

HIGH WATER POTENTIAL VERMICULITE AS AN INCUBATION MEDIUM FOR REPTILE EGGS

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ABSTRACT

Eggs with flexible and hard shells (N = 216) representing five lizard and seven snake species and subspecies, were incubated in ventilated incubation containers with a water reservoir and a saturated coarse grade vermiculite and water medium of 1: 4 (g/g). Hatching success was 95%. This was contrary to the widely used method of incubating reptile eggs in sealed containers with a vermiculite and water medium of 1: 1 (g/g), or less. The high hatching success was ascribed to the above factors as to well as the non disturbance of containers during incubation and the lack of fungal infections on fertile eggs. As a control, snake eggs (N = 42) were incubated under the same conditions using sealed containers and a fine grade vermiculite and water medium of 1: 1 (g/g). The control incubations resulted in a comparatively poor hatching success of 32%. The results and effects on the hatchability of reptile eggs in other studies where vermiculite with varying water potentials were used, are also discussed.

INTRODUCTION

The water content of an incubation medium may be expressed as either a ratio or a measurement. In the former, the ratio expresses the amount of water added to the incubation medium in either g/g or g/ml. In the latter, the water potential of the incubation medium is expressed as kiloPascal (kPa) (Tracy, 1980; Packard & Packard, 1987; Plummer & Snell, 1988). KiloPascal measurements are usually determined by the use of thermocouple hygrometry with a calibrated sample chamber (Wescor C-52) and a dewpoint microvoltmeter (Wescor HR-33T), or a water potential data system (Wescor HP-115) (Packard & Packard, 1987; Plummer & Snell, 1988). As water potential determination equipment is not always available, the vermiculite/water ratio method is preferred by many breeders. An explanation as to the use of these two water determining measurements in text, is given under the Methods section.

The incubation methods of many researchers (Morgan, 1988; Boycott & Morgan, 1988; Deeming, 1989) have been based on that of Tryon (1975), who used a vermiculite and water medium of 1: 1 (g/g) (-160 kPa). Incubation mediums with a water potential of below -160 kPa (V/WR 1: 1) have been used extensively. Water potentials of -200 kPa (V/WR 1: 0.71) to -2000 kPa (V/WR 1: <0.1) were used by Plummer & Snell (1988); -300 kPa (V/WR 1: 0.43) to -900 kPa (V/WR 1: 0.17) by Packard, Taigen, Boardman, Packard & Tracy (1979); -200 kPa (V/WR 1: 0.71) to -450 kPa (V/WR 1: 0.32) by Packard, Packard & Boardman (1982); -150 kPa (V/WR 1: 1) to -950 kPa (V/WR 1: 0.15) by Packard & Packard (1987); and -825 kPa (V/WR 1: 0.18) and -1500 kPa (V/WR 1: 0.10) by Packard, Packard & Benigan (1991). Mattison (1991) recommended a water potential of -270 kPa (V/WR 1: 0.48) for husbandry use. Using sand in ventilated vials, Ferguson & Snell (1986) used a sand/water ratio

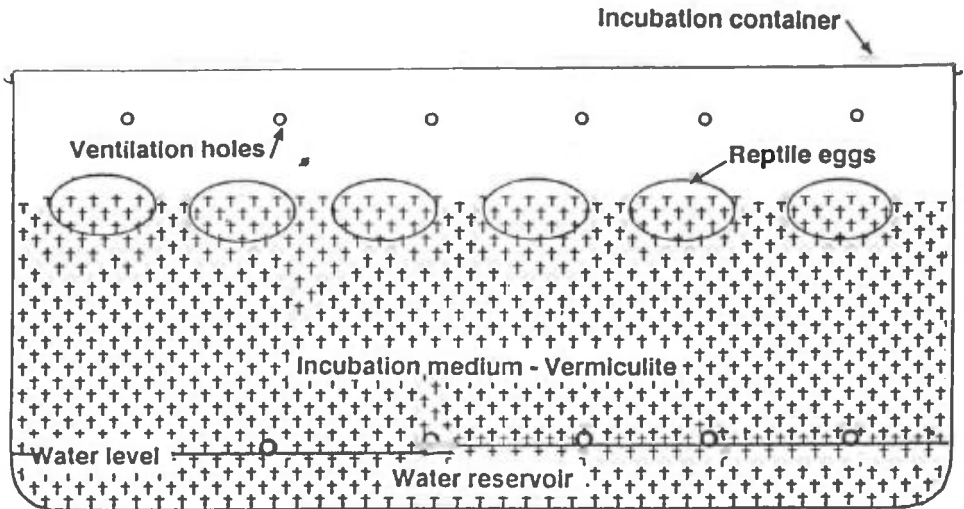


Fig. 1. A diagrammatic presentation of a ventilated incubation container showing the water reservoir (not to scale).

of 1: 0.05 (< -1500 kPa). Despite the extensive use of drier incubation mediums, evidence in the literature strongly suggests that there may be distinct advantages in using wetter incubation mediums, the advantages of which will be discussed later in this paper. However, the use of incubation mediums with water potentials of above -160 kPa (V/WR 1: 1) appear to be relatively uncommon, although becoming more popular. Packard *et al.* (1979) used a water potential of -50 kPa (V/WR 1: 3.6); Packard *et al.* (1982), -100 kPa (V/WR 1: 2.8); and Ford & Seigel (1989), and Seigel & Ford (1991), -70 kPa (V/WR 1: 3.1).

When using sealed incubation containers, researchers have found it necessary to open these repeatedly for ventilation purposes and sometimes the addition of water. Boycott & Morgan (1988) opened containers every two to three days. Anstandig (1984) weekly, and Deeming (1989) once or twice a week. Even when using commercially available incubators (Hova-Bator), it is recommended that the incubator be opened for occasional misting. Tracy (1980) also mentioned that the spread of fungal infections on incubating eggs may be a problem related to the use of sealed containers.

The choice of incubation mediums appears to be an individual choice, with a variety of mediums being utilized. Patterson (1987) suggested leaving soft-shelled eggs *in situ* and using wet paper towelling as an incubation medium. For the incubation of gecko eggs, Miller (1982, 1983) used either slightly damp sand, or peat and bark chips, with water at the bottom of the container. Mattison (1982) suggested the use of fine vermiculite, while Mattison (1991) gave sand, perlite, and sawdust as alternatives to vermiculite, and Ferguson & Snell (1986) used sand. Anstandig (1984) preferred wet, squeezed out vermiculite. However, in the majority of studies referred to in this investigation, vermiculite appears to be the most widely used incubation medium.

The primary aim of this investigation was to examine the hatching results of incubating reptile eggs in ventilated incubation containers with a reservoir of water and an initially saturated incubation medium of large grade vermiculite, and to compare these results to control incubations using fine grade vermiculite and water with a V/WR of 1: 1 in sealed containers. It was also deemed pertinent to examine the incubation results of other researchers who have used vermiculite and water in varying proportions.

MATERIALS AND METHODS

Plastic incubation containers with sealing lids, such as Tupperware (length (L) = 270 mm x width (W) = 270 mm x height (H) = 145 mm), or plastic ice cream containers (L = 315 mm x W = 210 mm x H = 105 mm, and L = 220 mm x W = 220 mm x H = 140 mm) were used for snake eggs, and similar, but smaller containers for lizard eggs. A series of 3 mm diameter holes (4 to 6 per side) were drilled around the bottom of the container, 5 mm up from the base, and another series of 3 mm holes (4 to 6 per side) were drilled around the top of the container, 10 mm down from the top edge (Fig. 1).

Tests were carried out to determine the saturation point of coarse (large) and fine (small) grade vermiculite, plus a small excess quantity of free water to form a reservoir at the bottom of the container. Saturation points were determined by gradually adding measured quantities of water to the vermiculite, and mixing until, after being left to stand a short while, a small quantity of water accumulated at the bottom of the container. This determination resulted in a saturation V/WR for large and fine grade vermiculite of 1: 3,8 (g/g) (0,0 kPa) and 1: 3,2 (g/g) (0,0 kPa) respectively. The incubation medium used was granular, large grade Mandoval Vicafil vermiculite with a large grain size average of 8 mm x 6 mm x 6 mm (although smaller grain sizes were in abundance). For practical use, vermiculite was simply soaked in a bucket of water for 30 minutes before use.

One-hundred and thirteen snake and lizard eggs, the latter consisting of both hard and soft-shelled eggs, were incubated by the above method between 1987 and 1990. A further 103 snake eggs were incubated between 1991 and 1992. As the number of eggs incubated during these two periods were similar, it was decided to regard them as separate samples. Eggs of species incubated during the 1987/90 period are marked *, eggs of species incubated during the 1991/92 period are marked **, and the eggs of species incubated during both periods are marked ***.

Sauria: *Lygodactylus capensis capensis* *, *L. ocellatus* *, *Pachydactylus capensis capensis* *, *Mabuya quinquetaeniata margaritifer* *, and *Gerrhosaurus flavigularis* *.

Serpentes: *Dasypeltis scabra* *, *Pituophis melanoleucus sayi* *, *Elaphe obsoleta quadrivittata* X *E. o. obsoleta* *, *Lamprophis fuliginosus* **, *E. guttata guttata* **, *E. o. quadrivittata* ***, *E. taeniura freisii* ***, and *E. o. quadrivittata* x *E. o. obsoleta* X *E. o. quadrivittata* *E. o. obsoleta* (F1) ***.

Incubation containers were filled with saturated vermiculite to a minimum depth of 6 cm, with maximum depth being determined by the upper level of vermiculite being from 0 cm to 1 cm below the level of the top row of holes. The latter always applied in this study. Soft-shelled eggs were loosely buried two-thirds into the medium and very lightly covered with a sprinkling of vermiculite, while hard-shelled eggs were buried one-third into the medium and left exposed. The lids were then firmly closed.

As no environmental control chambers were available for incubating the eggs, and in order to compensate for a summer ambient temperature variation of between 8°C and 38°C, a simple incubator was devised. This was constructed from a steel trunk (L = 850 mm x W = 470 mm x H = 340 mm), painted white, both inside and out, and lined with 12,5 mm polystyrene sheeting. A heater and wafer thermostat were fitted to the inside of the lid. Two (4 mm) ventilation holes were drilled just above the floor at each bottom corner of the trunk, and a 6 mm ventilation hole was drilled in the centre of the lid. The ventilation hole in the lid could be closed to

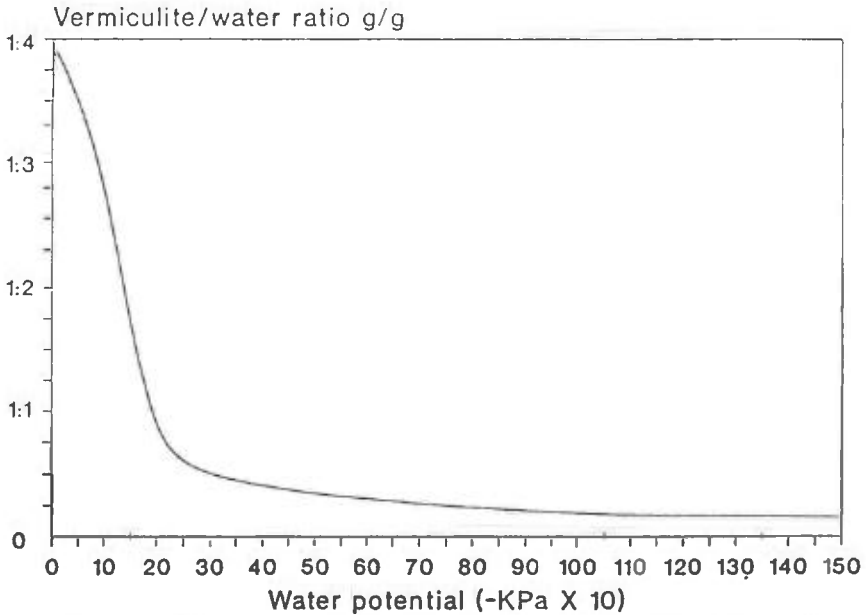


Fig.2. Water potential conversion chart for converting kPa to a vermiculite/water ratio where vermiculite is held constant at 1.

reduce air circulation when cooler conditions prevailed. In this manner incubation temperature was kept within a 25,5°C; to 29,5°C range. An open container of water was also placed inside the incubator.

For comparative purposes, an additional 42 snake eggs of *Dasypeltis scabra*, *Elaphe obsoleta quadrivittata*, and *Pituophis melanoleucus sayi*, from the 1987/90 period, were incubated using completely sealed containers, and incubation mediums of flaked and fine granular vermiculite. The mediums were mixed with water in a ratio of 1: 1 (g/g) (-160 kPa). During these incubations the containers were opened twice a week for aeration and sometimes the addition of water. These eggs were then incubated under exactly the same conditions as previously mentioned.

Both water determining values for vermiculite, kPa and V/WR, have been provided in order to accommodate both schools of use. Conversion values were extrapolated from the kPa to V/MR graph (Fig. 2). Because the capacity of vermiculite to hold water may not only vary with size, but also type (eg. flaked or granular); because different grades of vermiculite have been used by different researchers, with grade size often not being mentioned; and because grade size may vary from country to country; extrapolated comparative values should be considered as approximations.

RESULTS

Of the 113 eggs from the 1987/90, V/WR 1: 4 incubations, 22 eggs (19,5%) were presumed infecund due to there being no observable indication of embryonic development at the end of the incubation period. Of the remaining 91 fertile eggs, 86 eggs hatched successfully (94,5% hatching success), while five eggs containing well developed embryos failed to hatch (5,5% mortality).

Of the 103 eggs from the 1991/92, V/WR 1:4 incubations, seven eggs (6,8%) were presumed infecund due to there being no observable indication of embryonic development at the end of the incubation period. Of the remaining 96 fertile eggs,

91 eggs hatched successfully (94,8% hatching success), while five eggs containing well developed embryos failed to hatch (5,2% mortality). Further analysis revealed that in 60% of clutches (6) there was nil mortality, in 30% of clutches (3) there was one mortality, and in 10% of clutches (1) there were two mortalities.

Fungal infections remained confined to the infecund eggs during incubation, with no apparent infections spreading to, or developing on, fertile eggs. The spread of fungal infections to eggs containing dead embryos was rarely observed, and could be explained by the embryos only dying at a late stage of their development, with infections not having had time to become significantly established.

The incubations of eggs from the 1987/90 period with a V/WR of 1: 1, sealed containers, and fine vermiculite, resulted in the mortality rate being higher than the survival rate. Of the 42 eggs incubated, six eggs were presumed to be infecund due to there being no obvious indication of embryonic development at the end of the incubation period. Of the remaining 36 eggs, 13 eggs hatched successfully (36,1% hatching success), while 23 eggs contained developed embryos which failed to hatch (63,8% mortality). *D. scabra* recorded a 67% mortality, *E. o. quadrivittata* a 48% mortality, and *P. melanoleucus sayi* a 73% mortality.

DISCUSSION

There appeared to be numerous advantages in using a combination of coarse grade vermiculite, with either a V/WR of 1:4, or by simply saturating the vermiculite, and ventilated incubation containers. One apparent advantage was the minimal disturbance of containers during incubation. As the ventilation holes allowed for a certain amount of evaporation during incubation, it was necessary to keep the vermiculite moist without having to continuously open the containers. This was achieved by the lower row of holes providing a 5 mm deep reservoir of water at the bottom of the container. Owing to natural drainage, the upper layers of vermiculite became less saturated after standing for a short period, and therefore the eggs incubated in the drained upper layers, free from any hygroscopic influence of the reservoir. An advantage of using initially saturated vermiculite was that water potential, as of water/vermiculite ratios and kPa, become irrelevant. The containers were usually only opened 10 to 14 days after the eggs were set, as this appeared to be about the time it took for infecund eggs to become obvious, and again in the middle of the incubation period, but never within the last two to three weeks. When the containers were opened, the water level in the reservoir was also carefully checked, and water added down the side of the container if necessary. The series of holes in the incubation containers, and incubator, also allowed for an exchange of air through the coarse vermiculite. Trials indicated that containers could be left undisturbed for up to four months in the incubator without any appreciable desiccation of the medium.

A particular disadvantage of using fine granular or flaked vermiculite was that it tended to compact around the eggs during incubation, with flaked vermiculite sticking in layers to the eggs. This situation was aggravated by increases in the water portion of the medium and may have well contributed to the suffocation of the eggs by inhibiting the exchange of gasses between the egg and the atmosphere. It was concluded that fine granular and flaked vermiculite were unsuitable as incubation mediums. When using the aforementioned incubation mediums as comparisons to other studies, it should be noted that the difference between wet and dry will be relative to the moisture range being used, or compared to. In this investigation, and for convenience, a V/WR of 1: 1 (-160 kPa) has been taken as an arbitrary division between wet and dry incubation mediums.

The temperature fluctuation in the incubator was felt to be advantageous, in that when the eggs of environmentally sex determined (ESD) species were incubated, the temperature variation possibly allowed for a more 50/50 related sex ratio to develop. This latter statement may appear somewhat incongruous when referring to snakes, as the literature indicates that there is little or no ESD bias in snakes (Bull, 1980). However, a clutch of *E. o. quadrivittata* x *E. o. obsoleta* X *E. o. quadrivittata* x *E. o. obsoleta* (F1) eggs which were excluded from the incubator and allowed to reach 38°C, produced 100% females, 50% of which had chronically reduced eyes of only 10% normal size, while incubated clutches produced near to 50:50 males and females.

From the advanced developmental stages of dead embryos in the eggs, it was judged that the majority of embryo mortality occurred within the few weeks prior to hatching, and therefore could possibly have been related to the disturbance of the containers. It was also found over the numerous incubations that embryos of certain species appeared to react more adversely to the disturbance factor than others. From the V/WR 1:1 mortalities, it was noted that *P. melanoleucus sayi* eggs had a 73% mortality, *D. scabra* a 67% mortality, and *E. o. quadrivittata* a 48% mortality, all of which were incubated under the same conditions. This was contrary to the results of the undisturbed incubations where no particular pattern emerged.

Hatchlings were left in the incubation containers until their first slough, only after which were they removed to rearing containers.

Numerous studies have indicated that there is a strong relationship, particularly in soft-shelled eggs, between hatchability, size of hatchlings, and the water potential of the incubation medium (Tracy, 1980; Packard & Packard, 1987; Deeming & Ferguson, 1991; Packard *et al.* 1991; Christina, Lawrence & Snell, 1991). From these studies it would appear that there may be definite advantages arising from the use of wetter mediums. Packard *et al.* (1982, 1991) noted that the size of hatchling turtles was positively correlated with the net water-exchange experienced by the incubating eggs, with a wetter medium resulting in a higher hatching success and larger hatchlings, sometimes after a longer incubation period. As with this study, Packard *et al.* (1991) also found a correlation between hatching success and water potential, with incubations on the wettest medium (-150 kPa; V/WR = 1: 1.13) producing a hatching success of 87.9%, on the intermediate medium (-825 kPa; V/WR = 1: 0.18) 51.4%, and on the driest medium (-1500 kPa; V/WR = 1: 0.01) 25.7%. Galapagos iguanas, *Conolophus subcristatus*, from wet nests have been also been reported as having a higher survival rate than hatchlings from dryer nests (Christian *et al.*, 1991).

Eggs of the Cuban iguana, *Cyclura nubila*, showed a negative correlation between the percentage yolk and the percentage fat in respect to the whole hatching mass, although in this instance water potential in natural nests appeared to have no effect on hatchling size (Christain *et al.*, 1991). It was also noted that the more negative the nest water potential, the greater the retarding effect was on the conversion of yolk to fat, and consequentially the accrual of fat and the depletion of yolk was highest in hatchlings from wetter nests (Christian *et al.*, 1991). Although yolk was found to be an energy source utilized for growth, activity, and maintenance after hatching, the conversion of yolk to fat prior to hatching rendered fat as a long-term storage media which persisted in neonates for longer periods than absorbed yolk (Christian *et al.*, 1991). Water limitations during incubation may therefore result in the introduction of severe survival limitations for *C. nubila*, with negative ecological and energetic implications. Conversely, in species such as the Green iguana, *Iguana iguana*, and the Green sea turtle, *Chelonia mydas* yolk was found to be the quantitatively

greater storage medium with post-hatching energy being largely expended on activity (Christian *et al.*, 1991). Snapping turtles, *Chelydra serpentina*, have also been found to absorb energy reserves from yolk more rapidly when incubated in wetter environments (Packard *et al.*, 1982). Therefore, the conversion of yolk to fat may be dependent on whether energy expenditure in hatchlings is to be utilized for activity, as in *I. iguana*, or for growth maintenance, as in *C. nubila*. In these examples it would appear irrelevant as to whether yolk or fat is the greater storage medium, as life history strategies would appear to dictate that the most important energy source for a particular species will be greater when eggs are incubated on a wetter medium.

When the snake, *Ophedrys aestivus*, was given a choice of hydric environments in which to deposit eggs, preference was given to the wetter mediums of -200 kPa (V/WR = 1: 0.71) and -300 kPa (V/WR = 1: 0.45) (Plummer & Snell, 1988). Although these water potential may appear low when compared to this investigation, they were the highest presented to the snakes, and were also relatively high when considering that the driest environment presented was -2000 kPa (V/WR = 1: 0.01).

It was suggested by Tracy (1980) that fungi invaded viable *Sceloporus undulatus* eggs, and eventually killed the embryonic lizards, rather than invading eggs containing dead embryos. The highest mortality rate of 72% was recorded by Tracy (1980) on the wettest medium of -200 kPa (V/WR = 1: 0.71), and it was speculated that this was either due to the initial high influx of water into the eggs resulting in an increased invasion of microbes, or the suffocation of the embryo due to an environment in which the transport of respiratory gases was inhibited because the pore space between the vermiculite particles was more severely occluded by water. With the exception of the latter statement, this was contrary to all observations during this investigation where a considerably wetter medium (6 X) was used and fungal infections were primarily attributable to the infertility of the egg, and secondly, to the death of the embryo during development. It has been noted that the albumen of certain reptile eggs, such as those of *Testudo horsfieldii*, contain strong antibacterial properties which act against a wide spectrum of micro-organisms (Movchan & Gabaeva, 1967), and these properties may well be common in other reptile eggs as well. Broad & Fuller (1974) also found evidence of a variety of substances in the albumen of bird eggs, which either inhibit or kill bacteria and fungi. It would therefore appear that the high influx of water and microbes as a cause of fungal infections can largely be discounted. More feasible alternatives for the high mortality recorded by Tracy (1980) would be the use of fine or flaked grade vermiculite which would have resulted in the suffocation of the eggs, and the disturbance of the containers, which were opened at least twice a week. It is of interest to note, that with all other factors being equal, Tracy's (1980) second highest mortality (50%) was recorded on the driest medium of -590 kPa (V/WR = 1: 0.27) which would make the use of fine grade vermiculite and the disturbance factor common denominators.

There however does not appear to be a relationship between mortality, hatchling mass, and water potential of the incubation medium in hard shelled turtle eggs (Packard *et al.*, 1979). Packard *et al.*, (1979) noted that the hard-shelled eggs of the Softshell turtle, *Trionyx spiniferus*, incubated on substrata lower than -50 kPa (V/WR = 1: 1.36) resulted in a decrease in mass throughout incubation. Although the eggs of lizards which lay hard-shelled eggs (eg. *Pachydactylus* and *Lygodactylus*) are environmentally adapted to incubate in the surroundings in which they are laid, observations during this investigation showed that when removed to a drier environment, they may rapidly dehydrate. Under these circumstances it is therefore

necessary to incubate the eggs, and the above described method of using a high water potential incubation medium appeared to have no adverse affect on either the eggs, the hatching success, or the hatchlings.

The success of incubating reptile eggs may also be dependent on factors such as the composition of the egg shell, which in turn may be influenced by variables such as diet, health, geographic location, seasonality, habitat, climate and micro-environment. Initial clutch masses, clutch size, and relative clutch masses, which may be ascribed to factors such as geographic variation, may well be the result of phenotypic plasticity. Factors such as prey availability and diet have been found to influence phenotypic plasticity in reptile eggs (Seigel & Ford, 1991). However, Seigel & Ford (1991) found that although clutch mass and egg width were significantly higher in higher energy intake groups, as in the superior availability of prey and diet, there was no difference between egg mass, egg length, hatchling mass, and hatchling snout-vent length for either the high or low energy intake groups. The effects of factors influencing phenotypic plasticity on fertility and hatchability do not appear to have been researched in depth.

From the findings of this investigation and other similar studies, it would appear that wetter incubation mediums may have some distinct advantages over drier mediums. It is also indicated from the prevailing evidence that fine grade of flaked vermiculite may result in the suffocation of the eggs, and that the disturbance of the containers during the latter stages of incubation may also be a cause of death in developing embryos. The described method of incubating reptile eggs provided a successful incubating environment for a wide range of reptile eggs, and would appear to go some way in helping to eliminate certain adverse factors apparent in other incubating methods, thereby ensuring a higher hatching success.

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