Short Notes

Needles and haystacks: the location of lizard eggs in sand dunes

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Most reptiles do not demonstrate parental care. The female typically deposits her eggs in a burrow and leaves; unless environmental conditions (e.g. temperature, humidity and gas composition) remain within narrow limits, the egg will not develop successfully. Soft-shelled eggs, typical of lizards and snakes, have an especially low tolerance for dehydration, and require a nearly saturated environment throughout their incubation (Packard and Packard, 1988; Ackerman, 1991). Although the egg stage may be critical in understanding the life cycles of reptiles (Overall, 1994), information on the incubation conditions and egg requirements of many species is not available.

The fringe-toed lizard *Acanthodactylus scutellatus* (Lacertidae) is common in sandy soils in central and southern Israel, and is an important element of the dune fauna there (Werner, 1987). Many aspects of its biology and reproductive cycle have previously been studied (see Perry and Dmi'el, 1989a, 1994; Perry, 1990; Perry et al., 1990; Frankenberg and Werner, 1992). Exept for an inconclusive preliminary report (Perry and Dmi'el, 1989b), however, nothing has been published on egg requirements and environmental conditions in the dunes it inhabits. Although it is ubiquitous and despite a massive effort, Perry (1990) could not find any eggs in nature. In this study we combine data on egg physiology, conditions in the soil, and the ecology of adult lizards to create a simple model to predict the location of *A. scutellatus* eggs in nature. Because we were interested in studying realistic conditions, much of our laboratory work was conducted under naturally fluctuating temperature regimes.

Three dune sites along the coastal plain of Israel were chosen for this study: the dunes at Nitzanim (31'43'N 34°36', 400 mm annual rainfall, 1.8% plant cover, with *Artemisia* as the dominant plant) and Holon (32°02'N 34°45', 500 mm annual rain, 2.3% cover, *Scrophularia* dominant); both have large populations of *A. scutellatus*. At a third site, Hadera (32°24'N 34°54', 550 mm annual rain, 2.2% cover, *Artemisia* dominant), *A. scutellatus* is absent and the closely related *A. schreiberi* is common. Data were collected from July 1987 until July 1990. Most of the work was conducted at Holon, which was sampled at least once a month. Other sites were sampled at least every other month throughout most of the study period. All sites receive rain only during the winter and early spring (October to March). To allow comparisons and controlled observations we built a sand-filter enclosure (1.5 × 10 m wide and 0.5 m deep) in the Segals Garden for Zoological Research (SGZR), Tel Aviv University. Lizards were collected at Holon and introduced into the enclosure two years after construction was completed. Observations and measurements in the enclosure were carried out from March 1989 until July 1990.

Soil water contents were obtained in the field using a SoilMoisture Lord soil core sampler. Two samples were taken at each of 10 cm intervals from the surface to a depth of 60 cm. Samples were sealed in the field and weighed in the laboratory (using a Sartorius 1518 balance) to the nearest 1 mg within 5 hours of sampling. They were then dried to a constant mass at 105°C. The pressure plate technique (Klute, 1986) was used in the laboratory to determine the relationship between water potential and water content in the soil at each of the sites. In addition we permanently installed and used tensiometers (SoilMoisture 2725A) for measuring soil water potential at depths of 20 and 40 cm in the enclosure. Soil temperatures were measured using pre-calibrated copper-constantan thermocouples, buried at 10 cm intervals: from the surface down to a depth of 60 cm in the field, and to 40 cm in the enclosure. Temperature 20 cm inside a single burrow was repeatedly sampled using the same techniques. A datalogger (Campbell 21X) was used to record and store temperatures every 10 minutes.

Egg requirements were estimated in the laboratory from eggs laid in cages at the SGZR. Twenty seven eggs were individually incubated, buried in 100 cc vessels filled with sand. Three temperatures (26, 30 and 35°C) and three soil moistures (0.5, 3.0 and 5.5%) were used. Three eggs from separate clutches were exposed to each combination of humidity and temperature. To maintain soil moisture constant, we weighed the eggs and replaced the sand with a freshly-made mixture every seven days. Additionally, 48 eggs were incubated at different temperatures in moist (5% water) sand: ten eggs at 36°C, ten at greatly fluctuating temperatures (26-35°C daily), and 28 at slightly fluctuating temperatures (28-32°C daily). Shell water vapor conductance was measured and calculated using the method of Ackerman et al. (1985). *A. scutellatus* eggs were placed on an analytical balance in a chamber with known relative humidity and temperature. Each egg was weighed to the nearest 1 mg at five minute intervals, and its surface temperature was measured at regular intervals. Shell water vapor conductance was then calculated from rate of mass change, shell surface temperature, and air temperature and humidity. Osmotic concentration of the eggs was measured using a Wescor 5500

osmometer. Water vapor pressure of the egg and the environment were calculated from the measured values of water potential (Packard et al., 1985).

Surface water content at Holon was always low (fig. 1). As the dry season progressed, moist sand (water content > 1.0%) could only be found at greater and greater depths. By September, however, the soil profile was dry to a depth of at least 60 cm. Winter rains begin falling in October or November, at which time the soil profile becomes rapidly rehydrated. Similar values were obtained at the other sites, and in the enclosure, with the exception of maximum soil moisture at Hadera, which was 12% in early spring.

The relationship between water content (WC, %) and water potential (WP, kPa) at Holon is given by the equation

$\log WP = 2.18 - 1.78 \log WC$ ($r^2 = 0.77$).

Above 6% water content, water potential remained low. In dry sand (less than 0.5% water content), water potential climbed asymtotically. Between 6% and 0.5%, the change in water potential was intermediate. The relationships at other sites were similar;

$\log WP = 1.24 - 0.44 \log WC$, $r^2 = 0.83$ at Hadera
$\log WP = 1.05 - 0.44 \log WC$, $r^2 = 0.81$ at Nitzanim

These equations indicate that soil structures were similar at the three sites.

Temperature fluctuations at Holon decreased with depth. At the surface and at a depth of 10 cm, daily temperature fluctuations of 15°C or more were not unusual during
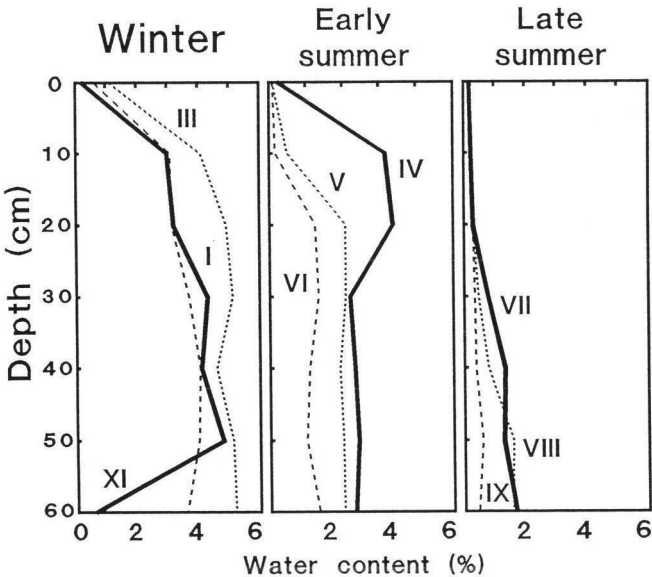

Figure 1. Changes in soil water content at different depths in a Holon sand dune. Roman numerals indicate months of the year.

the reproductive season. At a depth of 30 cm, however, daily variation in temperature did not normally exceed 4°C and was nearly constant at 31°C from May till the end of September. The situation was similar till a depth of 60 cm. Conditions at Nitzanim and in the enclosure were similar to those at Holon. No systematic temperature measurements were obtained at Hadera.

Twenty six of 28 eggs incubated at 5% water content and at 28-32°C hatched successfully. Egg shell conductance to water vapor was 1.0 g day^{-1} kPa^{-1}, a value very similar to that predicted by Ackerman et al. (1985; 1.03 g day^{-1} kPa^{-1}) for eggs of this size. Eggs increased in mass throughout incubation, and towards the end of the incubation period reached an osmotic concentration of 162.4 mmol kg^{-1} (SD = 24.11, n = 6) and an average water potential of 364 kPa. Eggs incubated in wet (>10%) or dry (<0.5%) substrates died rapidly, possibly because of the combination of handling and unfavorable incubation conditions. In wet substrates, eggs immediately started increasing in mass and volume; within a week the shell showed stretch marks, and those were immediately followed by the appearance of droplets of liquid on the shell, and death. In very dry substrates eggs dehydrated in less than a week. Under intermediate conditions, eggs survived over four weeks. No hatching was achieved in eggs incubated at 36°C or at greatly fluctuating temperatures (>10°C daily change).

Water and temperature cycles were similar in all study sites. We will therefore use the results obtained at Holon, the site for which we have most information, to illustrate our point. When laying its eggs the female should choose a location where humidity throughout the incubation period is no higher or lower than the eggs can tolerate. Even in winter, water content at the soil surface is low (fig. 1). As the season progresses, the amount of water in the soil column increases, then starts decreasing again in early spring. Drainage and evaporation act to decrease water availability, and at the end of summer the soil is practically dry to a depth of at least 60 cm. These patterns are typical of soils that lack a water table (Payne, 1988).

Temperature is important in controlling chemical, physical and biological processes in the soil. It has strong direct and indirect effects on incubation. Length of incubation and, in many reptile species, sex, are directly affected by soil temperature. Indirectly, temperature may affect incubation success by influencing water vapor pressure. The soil temperature profiles found in the present study were similar to those reported from other soils (Payne and Gregory, 1988). Temperature fluctuations were smaller as depth increased, and daily temperature fluctuations at depths greater than 30 cm were no higher than 2°C. Seasonal changes in soil temperature were smaller at greater depths, and were almost nonexistent at Holon during the reproductive season. Seasonal variation at Nitzanim was slightly more pronounced, but was nearly constant at 31°C from early July till late August. Due to different thermal inertia in the egg and its environment, fluctuations in egg temperature should be slightly different from those in its environment. In a saturated atmosphere, a change of one degree, from 29° to 30°C, will cause a change of 0.237 kPa in water vapor pressure (Diem and Lentner, 1970). Given

the water conductance values we measured, an *A. scutellatus* egg that is warmer than its environment by 1°C will lose over 20% of its mass per day.

Integrating egg requirements and prevailing temperature and water availability in the soil throughout the year allows us to predict where in the soil column *A. scutellatus* eggs should be located, depending on when in the season they are laid (fig. 2, crosshatched rectangle). Using our data for water content in Holon soil (fig. 1; June, 30 cm depth) and the corresponding values of water potential, we calculated the relative humidity of the soil atmosphere to be 99.95% and the water vapor pressure as 4.491 kPa, just above that of an egg at the same temperature (99.74% and 4.481 kPa). Thus, water vapor will move from the soil atmosphere into the egg. By September, however, the soil at a depth of 30 cm (99.24%, 4.459 kPa) is less saturated than the eggshell. Thus, an egg buried at 30 cm in September will lose water to its environment.

It is surprising that the minimal depth indicated, 30 cm, is greater than the maximum depth known for *A. scutellatus* burrows in the field (20 cm; Perry and Dmi'el, 1994). Adult activity cycles and juvenile emergence patterns, however, support our model. Given the small egg (1.1 g on average) and clutch (2.5 eggs; Perry and Dmi'el, 1994) sizes and the difficulties of working in a shifting sand dune, it is not surprising that no eggs were found in the field. However, finding burrows extending to the predicted depth in the enclosure, and especially finding an egg shell at a depth of 40 cm, validate our model. Though females and juveniles have been found together in the same burrow systems in captivity (Perry, 1990), maternal care remains unknown in *A. scutellatus*.
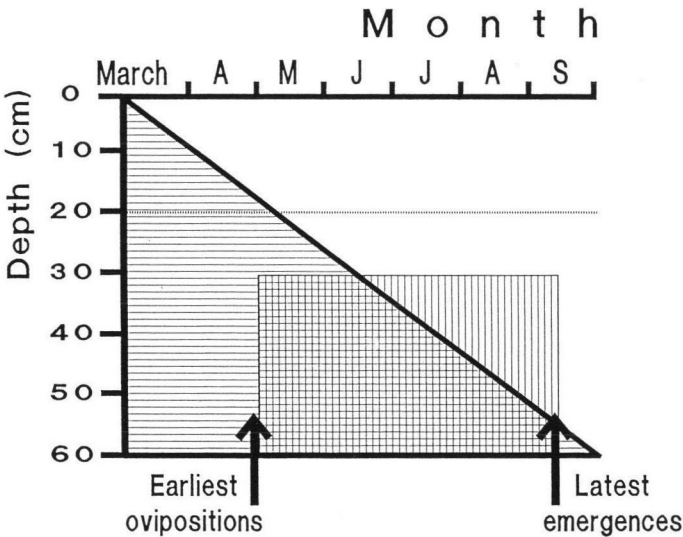

Figure 2. Water and temperature conditions in a Holon sand dune during the *Acanthodactylus scutellatus* reproductive season. Horizontal lines represent area with appropriate hydric conditions, vertical lines the area in which temperature is stable at 29-33°C throughout the day. Eggs can only develop where both humidity and temperature are appropriate (hatched rectangle). Dotted line depicts depth of deepest known burrows in the field.

The concordance between egg requirements and adult egg-laying behavior is impressive. However, adults emerge from hibernation considerably before the first eggs are laid, and even the last juveniles to emerge remain active for several months after hatching (Perry and Dmi'el, 1994). Why, then, is the reproductive season so limited? We suggest that eggs deposited outside this period are unlikely to hatch. Before the beginning of the season there is no shortage of water in the soil, but temperatures in the soil are too low (at 30 cm) or too variable (closer to the surface). After the season, temperatures below a depth of 30 cm are appropriate, but sufficient water can only be found at depths of over 60 cm.

Can these considerations be used to explain the distribution of *A. scutellatus* in Israel? *A. scutellatus* is not found at Hadera, where it is replaced by the closely related *A. schreiberi*. The water profile was very similar to those at Holon and Nitzanim; although we have not systematically measured soil temperatures at Hadera, surface conditions were very similar to those at the geographically close Holon site. Thus, we cannot attribute the lack of *A. scutellatus* at Hadera to egg physiology. Most likely, this absence is due to a physical barrier, probably the Yarkon river, that prevented the expansion of *A. scutellatus* north.

Egg physiology, soil conditions and adult reptile behavior are strongly linked. In the case of *A. scutellatus*, adult choice of reproductive season can be explained by egg requirements and the availability of water and heat in the sand profile. Although these factors could not explain the northern distributional border of *A. scutellatus*, they may be important in explaining microhabitat selection in other reptile species. Since different soil types have different thermal conductances and water holding capacities, some organisms may be limited to the vicinity of areas that provide adequate conditions for egg development. Population-level variation in reptile reproductive cycles (Overall, 1994) could partially be the result of differing conditions in the soil.

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Water flux in the Australian treefrog *Litoria caerulea* under natural and semi-natural conditions

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Rates of water flux can be measured by monitoring the decline of injected isotopically
labelled water in the body water of an animal over time (Nagy and Costa, 1980). This
technique has been used to assess the water fluxes of a number of species of arthropods,
retiles, birds and mammals in their natural habitats (Nagy, 1982; Nagy and Peterson,
1988). Although the technique can be used to study water fluxes of amphibians and
aquatic animals in captivity, the high water fluxes of these animals make it difficult to
use the technique under natural conditions. In an extensive review of studies of water
flux rates in different animal groups, Nagy and Peterson (1988) were not able to
determine the relationship between water flux and body mass in adult amphibians