REPTILES & AMPHIBIANS

Artificial Incubation of Eastern Montpellier Snake (*Malpolon insignitus*) Eggs and Physical Abnormalities in Hatchlings

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Snakes exhibit a wide variety of reproductive strategies (Bellini et al. 2019). More than 80 percent of modern reptiles reproduce by laying eggs (Shine 1985). However, some species retain eggs in the oviduct after ovulation, and others lay eggs after a large portion of the embryonic development has elapsed (Köhler 2005). The period during which the eggs remain in the oviduct after ovulation is called the pre-laying fraction (Shine 1983). After eggs are laid, ideal environmental conditions must be present to allow growth and development of the embryo. Embryonic growth rate is mainly affected by temperature and to some extent by the amount of available moisture (Packard et al. 1982). Unlike birds, the yolk of snakes settles on the bottom, and the embryo floats and attaches to the top of the egg; consequently, the rotation of eggs in the horizontal axis during incubation should be avoided (Köhler 2005). Also, the presence of moist substrate and organic matter can lead to the growth of pathogens such as mold (Mohammadalizadegan and Sardari 2022; Parmar and Limbachiya 2020).

For purposes of conservation or research, some species of wildlife are bred in captivity. For instance, when the number of individuals of a species is rapidly decreasing or the species is at risk of extinction, captive breeding could provide sufficient numbers for increasing natural populations, reintroduction to new or formerly occupied habitats, and for conservation-management studies (Kian 2011; Rasoulinasab et al. 2020). In addition, captive breeding can yield important information on the reproductive biology of the studied species, and captive-breeding programs allow the study of certain aspects of the physiology and behavior of endangered species without affecting wild populations (Fraser 2008).

The Eastern Montpellier Snake (*Malpolon insignitus*), a rear-fanged venomous psammophiid (Mezzasalma et al. 2023), is currently listed as Least Concern (LC) on the IUCN Red List (Aghasyan et al. 2021). The range of the species includes northern Italy, Croatia (including some islands), Bosnia-Herzegovina, Montenegro, Albania, Macedonia, southern Bulgaria, Cyprus, Turkey, Greece (including many islands), Armenia, Azerbaijan, southwestern Russia, northern Iraq, Iran, eastern Morocco, Algeria, Tunisia, northern Libya, northern Sudan, northern Egypt, Israel, Jordan, Lebanon, and southern Syria (Wallach et al. 2014). This species is diurnal, which renders it particularly susceptible to road mortality. Its large body size, fast movement, aggressiveness (when seized), and ophiophagous behavior (Safaei-Mahroo et al. 2017) contribute to its mistaken identification as a highly venomous snake; consequently, shepherds often attempt to kill it immediately when encountered. Recent studies indicate that global warming could lead to population decreases, particularly among adult males (López-Calderón 2017). In this context, expanding our knowledge of this species' captive breeding could prove instrumental in conservation plans, potentially aiding in the restoration of wild populations (Roe et al. 2015).

A female snake (SVL 88 cm) captured on 25 May 2020 in the Nazarabad area of Alborz Province was transferred to the first author's personal collection quarantine room in Karaj, Alborz Province, Iran. Despite a relatively severe external parasitic infection (mites), antiparasitic treatment was not performed to prevent the possible effects of antiparasitic drugs on egg development. The female was kept in a transparent plastic container (90 × 60 × 50 cm) until she laid eggs. Four holes (diameter 10 cm) covered with a metal mesh in the upper part of the container provided ventilation. The substrate was coconut husk chips with a depth of 3–4 cm and a sheet of dried tree bark was placed in the container as a hide. Room temperature varied between 25 and 35 °C and the duration of lighting was similar to the seasonal conditions in natural habitat. For basking, a silicon coated wire heater placed under one side of the container and controlled by a timer and a thermostat increased temperatures to 35 °C for four hours each day (1000–1200 h and 1400–1600 h). A clay

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water dish (diameter 25 cm, depth 10 cm) was placed opposite the heater. Humidity (~70% during the day and 50% at night)was provided by evaporation from the water dish.

Five days after capture and every three days after that, a 15–20 g euthanized mouse offered to the snake was not accepted. On 16 June 2020, the snake shed its skin. Two days after shedding, it became more active and seemed to be searching for a suitable egg-laying place. According to observations in nature during the egg-laying season, female eastern Montpellier snakes usually bask on large stones a short distance from water and, under which they quickly retreat if threatened. Soil under such stones is moist and it can provide a suitable environment for laying eggs. By 27 June 2020, when eggs were evident in the posterior third of her body, the female started rooting in the substrate around the water dish and this behavior increased substantially. At this time, we moved the snake to an egg-laying container, a semi-transparent plastic box (45 × 25 × 20 cm) with several small holes in the lid, in a room with ambient temperature of ~25 °C. Substrate was ~5 cm of perlite mixed with cooled, boiled water at a ratio of 1:1 by weight, which prevented dehydration of eggs until they are transferred to the incubator.

Egg-laying commenced at 0300 h on 29 July 2020 and continued until 0830 h. Eventually, 13 fertile eggs in seemingly good condition and one infertile egg were laid (Fig. 1A). The dimensions of eggs were approximately 35 × 20 mm. Fertile eggs were transferred to the incubator at 1000 h. A few hours later the snake ate two 18-g euthanized mice for the first time in captivity. The eggs, stuck together by a glue-like substance, were separated; but before separation, the upper surfaces were marked with an alcohol-free marker to avoid rotating the eggs during transfer and handling.

We used a prefabricated aluminum egg-incubator (model: Damavand-dq40sh) and a plastic box (28 × 21 × 7.5 cm) as the incubation container. The inside temperature of the incubator chamber was supplied by a wired placed in the walls and adjusted by a thermostat with an accuracy of 0.5 °C. In various references, the appropriate temperature for the incubation of snake eggs is was listed as 27–32 °C (e.g., Köhler 2005). Considering the successful experience of incubating eggs of a close related species, the Montpellier Snake (*M. monspessulanus*) at a temperature range of 28–32 °C (Coli 2007), we set the temperature at 29 °C. To prevent the formation of a temperature gradient in incubator, air was constantly circulated by a fan. A combination of cooled, boiled water and clean, dry perlite in a weight ratio of 1:1 and a depth of 3 cm was used as wet substrate. Seven of the eggs were placed directly on the substrate, with the level of immersion ~20% of volume. The other six eggs were placed on a plastic mesh a short distance from the substrate (Fig. 1B). The container was then placed in the incubator.

Every other day, the container was removed from the incubator and left open for a few seconds to allow air exchange. During the first days of incubation, some eggs began to rot, color changed from white to green-yellow, and drops of dew were evident on the surfaces. Rotten eggs were removed immediately. Eggs placed directly on the substrate absorbed more water and were deformed, whereas those placed on a plastic tray retained their shape. After ten days, blood vessels could be seen when the eggs were candled (Fig.

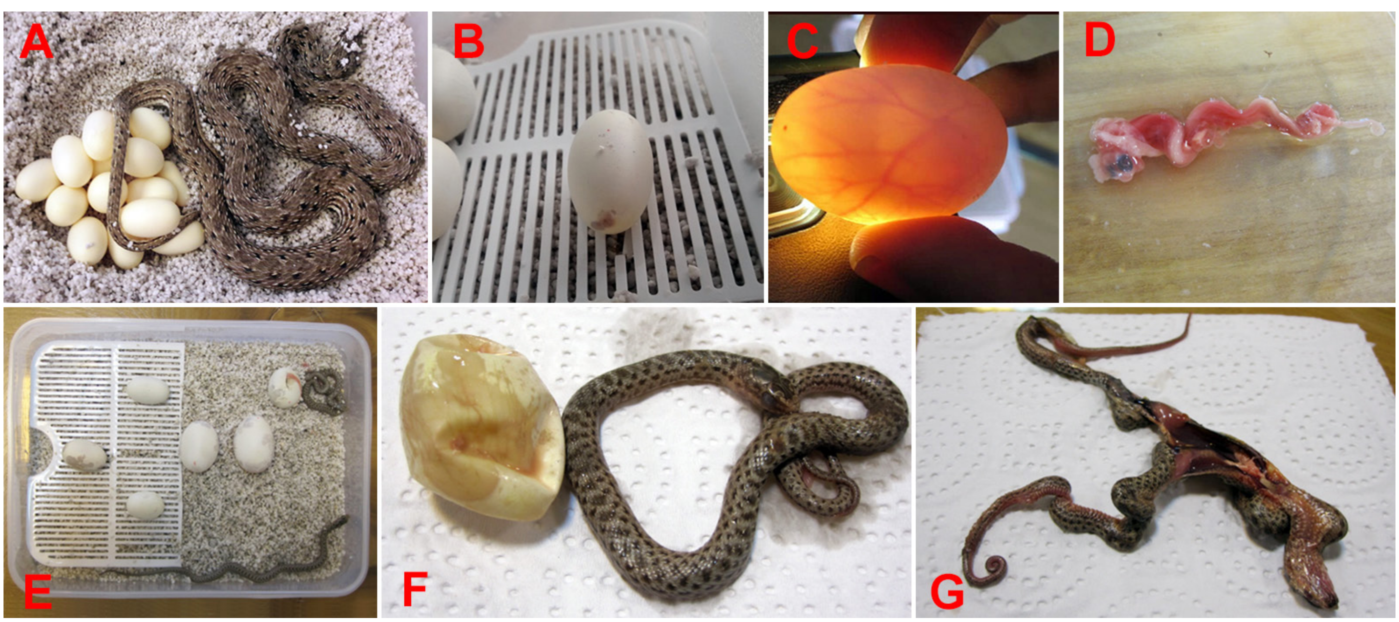

Figure 1. A female Eastern Montpellier Snake (*Malpolon insignitus*) one minute after laying a clutch of eggs (A); placement of eggs on a plastic tray in the incubation container (B); expansion of veins in eggs on the 15th day of incubation (C); a dead fetus with a physical abnormality (D); hatchlings a few hours after emergence from the egss (E); a fully-formed hatchling that died in the egg (F); and conjoined twins with physical abnormalities that presumably survived until the last day of incubation (G). Photographs by Mohammad Kaboli.

1C). During incubation, some fertilized eggs died at different stages of embryonic development and were removed from the incubator. After opening the dead eggs, one of the embryos exhibited a clear physical abnormality (Fig. 1D).

Eventually, five eggs remained, two of which hatched on 22 August 2020, 54 days after deposition (Fig. 1E). We were unable to find any data about the incubation period of eggs of Eastern Montpellier Snakes; however, the incubation period of eggs of Montpellier Snakes was reported to be 63–67 days temperatures of 28–31 °C (Coli 2007). A week after the first eggs hatched, one of the three remaining eggs hatched with our help but the hatchling died shortly thereafter. On the same day we opened the two remaining eggs, which were found to contain dead snakes (Fig. 1F). One was a fully developed hatchling that was normal in appearance. The other one was a conjoined twin with two bodies and one head (Fig. 1G). Its chest cavity was open and internal organs were visible.

The lengths of two living hatchlings were ~26.5 and 25 cm and weights were 8.6 and 7.9 g, respectively. Hatchlings were fed after first shedding with mouse tails (assisted feeding). After 3 weeks they started feeding actively on day-old pinky mice. On the night of 20 September, hatchlings were released into suitable habitat in Alborz Province.

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Literature Cited

Aghasyan, A., A. Avci, B. Tuniyev, P. Lymberakis, C. Andrén, D. Cogălniceanu, J. Wilkinson, N.B. Ananjeva, N. Üzüm, N.L. Orlov, R. Podloucky, S. Tuniyev, U. Kaya, M. Vogrin, and S. Baha El Din. 2021. *Malpolon insignitus*. *The IUCN Red List of Threatened Species* 2021: e.T157253A744783. https://dx.doi.org/10.2305/IUCN.UK.2021-3.RLTS.T157253A744783.en.

Bellini, G.P., V. Arzamendia, and A.R. Giraudo. 2019. Reproductive life history of snakes in temperate regions: what are the differences between oviparous and viviparous species? *Amphibia-Reptilia* 40: 291–303. https://doi.org/10.1163/15685381-20181076.

Fraser, D.J. 2008. How well can captive breeding programs conserve biodiversity? A review of salmonids. *Evolutionary Applications* 1: 535–586. https://doi.org/10.1111/j.1752-4571.2008.00036.x.

Kian, N., M. Kaboli, M. Karami, A. Alizadeh, S. Teymurzadeh, N. Khalilbeigi, J.B. Murphy, and E. Nourani. 2011. Captive management and reproductive biology of Latifi's Viper (*Montivipera latifii*) at Razi Institute and Tehran University in Iran. *Herpetological Review* 42: 535–539.

Köhler, G. 2005. *Incubation of Reptile Eggs: Basics, Guidelines, Experiences*. Krieger Publishing Company, Malabar, Florida, USA.

López-Calderón, C., M. Feriche, E. Alaminos, and J.M. Pleguezuelos. 2017. Loss of largest and oldest individuals of the Montpellier snake correlates with recent warming in the southeastern Iberian Peninsula. *Current Zoology* 63: 607–613. https://doi.org/10.1093/cz/zow112.

Mezzasalma, M., E. Brunelli, G. Odierna, and F.M. Guarino. 2023. Comparative cytogenetics of *Hemorrhois hippocrepis* and *Malpolon monspessulanus* highlights divergent karyotypes in Colubridae and Psammophiidae (Squamata: Serpentes). *The European Zoological Journal* 90: 201–210. https://doi.org/10.1080/24750263.2023.2180547.

Mohammadalizadegan, A. and P. Sardari. 2022. Artificial incubation and mold treatment of European Glass Lizard (*Pseudopus apodus*) eggs in a home-made incubator. *Reptiles & Amphibians* 29: 76–78. https://doi.org/10.17161/randa.v29i1.16315.

Packard, M.J., G.C. Packard, and T.J. Boardman. 1982. Structure of eggshells and water relations of reptilian eggs. *Herpetologica* 38: 136–155.

Parmar, D.S. and V.P. Limbachiya. 2020. Artificial Incubation of Buff-striped Keelback (*Amphiesma stolatum*) eggs. *Reptiles & Amphibians* 27: 335–336. https://doi.org/10.17161/randa.v27i2.14495.

Rasoulinasab, F., M. Rasoulinasab, D. Shahbazzadeh, A. Asadi, and M. Kaboli. 2020. Comparison of venom from wild and long-term captive *Gloydius caucasicus* and the neutralization capacity of antivenom produced in rabbits immunized with captive venom. *Heliyon* 6: e05717. https://doi.org/10.1016/j.heliyon.2020.e05717.

Roe, J.H., M.R. Frank, and B.A. Kingsbury. 2015. Experimental evaluation of captive-rearing practices to improve success of snake reintroductions. *Herpetological Conservation and Biology* 10: 711–722.

Safaei-Mahroo, B., H. Ghaffari, S. Salmabadi, A. Kamangar, S. Almasi, S.M. Kazemi, and A. Ghafoor. 2017. Eastern Montpellier Snake (*Malpolon insignitus fuscus*) ophiophagy behavior from Zagros Mountains. *Russian Journal of Herpetology* 24: 69–72. https://doi.org/10.30906/1026-2296-2017-24-1-69-72.

Shine, R. 1983. Reptilian reproductive modes: the oviparity-viviparity continuum. *Herpetologica* 39: 1–8.

Shine, R. 1985. The reproductive biology of Australian reptiles: a search for general patterns, pp. 297–303. In: G.H. Grigg, H. Ehmann, and R. Shine (eds.), *Biology of Australasian Frogs and Reptiles*. Australasian Herpetology Conference 1984. Surrey Beatty & Sons Pty Limited, Chipping Norton, New South Wales, Australia.

Wallach, V., K.L. Williams, and J. Boundy. 2014. *Snakes of the World: A Catalogue of Living and Extinct Species*. CRC Press, Boca Raton, Florida, USA.