

WATER LOSS FROM *TRIONYX TRIUNGUIS* EGGS INCUBATING IN NATURAL NESTS

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ABSTRACT

In Israel, the oviparous softshell turtle *Trionyx triunguis* lays its eggs in nests buried in elevated sand bars along the banks of the Alexander river. We measured the temperatures and humidity prevailing in natural nests throughout the entire incubation period (78 days), and found that the eggs' hatchability was dependent upon the substrate wetness. In dry sand (water content 0.11 per cent, water potential -2760 kPa), the eggs lost an average of 31 per cent of their initial mass and failed to hatch. In a wetter substrate, in which the water loss of the eggs did not exceed 15 per cent, the eggs hatched successfully.

INTRODUCTION

Oviparous reptiles lay their eggs either in burrows, crevices or nests dug out in the soil substrate. The eggs are then left to develop unguarded by their parents and, throughout the entire incubation period, they are exposed to the impact of the changing physical environment prevailing in their immediate surroundings. There has been much documentation about the effect of temperature and soil water potential on hatching success (Packard, Taigen, Packard and Boardman, 1981; Packard, Packard, Boardman, Morris and Shuman, 1983). Most of the findings, however, were observed in eggs incubated in laboratories under artificial conditions. In this study we aimed at determining the effect of temperature and, especially, humidity, on the eggs of the Nile softshell turtle *Trionyx triunguis*, which were laid and left to develop in natural nests.

MATERIALS AND METHODS

Today, only one viable population of *Trionyx* has been found in Israel. It inhabits the Alexander river which flows westwards into the Mediterranean Sea. Female *Trionyx* lay an average of 40 hard, calcareous-shelled eggs, in nests buried some 30-40cm deep in elevated sand bars along both sides of the river. The laying season is from mid-May to mid-July, incubation lasts some 10-11 weeks and hatching commences approximately in mid-August, lasting for 2-3 weeks.

Three nests were monitored. One (nest A) was located on the northern side of the river, 20m from the river edge. This south-facing sand bank had an incline of 45° . It was poorly covered with sparse vegetation and thus was fully exposed to the sun's impact. The other two nests (B and C) were located on the southern side of the river in a steeper sand bank with scattered but better vegetation cover (*Artemisia monosperma*). Nest A was found three days after the eggs had been laid. Nest B and C were found at the time of laying. The eggs were carefully removed from the nests and numbered according to their position in the nest. They were weighed, their dimensions were measured and

they were then returned to their original position in the nest.

Temperatures of the nests were measured by calibrated copper-constantan thermocouples which were connected to a portable thermometer Wescor TH65. The thermocouples were placed between the eggs at the bottom, centre and top of each nest. For measuring the water potential of the nest's soil we used ceramic-head probes of a Wescor Dew Point Microvoltmeter HR-33T. The probes were placed together with the thermocouples. Water potential values were obtained either from direct reading of the dew point probes, or by using a previously prepared calibration curve in which water potential values were directly related to the water content of the same soil. Soil samples were taken from the sides of the nests and at different depths adjacent to the nests. They were weighed, oven dried to a constant mass and their water content was determined. Known amounts of water were then added to the dry soil, and the water potential of the mixture was measured with the dew point probes (Horton, Wierenga and Nielsen, 1982). There was a good agreement (± 5 per cent) between the two methods.

The nests were measured once a week every four hours throughout a 24 hour cycle. After the first weighing, the eggs of nest A were weighed twice more, 10 and 40 days after being laid. The entire treatment (weighing and returning the eggs to their original position) lasted up to 45 minutes, the physical equilibrium of the nest (temperature and humidity) was restored within the next four hours. The eggs of nests B and C were taken from their nests after 70 days of incubation. They were weighed and incubated in the laboratory during the last seven days in incubation in the same soil substrate as the natural nest at a constant temperature of 30°C .

RESULTS

The initial mass of the 108 eggs of the three nests was 17.82 ± 1.1 g (SD), and their average size 30×30 mm. The incubation period, 76-78 days, was similar for the three groups as well as for other nests located in the same area. Hatching success, however, was quite

different. It was 99 per cent and 95 per cent in nests B and C, respectively, but only 64 per cent in nest A.

We miscalculated the hatching date of nest A and reached the nest site one day after the eggs had hatched. We found 15 dead hatchlings at various stages of hatching, from cracked eggs up to the stage of a body with only a few pieces of shell attached. Of the 15, seven were identified by numbers. These, and probably all of the eggs that failed to hatch, belonged to the top layer of nest A (Fig. 1). Five of the largest and least-damaged dead hatchlings had a mean carapace length of 33.5mm (32.0 to 36.3mm), whereas that of nest B and C's hatchlings was 38.5 ± 5.2 mm (SD).

A comparison among the physical environments of the three nests (Table 1) shows that the temperature of nest A was higher by 1.6 and 0.8°C than those of nests B and C, respectively. However, these temperature differences between corresponding layers exist along

the entire depth and not only between the top layers of the nests. Therefore, the relative hatching failure of nest A's eggs cannot be attributed to the temperature differences. On the other hand, the top layer of nest A is 6-7 times drier and its water potential is 4-11 times greater than the corresponding layers of nests B and C, whereas the bottom layers of the three nests are much the same. This indicates that the eggs in the top layer of nest A were susceptible to a much higher rate of water loss.

The rate of water loss was estimated from the mass changes of the eggs. The three weighings of nest A's eggs yielded a linear mass change for each egg. This allowed us to assume a constant rate of daily mass change and to predict the total mass loss of each egg on the day of hatching. Similarly, we calculated the total mass loss of the eggs from nests B and C. Fig. 1 shows that 12 out of the 13 eggs laid in the top layer of nest A, and one or two eggs in the next layer, would have suffered a total loss of 20-54 per cent of their initial mass, whereas those on the bottom might even have gained some water from their surroundings. The average mass loss of the top layer's eggs of nest A (31 per cent) is 14-15 times the total loss of the eggs laid in the top layers of nests B and C. In the bottom layers, however, the mass changes of all eggs are within a comparable range (Table 2).

DISCUSSION

The hatching failure of the top layer eggs in nest A can be attributed to several biotic (e.g. predation, egg-handling) and physical factors. Predation, however, can be excluded since marks of digging or footprints of jackals and mongeese, the most common predators in the research area, have never been seen in the nest vicinity. Also, the extra handling during mid-development of nest A eggs might not necessarily have a pronounced effect on hatching success. Since each egg was weighed and handled in the same manner, handling is expected to affect the entire clutch and not only the top layer eggs. Moreover, of the hundreds of metabolic measurements performed in our laboratory on *Trionyx* eggs, many were done on eggs which were weighed and handled in the same manner and frequently as those of nest A. Hatching success was about 90 per cent (Leshem, unpublished data).

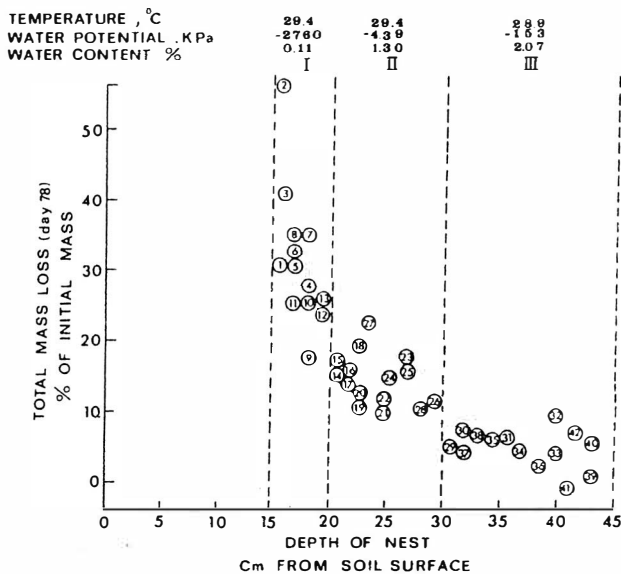


Fig. 1 Nest A. Water losses of *Trionyx* eggs (expressed as % of the initial egg mass) as a function of the depth at which they were buried. Encircled numbers depict the position of individual eggs as they were arranged in the nest when laid. Average temperature and soil humidity conditions at three depths (I, II, III) are given at the top of each layer.

Nest	Depth of layer from soil surface (cm)								
	A	15-20			25-30			35-40	
		B	C	A	B	C	A	B	C
Water content	0.11	0.62	0.75	1.30	—	—	2.07	1.50	1.00
% of dry sand	± 0.02	0.15	0.15	0.22	—	—	0.25	0.30	0.20
Water potential	2760	718	220	439	—	—	153	162	106
-kPa	± 208	85	43	50	—	—	62	40	16
Temperature	29.4	27.6	28.6	29.4	27.8	28.4	28.9	27.4	28.2
°C	± 2.9	1.8	1.0	1.8	2.0	1.1	1.6	2.1	1.1

TABLE 1: Environmental variables prevailing in three *Trionyx* nests. Values are mean ± SD.

Apparently, the excessive water lost from the eggs that were laid in the dry upper layer of nest A, was the prime factor determining their hatchability.

Layer depth cm		A*	Nest B	C
15-20		31.1	7.1	1.7
	±	9.6	5.4	4.2
25-30		14.2	2.7	0.5
	±	3.5	1.6	1.2
35-40		4.8	2.0	-0.4**
	±	2.9	1.4	1.4

TABLE 2: Total water loss (% of initial egg mass) of *Trionyx* eggs incubated in natural nests. Values are mean ± SD.

* Predicted values (see text)

** Net gain

The exact mechanism by which reptilian eggs exchange water with their immediate surroundings, whether it is via liquid (Tracy, Packard and Packard, 1978) or via water vapour (Ackerman, Dmi'el and Ar, 1985), has not yet been fully explained. It is known, however, that the rate of water exchange is lower in calcareous shelled eggs than in flexible or leathery shelled eggs (Ackerman *et al.*, 1985). Packard, Taigen, Boardman, Packard and Tracy (1979) and Packard *et al.*, (1981) showed that *Trionyx spiniferus* eggs declined in mass throughout incubation when artificially incubated on substrates of different water potential, and that rates of decline in mass were lower for eggs incubated on wetter than on drier substrates. Our measurements of the carapace length indicate that if the failed-to-hatch embryos of nest A would have hatched, they would be shorter than those of nests B and C. This is in accord with the observation made by Packard *et al.* (1983), that painted turtle eggs exposed to dry substrate produced shorter hatchlings than eggs associated with wet media. Our findings thus support earlier observations and further emphasise the importance of choosing the appropriate nesting site by the female turtle.

A total water loss of about 15 per cent (Fig. 1 and Table 2, centre layers of nest A) during natural incubation is probably the upper line separating hatching success or failure in *Trionyx triunguis* eggs. This value is very similar to that calculated for avian eggs (Ar and Rahn, 1980). The hatching of eggs which are laid in a dry substrate such as the top layer of nest A, would be seriously jeopardised.

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