

Successful Hatching of a Patched *Varanus beccarii* (Doria, 1874) Egg at the Saint Louis Zoo

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Abstract - Paraffin wax and a section of latex medical glove were used to patch a black tree monitor (*Varanus beccarii*) egg that ruptured after 118 days of artificial incubation. The embryo continued to develop following the procedure and the egg hatched successfully 39 days later. To date, the juvenile appears healthy with no ill effects from either the rupture or repair of the eggshell.

Introduction

The shell of a reptile egg has several vital functions, including protection of the embryo and regulation of the internal environment of the egg (Packard & DeMarco, 1991). As with most other squamates, monitor lizards (genus *Varanus*) lay eggs with flexible parchment-like eggshells. Pliability of the eggshell accommodates changes in the size and shape of these eggs (Packard *et al.*, 1982). However, there are constraints to this flexibility and damage to artificially incubated eggs may arise from improper nesting, handling during excavation or transport, and environmental conditions during incubation (Packard & Phillips, 1994; Köhler, 2005; Eidenmüller, 2007).

When reptile eggs are damaged during artificial incubation, manipulations of the incubation environment, such as reducing the level of moisture, may be useful in stopping further damage (Packard & Phillips, 1994). Sometimes, damaged eggs can hatch successfully without any additional interventions (Ackerman *et al.*, 2002). However, when severe cracking or rupturing has occurred, repairs to the shell can be attempted. Repairs should serve to stabilize the eggshell, preventing more structural damage, loss of contents from the egg, or infiltration by other organisms, while still permitting gas and water exchange.

Flexible-shelled eggs are particularly problematic to repair as the eggshell typically remains more elastic than the sealant (Ackerman *et al.*, 2002). Many materials

have been used for reptile egg repair (Ackerman *et al.*, 2002; Augustine & Saunders, 2015); published accounts of repairs on ruptured flexible-shelled eggs have utilized pieces of shell from infertile or previously hatched eggs (Maxwell, 2005; Adragna & Madden, 2009), cyanoacrylate adhesives (Ackerman *et al.*, 2002; Davis, 2014), transparent medical dressings (Augustine & Saunders, 2015), and waxes (Maxwell, 2005; Fischer, 2012).

Several cases of *Varanus* eggs rupturing during artificial incubation have been described, involving species from the subgenera *Odatria* (pygmy monitors) and *Euprepiosaurus* (tree monitors). For the repair of split *Varanus* eggs, multiple techniques have been detailed. In one report involving the subgenus *Odatria*, a ruptured *V. acanthurus* egg was mended with a section of shell from a previously hatched egg of another species (*V. tristis*), continued development, and eventually hatched successfully (Adragna & Madden, 2009). Published attempts at fixing eggs of species in the subgenus *Euprepiosaurus* have produced mixed results. One *V. prasinus* egg was successfully repaired using tissue (cyanoacrylate) glue (Davis, 2014). However, the embryo of a damaged *V. beccarii* egg sealed with wax died prior to hatching (Fischer, 2012). Herein, an additional account describing the patching of a ruptured *V. beccarii* egg is provided. This method of utilizing paraffin wax and a piece of latex glove resulted in a successful hatching.

General Husbandry

A pair of black tree monitors (*V. beccarii*) has been housed at the Saint Louis Zoo since 2007. The female hatched at the Milwaukee Zoo in January 2007; the male was imported from Indonesia in 2001 and is of unknown age. On 30 June 2017, the adult pair was moved into a 2.8 x 1.7 x 4 m (L x W x H) exhibit outfitted with live plants, concrete rockwork, fake vines, natural wood branches, and two artificial dead trees (Fig. 1). The enclosure received filtered sunlight through a skylight and two basking lights (160W PowerSun UV, Zoo Med Laboratories, Inc., San Luis Obispo, California, USA) provided supplemental heat and ultraviolet light. The thermal gradient ranged from 25 °C to approximately 40°C directly under the basking lights. A varied diet was offered, consisting of gut-loaded and supplement-dusted live invertebrates (*e.g.*, crickets, roaches, mealworms) once or twice a week and either neonate mice, hard-boiled egg, or quail chicks approximately once a week.

The artificial trees (approximately 1.5 and 2 m high) were constructed from fiberglass and wireframes covered by epoxy modeling compound (Apoxy Sculpt, Aves Studio, LLC, Hudson, Wisconsin, USA). A 5-gallon plastic bucket (26 x 33 cm) was concealed within the upper end of each tree. A removable lid was fastened to each bucket to provide keeper access, and a mixture of moistened coir, sphagnum moss, and hardwood mulch was added to the interior. Water was periodically added to keep the substrate inside the buckets slightly wet, but not overly saturated. Slightly below the top of each bucket, a roughly 8 cm diameter hole was cut into the side, creating an opening which allowed the *V. beccarii*

entry into the cavity for nesting and retreat.

Oviposition and Early Incubation

On 16 September 2017, the female buried a clutch of three eggs inside one of the nest chamber buckets. Two of the eggs were misshapen, deflated, and nonviable. The remaining egg (45.6 x 21.1 mm, 13 g) looked viable externally and was transferred to a clear plastic box (16.5 x 16.5 x 5 cm) for artificial incubation (Fig. 2). A single 2 mm diameter hole in the box lid provided air exchange. The egg was partially buried in medium coarse horticultural vermiculite that had been hydrated with aged tap water at a ratio of 1:0.9 by weight. The total mass of the box with substrate and the egg was recorded before the box was placed inside an incubator (Nature's Spirit, LLC, Vicksburg, Michigan, USA) controlled by a thermostat (Herpstat 1, Spyder Robotics, Rochelle, Illinois, USA) set at a constant temperature of 29 °C.

During incubation, the egg was frequently observed through the transparent incubator window and lid of the box. The box was periodically removed from the incubator and weighed to determine the amount of moisture lost through evaporation. Aged tap water was added to the vermiculite farthest from the egg until the box matched its initial mass. On occasion, while the box was out of the incubator, development of the egg was assessed using a candler (Powerlux SL-PL, Olba B.V., Coevorden, The Netherlands), typically without handling of the actual egg. The last addition of water to the substrate took place over a week prior to the date of the rupture, and the incubator was not opened nor was



Fig. 1. Left: A section of the adult *Varanus beccarii* enclosure showing live plants, artificial trees and other features. Right: The female emerging from a nest cavity in one of the artificial trees.



Fig. 2. The viable *V. beccarii* egg at the start of incubation.

the box moved during the two days before the incident.

Egg Rupture and Patching

On 12 January 2018, 118 days into incubation, the egg was visually inspected and a rupture was observed on the upper surface of the eggshell. Approximately 0.5 ml of albumen was protruding from a 3-4 mm hole in the egg. The albumen was slightly yellow, clear, and did not display any signs of decay. While the egg remained on the substrate, it was carefully candled. Movements of the embryo and an abundance of blood vessels were discernable.

For the repair, the egg was left in place within the box. A 10 x 10 mm section was cut from a disposable medical glove (Ambitex Powder Free Latex Glove, Tradex International, Inc., Cleveland, Ohio, USA). By the time patching of the eggshell was attempted, the surface of the protruding albumen had become slightly dry and viscous. Due to concern that wiping might apply too much pressure to the egg, small sterilized scissors were used to help separate the albumen from the egg. The area was blotted with tissue paper to absorb any remaining fluid. The latex patch was then quickly placed over the hole before more albumen could leak from the egg and carefully held in place with the tip of the scissors. Paraffin wax from a lit candle was then dripped on and around the latex patch to seal it in place. Once the repair was complete, the box was closed and returned to the incubator.

Late Incubation and Hatching

Following the repair, the egg was still occasionally

candled without being handled directly. No further movements were seen during candling, but healthy blood vessels continued to be observed. Water lost from the box through evaporation was no longer replenished and the substrate was allowed to dry slowly. Approximately two weeks prior to hatching, the egg began to dimple slightly. At this time, the egg also became more difficult to assess via candling. Deflation of the egg continued until it began to hatch on 20 February 2018, 39 days after the repair. The hatchling did not emerge from the site of the patched hole but rather at one end of the egg. During hatching, movements of the exiting lizard caused the patch to detach from the egg (Fig. 3). The egg was transferred to a box with moist paper towels and returned to the incubator until the hatchling had completely left the egg. Mass of the hatchling was 9 g on 24 February 2018.

The hatchling was set up in a small enclosure containing cork bark, a live plant, and natural wood branches. A basking spot (100W PowerSun UV, Zoo Med Laboratories, Inc., San Luis Obispo, California, USA) provided ultraviolet light and temperatures similar to the thermal gradient in the adult enclosure. Humidity was kept high (> 60%) through daily misting. Within a few days, the neonate began feeding on live invertebrates. Subsequently, the juvenile was offered food several times per week and readily accepted crickets, fresh newborn mice, mealworms, roaches, and other small prey.

Discussion

Many factors, including an inadequate oviposition site, handling during excavation or transport, and environmental conditions during incubation, can be responsible for damage to artificially incubated eggs (Packard & Phillips, 1994; Köhler, 2005; Eidenmüller, 2007). For the rupture reported here, the proximate cause is not immediately obvious. The nesting cavity appeared to be suitable and the female buried the egg normally in the middle of the chamber. During excavation, the egg was handled with great care. Once incubation began, the egg was rarely picked up directly. For candling, the egg was typically kept on the substrate and the light source gently placed along one side. Furthermore, during the two days prior to the incident, the incubator had not been opened nor had the box been moved.

Since neither nesting nor handling appears to be a likely cause, it seems probable that incubation conditions might have played a role in prompting the rupture. The external environment is capable of inducing substantial



Fig. 3. The *V. beccarii* emerging from the egg. During the hatching process, the patch of wax and latex fell off the eggshell and can be seen to the left of the egg.

changes in flexible-shelled reptile eggs, such as those of *Varanus*. In one instance, a rapid change in air pressure, produced by opening an incubator door, was presented as the possible cause of a *Varanus* egg rupture (Adragna & Madden, 2009). In the current paper, unlike the setup used by Adragna & Madden (2009), neither the incubator nor the box containing the egg was sealed tightly. Therefore, it is doubtful that an air pressure differential led to the rupture.

Most suggested causes of *Varanus* egg ruptures relate to hydric conditions during incubation; monitor eggs appear to be particularly sensitive to moisture levels (Moldovan, 2008). Availability of water is a key environmental condition for flexible-shelled eggs, as they are capable of taking in moisture from the surrounding substrate and will expand during this process (Packard et al., 1982). However, damage may occur if too much water is absorbed (Packard & Phillips, 1994). Bursting of *Varanus* eggs has been attributed to the use of an excessively wet substrate (Fischer, 2012) and to the rapid rehydration of partially desiccated eggs (Davis, 2014).

For the rupture documented here, sudden uptake of water is unlikely. The same level of moisture had been maintained throughout incubation until the rupture, and no water had been added to the substrate for at least a week prior to the incident. It is possible that too much water was initially added to the incubation substrate and the water potential (a quantification of the tendency of water to move from one area to another) of the substrate subsequently remained too high, as during additions of water to replace evaporation, no compensation was made

for the amount absorbed by the eggs. High water content in the substrate could have caused the egg to swell to the point where the eggshell weakened and was no longer able to contain the hydrostatic pressure, resulting in rupture. The ratio of substrate to water used here (1 part vermiculite to 0.9 part water) was slightly drier than the equal proportions generally recommended for most reptile eggs (Packard & Phillips, 1994). However, while water potential initially increases rapidly as small amounts of water are added to dry coarse vermiculite, it is comparatively less affected by additional water above a ratio of approximately 1:0.5 (Packard & Phillips, 1994).

Therefore, the substrate in this account likely had a water potential only slightly below those of substrates implicated in previous ruptures of *Varanus* eggs, which had been hydrated at ratios of 1:2 and 1:1 (Fischer, 2012; Davis, 2014). This suggests that, if excess water absorption was the cause of the rupture, it may be desirable for the incubation of tree monitor eggs to either use vermiculite with a further reduction of water or a substrate-less technique as suggested by Fischer (2012). However, there are two caveats to this approach: first, water could be drawn from the eggs under dry conditions, requiring careful monitoring for dehydration and possibly increasing the risk of damage from frequent handling; and second, *Varanus* eggs have been reported to rupture even when not in contact with moist substrate (Adragna & Madden, 2009; Fischer, 2012).

Another possible factor is that the ultrastructure of the eggshell could have been inherently weak due to a physiological issue with the female. The misshapen

and nonviable nature of the other two eggs in the clutch supports this idea. Although flexible, the parchment-like eggs of most squamates typically possess a thin calcareous layer (Packard & DeMarco, 1991). Shelling of eggs occurs within the glandular uterus region of the oviducts (Siegel *et al.*, 2014). Dysfunction of the oviducts can cause abnormalities in eggshells; associations between defects in eggs and environmental stress, oviductal infections, or genetics of the mother are well established in poultry (Reynard & Savory, 1999; Feberwee *et al.*, 2009; Wolc *et al.*, 2012). Egg quality is also affected by nutrition of the female (Köhler, 2005). If calcium or another maternal contribution to the egg was insufficient in the diet of the female, the integrity of the eggshell could potentially have been impacted.

In this case, however, the female seemed to acclimate quickly to the exhibit and be in good overall health. The diet provided to the pair of *V. beccarii* appeared to be nutritionally complete and similar in composition to what is typically offered this species (Eidenmüller & Wicker, 1993; Mendyk & Horn, 2011; Fischer, 2012). No noticeable defect in the eggshell was apparent upon initial recovery of the egg from the nest site or throughout the incubation period before the rupture; yet, a weakness in the eggshell ultrastructure cannot be definitively excluded. If a flaw was present, environmental conditions could have still contributed to the rupture. However, if the eggshell was sufficiently weak, the rupture could have occurred even under incubation conditions that would have typically been ideal for the species.

When reptile eggs break during artificial incubation, modifying the incubation environment can prevent further damage (Packard & Phillips, 1994). For incubation of the egg here, the substrate was allowed to slowly dry following the repair. Regardless of the cause of the initial rupture, lowering of the water potential in the substrate would have reduced pressure within the egg, decreasing stress on the eggshell, and possibly keeping other ruptures from occurring. However, the wax portion of the patch was less yielding compared to the shell; this is a shortcoming with many sealants used in the repair of flexible-shelled eggs (Ackerman *et al.*, 2002). A sudden decrease in moisture could have caused deflation to occur too quickly, leading to the repair material falling off prematurely. Due to the responsiveness of *Varanus* eggs to water, it seems that any changes in hydric conditions during incubation should be gradual, avoiding rapid fluctuations which may damage eggs (Davis, 2014). Additionally, drier conditions at the end of incubation may prove helpful

in preventing full-term embryos from dying within the eggs just prior to hatching (Fischer, 2012).

Damaged eggs will sometimes hatch successfully without major intervention (Ackerman *et al.*, 2002). However, in this case, repair was deemed necessary given the severity and timing of the break. The *V. beccarii* egg reported here ruptured three-quarters of the way through incubation but still successfully hatched after a total incubation length of 157 days. The timing of hatching was slightly less than the period of 172-203 days reported by Eidenmüller and Wicker (1993). However, it was within the range of 157-162 days given by Wanner (1991) and variation in incubation length is frequently observed in monitor lizards (Eidenmüller, 2007). The neonate appeared normal and was active and feeding soon after hatching. To date, it has appeared to be healthy and vigorous (Fig. 4). Despite the rupture and subsequent repair of the egg, there do not seem to have been any ill effects on the juvenile.

Further refinements in the husbandry and artificial incubation of *V. beccarii* are needed, including investigations on appropriate nutrition, the water dynamics of eggs, and the ideal level of moisture in the incubation substrate. Special attention should be given to the cause and prevention of egg rupture during



Fig. 4. The juvenile *V. beccarii* at one month of age.

artificial incubation. However, when ruptures occur in the flexible-shelled eggs of *V. beccarii*, and other monitor lizards, the wax and latex method presented in this account offers another viable method of successfully repairing the eggshell.

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