing on the viability of the collections of World Association of Zoos and Aquariums (WAZA) member institutions (Lees & Wilcken, 2009). To respond to the global extinction crisis and to fulfil the conservation potential of zoos a combination of wild and zoo population management and other *in situ* efforts have to be considered to sustain threatened species (Conway, 1995; Conde *et al.*, 2013).

Collective information about reptilian diversity has not been readily available for a long time. However, the first global analysis of extinction risk in reptiles was recently reported by Böhm *et al.* (2013), highlighting habitat loss and harvesting as the major threats to reptile species. Turtles, considered as target harvesting species, represent the largest portion of the 87% of freshwater and marine reptiles threatened by biological resource use and, overall, the Geoemydidae constitute the most threatened (88%) reptile family (Böhm *et al.*, 2013).

It was not only the alarming trend of biodiversity loss in reptile species but also an opportunistic and collaborative approach that substantially contributed to the development of Project-Batagur. This study reports on the cooperative initiative of international and local experts rounding up the last few surviving specimens of the Northern river terrapin to establish a sustainable *in situ* breeding programme and preserve the species for future generations.

TARGET SPECIES

The Northern river terrapin (family Geoemydidae) is a large river terrapin with a carapace length up to 59 cm. The distribution of the species is restricted to regions from coastal north-east India and adjacent Bangladesh to the Ayeyarwady and Bago estuaries in Myanmar (Praschag *et al.*, 2007, 2008). Formerly known as a single species, *B. baska* actually comprises at least two genetically distinct species (Praschag *et al.*, 2007). Populations of river terrapins occurring in South East Asia, which are usually treated as



Plate 1. Male Northern river terrapin *Batagur baska* in breeding coloration. Peter Praschag, Turtle Island, Graz.

conspecifics, are now considered to be the Southern river terrapin *Batagur affinis*, a distinct but closely related species. Apart from genetic differences, *B. baska* has a narrower and more pointed snout than *B. affinis*, and the male breeding coloration differs (Moll, E. O., *et al.*, 2009; Praschag *et al.*, 2009). *Batagur baska* males display a black coloration on the head and ventral neck, and bright red–orange on the dorsum of the neck during the breeding period (Moll, E. O., *et al.*, 2009) (Plate 1).

The distribution of *B. baska* is confined to brackish water. Individuals inhabit mangrove belts, estuaries and inshore beds of marine vegetation. Not even 100 years ago the Northern river terrapin could be encountered in high population densities in rivers and estuaries in East India, Bangladesh and Myanmar. Hundreds of females could be

observed sun basking along the rivers (Maxwell, 1911). Harvesting of eggs in large numbers (Moll, D., & Moll, 2004) and habitat degradation, caused by the building of dams and depletion of sand, led to *B. baska* reaching fourth place in *Turtles in Trouble: The World's 25+ Most Endangered Tortoises and Freshwater Turtles* (Rhodin *et al.*, 2011). In Bangladesh, nesting beaches were reported in coastal areas in the southern part of the Sundarbans (Whitaker, 1983); however, a survey carried out in 1990 was unable to confirm the occurrence of *B. baska* along the suggested sites (Moll, E. O., 1990). In Myanmar, *B. baska* is considered regionally extinct and surveys conducted in 2004 verified the occurrence of small populations of *B. baska* at scattered localities in Mon State, and Tanintharyi and Ayeyarwady Divisions (Platt *et al.*, 2008).

At the time of writing the worldwide known *B. baska* population comprises fewer than ten breeding females. Twelve individuals [7.5 (♂♂.♀♀)] are maintained at the Sajanakhali Interpretation Center in the Sundarbans Tiger Reserve, India, one of which was rescued in April 2013. Two adult females originating from Howra market, India, collected in the 1980s were kept without a male at the Madras Crocodile Bank Trust (MCBT) in Tamil Nadu, India. In spite of a series of attempts to legally transfer one male from West Bengal to the females, so far permission has not been obtained. Consequently the Vienna Zoo, Austria, donated a male to the MCBT in March 2014. At the time of writing, Vienna Zoo houses 1.1 plus two juveniles, while the private conservation centre Turtle Island in Graz, Austria, has another 1.2 Northern river terrapins (P. and R. Praschag, pers. comm.).

EX SITU BREEDING VIENNA ZOO

In March 2010, P. and R. Praschag transferred 2.2 individuals from their collection in Graz to Vienna Zoo. Later, one female was returned to the conservation centre in Graz. Upon arrival at Vienna Zoo a series of veterinary and radiographic tests were

	WEIGHT (kg)	CARAPACE			PLASTRON LENGTH (cm)
		LENGTH (cm)	WIDTH (cm)	HEIGHT (cm)	
Female 1	17.89	48.4	39.5	20.1	44.1
Female 2	23.97	54.1	44	22.6	50.6
Male 1	10.4	41	32.8	16.3	35.3
Male 2	9.3	39.2	31.6	15.1	34.2
Juvenile 1	0.046	5.9	2.7	5.7	5.6
Juvenile 2	0.045	5.9	2.8	5.7	5.6

Table 1. Measurements of Northern river terrapins *Batagur baska* at the Vienna Zoo, Austria. The males and females are adults, while juveniles were measured one day after hatching in May 2010.

carried out showing that both females were gravid and had mature eggs. There was no detailed knowledge about breeding in *B. baska* at that point so information about a related species, *B. affinis* (Miller *et al.*, 2004), was used as the baseline for trials with different temperatures and humidity levels. Both females were observed digging for several nights but no eggs were laid. In a further X-ray the eggs were shown to have already moved towards the back and the decision was made to induce egg deposition hormonally. Veterinarians treated the females with oxytocin $10 \mu \text{kg}^{-1}$ and prostaglandin 1.0mg kg^{-1} , and the terrapins were placed in a tank with a gridiron base to protect any eggs laid from damage. The smaller female (Female 1: Table 1) laid 21 eggs with a mean length of 68 mm, mean width of 40 mm and mean weight of 66 g. The eggs were incubated at three different temperatures (29.5°C , 31.5°C and 33.5°C) although it was suspected that they were unfertilized as no embryonic disk was visible at the point of removal. The second female (Female 2: Table 1) laid 24 eggs with a mean length of 74 mm, mean width of 43 mm and mean weight of 77 g. All eggs except three had a visible embryonic disks and these were incubated at 29.5°C , 31.5°C or 33.5°C and a humidity of $>92\%$. In these early stages, incubation was a critical period. It was difficult to ensure the survival of the embryos because no information was available pertaining to the most appropriate moisture and humidity levels for the incubation substrate. The eggs were put onto a Ver-

miculite substrate, a high-absorption material that binds up to four times of its net weight. However, the eggs also absorbed water creating a counter pressure and sank into the substrate, which in turn led to a decrease in respiratory surface and a collapse of the oxygen supply. When it was noticed that the eggs were covered with a film of water and were caved in at the bottom, they were immediately moved onto a coco peat substrate.

In May 2010, 62 and 63 days after egg deposition, two individuals hatched from Female 2's clutch incubated at 29.5°C (Table 1). This was the first successful breeding of *B. baska* in captivity worldwide.

The official announcement of the breeding success of a highly threatened species at Vienna Zoo received extensive media coverage and the Northern river terrapin became famous overnight. In August 2010, during the annual wildlife conservation days held at Vienna Zoo, facts and figures about *B. baska* were presented to a large audience and a fundraising campaign to support the conservation of the species was started. The positive response of visitors and supporters led to Vienna Zoo extending its conservation work into a sustainable long-term engagement in *in situ* conservation and a key project to ensure the survival of *B. baska* in its natural range.

In May 2014, the only living Northern river terrapins outside of the countries of origin are the seven individuals in Austria. At the time of writing, Vienna Zoo houses 1.1 and the two juveniles hatched in 2010, and 1.2 are housed at Turtle Island.

IN SITU BREEDING BANGLADESH

Project development and implementation

The Centre for Advanced Research in Natural Resources & Management (CARINAM), a non-governmental organization in Bangladesh, the Forest Department of Bangladesh and international turtle experts had failed to locate the Northern river terrapin in the wild. After 3 years of field surveys, conducted by R. Ghosh and P. Praschag, a few individuals were discovered in fish breeding ponds. Eventually, and in close cooperation with the Turtle Survival Alliance (TSA), Fort Worth, TX, USA, 14.6 *B. baska* were acquired, all of which had been kept for many years as talismans for the breeding success of pond-fish species. The male bias was probably because males never leave the water, while females leave the ponds when looking for nesting sites and there is a local preference for harvesting females because their meat, which contains more fat, is supposed to be more flavoursome. The newly acquired individuals were placed in breeding ponds in the Bhawal National Park, a protected area close to Dhaka, managed and provided by the Forest Department in Bangladesh.

Project-Batagur is a joint initiative of the Bangladesh Forest Department, the TSA, Zoo Vienna and IUCN Bangladesh. The primary responsibilities of the Forest Department have been to provide the location for the assurance colony and issuing permits. IUCN Bangladesh is responsible for managing and administrative processes. The TSA and Zoo Vienna provide fundraising, management and expertise. The Project is approved by the Bangladesh Wildlife Advisory Board under the Ministry of Environment & Forest, Government of the People's Republic of Bangladesh. Field coordinator R. Ghosh and supervising wildlife biologist A. G. J. Morshed are responsible for on-site maintenance and monitoring.

Breeding programme

In December 2010, a breeding group was separated into an additional pond with a

newly established deeper-reaching sand beach and a fence to ensure egg deposition on the beach and protect the terrapins from predators. Effective fencing is of particular importance as predators, such as monitor lizards *Varanus* spp and mongoose *Herpestes* spp, hunt for the eggs of terrapins. The breeding group comprised five females and only two males to minimize any stress for the females.

In spring 2012, for the first time, all females nested (Table 2). The number of eggs found in a clutch ranged from 11 to 26 with a total 92 eggs laid. There was a flood risk for one nest containing 15 eggs and this was removed from the sand beach. Eight of these eggs were relocated to a different position on the beach and seven eggs were placed in a tank filled with sand and covered with protective mesh. The other four nests remained on the sandy beach and were covered by mesh to prevent predation. Nest temperatures recorded with a digital probe remained constant at around 27–30°C, whereas outside temperature varied greatly between 28°C and 41°C in the month of May.

A total of 26 juveniles hatched after a period of 59–77 days. Three juveniles died within the first 3 months but the other 23 (25% of the eggs laid) were reared successfully in separated nursing tanks. These tanks were maintained at a mean temperature of 30.1°C (SD ± 1.3°C; range 27.1–31.7°C) and a mean pH of 10.5 (SD ± 0.3; range 9.8–11.7).

The diet offered to the juvenile Northern river terrapins at the beginning mainly consisted of water cress, and in the second month after hatching small shrimp, fish and fruit (bananas and jack fruit) were offered on a daily basis.

In 2013, the brief nesting season started in April, similar to the year before. Within one week all five females had nested. Clutch size ranged from 14 to 24 eggs per nest, with a total 99 eggs laid on the beach (Table 2). In contrast to the previous year, on examination 11 broken eggs and three infertile eggs were found. Low nesting temperatures were regarded as one of the problems of the previous breeding season so the nests in 2013 were

NEST	2012							2013							TOTAL
	1	2	3	4	5	TOTAL	1	2	3	4	5	TOTAL			
Laying date	22 Mar	30 Mar	4 Apr	10 Apr	15 Apr		21 Mar	23 Mar	23 Mar	24 Mar	26 Mar				
Eggs found	26	16	15	24	11	92	19	22	14	24	20	99			
Eggs broken	-	-	-	-	-	-	1	-	8	-	2	11			
Not hatched	25	5	3	22	11	66	-	-	4	2	18	24			
Infertile eggs	-	-	-	-	-	-	2	1	-	-	-	3			
Hatchlings	1	11	12	2	-	26	16	21	2	22	-	61			
Hatching days	77	70	64-74	59-65	60		63	64	65	62	-				

Table 2. Record of *in situ* breeding of Northern river terrapin *Batagur baska* in two consecutive years in Bhawal National Park, Bangladesh, as part of the collaborative conservation-breeding programme of Project-Batagur.

slightly rearranged. The eggs were transferred into plastic containers inside a metal mesh hatchery at a higher level on the same beach. To attain warmer nesting temperatures the surrounding trees were trimmed and one nest was periodically covered with glass to generate additional heating. The mean temperature in the nests reached 29.3°C (SD ± 1.2°C); however, because of the elevated clutch positions the eggs were submitted to a wider temperature range of between 26.1°C and 33.4°C (disregarding nest 5, which had no hatchlings). The strategy proved to be effective when 61 individuals (62% of the eggs laid) hatched after 62–65 days. To date, 84 juveniles from two breeding seasons are still maintained. Immediately after hatching individuals from the 2012 breeding season ($n = 23$) had a mean weight of 40 g and mean carapace length, width and height of 60 mm, 56 mm and 26 mm, respectively. The weight of the hatchlings more than doubled within the first month. Enhanced growth was also observed in months 11–16 (Fig. 1). Comparisons between hatchling size and weight for the 2012 and 2013 generations showed that the 2013 individuals at the age of 3 months (Mann–Whitney $U = 55$; $P < 0.001$) and 4 months (Mann–Whitney $U = 25$; $P < 0.001$) weighed more than the hatchlings from the previous year (Fig. 2).

During a certain period in 2013 all juveniles [both those from 2012 (11–12 months of age) and those from 2013 (3–4 months of age)] grew rapidly. The enhanced growth could have been the result of the higher food supply offered to the hatchlings during this period. The caretaker adjusted the amount of food offered according to the size and abundance of juveniles, and thereby offered more food when there were more young terrapins to feed. Once the enhanced growth of juveniles was noticed the food schedule was immediately modified and no food was given every fourth day, to avoid problems with bone calcification resulting from rapid growth rates.

The key question that remains to be answered is whether females can be bred and

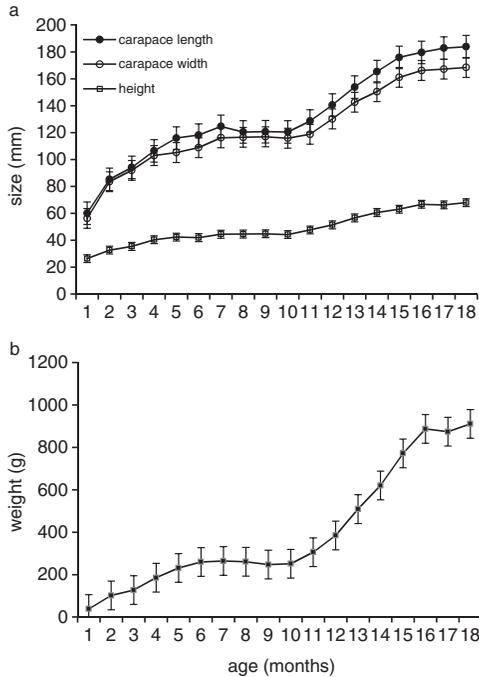


Fig. 1. Northern river terrapin *Batagur baska* (a) sizes and (b) weight of individuals hatched in 2012 in Bangladesh. Dots represent mean carapace length, width, height and weight \pm SD over a measurement period of 18 consecutive months.

raised, and this will not be known until the gender of the hatchlings can be established.

CONCLUSION

The key role of zoos in the conservation of threatened species is undisputed and captive-breeding programmes have prevented the extinction of species of several taxa (Stüwe & Nievergelt, 1991; Butchart *et al.*, 2006; Hedrick & Fredrickson, 2008; Conde *et al.*, 2011a). *In situ* breeding programmes have improved the conservation status of threatened species (Hutchins & Conway, 1995; Conde *et al.*, 2011a), nevertheless the major goal of any species-conservation project is to establish, or re-establish if necessary, threatened species in their natural habitat. Project-Batagur emphasizes a first step by which zoos can contribute to seminal field-

conservation efforts. Institutional members of WAZA are the third largest contributors to field-conservation projects worldwide (Conde *et al.*, 2011b). Zoological facilities are fundamental to such projects, not only as fundraisers but also by making the experience of staff available, especially successful breeding techniques that can be key factors in *in situ* conservation programmes. The knowledge gained from zoo-based research is particularly important, especially for species for which data about the natural population is limited because of their threatened status, and can contribute significantly to the survival of offspring hatched or born in the natural habitat.

The achievements of Project-Batagur are best reflected by the 84 juveniles hatched and reared in the past 2 years. An environment of secure on-site reproduction and survival of offspring has been successfully established. At this point, however, it is regarded as irresponsible to simply release these turtles into the wild. Several further steps have to be taken to lead the way for '*Batagur* going home'.

The sensational discovery of a small wild population of *B. baska* in the Bangladeshi Sundarbans in 2013 makes it possible that a few adults can be captured in the wild and followed using satellite telemetry to learn about their natural history. Females and hatchlings are sometimes found by shrimpers along the shores of the mangrove forest in the Sundarbans. Sightings of males remain rare, probably because of their strictly aquatic life and a flight distance of at least 500 m. Without clarification about the reproduction biology, nesting locations and overall natural history of the Northern river terrapin, the release of hatchlings seems inappropriate. Several biological and habitat constraints have to be considered before repatriation of the species can be contemplated (reviewed in Dodd & Seigel, 1991). The preferred approach is a combination of *in situ* conservation techniques (i.e. hatcheries, captive breeding and head-starting) coupled with protected areas to maintain the wild population and its habitat (Praschag *et al.*, 2008).

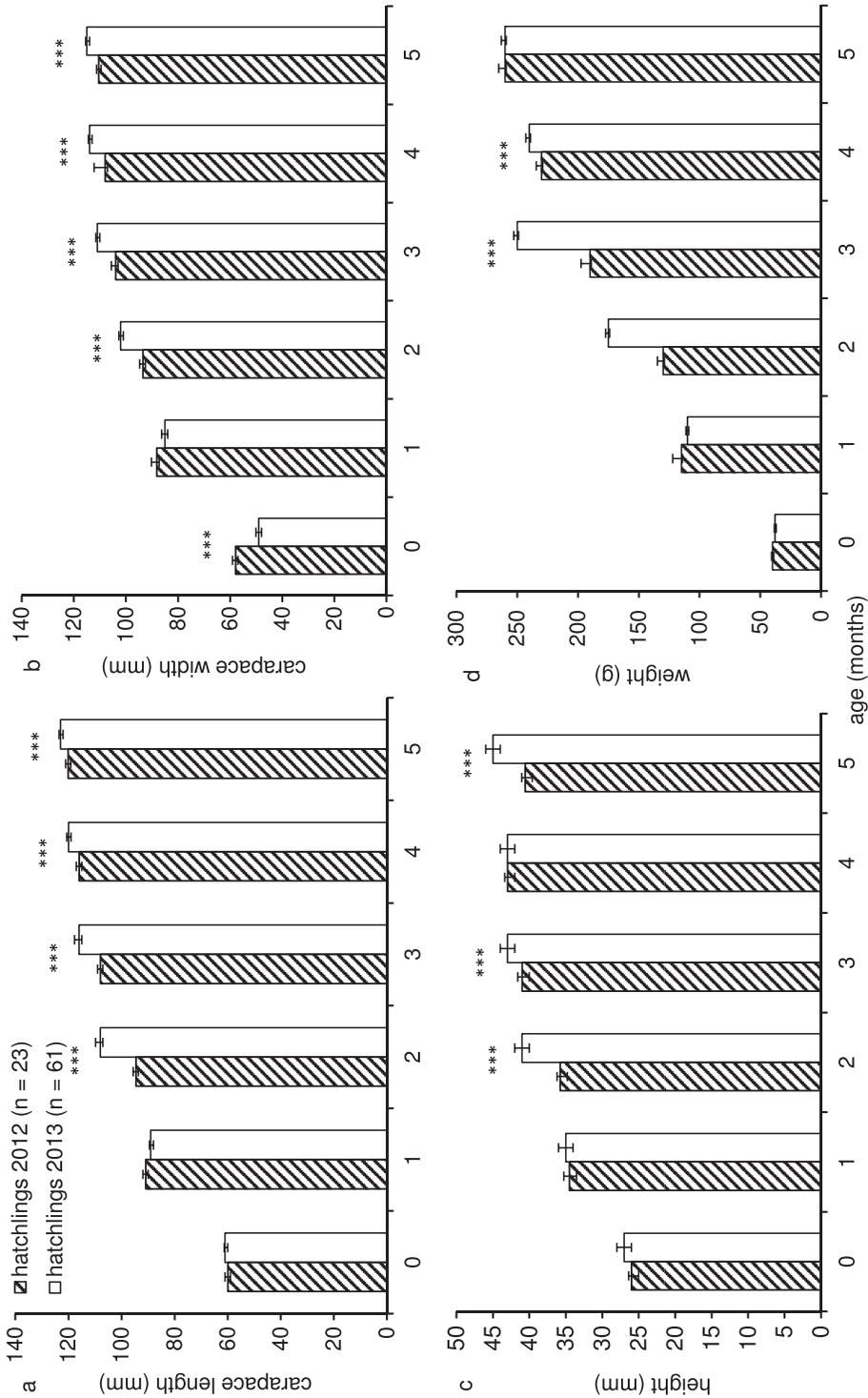


Fig. 2. Comparison of (a) carapace length, (b) width, (c) height and (d) weight between Northern river terrapin *Batagur baska* hatchlings from 2012 and 2013: *** $P < 0.001$, statistically significant differences between age classes (Mann–Whitney U -test).

Unlike the *B. trivittata* recovery programme in Myanmar, where nesting sites are protected and monitored along the Chindwin River, and juveniles are collected and reared (head-started) until they are old enough for release (Platt *et al.*, 2013), wild populations of *B. baska* cannot be protected in a similar way because no information about active nesting beaches has arisen from surveys in the only seasonally accessible Sundarbans. All information about *B. baska* is obtained from fishermen and this suggests scattered off-coast nesting on small islands. Juveniles that travel upstream from possible off-coast beaches are caught by the wide-area nets of fishing crafts or the local fishermen. The Shan villagers in Myanmar do not harvest adult *B. trivittata* (Kuchling *et al.*, 2006), which helps conservation of the species. In Bangladesh, however, adults, juveniles and eggs of *B. baska* are considered delicacies and, therefore, are subject to high harvesting pressure as they are collected and sold.

The future aims of the current *B. baska* Project are (1) to establish a second facility further south amidst the distribution area of *B. baska*, and (2) to establish a studbook based on genetic data including all available specimens in order to avoid a genetic bottleneck and document defined breeding lines. In general a minimum effective population size (reproducing specimens) of 25.50 ($\delta\delta$ \cdot ff) is suggested but experience shows that vertebrate species with small population sizes that include effectively reproducing individuals of even fewer than ten females can contribute to the overall security of the species (Yamada & Kimura, 1984; Bell *et al.*, 2010; Shoemaker *et al.*, 2013). A carefully considered breeding strategy (using the newly developed studbook) and a quickly increasing population size are essential.

To operate a pedigree breeding programme permits were obtained for collecting tissue and/or blood samples from *B. baska* out of Bangladesh and first analyses will be carried out in 2014. Additionally the pond and the adjacent beach at the current location in Bhawal National Park will be modified to

provide nesting areas for separated breeding lines.

However, the viability of a population of the given size is strongly dependant on the interaction between the environmental context and life history, and the threats facing certain species (Flather *et al.*, 2011). Population size alone is no guarantee against extinction. Treating the mechanisms behind the decline of the species in its natural habitat remains important but admittedly rests at the disposition of local governing authorities. Therefore, zoological facilities can only assist, promote and support these conservation processes.

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