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Captive breeding and ex-situ conservation of the Caucasian pit viper *Gloydius caucasicus*

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ABSTRACT - The Caucasian pit viper *Gloydius caucasicus* is one of six snake species that is frequently reported as a cause of venomous snakebite in Iran. We present the results of successful captive breeding of 20 Caucasian pit vipers (10 males and 10 females) collected in August 2018 from the Lar National Park, northern Iran. Mating and copulatory behaviours were observed from mid-June to early July 2019. Five of the ten females gave birth with parturition occurring from 10 to 13 September, when 17 vipers were born in litter sizes ranging from 2 to 5. The present captive breeding programme has been successful and shows potential for both venom production and support for conservation by reducing the demand for wild caught specimens.

INTRODUCTION

In the Middle East, vipers are a significant cause of snakebite, as well as of importance to medical research and development, but they have been relatively little studied (Stümpel & Joger, 2009; Rima et al., 2018). One approach to securing a ready supply of vipers for the extraction of venom for therapeutic antibody production and pharmaceutical purposes is captive breeding; this also has the advantage of reducing the demand for the collection of vipers from the wild (Újvári et al., 2002).

The only pit viper species occurring in Iran is the Caucasian pit viper *Gloydius caucasicus* (Nikolsky, 1916) (Asadi et al., 2019). This is found in montane habitats at altitudes up to 3,000 m a.s.l. and belongs to the *Gloydius halys*/*Gloydius intermedius* species complex, a group of closely related vipers in the subfamily Crotalinae (Alencar et al., 2016). The Caucasian pit viper is a viviparous, diurnal snake distributed from the northwest of Afghanistan and southern Turkmenistan to Azerbaijan and northern parts of Iran (Rastegar et al., 2008; Khani et al., 2017; Asadi et al., 2019; Uetz et al., 2022). In Iran, the species is distributed along the Alborz Mountains, from the Hezar Masjed Mountains in Khorasan Razavi province to the western regions of Gilan province (Mozaffari et al., 2016).

The Caucasian pit viper is threatened by several anthropogenic impacts that include habitat loss due to agricultural development, urbanisation, livestock overgrazing, indiscriminate and irrational killing by local people, and most importantly, excessive collection and over exploitation by antivenom co-operatives in Iran (Rastegar-Pouyani et al., 2018; Asadi et al., 2019; Kaboli, pers. obs.). Overharvesting of this viper for antivenom production has dramatically reduced wild populations of *G. caucasicus*, especially in the Lar National Park. For instance, in 2020, out of 184 Caucasian pit vipers captured from the Lar National Park, few had reached full adult size. Each year, the co-operatives harvest many snakes directly from the wild yet remain firm in arguing that the extent of wild capture has been overstated and that, in any case, the snakes have been released back into the wild after venom extraction. This claim has gone unchallenged by the Department of Environment of Iran (DoE) for decades (Kaboli, pers. obs.) and in any case it would be expected that the survival rates of these snakes when released back into the wild would be drastically reduced (Roe et al., 2010; Harvey et al., 2014; Wolfe et al., 2018). Furthermore, such practices are not recommended as, according to the latest protocols, venom should be collected from infection-free captive bred snakes reared under controlled environmental conditions in order not to compromise the quality of the venom extracted. Manufacturers should fulfil a number of standards, including venom traceability, quality control, and animal welfare during snake milking, handling and maintenance in captivity (WHO, 2017).

To date, there is only one published research study on the captive husbandry and breeding of *G. caucasicus* that of Shakoori et al. (2015). The most fundamental requirement of a captive breeding programme is successful reproduction. However, the methods employed in that study appear to have resulted in low reproductive success and low offspring survival rates, which challenge the long-term sustainability and commercial viability of a breeding programme. Establishing a large-scale captive breeding programme for venom production or conservation breeding projects calls for a comprehensive assessment of captive breeding requirements as well as comparison of venom composition between wild and captive snakes (Leloup, 1984; Braz et al., 2012). With regard to venom composition, an investigation of the properties of venom obtained from our viper specimens at the time of capture, and subsequently, has confirmed that antivenom from the captive Caucasian pit vipers would be an effective treatment against bites from this species (Rasoulinasab et al., 2020). On that basis it has been recommended that capture of wild snakes for their venom be discontinued to reduce their risk of extinction (Rasoulinasab et al., 2020). In light of these findings, we present here data collected at the Laboratory of Herpetology of the University of Tehran that contribute towards the development of guidelines for the husbandry and captive breeding of Caucasian pit vipers.

CAPTIVE BREEDING METHODOLOGY

Collection of breeding stock from the wild

This project was authorised by the Iranian Department of Environment under license number 9061/97. In August 2018, a total of 20 Caucasian pit vipers, 10 females and 10 males, were collected from Lar National Park (central Alborz, northern Iran; Fig. 1) to be used as breeding stock for the captive breeding programme (Fig. 1). The park is located on the south-western slopes of the highest peak in Iran, Mount Damavand (5,671 m). The region is characterised by a cold, humid mountain climate marked by high precipitation, mostly in the form of snow that covers the landscape for approximately half of the year, during which time it is inaccessible to humans. During the day when temperatures rise, reptiles of this region are very active but at night when temperatures fall reptile activity decreases. Detailed records were made of geographical locality, habitat, season and date of capture. Venom was collected immediately from the snakes at the sites of capture to be stored as wild venom for future comparisons, reported by Rasoulinasab et al. (2020). The vipers were transferred to the captive breeding facility at the University of Tehran designed according to WHO guidelines. The facility comprises an administrative office, snake housing room, quarantine room, milking room, venom production and storage room, control laboratory, animal (rodent) housing room, and cleaning room (WHO, 2017).

Quarantine and disinfection

On arrival, the 20 snakes were kept for three months in the quarantine room which is located away from the maintenance and production rooms, to minimise transmission of parasites and diseases. During this period, the individuals were housed in separate boxes with good ventilation, warm and cool sides, cover objects (e.g. broken flower pots or small hollow logs), and a water dish in the cooler part of the box. Ventilation of the quarantine room itself was supplied using fans and air conditioning. On arrival, biometric measurements for all snakes including weight and other morphological characteristics were recorded. They were then all examined by an experienced veterinarian familiar with reptiles for any ectoparasites, wounds or fractures. Ectoparasites (e.g. lice, mites, and ticks), endoparasites (e.g. nematodes, protozoans, cestodes and pentastomids), and infections, if present, were treated using antiparasitic and anti-inflammatory drugs, including diluted ivermectin spray for the ectoparasites. The snakes were inspected every 15 days by the veterinarian for any unwanted symptoms. To prevent pathogen transmission, personnel footwear was treated using a disinfectant before entrance.

Design and maintenance of the routine enclosure

Each snake was housed separately in a semi-transparent plastic container (120 cm long × 60 cm wide × 50 cm high) with perforations in the sides. Clean sawdust was used as a substrate which was replaced monthly. A water dish, two hides (one in the warm and another in the cool end of the box), and a piece of stone were provided in the container. Each container was assigned a unique number on a label stating the animal's sex, feeding times, venom collection and cleaning schedules. The enclosures, the hides and the production room were cleaned and disinfected twice a week.

Temperature and humidity

The temperature regime was arranged to heat to 30–32 °C one-third of the posterior end of the box during the day using six 32-watt heating strips and cooling to 20–24 °C along one-third of the anterior end of the box at night. The average relative humidity was maintained between 30–45 % by placing a water dish in the box. When the ambient humidity dropped to 30 %, the boxes were sprayed with warm water at least twice per week, increasing humidity up to 55 %. Water should be sterilised with ultraviolet light (WHO, 2017); however, we used regular tap water.

Sexing and marking

The snakes were initially sexed using tail/bodylength ratio and later confirmed by applying gentle pressure on the tail base to evert the hemipenes.

Feeding

In the wild, Caucasian pit vipers feed on a variety of lizards, rodents and insects (Mallow et al., 2003). In captivity they

Figure 1. The Caucasian pit viper *Gloydius caucasicus* - **A.** Habitat of Lar National Park in central Alborz, northern Iran, **B.** Conducting field searches during the early hours of the day using snake tongs, **C.** The Caucasian pit vipers captured from Lar National Park in August 2018

were offered laboratory mice (10–15 g). In accordance with ethical principles and to prevent the snake from sustaining any potential rodent bite injuries and to reduce venom usage, laboratory mice *Mus musculus albinus* were euthanised with carbon dioxide (CO_2) prior to feeding. For euthanasia, a cage (50 cm long x 25 cm wide x 25 cm high) with a CO_2 flow rate of 5.6 L/min was used. We maintained CO_2 flow for 2–5 minutes to induce anesthesia and death and continued for at least one minute after observing death signs including lack of respiration and faded eye colour.

After euthanasia, the mice were stored at -20 °C for a week to eliminate any possible pathogens or parasites. After completion of an initial adaptation period in the enclosures, the vipers were fed once a week with a diet of newborn and adult mice. We used small prey at the beginning of feeding and after several acceptances of small prey graduated to larger prey. After a few hours, any uneaten or regurgitated food and faeces were removed. Details of food and feeding frequency for each individual snake were recorded on an information sheet attached to each cage and stored electronically. The captive vipers were weighed monthly to estimate their body condition. Freshwater was provided at all times and was changed every three days but if it was contaminated with faeces, it was changed immediately.

Hibernation

Prior to lowering temperatures, all food was withheld from the snakes for six weeks during which time lighting and heating were gradually reduced. The temperature was lowered by 1 °C every week and light was gradually reduced. This started in early October 2018 and lasted until the end of November until the amount of daily light reached zero and temperature had fallen to 8–10 °C. In order to reach such a temperature in the laboratory, cooling was provided by a freezer that gave temperature control through the operation of a small fan in the top of the freezer that was in operation for 5 minutes every hour to exchange the air between the inside the freezer and the room. Humidity was controlled for hibernating snakes at around 60–80 % as a decrease in humidity could lead to water loss and eventually death. To control humidity, a water dish and a clean, wet frozen cloth were placed in the box. During hibernation, water was available to snakes at all times. Also, regular visits were made to check the conditions and change the water. In late February, light and temperature were gradually increased and the snakes emerged from hibernation in the third week of March 2019. The same hibernation procedure was followed in 2020.

OBSERVATIONS ON REPRODUCTION

Pairing the snakes

To avoid outbreeding, we paired only males and females from the Lar National Park (Chanhome et al., 2001; Mitchell, 2004; WHO, 2017). In the spring of 2019, the ten breeding pairs of Caucasian pit vipers were placed together in the boxes for courtship and copulation, although the pairings were not simultaneous. To stimulate mating activity, we placed two male snakes with a female following her postovulatory slough (Siegel & Ford, 1987). Male-to-male combat behaviour was observed prior to mating. After a couple of minutes, the weaker male escaped to the corner of the box. We immediately removed the defeated snake from the box. If a female showed no interest, the remaining male was removed from her box after 3–4 days. To witness copulation, the vipers were checked on an hourly basis. After mating, the male vipers were kept with the females for about two weeks and then returned to their own boxes to reduce any stress during the period of embryonic development. Gravid female snakes are known to consume less food and to have a reduced appetite during pregnancy (Gregory et al., 1999) and so during this period we gave smaller prey to the gravid snakes to avoid any possible harm to the embryos (Osborne, 1982). The diet of the gravid vipers consisted of 2–3 baby white mice weighing less than 10 g per week.

During the last week of pregnancy, gravid vipers ceased feeding and spent their time in close contact with the heat source. In the final stages of pregnancy, we put a wet sponge in the cage to provide adequate humidity for the female viper to give birth; it also helped neonates to emerge from the amniotic sac more easily. We only used sawdust as the substrate rather than other fine particles (e.g. sand, soil etc.) because the particles could stick to the amniotic sac and make it difficult for neonate emergence and may enter the eyes and mouths of the newborn snakes (Gregory et al., 1999).

Reproduction and neonatal care

Mating and copulatory behaviours were observed from 18 June to 5 July. Copulations lasted for up to three hours and of the ten females five were impregnated successfully (Fig. 2A). From 10 to 13 September, 17 Caucasian pit vipers were born (Fig. 2B), only one of which was stillborn. They were kept separately in boxes with perforated sides (30 cm long × 10 cm wide × 10 cm high) with a water dish, a hide, and a sheet of paper as substrate. After parturition, the female moved to the darker and cooler side of the box. Upon emergence from the amniotic sac, the neonates took shelter in the warmest area of the box. The mean (±SD) litter size was 3.40 ± 1.14 (range 2–5), similar to Shakoori et al. (2015) who reported a litter size ranging from 3–5. Normally, on the first day after birth, the young slough but few had sloughed even ten days after birth. Following the first slough, the neonates refused to feed readily and were therefore stimulated to eat or gently force-fed every ten days with newborn mice weighing 2 g. We removed bones from the newborn mice and only fed the soft tissue to the neonates. We also supplemented their food with calcium and vitamins A, C, and D3. It took the neonates approximately three to four weeks to accept food (pre-killed newborn mice) readily. The neonates were not put into hibernation and we continued to feed them during the autumn and winter of 2019.

As parturition occurred in early September, the gestation period for the Caucasian pit viper can be estimated to be 70–90 days. A similar gestation period (2.5 months) for the Caucasian pit viper was reported by Shakoori et al. (2015). Of the 18 females used in their study, 14 were gravid at the time of capture but their reproductive output was poor as

Figure 2. Captive Caucasian pit vipers *Gloydius caucasicus* - **A.** A gravid female, **B.** A female (bottom left) that gave birth to three young, two of which are visible

Table 1. Length and weight measurements for the adult male and female Caucasian pit vipers in the breeding study

Sex	Number	Total length range and mean ± SD (cm)		Tail length range and mean ± SD (cm)		Weight range and mean ± SD (g)	
Male	10	40.50 – 53.70	45.67 ± 1.39	4.50 – 7.00	5.90 ± 0.21	27.31 – 61.44	39.34 ± 3.41
Female	10	41.50 – 47.70	45.42 ± 0.73	4.50 – 5.30	4.86 ± 0.14		
	5 (gravid)					51.85 – 67.84	58.41 ± 2.64
	5 (not gravid)					36.40 – 50.35	42.14 ± 3.21

Table 2. Length and weight measurements for neonate Caucasian pit vipers

Postnatal age	Total length Range and mean ± SD (mm)		Tail length range and mean ± SD (mm)		Weight range and mean ± SD (g)	
Newborn	150.00 – 219.00	189.29 ± 14.57	18.00 – 26.00	22.00 ± 1.70	2.57 – 4.34	3.49 ± 0.49
1 month	181.00 – 224.00	198.17 ± 11.41	20.00 – 28.00	23.41 ± 2.67	3.19 – 4.62	3.83 ± 0.41
2 months	191.00 – 228.00	205.70 ± 10.17	20.00 – 29.00	25.00 ± 2.50	3.91 – 5.66	4.52 ± 0.44

they state that "…. only three young were able to survive for three months", which suggests the need for revised captive breeding protocols. The remaining four females were subjected to hibernation in the hope that they would breed in the following year, but as yet no information has been provided on whether this was successful.

Growth and development of neonates

Total length (from the tip of the snout to the end of the tail), tail length and weight were measured for adult male and female snakes in the breeding study (Table 1). To avoid stressing gravid females and harming developing embryos, the last weighing was done on 29 August (two weeks before parturition). We used an independent t-test to compare differences between adult male and female measurements. Adult females were significantly heavier than male vipers at the time of mate seeking and mating behaviours (t = -3.55, p = 0.003). There was no significant difference between male and female vipers in total length (t = 0.14, p = 0.890), although males had significantly longer tails (t = 3.27, p = 0.005). Shakoori et al. (2015) report that the mean weight for gravid females was 68.92 ± 10.41 g and snout-vent length (SVL) 48.39 ± 2.69 cm, indicating that their female snakes were larger than ours, which were on average 58.41 g and SVL 40.56 cm.

Neonates were measured for total length, tail length and weight at birth, one month after birth (mid-October) and two months after birth (20 November) (Table 2). Shakoori et al. (2015) reported a mean birth weight as 2.81 ± 0.39 g and SVL 148.2 ± 8.4 mm, values that on average were lower than ours which were on average 3.49 g and 167.29 mm SVL.

DISCUSSION

The demand for venom from the Caucasian pit viper for medical purposes and the decline of wild populations of this species make a captive breeding programme essential. A venom comparison of our wild-caught adults, long-term captive adult and captive-born neonates has demonstrated that the venom obtained from captive-bred Caucasian pit vipers is an appropriate source for the production of antivenom in Iran (Rasoulinasab et al., 2020). We believe that the continuation of captive breeding programmes should be a high priority for the Iranian Department of the Environment, to ensure an adequate supply of pit vipers in light of the serious population declines and habitat loss over recent years (Ahmadi et al., 2019; Asadi et al., 2019). Furthermore, this will support the conservation of wild populations by reducing the demand for wild caught specimens.

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