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TEMPERATURE SELECTION FOR EGG INCUBATION BY THE LIZARD *PODARCIS HISPANICA ATRATA*

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This paper reports on the selection of substrate temperatures at oviposition sites by female lizards *Podarcis hispanica atrata* under laboratory conditions. Seven females deposited 11 clutches (a total of 34 eggs) in very small areas, representing 20 % of the available surface. The mean temperature of the sites used for egg-laying was 25.7 °C. Results are compared with incubation temperatures of the same species under natural conditions (Columbretes islands, Mediterranean, Spain).

INTRODUCTION

Temperature has an important and instantaneous effect on the rate of embryo development, and hence on the duration of egg incubation, and can have long-lasting effects on the morphology, physiology, behaviour, and survival of hatchling reptiles (e.g., Packard & Packard, 1988; Van Damme *et al.*, 1992; Janzen, 1993; Overall, 1994; Castilla & Swallow, 1996). Thus, the decisions that female reptiles make while selecting an appropriate egg deposition site have important consequences on their reproductive success. Notwithstanding their importance, the factors that influence the choice of egg-laying sites are a neglected part of lizard biology. This is hardly surprising, given that lizard clutches are extremely difficult to locate in the field. Consequently, little information is available about the environmental conditions in natural nesting sites (but see Bock & Rand, 1989; Christian, Lawrence & Snell, 1991; Christian & Lawrence, 1991; Burger, 1993; Castilla & Bauwens, 1996). Therefore, laboratory studies should provide a fruitful alternative, because eggs are easier to locate and environmental factors can be controlled more readily and precisely.

In this study, selection of substrate temperatures for egg deposition by *Podarcis hispanica atrata* are examined in a laboratory photo-thermal gradient. Confinement to a terrarium undoubtedly produces unnatural stress. However, individuals of this species have repeatedly shown (e.g., during this study) to tolerate captive conditions well enough to perform normal activities (e.g., thermoregulation, foraging, social interactions, copulation). Hence, egg-laying behaviour by *P. hispanica atrata* is considered to be largely unaffected by captivity. In addition, captive conditions enabled lizards to select the substrate temperature for egg deposition from a wide range of available temperatures. Thus, although the results do not necessarily apply to field conditions, they do indicate temperature selection in a laboratory situation.

MATERIALS AND METHODS

P. hispanica atrata is a heliothermic lizard endemic to 20 ha of the Columbretes archipelago (Mediterranean Sea, Spain; Castilla & Bauwens, 1991*a*, 1991*b*, 1996). In their natural habitat, lizard density is high (up to ca. 800 ind/ha; Castilla & Bauwens, 1991*b*). Females attain sexual maturity within their first year of life at a snout-vent length (SVL) of 50 mm and, within a single reproductive season (April-June), they may produce up to at least three clutches composed of 1 - 5 eggs (Castilla & Bauwens, 1996). During the egg-laying period, dense concentrations of pregnant females were observed in several very restricted areas (Castilla & Bauwens, 1996). In addition, the only nesting site found in the Columbretes islands is a communal nest where 240 eggs were found over two consecutive years (Castilla & Bauwens, 1996). Together, these data indicate that communal laying behaviour could be a common behavioural characteristic of this species. As *Podarcis hispanica* possesses sex chromosomes (Odierna, Kupriyanova, Capriglione & Olmo, 1993), the thermal conditions during incubation do not affect sex determination.

During June-July 1995, seven females (mean SVL ± 1SE: 60.8 ± 1.9 mm) and four males (mean SVL ± 1SE: 62.8 ± 3.0 mm) were kept in two terraria (100 x 40 cm) at a laboratory in Hasselt (Belgium). Several lizards were intentionally kept in the same cage (3 females + 2 males, and 4 females + 2 males), considering the high population densities and aggregations of gravid females in the field. Lizards in terraria were provided with water (supplemented with vitamins) and food (meal-worms and crickets) *ad libitum*. The substrate of the terraria consisted of a 7 cm thick layer of eolic sand of very fine grain. The entire sand surface was covered with litter, and flat stones and pieces of wood were scattered over the entire surface. Illumination and heat were provided by a 150 W light bulb suspended 25 cm above the substrate near one end of the terrarium (Fig. 1). The

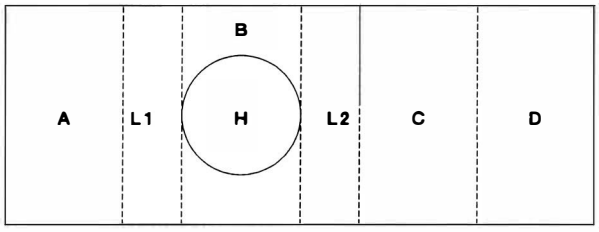

FIG. 1. Thermogradient (100 x 40 cm) designed to examine temperature selection for egg incubation by the lizard *Podarcis hispanica atrata*. Shown are the different sections where substrate temperature was measured (n = 6 records/section). L1 and L2 are the sections where all clutches were found; H is a circle of 15 cm diameter at 25 cm below a 150 W bulb.

light bulb was switched on daily from 0900 to 1800 h; within this interval, the bulb was disconnected for 15 min periods at 1015, 1130, 1345, 1500 and 1600 h. As the substrate was heated by the light bulb only, substrate temperatures followed a cyclic regime. I deliberately chose a cyclic temperature regime, because it more closely simulates natural conditions. I also chose an interrupted light cycle, because lizards in the field may not be active continuously.

During a single day, substrate temperatures were measured at a depth of 7 cm, using a thermocouple connected to an electronic thermometer (Digitap Therma 3; to the nearest 0.1 °C), during nine hourly intervals (0700-0800, 0900-1000, 1000-1100, 1200-1300, 1300-1400, 1500-1600, 1700-1800, 1900-2000, 2000-2100 h). During each hourly interval, temperature was measured atsix different sites within each of seven sections of the terrarium (Fig. 1). The daily average substrate temperature was estimated in each section by calculating the mean of the readings obtained at 0700-0800 h and 1700-1800 h, which correspond to the minimum and maximum daily temperatures. This approach is considered reliable on biophysical grounds (W. P. Porter, pers. comm.). In addition, in a previous study of artificial egg laying sites for *P. hispanica atrata* (Castilla & Swallow, 1996), a significant correlation was found between the average of the two extreme temperatures and the mean value obtained from measurements made at 3 h intervals throughout a day. At the very least, the approach provides an index of the

TABLE 1. Descriptive statistics of substrate (7 cm deep) temperatures (in °C) in the different sections of the thermogradient (see Fig. 1 for section codes).

Section	Mean	Variance	Minimum	Maximum	n
L1	26.11	4.92	22.1	30.0	54
L2	25.77	3.75	22.1	28.8	54
B	26.61	5.43	22.1	29.9	54
H	29.02	13.05	22.1	33.7	54
A	24.60	2.01	22.1	27.1	54
C	24.37	1.37	22.1	26.0	54
D	23.63	0.58	22.1	24.6	54

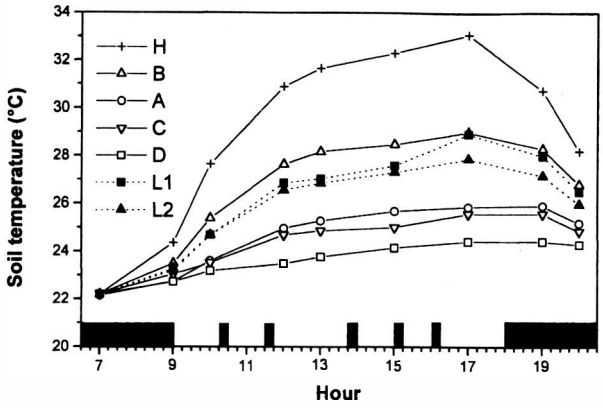

FIG. 2. Diel variation of substrate temperatures (depth: 7 cm) recorded on 27 July 1995 in different sections within a thermogradient (see Fig. 1). Shaded areas indicate the time intervals when the lightbulb was switched off. L1 and L2 denote the sections when all clutches were laid.

relative temperature differences among various sections within the terrarium. Note that "daily average temperature", i.e., the mean of the records obtained at 0700-0800 h and 1700-1800 h, differs slightly from the arithmetic mean temperature, i.e., calculated by averaging the readings obtained during all seven hourly intervals (Table 1).

Humidity potentially influences the females' searching behaviour for laying sites, because extreme dry and extreme wet conditions affect egg development in some lizard species (Muth, 1980; Packard, 1991). Water was sprayed uniformly over the entire surface of the terraria every other day. Despite its apparent importance, humidity could not be measured in this study. Only the soil water content was estimated (0.6 g of water per 10 g of dry sand) by weighing wet sand, drying it in an oven (90°C) for 1 h and reweighing it.

To avoid disturbance of the terraria, no attempt was made to locate the clutches until all females had deposited their eggs. To confirm that all clutches were laid, both terraria were regularly inspected for the presence of post-reproductive females.

RESULTS

A total of 11 clutches (one to three per female), comprising a total of 34 eggs, were found. All were discovered at a depth of ca. 7 cm (the maximum depth of the substrate) in two very narrow (10 x 40 cm each) sections near the bulb (L1 + L2, Fig. 1). The null hypothesis was examined, that females used different zones in proportion to their availability. As all clutches were found in 20% of the available surface, and none in the other areas of the terrarium, the observed distribution differed significantly from the expected one (χ^2 = 44.0; 1 df; $P < 0.001$). Overall substrate temperatures fluctuated between 22.1 and 33.7 °C (Table 1, Fig. 2). Hourly variation of substrate temperature was significant in all sections of the thermogradient (ANOVA, $P < 0.001$ in all cases; Fig. 2). In the two sections where all clutches were located, substrate temperatures varied

between 22.1 and 30.0 °C (L1) and 22.1 and 28.8 °C (L2). *P. hispanica atrata* avoided the lowest and highest substrate temperatures available in the thermogradient (Fig. 2) and laid their eggs at sites that experienced a daily average substrate temperature of 25.7 °C.

DISCUSSION

Because several females were housed together in single terraria, the first ovipositing female could have guided the others to lay at the same site. However, this cannot explain why the same areas were chosen in both terraria. Therefore, the deposition of all clutches in a restricted area, corresponding to a narrow range of substrate temperatures, was interpreted as evidence for the existence of very precise cues of temperature selection for egg incubation.

The precise selection of substrate temperatures must be seen in the light of the pervasive effects of temperature on the rate of embryo development and characteristics of hatchlings in lizards. Very low (20 °C) and very high (> 32 °C) mean constant incubation temperatures have lethal effects on the eggs of some closely related lacertid lizards (Van Damme *et al.* 1992; D. Bauwens, pers. comm.). It is evident that lizards will avoid laying their eggs at such extreme temperatures. However, the daily mean substrate temperatures in all sections of the thermogradient, as well as the minimum and maximum values (Table 1), were well within the range of non-lethal incubation temperatures for the mainland subspecies *P. h. hispanica* (Van Damme *et al.* 1992, D. Bauwens, pers. comm.). Thus, the present observations suggest that very precise mechanisms for the selection of substrate temperatures exist, even within the non-lethal range for incubation.

The daily average substrate temperatures at the laying sites in the thermogradient (25.7 °C) corresponds closely to the mean temperature recorded near a clutch deposited by a field-active female in an artificial nesting site (25.3 °C; Castilla & Swallow, 1995), and was somewhat lower than the temperature recorded in a natural communal nesting site (28.3 °C; Castilla & Bauwens, 1996). Relatively low substrate temperatures prolong the duration of incubation in this and other lizard species (e.g., Packard & Packard, 1988; Van Damme et al., 1992; Castilla & Swallow, 1996). However, eggs incubated at relatively low temperatures produce larger-sized hatchlings than those kept at higher temperatures (e.g., Beuchat, 1988; Phillips *et al.*, 1990; Van Damme *et al.*, 1992; Phillips & Packard, 1994). In the closely related *P. h. hispanica*, a constant incubation temperature of 26 °C maximises hatchling size as compared to temperatures of 29 °C and 32 °C (Van Dijck, 1993). Being big at hatching may have positive fitness consequences (Van Damme *et al.*, 1992 and references therein), especially in the populations of *P. hispanica. atrata* where cannibalism appears to constitute an important cause of egg and juvenile mortality (Castilla, 1995; Castilla & Van Damme, in press).

Several factors may influence a female's decision to deposit its eggs at a given site (i.e., a variety of physical factors, protection of eggs and females from predation, etc.), but the design of the present study did not allow identification of these. The results only suggest that substrate temperature is an important physical factor guiding females in the selection of a laying site. Nevertheless, more research is needed in this field. In particular, integrated field and laboratory studies that examine the effect of both substrate temperature and soil water content on the duration of incubation, hatching success, morphological characteristics and physiological performances of juveniles are needed to enhance our understanding of the relevance of incubation environments and female parental care. In addition, the distribution of some lizard species is apparently limited by the combinations of soil temperature and water content that allow successful egg incubation (Porter & Tracy, 1983). Thus, studies on the characteristics of nest environments will also help to understand the geographical distribution of lizards.

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