THE EFFECTS OF HYDRIC AND THERMAL PROPERTIES OF INCUBATION SUBSTRATE ON EMBRYONIC DEVELOPMENT IN THE WATER SNAKE, *NATRIX TESSELLATA*

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ABSTRACT

We studied the effects of incubation substrate and its water content on egg water uptake, incubation duration, and hatchling mass in the diced water snake *Natrix tessellata*. Egg water uptake was highest in sand (the substrate with the highest thermal conductivity) and lowest in air (which had the lowest thermal conductivity). Water uptake was independent of initial egg mass. Incubation duration was correlated with substrate type and water content. Hatchling mass was independent of initial egg mass, but there was a weak negative correlation with total water uptake and with length of incubation. Wet mass of hatchlings incubated in air was greater than those incubated in sand or vermiculite. A greater increase of water uptake by eggs incubated in the most thermally conductive substrate (moist sand) suggests transport of water vapour, rather than liquid water, as the mechanism of transport across the membranes of parchment shelled eggs.

INTRODUCTION

The egg is the most vulnerable stage in a reptile's life. Immobile and often lacking parental care, it is susceptible to predation and exposed to the prevailing environmental conditions. Suitable substrate temperature and moisture are vital for successful development of the embryo and subsequent hatchling performance and behaviour (Packard & Packard, 1988; Burger, 1991).

It has been shown that incubation temperature not only affects incubation duration, but also sex ratios and juvenile behaviour in the pine snake (Burger & Zappalorti, 1988; Burger, 1989) and juvenile behaviour in black racers and king snakes (Burger, 1990). The effects of egg water uptake on hatchling size and physiological characteristics are, however, not clear. Miller, Packard, & Packard (1987) found that hatchling snapping turtles (*Chelydra serpentina*) incubated in moister media (-150 kPa) were larger and heavier and displayed superior locomotory ability relative to those incubated in dryer media (-850 kPa). Similarly, pine snake (*Pituophis melanoleucus*) hatchlings incubated in moister substrates were larger than ones incubated in dryer substrates (Gutzke & Packard, 1987). Some other species, however, showed no such effects (Packard & Packard, 1988). Moreover, the total energy reserves of the hatchlings were only sometimes affected (Gutzke & Packard, 1987), and the incubation period was generally longer for hatchlings incubated in wet substrates (Packard & Packard, 1988). Ratterman & Ackerman (1989), however, found that hatchling mass of the painted turtle *Chrysemys picta* was affected only by the initial egg mass and by incubation duration, but not by the water exchange rate of the egg.

The mechanisms of egg water uptake are controversial. Ackerman, Dmi'el. & Ar (1985), and Ackerman, Seagrave, Dmi'el & Ar (1985) considered water vapour diffusion to be the major mechanism for water transport across reptilian egg membranes. Kam & Ackerman (1990) concluded that the water uptake by *Chelydra* eggs could only be by vapour. Other workers (see Packard & Packard. 1988) have assumed a predominance of liquid water exchange. and Thompson

(1987) maintained that "in natural nests ... sometimes exchanges in the form of water vapour and sometimes exchanges in the form of liquid water would be more significant". Thompson (1987) however, did not demonstrate that liquid water exchange actually occurred, but only that a liquid water connection was present very early in incubation. Whereas experimental evidence (Ar, Koltai, Belinsky & Ackerman, 1990; Ar, Ackerman, Belinsky, Koltai, Blumberg-Binyamini & Dmi'el, in press; Kam & Ackerman, 1990) supports the importance of water vapour exchange, the relative importance of liquid water transport has not yet been established (Packard & Packard, 1988).

Ackerman, Seagrave *et al.* (1985) predicted that the thermal conductivity of the substrate, affected by both its physical properties and its water content, would greatly affect egg water uptake. This prediction was confirmed by Kam & Ackerman (1990) but not supported by Packard *et al.* (1987). If true, maternal choice of oviposition site (e.g. soil type, soil water content and temperature) could profoundly affect the hatchling.

To investigate the relative importance of water vapour versus liquid water transport in parchment shelled eggs, we incubated eggs of the diced water snake *Natrix tessellata* in substrates containing different amounts of water but of similar water potentials. We studied the effects of the different incubation regimes on incubation duration, hatching success and hatchling mass. We also examined the relationship between initial egg mass and hatchling mass under different incubation conditions.

MATERIAL AND METHODS

Gravid female *Natrix tessellata* were collected at Kibbutz Hazorea in northern Israel and transferred to the Canadian Centre for Ecological Zoology at Tel Aviv University. Snakes were housed in large enclosures and fed on fish. A large water bath and ample hiding places were provided in each cage. Cage floors were covered with sawdust; areas that were kept constantly moist served as oviposition sites and were inspected every 1-2 days for fresh eggs.

Thirty one eggs which were obtained from two clutches, were used in this study. The initial egg masses of the two clutches differed significantly ($P<0.001$, *t*-test). The 15 eggs of the first clutch (mean±SD: 5.98±0.33 g) were distributed randomly to incubate in either sand or vermiculite (sand 1 vs. vermiculite treatment, Table 1), and the 16 eggs of the second clutch (mean±SD: 7.52±0.29 g) were incubated in either sand or air (sand 2 vs. air treatment, Table 1). The relative humidity of all substrates was 100%; both sand 1 & 2 and the vermiculite had similar water potentials (-6 and -12 kPa, respectively). Liquid water contents, however, were drastically different: 0% for eggs incubated in air, 9% in the sand and 200% in vermiculite.

The eggs were incubated in eight plastic containers (20x14x8 cm) with pierced covers to allow adequate gas exchange. Each container was filled with the appropriate substrate. After recording their initial mass, three to four eggs were placed in each container. The eggs assigned to incubate in sand and vermiculite were buried completely in the substrate. Those incubated in air were placed on a plastic mesh, in a trench dug in the surrounding wet (-6 kPa, 9% water content) sand. The eight containers holding the eggs were placed in a temperature-controlled cabinet at 29±0.5°C. During incubation they were weighed (±0.01 g) at intervals of three to four days, and, depending on the change in mass, water was sprayed on the surface to supplement the amount lost. Every seventh day, after weighing the whole container, the eggs were taken out, carefully cleaned and weighed individually, using a Sartorius 1518 (±0.001 g) balance. Before returning the eggs to their containers, the substrate in each container was replaced by a newly prepared one, and water content and water potential were determined in the old substrates.

Sand containing 9% water was prepared by mixing 5 kg oven-dried sand with 450 g distilled water. The vermiculite (heat expanded mica) substrate was prepared by mixing 1.2 kg coarse vermiculite with 2.4 kg distilled water. To determine water contents during incubation, two samples were taken from each container at the end of every week, and were dried to a constant mass at 105°C. The water content level of each experimental medium was fairly stable during the entire incubation period (8.5%-9.3% for sand, 192%-207% for vermiculite). Also, no statistically significant differences were found between the values obtained for the containers of sand 1 and sand 2, nor between them and the sand of the "air" substrate ($F_{2,29}=0.21$, $P>0.8$). The water potential values were obtained by two methods, water retention curve (water potential as a function of water content) and tensiometry (Cassel & Klute, 1986). The characteristic water retention curves of the sand were constructed using the pressure plate method (Klute, 1986). Twelve such curves (two for each container of sand substrate) were constructed in the first and at the end of the fifth week of incubation. The water potentials were estimated from these curves using the measured water content values. In the second method we measured directly the water potential of the sand immediately after the eggs were taken out. This was done several times in each container, using tensiometers with a small, 3 cm long, ceramic cup (Soil Moisture Corp). There was good agreement (±2 kPa) between the two methods. For vermiculite, however, a reliable characteristic water retention curve could not be established. We therefore prepared different mixtures of vermiculite and water, sealed them in wide-mouth 1000 cm³ bottles together with jet-filled tensiometers (Soil Moisture Corp), and measured their corresponding water potential.

We did not measure the thermal conductivities of the

	Number of eggs	Initial egg mass (g)	Average mass change (mg d⁻¹)	Hatchling mass (g)	Ratio of hatchling to initial egg mass	Incubation duration (days)	Hatching success (%)
Sand 1	7	6.051 ±0.315	205 ±29	4.750 ±0.257	0.786 ±0.053	41.90 ±0.38	86
Vermiculite	8	5.920 ±0.423	103 ±17	4.781 ±0.185	0.785 ±0.015	41.10 ±0.38	88
P		NS	<0.005	NS	NS	<0.01	
Sand 2	8	7.477 ±0.223	231 ±28	5.203 ±0.393	0.690 ±0.046	43.00 ±1.15	88
Air	8	7.503 ±0.318	-8 ±0.4	5.597 ±0.358	0.749 ±0.044	42.10 ±0.38	88
P		NS	<0.001	NS	<0.05	<0.05	

TABLE 1. Effects of incubation substrate on egg water uptake, incubation duration, hatchling mass and hatching success in the water snake *Natrix tessellata*. Water potentials and water contents were -6 kPa and 9% in sand 1 & 2, and -12 kPa and 200% in vermiculite. All substrates were saturated with water vapour (100% RH). Incubation temperature was 29°C. Values are mean ± SD; NS = not significant.
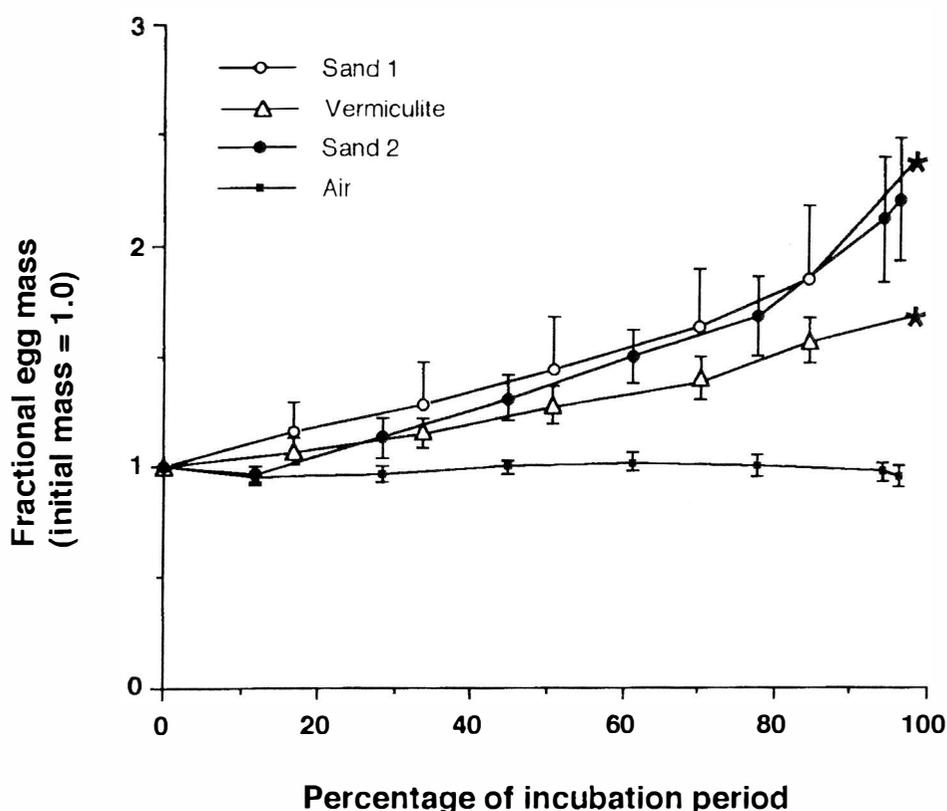
Fig. 1. Increase in the mass of *Natrix tessellata* eggs during incubation in air, sand and vermiculite. Mass change is expressed as egg mass relative to that at laying (initial mass = 1.00). Values are means±1 SD. (vertical bars). Most of the eggs incubated in sand 1 and in vermiculite hatched on the morning they were to be weighed for the last time; the last data point (*) is the mean of two eggs only.

different substrates. From values given in the literature (Ackerman & Seagrave, 1987; Kam & Ackerman, 1990) we estimate, however, that the sand thermal conductivity in our experiments was 6-8 times greater than that of the vermiculite (about 1.6 vs 0.2 W m^{-1} °C^{-1}).

RESULTS

Eggs developed and hatched successfully (88%) in all incubation substrates (Table 1). Water uptake by the eggs was independent of initial egg mass ($r=-0.21$, $P>0.1$) but it was dependent on the incubation substrate. The total and daily mass gain was significantly lower in eggs incubated in air than in those incubated in either sand 1 & 2 or vermiculite (Fig. 1; $P<0.05$. Wilcoxon's signed-ranks test); egg mass gain was highest in sand. The difference between the average rate of mass change (mg day^{-1}) of the eggs incubated in sand 1 and sand 2 (Table 1) was not significant ($t=1.64$, $P>0.1$). During the last third of incubation the difference in mass increase between eggs incubated in sand and vermiculite was very marked (Fig. 1). During this period, however, the difference in mass increase between eggs incubated in sand and vermiculite increased (Fig 1).

Incubation duration was related to substrate type and its water content. Eggs incubated in sand 1 hatched significantly later than those incubated in vermiculite (t-test, $P<0.01$), and those incubated in sand 2 hatched significantly later than those incubated in air (Table 1). These

differences, however, are rather small: less than a day for both treatments (i.e. about 2.5% of the incubation period). Hatchling mass was weakly negatively correlated with egg water uptake during incubation ($r=-0.42$, $P=0.05$). Hatchling mass, however, was not correlated with either initial egg mass ($r=0.31$, $P>0.1$) or duration of incubation ($r=0.17$, $P>0.1$).

DISCUSSION

The model suggested by Ackerman, Seagrave *et al.* (1985) presupposes a predominance of water vapour over liquid water transport across the egg membranes. In this model, environmental and internal water vapour pressures will determine the net movement of water to or from the egg. Water vapour pressure is especially dependent on temperature. If embryonic heat production raises the egg temperature, water vapour pressure inside it will be higher and the egg will lose more (or gain less) water (Ackerman, Seagrave *et al.*, 1985). The temperatures of the egg and its immediate vicinity depend, to a large extent, on the thermal conductivity of the substrate. This, in turn, is mainly determined by substrate moisture content. It is expected, therefore, that eggs incubated in substrates having similar water potentials but different thermal properties should absorb water at different rates, and that at the higher levels of embryonic heat production the differences in water absorption will be greater.

Our data support this model, although we cannot rule out

some exchange of liquid water across the egg membranes. As in Dmi'el (1967) and Ackerman, Seagrave *et al.* (1985), the incubation substrate affected the rate of egg mass increase (Fig. 1). Since all substrates were saturated with water vapour and had similar water potentials, differences in their thermal conductivity probably were responsible for the different rates of mass increase. The greatest change in mass occurred in sand, the most conductive substrate used in this study. Moreover, the highest mass increase was observed during the last third of incubation, when the metabolic heat production of *Natrix* embryos is highest (Dmi'el, 1970). The lowest mass increase occurred in air, the most thermally insulating substrate. Eggs incubated in moist vermiculite, intermediate in conductivity, showed an intermediate mass gain. Parchment-shelled reptile eggs, including those of *Natrix*, have a boundary layer of "dead air", whose resistance to water vapour transport is quantitatively equal to the egg-shell resistance. This layer, however, disappears when the egg is buried in the sand (Ackerman, Dmi'el *et al.*, 1985). The reduced water uptake shown by the eggs incubated in air is therefore probably due to two factors: (1) the low thermal conductivity of the air and (2) high resistance of the air boundary layer around the egg to vapour transport.

The inverse, though weak, relationship of *Natrix* hatchling mass to egg water uptake differs from the results of Gutzke & Packard (1987), who found that bull snakes hatched from eggs which were incubated in wet substrates (and therefore had high rates of water uptake) were heavier than those hatched from eggs incubated in dry conditions. It is also in contrast to Packard & Packard (1988, p. 567), who reported a positive correlation between egg water uptake of parchment shelled eggs and hatchling size. Dmi'el (1967), on the other hand, found no correlation between egg water uptake and hatchling size. Plummer & Snell (1988) also found that hatchling mass of the snake *Opheodrys aestivus* was not related to changes in egg water uptake during incubation.

We found hatchling mass to be independent of initial egg mass. This is in agreement with the findings of Packard & Packard (1987) for the snake *Coluber constrictor*. In the present study, the ratio of hatchling mass to initial egg mass was significantly higher for eggs incubated in air than for those incubated in sand 2 (Mann-Whitney U-test, $P<0.01$). If body size is indeed important to hatchling survival (Ferguson & Fox, 1984; Jayne & Bennett, 1990; Sinervo & Huey, 1990), depositing eggs in a moister substrate than necessary for egg development may be deleterious to the survival of the hatchlings. Excessive water uptake can, however, be avoided, if the female places the egg in saturated air on the surface rather than burying it. Subjecting the egg to dry conditions, on the other hand, will also have adverse effects. Thus, when laying parchment shelled eggs, the female reptile should choose a location where humidity conditions throughout the incubation period are (1) no higher than is necessary for normal development, and (2) not below the tolerance of the egg to dry substrates. These predictions are supported for pine snakes (Burger & Zappalorti, 1988), and by the findings of Perry (1990) and Perry & Dmi'el (1989) for the lizard *Acanthodactylus scutellatus*.

ACKNOWLEDGEMENTS

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